A PHOTOMETRIC METHOD FOR ESTIMATING THE OXYGEN SATURATION OF SMALL BLOOD SAMPLES

BY

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Since Kramer (1933) investigated the optical properties of hemoglobin/oxyhemoglobin solutions, many methods have been proposed for the photometric estimation of the oxygen saturation of blood. A new method can only be advocated if it offers economy of apparatus or reagents, greater ease of performance, or the elimination of sources of error. It is upon these grounds that the following method is proposed for consideration.

METHOD

(1) COLORIMETER AND CUVETTES. Any simple photoelectric colorimeter that employs small cylindrical cuvettes of about 0·5/0·6 cm. internal diameter could be used. We have used the “EEL” portable colorimeter with the “EEL” Filter No. 205 (6500° A).

As will be explained, hemolyzed but minimally diluted blood is used for the estimation and if the cuvette was filled with this solution, its optical density would be too great for evaluation in a simple colorimeter. The optical thickness of the cylindrical cuvette (“EEL” No. 305) is therefore reduced by inserting a glass rod of 5·32/5·40 mm. maximum diameter and with ovality of not more than 0·15 mm.

The rod is located centrally in the cuvette by (a) grinding its lower end flat, and (b) wrapping around its upper end waterproof self-adhesive tape to form a sleeve which fits snugly into the cuvette (Fig. 1).

We obtained the glass rod by purchasing Pyrex rod of nominal 5–6 mm. diameter. From the delivery, a considerable quantity of suitable rod was selected, using a micrometer. After the rods had been cut and prepared they were indexed as follows: About ½ ml. of a light blue solution (1:80 dilution of Waterman’s Royal Blue Ink) is put into one of the cuvettes and a rod inserted. The rod is slowly rotated while watching the meter. The meter reading varies, but over a portion of the circle considerable angular rotation of the rod produces only small changes in the meter reading. With the rod in this position, a mark is made upon it to line up with the locating mark on the cuvette tube. Thereafter, the rod is always lined up in this way whenever it is used. The purpose of this adjustment is to make sure that errors in the plane of the rod will have a minimal effect upon the accuracy of readings.

A final check upon the satisfactory size of the glass rod should be made with blood. A fully reduced hemolyzed specimen of blood of normal hemoglobin concentration is prepared as described in a later paragraph. This is put into the cuvette and the rod inserted. The meter reading should lie between 84 and 91 on the logarithmic scale. The purpose of this selection is to ensure that blood containing up to 110 per cent of hemoglobin can conveniently be estimated with minimal dilution.

It is convenient to provide 3 rods for each set of 12 tubes, one tube and rod are kept filled with distilled water for zero setting, one rod is in use, and one is held as a spare.

The cuvette tubes must fit snugly and firmly in the holder of the colorimeter. To achieve this, some form of spring clip has to be fashioned to press the cuvette against one side of the holder. The holder for the “EEL” colorimeter is an ebonite...
rod drilled axially to accept the cuvette tube and with slots through which the light passes. Regarding the top of this holder as a clock face and the light path as passing through 6 to 12 o'clock, we drilled two small holes downwards into the holder at 3 and 9 o'clock. Into these holes were dropped the limbs of an inverted U of spring wire so that the centre of the U stood about \( \frac{1}{2} \) cm. above the top of the holder. The U must be thrust a little aside when the tube is inserted and it then holds the tube firmly.

(2) **Haemolyzing Syringe.** A 1-ml. long tuberculin syringe is used and it contains a stainless steel ball about 5/32 in. in diameter. A horseshoe pocket magnet (Eclipse) is used to move the ball around within the syringe in order to mix its contents quickly.

**Reagents.**

1. A synthetic detergent solution is used to secure haemolysis. Probably many detergents would serve but we have employed undiluted "Lissapol."

2. Sodium hydrosulphide is used to secure complete reduction of the haemoglobin solution. Hydrosulphide seems to be an impermanent substance and it should be stored either in sealed ampoules or in small, well-filled and well-closed tubes.

3. Heparin has been used to prevent coagulation of the blood sample which is stored, anaerobically, in a capped syringe.

**Procedure.**

1. Draw into the haemolyzing syringe a little detergent solution, expel all air bubbles and push the plunger down until it is stopped by the stainless steel ball. The dead space of the syringe and its hub are now filled with detergent.

2. Ensure that the blood sample is well mixed, expel the blood held in the hub of the sample syringe, and join the sample syringe to the haemolyzing syringe with a very short piece of elastic tubing.

3. Drive blood gently into the haemolyzing syringe until the plunger stands at the 0.8-ml. mark.

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**Fig. 2.**—Comparison of results of oxygen saturation with simultaneous determinations by Exton's method.
(4) Disconnect the haemolyzing syringe and, with the magnet, move the steel ball briskly for 15 to 30 seconds to secure haemolysis. The haemoglobin solution will become quite clear.

(5) Expel 0-1 ml. from the haemolyzing syringe to waste in order to clear the hub of unhaemolyzed blood, and attach a blunted needle 10 cm. long.

(6) Expel the haemolyzed blood gently into the cuvette holding the tip of the needle on the bottom of the cuvette. The haemoglobin solution will become quite clear.

(7) Set the instrument to zero using a similar rod and cuvette containing distilled water. Place the sample cuvette and rod into the instrument and take a reading $R_b$.

(8) Withdraw the glass rod from the cuvette and wipe it clean. Place a finger over the top of the cuvette and shake it in a horizontal position for one minute. This suffices fully to oxygenate this small quantity of haemoglobin solution.

(9) Restore the glass rod and take a second reading in the colorimeter $R_o$.

(10) Spread a little hydrosulphide in a thin but continuous layer on a tile. Remove the glass rod and touch its moist flat end upon the hydrosulphide so that crystals adhere to the flat surface. Return the rod to the cuvette and work it up and down for 15 seconds. This suffices fully to reduce the haemoglobin solution.

(11) Wait half a minute to allow any bubbles to come to the surface, and return the cuvette to the colorimeter. Take a third reading $R_h$. If the blood sample contains more than 110 per cent haemoglobin, it must be diluted slightly. This is accomplished by completing step (1) and then drawing boiled cooled distilled water into the syringe to the 0-3-ml. mark. Thereafter, proceed as described. This small extra dilution serves to bring within the operating range of the colorimeter blood containing up to 150–160 per cent of normal haemoglobin.

**Calculations**

The colorimeter we employ has a logarithmic scale so that readings may be taken directly from it. The calculation is then as follows:

$$\text{Percentage oxygen saturation} = 100 \times \frac{R_h - R_b}{R_h - R_o}$$

Alternatively, the mechanical nomograph described in the Appendix may be used (Fig. 3).

![Fig. 3.—Method of construction of nomograph for calculating the percentage oxygen saturation.](http://heart.bmj.com/)

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DISCUSSION

We have examined the accuracy of the method by comparing its results with simultaneous determinations of oxygen saturation of blood using Exton's method (1945), slightly modified for convenient operation (Pask, 1953). The results of the comparison are presented in Fig. 2.

Blood samples were drawn from the veins of five subjects after varying periods of occlusion and at various temperatures. There was thus considerable variation both in haemoglobin and oxygen content.

The rather good agreement of figures for low oxygen saturations is noticeable since this is not always achieved by photometric methods. The fact that the line drawn through most experimental points also passes through 0 and 100 per cent on both scales, is additional confirmation that the two methods agree in the low range as well as at higher percentage saturations.

The advantage of this method seem to us to be that it is easy and rapid to perform. An estimation can be completed in four minutes. The apparatus needed is not expensive and quite small blood samples can be used.

The blood is only slightly diluted, even when high haemoglobin concentrations are encountered. The effect on the oxygen content of the added diluent is therefore very small and no attempt need be made to correct for the change in saturation that might result from a considerable dilution such as is used in some other methods.

The three readings necessary for the construction of a calibration line for a particular sample, are all obtained in the same cuvette on the same sample within a short space of time. Thus, no assumptions need be made about the slope of this line.

In common with all methods employing haemolysis, variations in corpuscle size and shape do not affect the results nor is sedimentation a source of difficulty.

We are grateful to Evans Electroselenium Ltd., of Harlow, for their assistance in developing this method. Readers who wish to purchase suitable colorimeter tubes and glass rods should make enquiry of this firm.

REFERENCES


APPENDIX

A Simple Nomograph. A piece of plywood has drawn on it a scale "A" graduated 0–100 per cent. From each end of this scale lines are drawn which meet at "D1," the centre for a movable perspex cursor "C." Scale "B" is the same length as scale "A" but can be moved laterally and vertically (Fig. 3).

Operation. Scale "B" is adjusted so that while it is parallel to scale "A," Ro lies on line (a) while at the same time Rh lies on line (b). The cursor line is set to Rh on scale "B" when the percentage saturation is read from the cursor line on scale "A."
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