

TECHNICAL METHODS

A Spectrophotometric Method of Estimating Blood Oxygen Using the Unicam SP 600

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Accurate estimations of percentage oxygen saturation and oxygen capacity are frequently needed in research and routine investigation of patients suffering from diseases of the heart and lungs. Blood gas analysis for this purpose is time consuming and requires the services of a highly skilled technician. Further, the methods need to be in constant use if reliable results are to be obtained. Accurate results require duplicate or triplicate estimations.

The increasing use of cardiac catheterization in diagnosing congenital heart disease has created a need for a simple and reliable method for rapid estimations. A number of authors have described spectrophotometric methods designed to suit different types of instrument (Nahas, 1951; Wade, Bishop, Cumming, and Donald, 1953; Stainsby, Fales, and Lilienthal, 1955). In the present paper we describe a modification of the method of Holling, MacDonald, O'Halloran, and Venner (1955) suitable for the "unicam" SP 600. Individual determinations can be made in less than two minutes and the method is suitable for use in the dark at catheterization. It thus provides a simple and reliable technique for estimations of oxygen saturation on blood samples during the course of catheterization.

Methods

Blood gas analysis was performed by the method of Van Slyke and Neill (1924). Duplicate estimations of the oxygen content were made on each sample. Where a number of samples was taken the oxygen capacity was measured on one sample. A cuvette similar to that described by Holling *et al.* was constructed out of heavy gauge brass sheeting and $\frac{1}{4}$ in. perspex sheet. Two cells were constructed by making suitable holes in an "alkathene" sheet 5/1,000 in. thick (Fig. 1). The cell was mounted rigidly beneath a carriage similar to that used for the standard liquid cells. Filling and drainage tubes connected each cell to the upper surface of the carriage, the filling tube ending in a Luer socket and the drainage tube in a convenient bevel (Fig. 2). When the carriage was

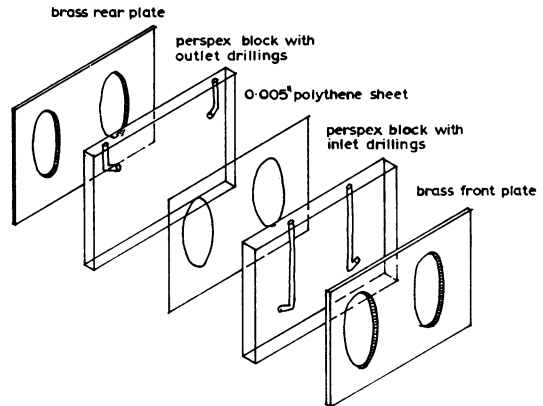


FIG. 1.—Exploded view to show construction of cell. The depth of the chamber in which the blood is contained is determined by the thickness of the polythene sheet. The fixing screws have been omitted from the drawing.

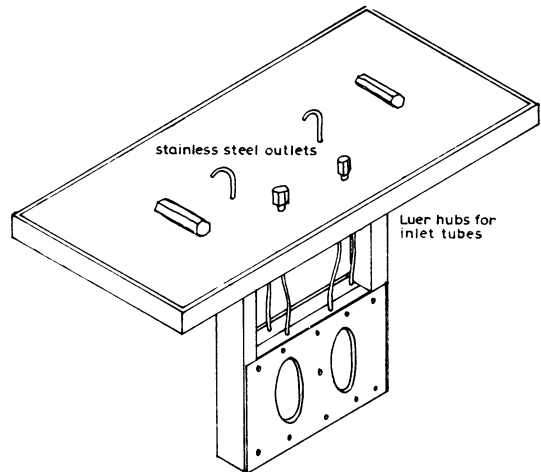


FIG. 2.—The chamber attached to the carriage. Only a few of the fixing screws are shown.

placed in position on the instrument the cells coincided with the light beam of the absorptiometer. Blood for estimation by blood-gas analysis and by spectrophotometry was taken into 10 ml. syringes suitably greased containing a small quantity of liquid heparin. Mercury was introduced into the syringe, which was then capped and the blood and heparin mixed by agitating the mercury. The samples were kept cold until estimations were made. Blood gas analysis by the Van Slyke method was made by using 2 ml. of blood for each estimation. All estimations were made in duplicate, and, in those in which agree-

ment was not within 3%, a third estimation was made. For the spectrophotometric estimation a 1 ml. syringe was first rinsed with a freshly prepared saturated solution of white heparin and the dead space filled with this solution. A short length of alkathene tubing (about 3 mm.) was passed over the nozzle of the Luer syringe containing the blood and the 1 ml. syringe fitted on to the other end of the alkathene tube. Approximately 1 ml. of blood was then passed into the small syringe and a small quantity of mercury added. The saponin solution and the blood were then mixed by agitation. Haemolysis was usually complete in 15 to 20 seconds and the haemolysed blood solution was introduced into the cuvette through the lower socket in the top of the carriage. It was found most convenient to remove the carriage from the spectrophotometer and place it horizontally against a piece of white paper to ensure that no large bubbles were left in the cuvette. The cuvette not filled with blood was filled with 0.4% ammonia solution as a reference cell. The optical density was then measured at 480 m μ and 506 m μ and the percentage oxygen saturation calculated by the method described by Holling.

%saturation =

$$\frac{x \text{ unknown sample} - \text{reduced haemoglobin}}{x \text{ oxyhaemoglobin} - x \text{ reduced haemoglobin}} \times 100$$

where x is the ratio $\frac{\text{optical density at } 480 \text{ m}\mu}{\text{optical density at } 506 \text{ m}\mu}$

The optical densities of reduced haemoglobin and oxyhaemoglobin need only be calculated for a single sample of blood as this ratio is independent of the haemoglobin concentration. It follows that in the equation for calculating the percentage saturation only the ratio of the unknown sample is a variable and the remaining terms are constants. It is therefore possible to simplify the calculation by constructing a nomogram relating the ratio for the unknown sample of optical density at 480 m μ to the optical density at 506 m μ to its percentage oxygen saturation. This is shown in Fig. 3.

After use the instrument is cleaned by rinsing through with 0.4% ammonia solution which may be left in the cuvettes. Should it be found necessary to dismantle the cuvette for cleaning, the perspex surfaces should be wiped with lens paper moistened with ethanol. The presence of one or two minute bubbles in the blood contained by the cuvette does not disturb the estimations. Small particles of mercury should be removed by rapid flushing with ammonia solution. Blood clots render the estimations unreliable and may necessitate dismantling and cleaning. They should not occur if the saponin and blood are adequately mixed.

Results

The absorption curves for reduced haemoglobin and oxyhaemoglobin were first determined in the cuvette. Using two different samples of blood no significant difference from the curves shown by Holling *et al.* was found.

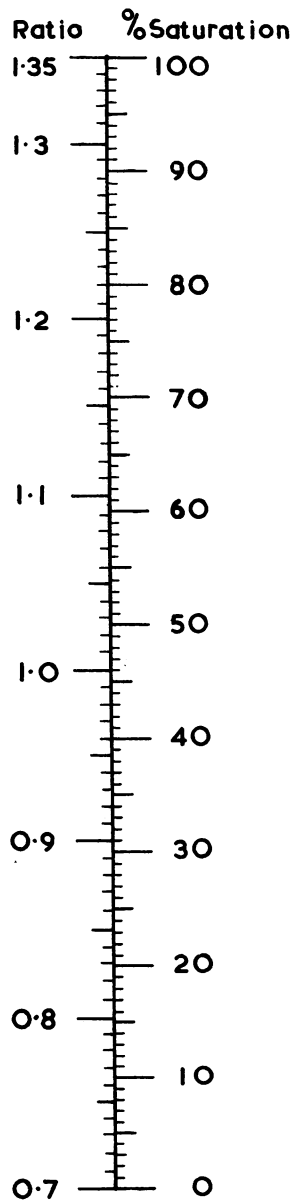


FIG. 3.—Nomogram relating percentage saturation to x, the ratio of the optical density of the unknown sample at 480 m μ and 506 m μ . This ratio is first calculated with a slide rule and the percentage saturation read from the nomogram; e.g., a ratio of 1.09 indicates an oxygen saturation of 60%.

Simultaneous estimations of percentage oxygen saturation were made on 50 samples of blood taken during cardiac catheterization using the Van Slyke and spectrophotometric methods. In three cases the results were discarded because the samples were too small to permit duplicate Van Slyke measurements. The results are shown in Fig. 4. The majority of the results agreed closely.

Those in which a divergence was found from the Van Slyke estimations had been unsatisfactory because only a small quantity of blood was obtained so that the calculation of percentage saturation

TABLE I

Percentage Difference between Estimates	Number of Estimations
0	10
1	6
2	15
3	5
4	4
5	3
6	0
7	1
8	1
9	2
Total	47

had been made on the oxygen capacity of another sample taken during the same catheterization. The percentage difference between the results obtained by Van Slyke and the SP 600 are shown in Table I. In 31 cases the difference was 2% or less and in only four was it greater than 5%.

The injection of coloured dyes (Evans blue, Coomassie blue, and Fox green) in the course of cardiac catheterization is becoming an increasingly common practice. It seemed desirable therefore to investigate the effects of concentrations of these dyes upon the estimate of percentage saturation with the SP 600. Coomassie blue was added to whole blood in varying concentrations up to one part of 4% solution in 250 parts of blood. This is equivalent to the injection of 20 ml. of 4% solution into an average human subject. Although there was a marked increase in optical density of the blood the determination of percentage saturation was unaffected. Similar results were obtained with Evans blue and Fox green.

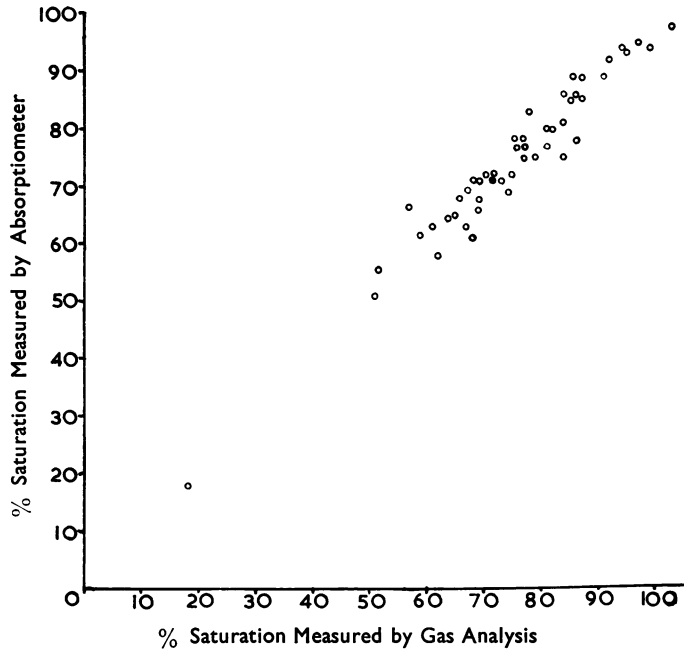


FIG. 4.—Graph relating the estimations of percentage saturation by absorptiometry and by gas analysis.

Conclusion

The method described here provides a rapid and convenient means of determining percentage oxygen saturation using a reliable spectrophotometer which is available in most laboratories. This has been shown to be unaffected by the addition of coloured dyes to the blood and is suitable for immediate use during cardiac catheterization.

In subsequent comparisons of the two techniques 29 samples were tested. Of these, 16 gave identical results in both methods, seven showed a 1% difference, four a 2%, and two gave a 3% difference.

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