

## Photometric Determination of O<sub>2</sub> Concentration

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### *Introduction*

The diagnostic value of measuring arterial O<sub>2</sub> concentration (O<sub>2</sub> content) (caO<sub>2</sub>), a universal value that includes all changes in partial pressure, saturation and Hb concentration, is in strong contrast to the actual methods currently available. Both Van Slyke's classical manometric procedure and the use of galvanic cells to determine the blood O<sub>2</sub> concentration remains the province of special laboratories, since the analysis requires special technical knowledge, complicated equipment and a relatively large sample volume.

A simple and portable procedure with a high degree of accuracy and precision will be described here, which allows fast measurement of the O<sub>2</sub> concentration (cO<sub>2</sub>, ml/dl) of the blood in everyday practice, using only 20 µl of blood. If the Hb concentration (cHb) is also determined with the same system, then a complete analysis of the O<sub>2</sub> status can be performed, i.e. determination of cO<sub>2</sub> (ml/dl), cHb (g/dl) and calculation of the O<sub>2</sub> saturation (sO<sub>2</sub>, %).

### *O<sub>2</sub> Cuvette*

The procedure, called the O<sub>2</sub> cuvette by the original authors [2], is based on a photometric O<sub>2</sub> determination, in which the oxygen reacts quantitatively with a specific and highly sensitive reagent (alkaline solution of catechol with iron ions), causing a clearly visible change in color

which can be recorded with a photometer as an increase in absorbance (broad absorbance maximum). The prototype of the O<sub>2</sub> cuvette was a disposable cuvette made of glass which was filled with the liquid reagent after exclusion of air and sealed with a membrane.

The procedure was simple and is described in detail in [3]. The sample to be investigated was measured with a precision syringe and injected into the disposable cuvette through the membrane. The change in absorbance was obtained by measurement with a photometer before and after the injection. In the case of blood a blank had to be taken into account. To this end, the same sample volume was injected into a second cuvette containing a liquid that does not form a color with oxygen (sodium dithionite solution). The calibration of the O<sub>2</sub> cuvette (described in detail elsewhere in this volume) was performed by injecting a gravimetrically adjusted solution of KIO<sub>3</sub> of defined O<sub>2</sub> content.

The O<sub>2</sub> cuvette procedure has been tested in detail [3]. For gas samples (air, 20.95% O<sub>2</sub>) a strictly linear relationship between the absorbance and the amount of O<sub>2</sub> in the cuvette was found up to an absorbance value of 2.0. For blood samples with different Hb concentrations, the O<sub>2</sub> concentration was also determined after equilibration with 100% N<sub>2</sub> or O<sub>2</sub>.

Within a concentration range of between 0 and 29 ml/dl a strictly linear relationship was found between the measured and calculated O<sub>2</sub> concentrations. In the middle (physiological) O<sub>2</sub> concentration range the O<sub>2</sub> concentration of the blood could be measured with an accuracy of 2% and a reproducibility of 2%.

The same results with regard to accuracy and reproducibility were obtained in a methodological study by Willis and Clapham [1].

Because of the extremely low limit of detection for oxygen – the O<sub>2</sub> cuvette procedure described here can detect 0.02 μl O<sub>2</sub> ( $\cong$  1 nmol) – this procedure can be considered to have the highest sensitivity to O<sub>2</sub> of all methods currently available. Thus a modification would be feasible, for example, which would allow measurement of the O<sub>2</sub> concentration in a 2 μl blood sample [4].

### *Dosing Attachment*

As a further simplification of the O<sub>2</sub> cuvette procedure, in which the blood sample had to be measured out and injected with a precision syringe, a special dosing attachment was developed by Wolf and Zander

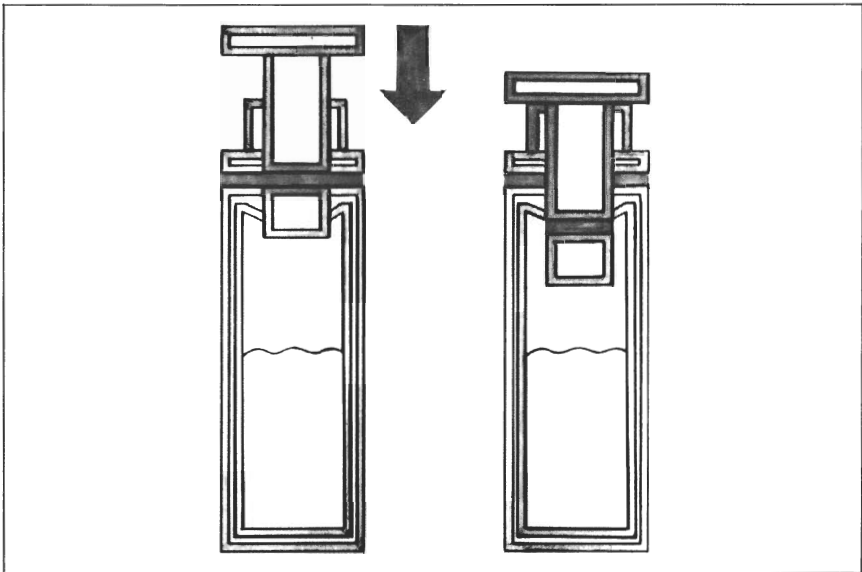
(unpublished data) that can be affixed to a rectangular disposable cuvette. The principle of this dosing attachment is shown schematically in figure 1.

About 20  $\mu\text{l}$  of blood (from the ear lobes or fingertips), i.e., a drop of blood, fill the lumen of the dosing attachment by capillary action. By displacing a piston, exactly 10  $\mu\text{l}$  are measured and inserted, with the exclusion of air, into the inner space of the cuvette and thus brought into contact with the liquid reagent.

The use of the cuvette is thus simplified as follows: application of a drop of blood, absorbance measurement, displacement of a piston, shaking, second absorbance measurement.

### *Oxystat Procedure*

The logical extension of the described  $\text{O}_2$  cuvette procedure with a dosing attachment is to measure the Hb concentration with a second



*Fig. 1.* Schematic presentation of a dosing attachment affixed to a disposable cuvette according to Wolf and Zander. A dose of exactly 10  $\mu\text{l}$  of blood takes place into the cuvette with the exclusion of air, when capillary force causes blood (about 20  $\mu\text{l}$ ) to enter the lumen, the center of which, a moveable piston, can be displaced. Tipping the cuvette then washes the contents of the lumen into the liquid reagent.

cuvette, in addition to the O<sub>2</sub> concentration (for example, using alkaline hematin D-575). This second, Hb cuvette renders the blank cuvette superfluous. It means that from only  $2 \times 20\mu\text{l}$  of blood ( $10\mu\text{l}$  serve to seal off the air and are rejected) and using 2 disposable cuvettes (O<sub>2</sub> cuvette and Hb cuvette) with a dosing attachment, the following values for the O<sub>2</sub> status are obtained:

O<sub>2</sub> concentration (cO<sub>2</sub>, ml/dl),  
Hb concentration (cHb, g/dl), and  
O<sub>2</sub> saturation (sO<sub>2</sub>, %, calculated).

Using a photometer with a built-in calculator (fig. 2) the data for O<sub>2</sub> status can be calculated quickly and accurately from the measured absorbance difference (only one wavelength) and recorded.

Since a crystalline standard exists for both procedures, the O<sub>2</sub> cuvette and the Hb cuvette, which allows gravimetric calibration, an accuracy of

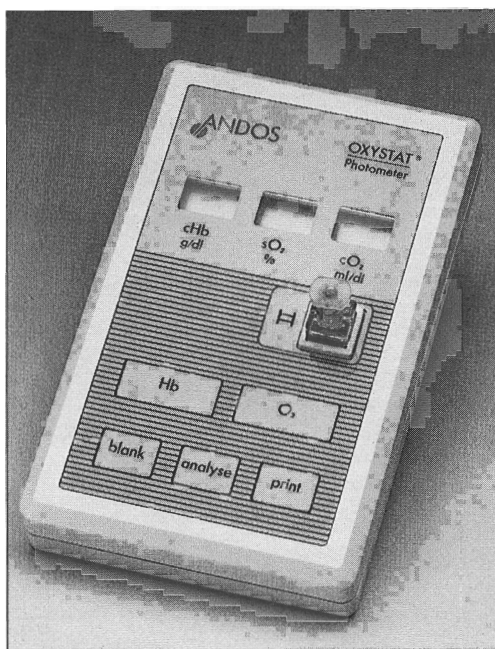


Fig. 2. The Oxystat™ photometer. A battery-operated mini-photometer for measuring the absorbance difference of a Hb and an O<sub>2</sub> cuvette, before and after injection of  $10\mu\text{l}$  of blood in each case. A built-in calculator gives the Hb concentration (g/dl), the O<sub>2</sub> concentration (ml/dl) and calculates the O<sub>2</sub> saturation (%) from these two values.

2% is obtained in both procedures. Examination of this procedure led to positive results [1].

Using the Oxystat procedure [5], consisting of disposable cuvettes (for O<sub>2</sub> and Hb) with a dosing attachment and a battery-operated miniphotometer with built-in calculator, all the necessary data for the O<sub>2</sub> status (cO<sub>2</sub>, cHb, sO<sub>2</sub>) can be obtained quickly and simply anywhere with an accuracy and reproducibility of  $\pm 3\%$ .

The user can perform an optimal quality control of the entire procedure – namely, cuvette, dosing system, photometer and calculator – using concentrated solutions (Hb, O<sub>2</sub>) of defined composition, which are treated the same way as blood. This way the quality is optimally guaranteed.

Since only 30–40  $\mu\text{l}$  (1–2 drops) of blood are required for a complete analysis, the puncturing of arteries is unnecessary. With a few exceptions (extreme drop in blood pressure), the arterial blood can be obtained from the ‘arterialized’ ear lobes.

### *Summary*

The O<sub>2</sub> cuvette procedure, a highly sensitive photometric oxygen determination using disposable cuvettes, allows a quick, simple and portable measurement of the O<sub>2</sub> concentration of blood with a sample volume of only 20  $\mu\text{l}$  and an accuracy and reproducibility of 2%.

Using two disposable cuvettes, an O<sub>2</sub> cuvette and a Hb cuvette, the following data on the O<sub>2</sub> status can be obtained from only 40  $\mu\text{l}$  of blood: cO<sub>2</sub> (ml/dl), cHb (g/dl) and sO<sub>2</sub> (%). A special dosing attachment connected to the disposable cuvettes, simplifies the procedure considerably.

With the aid of the Oxystat procedure, all the necessary data on the arterial O<sub>2</sub> status can be obtained anywhere simply and within a very short time with an accuracy and reproducibility of  $\pm 3\%$ .

### *References*

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