

MICROSPECTROPHOTOMETRIC DETERMINATION OF THE HAEMOGLOBIN OXYGEN SATURATION IN HAEMOLYZED ARTERIALIZED CAPILLARY BLOOD

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SUMMARY

A description is given of a rapid spectrophotometric micromethod of determining the oxygen saturation in arterialized capillary blood. During sampling, the blood was haemolyzed in a glass capillary with the aid of the haemolytic agent Sterox SE.

Extinctions were measured at 795 nm and 655 nm in a cuvette made up of slides ($d = 1$ mm). A single determination is sufficiently exact. Capillary blood from the arterialized earlobe was found to be an adequate substitute for blood from an artery. This method showed good agreement with the haemoreflexor method.

INTRODUCTION

For diagnostic purposes, *e.g.*, heart catheterization, exercise tests and for the correction of the determination of the acid-base balance, rapid and simple determination of the oxygen saturation of the blood is an important requirement.

DRABKIN AND SCHMIDT¹ developed a spectrophotometric four-wavelength method of determining oxygen saturation, which is exact from a theoretical point of view but technically too complicated for routine use. Many publications have since been presented in which modifications and simplifications of method were described. Reviews on this subject were published by REFSUM AND SVEINSSON² and by GEUBELLE³.

In studies involving repeated determinations of oxygen saturation, it is useful that small quantities of blood should be sufficient for determination, so that the blood loss can be limited (particularly in children), and that capillary blood can be used to avoid arterial puncture.

SIGGAARD ANDERSEN, JØRGENSEN AND NERAN⁴ developed a spectrophotometric micromethod for capillary blood, by which the O₂ saturation is determined in erythrocytes haemolyzed by freezing and thawing, rather than in haemolyzed whole blood.

In selecting such a method, it seemed simpler to haemolyze whole blood directly while obtaining it. To ensure a more exact result, we used the spectrophotometric isosbestic point method with wavelengths 795 nm and 655 nm (GEUBELLE³; GORDY AND DRABKIN⁵). A special cuvette with a 1-mm path length was designed for this

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purpose. To verify the reliability of the method, we compared the oxygen saturation of arterial blood with that of capillary blood. In addition, we compared the method with the haemoreflector method of ZIJLSTRA⁶ employed at our clinic.

MATERIALS AND METHOD

Principle

Analogous to the procedure of GEUBELLE³ and GORDY AND DRABKIN⁵, we determined the oxygen saturation of blood haemolysed with the detergent Sterox SE, by the isosbestic point method of NAHAS⁷ from the extinction (D) measured at an isosbestic wavelength (795 nm) and that at a wavelength at which the light absorption of reduced haemoglobin far exceeds that of oxygenated haemoglobin (655 nm). For the oxygen saturation of the blood (S) we then obtain the equation:

$$S = a \frac{D_{655}}{D_{795}} + b, \quad (1)$$

in which a and b are constants.

Reagents and apparatus

Trafuril paste (Ciba) and plasticine. A buffered heparin Sterox SE solution, pH \approx 7.4 (1 ml heparin solution 5000 I.U./ml; 20 ml Sterox SE (Hartman Leddon Co., Philadelphia, Pa., USA); 0.949 g $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ and 0.182 g KH_2PO_4 , with distilled water up to 100 ml).

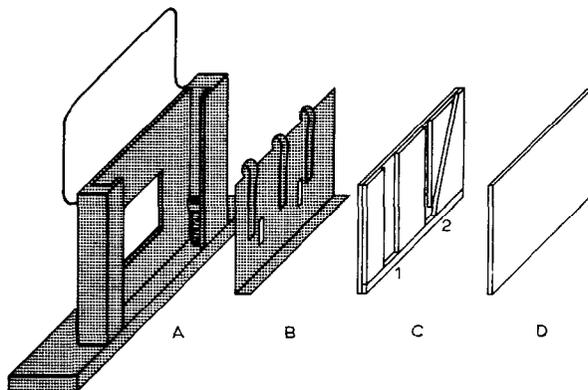


Fig. 1. Microcuvette with holder for Beckman model DU spectrophotometer.

Clean glass capillary tubes (length \approx 12 cm, capacity \approx 70 μl), with fitting fragments of steel wire (length 1.5 cm) and a magnet (Radiometer, Copenhagen). A Beckman model DU spectrophotometer.

A microcuvette*, path length 1 mm, made up of three flat slides (each 1 mm thick) (Fig. 1).

The parts of one slide are glued onto the other in such a way as to form two

* Special microcuvettes (3 \times 1 \times 25 mm, No. 105-OS), which can be used for each spectrophotometer are available from Hellma (Freibourg, Germany).

lumina, *viz.* C₁ (chamber for reference fluid, water) measuring 5 × 1 × 22 mm, and C₂ (filling canal ø 1 mm and chamber for measuring fluid), of 2 × 1 × 22 mm size.

For closure we used slide D which, with the aid of copper clamp B, was firmly pressed onto C, the contact surfaces of which had been greased with a thin layer of silicone grease. The cuvette fits in holder A, which for the Beckman spectrophotometer was designed by Siggaard Andersen (Dansk Laboratorie Udstyr, Copenhagen).

Tonometer equipment according to VON LAUE⁸ (Eschweiler, Kiel).

Procedure

Blood sampling with haemolysis. The inside wall of two glass capillaries was moistened with Sterox SE buffer solution by repeatedly filling and emptying with the aid of a rubber tube. The earlobe was then cleaned with alcohol, and hyperaemia was produced by rubbing with Trafuril paste. The paste was allowed to exert its influence for at least 10 min., after which the remnants were removed with alcohol. After puncture with a snapper and wiping off the first drop of blood, a capillary was held into the next drop and gently pressed against the earlobe. The capillary was filled by slight suction with the rubber tube. This tube was then removed, and the capillary was sealed on this end with plasticine. On the other end, a small nail was inserted into the capillary, which was then also sealed with plasticine. With the aid of the magnet, the nail was carefully moved up and down until haemolysis was complete.

Arterial blood from the brachial artery was sampled as described by MAAS AND VAN HEIJST⁹. Heparinized venous blood was used in making the calibration line.

Measurement of the oxygen saturation. The Beckman model DU spectrophotometer was adjusted on the red photo-tube, slit 0.03 and wavelengths 655 nm and 795 nm, respectively. With a glass knife, the two plasticine seals were cut off the capillary, and the nail was removed with the aid of the magnet. The measuring chamber of the cuvette (C₂) was then filled with the haemolysate by placing the capillary on the filling canal in vertical position, allowing it to empty. Chamber C₁ was filled with distilled water. At wavelength 655 nm and 795 nm, respectively, the extinctions relative to distilled water were determined. After completion of measurements the cuvette was washed out with distilled water and air-dried by suction.

Calculation. For the determination of the constants *a* and *b* (eqn. (1)), 2 ml heparinized venous blood from 15 different fasting patients was equilibrated at 38° (1 h) with water vapour-saturated gas mixtures of composition 5% CO₂, 95% O₂, and 5% CO₂, 95% N₂ (oxygen-free). After this, 0.2 ml Sterox buffer solution (air-free) was added. For the extinction ratio $\frac{(D_{655})}{(D_{795})_{\text{HbO}_2}}$, we found as a mean of 15 determinations 3.898

(S.D. = 0.016); for the extinction ratio $\frac{(D_{655})}{(D_{795})_{\text{HbO}_2}}$ we found 0.437 (S.D. = 0.010).

From this we calculated the calibration line for a blood sample:

$$S = -28.89 \frac{(D_{655})}{(D_{795})} + 112.61 \quad (2)$$

TABLE I

OXYGEN SATURATION VALUES OF BLOOD SIMULTANEOUSLY OBTAINED FROM THE BRACHIAL ARTERY AND EARLOBE, AS MEASURED BY THE MICROMETHOD DESCRIBED AND BY THE HAEMOREFLECTOR METHOD

No.	Percentual oxygen saturation			Difference in percentual oxygen saturation				
	Blood from the earlobe			Blood from the brachial artery		of the duplicate determination of blood from the earlobe a - b	of blood from the earlobe - brachial artery Micromethod (1) - (2)	of blood from the brachial artery Micromethod (2) - Haemo (3)
	Micromethod	(1) mean		Micro-method (2)	Haemo-reflector (3)			
a	b							
1	96.5	96.6	96.5	96.4	97.5	-0.1	+0.1	-1.1
2	83.7	83.5	83.6	82.8	83.0	+0.2	+0.8	-0.2
3	98.5	98.5	98.5	98.6	98.0	0.0	-0.1	+0.6
4	95.9	96.3	96.1	96.2	97.0	-0.4	-0.1	-0.8
5	92.8	92.9	92.8	92.9	94.5	-0.1	-0.1	-1.6
6	93.0	93.0	93.0	91.8	94.0	0.0	+1.2	-2.2
7	96.8	96.8	96.8	97.1	97.5	0.0	-0.3	-0.4
8	93.1	93.1	93.1	94.4	95.5	0.0	-1.3	-1.1
9	96.2	96.2	96.2	96.3	95.5	0.0	-0.1	+0.8
10	94.5	94.6	94.6	94.6	94.5	-0.1	0.0	+0.1
11	98.3	—	98.3	99.2	98.5	—	-0.9	+0.7
12	84.7	—	84.7	83.7	81.5	—	-1.0	+2.2
13	94.7	94.5	94.6	95.9	95.5	+0.2	-1.3	+0.4
14	94.3	—	94.3	94.3	93.5	—	0.0	+0.8
15	96.7	96.8	96.8	96.3	97.5	-0.1	+0.5	-1.2
16	92.8	92.4	92.6	93.9	95.5	+0.4	-1.3	-1.6
17	95.7	95.8	95.8	94.8	97.0	-0.1	+1.0	-2.2
18	97.9	98.0	98.0	96.6	95.0	-0.1	+1.4	+1.6
19	95.9	95.7	95.8	96.9	96.2	+0.2	-1.1	+0.7
20	94.9	94.9	94.9	94.2	96.5	0.0	+0.7	-2.3

$\bar{X} = 0.0$ $\bar{X} = -0.1$ $\bar{X} = -0.34$
S.D. = 0.16 *S.D.* = 0.8 *S.D.* = 1.31

RESULTS

Blood was taken simultaneously from the earlobe and the brachial artery of 20 patients, and the oxygen saturation of both samples was spectrophotometrically determined. In addition, blood from the brachial artery was used in comparative measurements with the haemoreflector. The results are presented in Table I.

DISCUSSION

Our method is a modification of the method of GEUBELLE³ and GORDY AND DRABKIN⁵, who haemolysed whole blood with saponin, and measured at wavelengths 650 nm and 805 nm. Our experience has shown that it is difficult to ensure complete haemolysis of blood in capillaries with saponin.

DEIBLER¹⁰, therefore, used Triton-X as haemolytic agent in his oxygen-saturation determinations. We have examined the action of this agent and compared it with that of the detergent Sterox SE. The ultimate results were identical, but Sterox SE haemolyzed more rapidly and more effectively than Triton-X. The Sterox SE buffer solution used in our study is clear and stable and need not be freshly prepared every day. In order to inhibit displacement of the oxygen dissociation curve as much as possible, we chose a phosphate buffer of pH \approx 7.4 (normal blood pH value). The great advantage of this haemolysis method over the method of freezing and thawing lies

in the fact that haemolysis is effected immediately during blood sampling, which makes this determination a rapid routine analysis.

GEUBELLE³ and GORDY AND DRABKIN⁵ used wavelengths 805 nm (isosbestic point) and 650 nm. From 12 absorption spectrum curves of oxygenated and reduced blood we established the value 797 ± 2 nm for the isosbestic point. Since the monochromator in routine is better adjusted to 795 nm, we chose this value. This point, however, is not critical because the two absorption curves intersect at a narrow angle at this point. The fact that we found a value lower than 805 nm must be ascribed, we think, to the method of reduction and the pH value of the haemolysate. The above mentioned authors reduced with $\text{Na}_2\text{S}_2\text{O}_4$, and worked at pH 8–9. Wavelength 655 nm instead of 650 nm was chosen after FEINBERG¹¹, because this value is nearer the isosbestic point of haemoglobin (Hb) and methaemoglobin (MHb) (657.5 nm). Adjustment to wavelength 655 nm with the monochromator, however, is critical and must be done exactly, because the absorption curves of oxygenated and of reduced haemoglobin are steep at this wavelength. The reliability of the method was studied by comparing the oxygen saturation of arterialized blood from the earlobe with that of blood simultaneously obtained from the brachial artery. We found a difference of 0.1%, which is not significant (*cf.* Table I). The excellent efficiency of Trafuril makes it possible to obtain blood equivalent to arterial blood.

Comparative measurements of arterial blood with the haemoreflexor (reflection method) routinely used at our clinic, showed no essential difference (0.3%).

By this two-wavelength method an apparent oxygen saturation is determined, because the extinctions of MHb and HbCO are also measured. Normally, the concentration of both components in blood is about 0.5%, and consequently this produces a constant error. A detailed discussion of this subject has been presented by GORDY AND DRABKIN⁵. Special attention must be given to smokers among the test subjects (REFSUM¹³).

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