DETERMINATION OF THE OXYGEN CAPACITY OF THE BLOOD AND OXYHEMOGLOBIN DISSOCIATION CURVES BY POLAROGRAPHIC COULOMETRY

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A method of obtaining the specified parameters by connecting Épshtein's coulometric cell to a polarograph is developed, giving automatic recording of current strength – time curves at a constant potential of -1.375 V. Blood was saturated with the corresponding gas mixture and the quantity of electricity and, consequently, the quantity of oxygen in the sample was calculated from the area beneath the recorded coulogram. Equations for the calculations are given. The results of the investigations confirm the accuracy of the method (2-3%). The time for one determination of the blood oxygen concentration is 20-30 min. The second is also suitable for determining the physically dissolved oxygen in fluids, including biological fluids, and also for determining the concentration of inactive (unable to carry oxygen) hemoglobin.

To obtain oxygen dissociation curves (OD) of the blood oxyhemoglobin (HbO_2) it is suggested that the laborious method of mercury gasometric analysis with Van Slyke's apparatus be replaced by the no less accurate polarographic determination of the oxygen concentration in the blood sample. The appropriate mathematical equations for the calculation have been deduced.

Unlike the method described previously [2], Épshtein's coulometric cell [1] is connected to the negative terminal of the polarograph. A type LP-60 (Czechoslovakia) polarograph with automatic recording of current strength–time (I/t) curves was used. In addition, whereas in the method described earlier [2] only one blood sample saturated with air was tested, in order to determine the OD of HbO_2 from five to six blood samples saturated with different gas mixtures at known partial pressures of oxygen (PO_2) from 0 to 160 mm Hg, and with the same partial carbon dioxide pressure (40 mm) in all the saturators, must be analyzed. The gas mixtures are most conveniently prepared, not in the saturators, but in gas cylinders in which they can be stored*. Simultaneously with introduction of the blood sample for testing into the electrolysis cell, and before electrolysis is carried out, measurements are made of the pH of this blood (with an Astrup microelectrode) and the hemoglobin concentration (C_2) by a spectrophotometric method [3, 4].

The oxygen capacity of the blood in milliliters oxygen per 100 ml blood (vol.%) was calculated by the equation deduced previously:

$$OC = \frac{\Sigma I_{bl} - \Sigma I_0}{v} \cdot \frac{c_1}{c_2} \cdot 1.740 \cdot 10^{-4} \text{ vol.\%} \quad (1)$$

In this equation ΣI_{bl} represents the total strength of the current (in μA) produced as the result of electrolysis of the blood, obtained by graphic integration of the area beneath the I/t curve, as described previouly

*The cylinders can be charged with gas mixtures of the desired composition at the Balashikhino (Moscow Region) Oxygen Factory.

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TABLE 1. Comparison of Results of Investigations of Blood Oxygen Saturation by the Proposed (A) and Spectrometric (B) Methods

A (in%)	B (in%)	Δ	%=Δ/B ×100
17,0	17,4	—0,40	—2,3
24,0	23,55	+0,45	+1,9
36,5	36,7	—0,20	—0,55
51,0	51,3	—0,30	—0,6
67,0	67,3	—0,30	—0,45
76,2	75,9	+0,30	+0,4

[2], or by accurate weighing of the paper between the I/t curve and the line corresponding to the residual current, followed by proportional comparison of the weight thus obtained with that of a standard square or rectangle of the same paper of known area (since this area corresponds to a known quantity of electricity I: t); ΣI_0 is the same as ΣI_{bl}, but produced as the result of electrolysis of oxygen dissolved physically in physiological saline saturated with air under the same conditions as the blood; v is the volume of the electrolysis cell (in ml) and, consequently, also the volume of blood undergoing electrolysis (with a correction for the volume of the magnetic mixer); c_1 the hemoglobin concentration in whole blood (in g%); c_2 the working concentration of hemoglobin in the blood after its dilution with 0.85% NaCl solution and saturation with air; the ratio c_1/c_2 in a simplified and less accurate alternative form of the method can be replaced by the degree of dilution of the blood with physiological saline before electrolysis, making spectrophotometric determination of the hemoglobin concentrations in the blood samples to be tested essential; factor $1.740 \cdot 10^{-4}$ is used to convert from the total current strength ΣI_{bl} to the number of milliliters oxygen in 100 ml blood, given by $GMV \cdot 30 \cdot 10^{-6} \cdot 10^2/4F$, where GMV is the gram-molecular volume of a gas ($22.4 \cdot 10^3$ ml) 30 the time interval (in sec) after which the strength of the current is measured on the I/t curve for graphic integration; 10^{-6} is a coefficient for conversion from microcoulombs to coulombs; 10^2 a coefficient for expressing the oxygen capacity (OC) in ml O_2/100 ml blood (i.e., in vol. percent); F is the faraday (96,495 coulombs); 4 F is the quantity of electricity theoretically required for the electrolysis of 4 g-eq of the substance (1 gram-molecular volume of oxygen contains 4 g-eq O_2).

The quantity (vol.%) of oxygen (A_i) bound by equal samples of blood saturated with the corresponding gas mixtures at known PO_2, is calculated by the same equation (1), which now gives A_i and not OC, because the blood sample is not saturated with air.

The degree of saturation (S%) of each blood sample with oxygen is calculated by the equation

$$S\% = (A_i/OC) \cdot 100\%. \quad (2)$$

The value of PO_2 must be reduced to its value at pH 7.4 (for electrolysis of blood with whole erythrocytes) or at pH 2 (for electrolysis of blood after hemolysis, when the erythrocytes are disintegrated:

$$PO_2 \text{ of blood at } \underset{pH=7.4}{} = PO_2 \cdot 10^{-0,48\,(7,4-pH)} \quad (3)$$

$$PO_2 \text{ of hemolyzate} = PO \cdot 10^{-0,48\,(7,2-pH)}, \quad (4)$$

where pH is the value measured before electrolysis and immediately after saturation with the gas mixture.

To construct the OD of HbO_2, percentages are plotted along the ordinate and values of PO_2 at pH 7.4 (for blood) or 7.2 (for hemoglobin solution) are plotted along the abscissa.

The method can also be used to determine the quantity of "inactive" (unable to carry oxygen) hemoglobin from the ratio between the oxygen capacities determined polarographically (OC_p) and spectrophotometrically (OC_S):

$$C_{\text{inact.Hb}} = c_1 \cdot (1 - OC_p/OC_S)\,g/100\text{ ml}, \quad (5)$$

where $OC_S = c_1 \cdot 1.39$.

Results of parallel investigations of the degree of oxygen saturation of the blood (by plotting OD of HbO_2) by the proposed method of polarographic coulometry (A) and by the spectrometric method (B) on the OSM-1 (oxygen saturation meter; Radiometer, Copenhagen, Denmark) apparatus are given in Table 1.

The value of Δ denotes the absolute difference between the results (A—B), and in the next column the relative error of the difference is given in percent (Δ/B · 100%).

Comparison of OC determined polarographically and calculated as the product of the spectrophotometrically determined hemoglobin concentration and Hueffner's* coefficient of 1.39 ml O_2/g Hb, showed that the accuracy of the determination likewise is 2-3%. The accuracy of construction of the whole OD of HbO_2 is of the same order.

*The value of the coefficient given in this paper is not 1.34 ml/g as given by Hueffner, but 1.39. This is more accurate, having been obtained on the basis of the most recent work on the molecular mass of hemoglobin.

Unfortunately Épshtein's polarographic cell does not allow the blood to be introduced into it anaerobically, which would enable the degree of oxygen saturation of arterial or venous blood to be determined after a single electrolysis.

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