MODIFICATION OF THE SPECTROPHOTOMETRIC METHOD OF DETERMINING OXYGEN DISSOCIATION CURVES OF HEMOGLOBIN

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UDC 612.111.11-087.4 : 543.42

A method of introducing an accurately measured volume of air by means of a dosage device into the spectrophotometer cell to obtain oxygen dissociation curves of hemoglobin solutions has been developed. The dose is measured with an accuracy of ±0.15%. Equations needed to calculate oxygen partial pressures in the cell are given.

KEY WORDS: oxygen dissociation curve; hemoglobin; partial pressure of oxygen; spectrophotometric cell; dosage device.

To study the functional properties of hemoglobin as an oxygen carrier the spectrophotometric method of plotting oxygen dissociation curves (ODCs) is frequently used [1-4]. The main difficulty in this case is in measuring the exact quantity of air supplied from the pipet [4]. The accuracy of dosage, especially at low oxygen partial pressures, is too low, being only ±1.5%.

A spectrophotometric cell with a special atmospheric oxygen dosage device is suggested; the volume of the device is known and can be changed by introducing glass inserts of known volume into it (Fig. 1). Hemoglobin in the deoxy form is transferred to this cell by blowing inert gas above the solution and then connecting to a vacuum, after which the partial oxygen pressure in the cell is zero. The dosage device is closed with a rubber stopper, smeared with vacuum grease, the cap connecting the dosage device with the cell is then opened for 2-3 sec and, as a result, the partial oxygen pressure in the cell and dosage device is balanced. The tap connecting the dosage device with the cell is closed and the spectrophotometric cell is fixed in a tonometric apparatus in a water bath. After tonometry the cell is transferred to the spectrophotometer and optical density readings are taken at $\lambda = 558$ nm. By increasing the partial oxygen pressure in the cell with the aid of the dosage device and taking the optical density reading (D) every time at the given wavelength, five or six points are obtained, from which the ODC of the hemoglobin solution can be plotted.

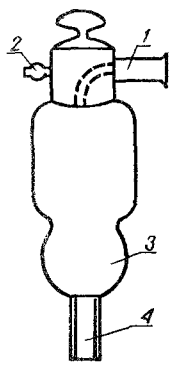

Fig. 1. Spectrophotometric cell: 1) dosage device; 2) connection to vacuum; 3) tonometric part of cell; 4) spectral part of cell.

The following procedures are carried out in succession during calculations with the ODC:

a) The percentage saturation of hemoglobin is calculated by the equation:

$$\%HbO_2 = \frac{D_{Hb} - D_x}{D_{Hb} - D_{HbO_2}}, \quad (1)$$

where D_{Hb} is the optical density of the deoxy form of hemoglobin; D_{HbO_2} the optical density of oxyhemoglobin; D_X the optical density of the hemoglobin solution at the given partial pressure of O_2;

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(Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 11, pp. 122-123, November, 1975. Original article submitted December 18, 1974.

b) To determine the partial pressure in the spectrophotometric cell, the partial pressure of O_2 after the first single addition of air from the dosage device is first calculated by the equation:

$$pO_2 = \frac{apO_2 \cdot V_{dos}}{V_{cell} + V_{dos}} \text{ mm Hg}, \quad (2)$$

where V_{dos} is the volume of the dosage device (in ml); V_{cell} the volume of the gaseous phase of the cell (in ml); apO_2 the partial pressure of O_2 in the air.

If it is necessary to determine the partial pressure of oxygen at a certain point k, at which the same dose of air has been introduced m times, the calculation is carried out by the equation:

$$kpO_2 = pO_2 \cdot f^m + pO_2 (f^{m-1} + f^{m-2} + \ldots + f + 1) \quad (3)$$
$$k-1$$

where $f = V_{cell}/(V_{dos} + V_{cell})$.

The method of calculation given above is sufficiently precise for work with a cell with a volume of more than 50 ml. However, if the volume is smaller, collections must be introduced for physically dissolved and chemically combined oxygen.

A series of ODCs for a 0.15% solution of native hemoglobin in 0.1 M phosphate buffer at pH 7.315 and at a temperature of 20°C was obtained with a cell of the writer's own design. Hill's coefficient (n) varied in these experiments from 2.68 to 2.81 and the affinity of hemoglobin for oxygen at 50% saturation ($\log_{50} pO_2$) was 0.731-0.740, in agreement with data in the literature for native hemoglobin.

The writer is grateful to Professor G. Ya. Rozenberg for help with the work.

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