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Title : COMPUTERIZED DETERMINATION OF OXYGEN DISSOCIATION CURVE

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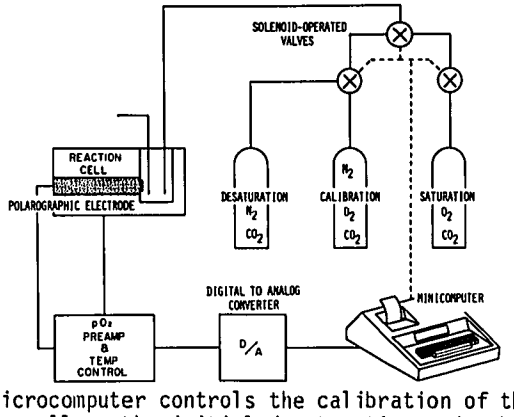
Introduction: A fully automated, computer controlled, yet portable method for determining and plotting the entire hemoglobin-oxygen dissociation curve on less than 0.5 ml of blood is being introduced to clinical practice. Determination of a p50 is now possible in the operating room within 20 minutes.
Method: The whole system mounts on a small cart and is depicted below:



The microcomputer controls the calibration of the system as well as the initial desaturation and subsequent saturation of the blood specimen. The blood sample is loaded into the reaction cell, much like a traditional blood gas machine. Initially the specimen is desaturated by exposure to the CO_2/N_2O mixture. Once the pO_2 falls below 0.5 torr, the computer automatically starts the saturation with the O_2/CO_2 mixture. Gas is exchanged with the sample in the reaction cell across a silicone rubber membrane which mechanically separates the two phases. The silicone rubber membrane is formed by hot compression molding in the shape of a thin walled (0.05 mm) circular cylinder 1 cm in diameter by 2.5 cm in length. The membrane is mechanically fastened to the gas delivery manifold which serves as the lid of the sample well. The gas delivery manifold is sealed to the sample well with a neoprene O-ring. When the gas delivery manifold is inserted into the sample well, the membrane is automatically positioned concentrically in the cylinder cavity. The sample solution is then contained within the annular region formed by the membrane and the sample cavity walls and stirred by a thin-walled, cup-shaped stirrer, magnetically coupled to an outside motor-driven rotor. A conventional polarographic oxygen electrode is interfaced to the sample solution through the cavity wall. The electrode continuously monitors the partial pressure of oxygen in the sample. The entire reaction cell is controlled to 37 C.

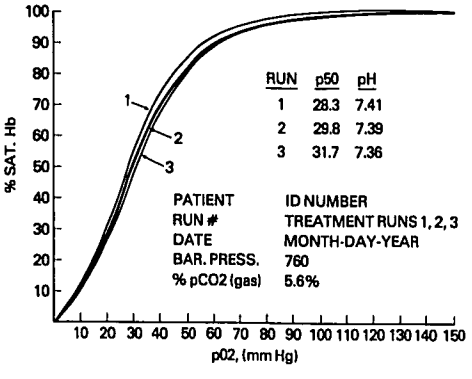
The amount of oxygen bound to hemoglobin at any time is expressed by the function:

$$HbO_2 = \int_0^t (P^*(1 - e^{-bt}) - P(t) - kP(t))\, dt$$

P^* Partial pressure of O_2 on gas side of membrane
$P(t)$ Partial pressure of O_2 in sample as a function of time
b Membrane equilibrium time constant
k Volume solubility coefficient

The amount of oxygen bound by hemoglobin is calculated from the equation using the k value obtained when P(t) equals 150 mm Hg. This function is then scaled by dividing the value of oxygen bound by hemoglobin at P(t)=150 and then multiplying by 100%. This function is then plotted against P(t) and represents the hemoglobin oxygen equilibrium curve, from which the p50 is then derived.
Results: The illustration below shows three successive curve determinations in an anesthetized patient with an increasing metabolic acidosis, who was studied with informed consent and institutional approval. The curve consistently shifted to the right and the p50 increased as the pH fell and lactic acid levels rose. While not a surprising finding, it does illustrate the sensitivity and precision of this computerized system.

HEMOGLOBIN-OXYGEN EQUILIBRIUM CURVE



Discussion: Manometric methods to determine the curve as first used by Van Slyke are time consuming and given to great error. Present clinical methods now involve the tonometering of blood with a known gas concentration and then estimating the percent saturation from measured changes in the optical absorbance spectrum of the hemoglobin. Optical methods, however, cannot deal with changes in the absorbance spectrum that are independent of changes in oxygenation of the samples. A more complex non-optical method[1] progressively oxygenates the blood samples by the injection of H_2O_2 in the presence of catalase. The accompanying release of H^+ is titrated by NaOH and plasma pO_2 measured with the traditional polarographic electrode. This too is a complex and time consuming method. In the new method described above, the amount of oxygen delivered to the blood sample is calculated from the diffusion equation and the measured pO_2 gradient across a thin silicone membrane.
References: 1. Rossi-Bernardi L, Luzanna M, Samaja M, et al: Continuous determination of the oxygen dissociation curve for whole blood. Clin Chem 21:1747-1753, 1975