Use and Application of the Cuvette Densitometer as an Oximeter

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A method has been developed for the continuous recording of oxygen saturation in blood by the use of a cuvette densitometer. The device consists of a light source, a cuvette through which the blood flows and a photosensitive element. It is simple to operate and has an acceptable sensitivity and stability. The instrument is applicable to a number of investigative problems.

THE oximeter is a device capable of providing a continuous indication of the percentage oxygen saturation of blood. Matthes in 1935 was the first to describe an instrument of this type. An impetus to the further development of this field was created by World War II in association with the problem of arterial oxygen saturation in high altitude aviation. A number of investigators, including Kramer, Millikan, Goldie, Hartmann, Wood, and others, have contributed much toward the elucidation of this subject. The work of Wood and associates has been especially noteworthy in the development of oximetry for investigative work, particularly in clinical medicine.

The fundamental principles upon which oximetry is based are the facts as demonstrated by Kramer that (1) oxyhemoglobin transmits visible red light (620-770 mμ) to a much greater degree than does reduced hemoglobin, and (2) that Beer's law of optical absorption may be applied to the hemoglobin of whole blood in various saturations with oxygen. These facts are applicable providing the hemoglobin concentration remains constant. Modifications have been introduced which correct for variations in hemoglobin content by the measurement of light absorption in two spectral regions.

In the present study the use of a cuvette densitometer as a direct recording oximeter has been evaluated. The device was originally developed for the continuous determination of the concentration of Evans blue dye in arterial blood for measurement of cardiac output by the Stewart-Hamilton principle.

METHOD

The instrument employed consists basically of a light source with transmission of light through a cuvette containing blood to a photosensitive element. The details of this device have been previously recorded and it is now commercially available.*

A diagrammatic illustration of the instrument is shown in figure 1. The light source is of a wavelength of 630 mμ, and at this wavelength the transmission of light through blood is directly related to its oxygen saturation. The photosensitive element consists of a multiplier tube capable of a gain of one million and with an output sufficiently high to permit the use of a fast-response galvanometer (125 c.p.s.).

The cuvette is constructed so that the entering blood changes its course in a 90° turn just prior to reaching the photosensitive area. This creates turbulence in the flow and prevents streaming of the blood. The light source, cuvette and phototube form a small unit remote from the power supply, permitting the use of the densitometer close to the field of operation.

A number of tests for stability, linearity, sensitivity and accuracy have been performed. The thermal equilibrium of the instrument is reached approximately 30 min. after the power is supplied. The baseline fluctuation at this time is less than 0.5 mm. at the sensitivity generally employed, and the random drift does not exceed 1 mm./hour. The reproducibility and linearity of the instrument may be tested by the interposition of calibrated neutral gelatin filters in the light path. The linear relationship of optical density to deflection of the densitometer is shown in figure 2. The galvanometer deflection for any given filter is reproducible to within 0.5 mm. and the range of linearity is at least 2.5 optical density units. The change from completely reduced to completely oxygenated blood with a hematocrit of 45 in the cuvette used is equivalent to approximately 1.2 optical density, which is well within the linear range of the instrument. Sensitivity is adjustable through a step attenuator. For stable operation with the recorder employed (Hathaway Model

* The Colson Corporation, Elyria, Ohio.
CUVETTE DENSITOMETER AS OXIMETER

FIG. 1. Diagrammatic illustration of densitometer.

Fig. 2. Calibration of densitometer with neutral density filters showing relationship of optical density (abscissa) to deflection of the densitometer (ordinate, min.).

FIG. 3. Calibration curve showing relationship of oxygen saturation determined by the Van Slyke manometric method (abscissa) compared with the deflection of the densitometer (ordinate, mm.) in blood from three separate experiments. A different gain of the densitometer was used for each experiment.

Results

Calibration of the instrument has been both in vivo and in vitro on blood of varying oxygen saturation (5–100 per cent) and satisfactory results were obtained in both with no significant differences. For the in vitro studies blood with a low oxygen content was obtained from the coronary sinus of dogs rendered anoxic by complete tracheal occlusion for a period of 4 to 6 min. Blood obtained in this manner was kept under 100 per cent nitrogen until used. A sample was then drawn through the cuvette into an oiled syringe and the galvanometer deflection was recorded. Further samples of the same blood were oxygenated in a step-wise manner and galvanometer deflections were recorded until a saturation of 100 per cent had been reached. The samples, which had been kept under anaerobic conditions, were then analyzed for oxygen content by the Van Slyke manometric technic. A comparison was then made with the densitometer deflection. In figure 3 the results are shown of the values obtained in three separate experiments with different gain settings of the densitometer of the correlation of oxygen saturation determined by densitometer and by the manometric technic. It may be observed that the maximum deviation of any point is 3 to 5 per cent.

A number of in vivo studies were done using anesthetized dogs maintained with artificial respiration. The animals were placed on 100 per cent oxygen. Following equilibration, blood from the carotid artery, external jugular vein and coronary sinus was successfully drawn directly through the densitometer into oiled syringes. The oxygen content of these samples was then determined by the manometric method and compared with the densitometer deflection. The same procedure was repeated after equilibration of the animal.
with oxygen in concentrations of 20 per cent, 16 per cent, 12 per cent and 8 per cent in the inspired air. Correlation between the two methods was good in each instance, all points of the densitometer deflections being within 5 per cent of the values obtained by the Van Slyke method. An example of figures obtained for a number of samples in the higher ranges of oxygenation from two different blood samples is shown in figure 4.

The studies revealed that the rate of blood flow through the cuvette exerted a mild effect on the galvanometer response. For example, a change in flow from 10 to 40 ml./min. was associated with a change of 2 mm., approximately equivalent to a 2 per cent change in oxygen saturation. A constant withdrawal apparatus therefore was designed and used for all calibrations. It was further observed that changes in hematocrit affected the linearity of the curve. A number of experiments were done with hematocrit values of 25 to 50, and it was found that increasing hematocrit results in a deviation in the direction of decreasing oxygen saturation. Therefore, the hematocrit was held essentially constant during the calibrations.

**Discussion**

A variety of applications exist for the use of a continuously recording oximeter in clinical and experimental studies. This pertains particularly to an instrument with an acceptable accuracy and stability. With such a device it is possible to determine continuously changing values of oxygen saturation in blood with reasonable precision. The results of the method described have been quite satisfactory as applied to a number of experiments related to myocardial oxygen consumption under various circumstances.

**Summary**

The cuvette densitometer has been used as a direct recording oximeter for use in the continuous determination of the oxygen saturation of whole blood. It is simple to operate, and its stability, sensitivity and response time are within acceptable limits. The range of linearity of the instrument exceeds the change in optical density encountered from completely reduced to completely saturated blood. Calibration curves are linear with a maximum deviation of 3 to 5 per cent. The instrument has been successfully used in the measurement of changing oxygen saturation in blood from the coronary sinus under varied physiologic conditions.

**Summario in Interlingua**

Le densitometro a cuvette esseva usate como oxymetro a registration directe pro le objectivo del continue determination del saturation oxygenic de sanguine integre. Illo es simple a manipular. Su stabilitate, sensibilitate, e tempore de responsa es intra limites acceptable. Le linearitate del instrumento excede le alteraciones del densitate optic que es incontrate in sanguines inter reduction complete e saturation complete. Curvas de calibration es linear con un deviation maximal de 3 a 5 pro cento. Le instrumento ha essite usate con bon successo in le mesuration del alterationes del saturation oxygenic in sanguine ab le sino coronari sub varie conditiones physiologic.

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A Tip on Tip Potentials of Microelectrodes

The development of any new technic brings with it chances of unrecognized errors as the new procedure comes into more general use. This appears to be the case of microelectrodes for detection of membrane potentials.

In his usual meticulous manner, Adrian found that conventional microelectrodes filled with 3M KCl solution can develop tip potentials after insertion into cells, because some substance may plug the tip during puncture of the membrane. Such a plug seems to act as a membrane which reduces the mobility of Cl ions and enhances the difference between the mobility of Na and K ions.

The potentials developed may result in (1) low readings of resting potentials when electrode resistance is large, (2) statistically significant differences in recorded resting potentials measured by two different electrodes and (3) changes in recorded potentials after an electrode is broken.

The uncertainty of measurements can be minimized by using only electrodes in which the tip potential is less negative than —5 mv. "This selection procedure involved a large wastage of electrodes—just over half of all electrodes with a resistance greater than 5 megohm had to be discarded at the first testing—but the increased reliability of the results justified the time spent on it."

For details see R. H. Adrian, J. Physiol. 133: 631, 1956.
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