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A SIMPLE PHOTOELECTRIC COLORIMETER.

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Ordinary colorimetric methods of measurement suffer from the disadvantage of requiring subjective judgments depending on the colour sensitivity of the eye of the observer. Their accuracy is limited by the eye fatigue which they engender and is dependent upon the quality of the light by which they are made. Most of them are also subject to other limitations: they require the use of complex and empirical calibration curves, are relatively slow, or necessitate fairly large quantities of material. The improvements brought about by the use of spectroscopic or photometric methods are still limited by the basic disadvantage of subjective measurement, intensities of two fields having to be balanced against each other or spectral bands located. This paper describes a simple colorimeter, using a differential copper copper-oxide photoelectric cell and two colour filters, which possesses none of these disadvantages. It is relatively cheap, simple, and quick to use, can be adapted to very small quantities of fluid, and has a linear calibration curve. It is especially well adapted to the measurement of the degree of oxygenation of hæmoglobin solutions, and is not subject to the usual disadvantages of the other optical methods (such as inaccuracies due to inactive hæmoglobin) which have been used for this purpose.

Principle of the method.

The principle of the method can be seen from the diagram at the bottom of Fig. 1, which illustrates its applicability to the oxygenation reaction of hæmoglobin. Light from a suitable source passes through the absorption trough containing the solution being tested. Behind the trough two colour filters are fastened side by side in front of a differential photoelectric cell, so that part of the beam goes through each. The differential type of cell measures directly the difference in the amount of light falling on each half of its light-sensitive surface, and acts in effect

like two photoelectric cells working against each other. The two colour filters are so chosen as to match in a rough way the colours of the two end-points of the reaction being measured, so that when the colour goes from one extreme to the other there will be an increase in the amount of light going through one filter and a decrease in the amount going through the other. This reverses the distribution of light falling on the two sides of the cell, and with it the galvanometer deflection.

The working of the device is well illustrated by the hæmoglobin reaction for which it was specifically developed. As can be seen from the

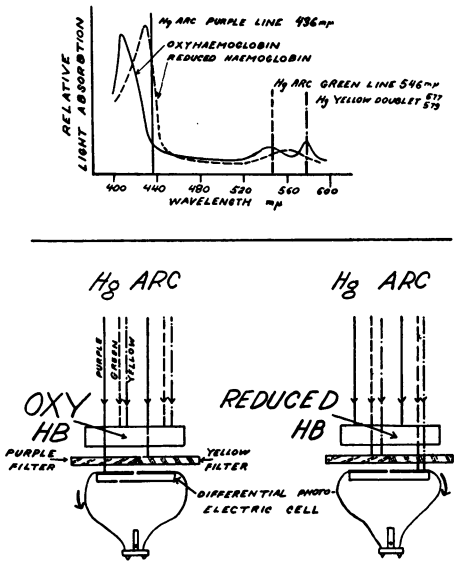

Fig. 1. Principle of photoelectric colour analyser applied to *HB*.

absorption curves plotted at the top of Fig. 1, the mercury arc is a peculiarly well-adapted source for this colour change. Violet light of wave-length 436 mμ, where there is a very strong emission line is very much more strongly absorbed by reduced than by oxyhæmoglobin, while the yellow and green lines (579, 576, and 546 mμ) are almost identical in wave-length with the peaks of the characteristic alpha and beta bands of oxyhæmoglobin, the position of which is used as the criterion for oxygenation by the Hartridge reversion spectroscope method. The Wratten D colour filter allows the violet light to pass almost undiminished and cuts out the yellow and green completely, while the yellow Wratten G filter allows these two colours to pass but is opaque to the violet. The

change from oxyhæmoglobin to reduced hæmoglobin in the absorption trough reverses the direction of the galvanometer current as shown in the diagram, partially oxygenated solutions producing intermediate deflections.

Description of the colorimeter.

A cross-section of the instrument is given in Fig. 2. It consists of light source, absorption trough, colour filters, photoelectric cell and galvanometer. The choice of source, trough, and galvanometer will depend upon the particular reaction being measured, the amount of material available, and the degree of sensitivity required. For most ordinary uses an incandescent lamp bulb, and a flat-sided trough about 1 cm. thick, with a low sensitivity galvanometer, or even a micro-ammeter, will prove

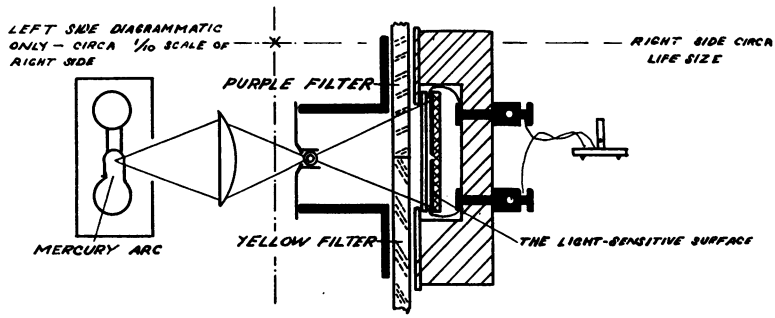

Fig. 2.

satisfactory. As illustrated the instrument is adapted to measuring the oxygen saturation of small quantities of hæmoglobin, flowing down a tube. A KBE 110-volt mercury arc lamp is used, focused on the absorption trough with a condensing lens. The trough itself is a 1 cm. length of 1 mm. glass capillary.

The photoelectric cell is the differential type of copper copper-oxide cell manufactured by the Cambridge Instrument Co. under Westinghouse patents. It consists of a copper disc about 2 cm. in diameter, with a layer of copper oxide on the front surface, and a thin metallic film on top of this. The surface is divided into two halves by a scratch along one diameter, and the terminals rest lightly on the metallic film, one on each half. This cell measures directly, and without driving potential, the difference in the amount of light striking the two halves. The colour filters are fastened to the photoelectric cell. They are adjustable from side to side, enabling the galvanometer to be brought to a convenient

balance during calibration, and are then clamped fast. The adjustment is so made that the two end-points give deflections which are approximately equidistant from the null point on either side. This use of a balanced system very greatly simplifies operation, and reduces to a minimum the ill effects of fluctuations in light source and lack of mechanical rigidity in the apparatus. It has proved of the utmost importance in getting reliable results.

With this apparatus, using the 1 mm. observation tube, and the mercury arc source, I have obtained galvanometer currents of about 3×10^{-7} amperes for the change from 0 to 100 p.c. oxygenation of a hæmolysed blood solution diluted 200 times.

Calibration.

The galvanometer deflections corresponding to the two end-points are obtained directly either by successively running the fully oxygenated and the fully reduced hæmoglobin through the observation tube, or by using a three-compartment absorption trough, one compartment of which contains the unknown fluid, and the other two the end-point standards. The frequency with which these standards must be referred to depends upon the stability of the set-up, the steadiness of the lamp source, and the desired accuracy of reading.

The intermediate concentrations of the different pigments can be calculated by linear interpolation from the two end-points, when sufficiently dilute solutions, such as the method is well suited to, are used. The condition of linearity is that the amount of light absorbed by each substance be directly proportional to its concentration in the solution. In a parallel-sided trough, light absorption actually follows the well-known logarithmic expression:

$$\text{Amount of light absorbed} = \text{amount of incident light} \times (1 - e^{-k \times c \times d}),$$

where k is the specific light absorption of the substance, c its concentration, and d the thickness of the absorption trough. A simple calculation shows, however, that if a certain concentration absorbs 4 p.c. of the total light, then half that concentration will absorb 2·02 p.c. of the total light, which is only $\frac{0{\cdot}02}{2{\cdot}02} = 1$ p.c. deviation from linearity. If an accuracy of 1 p.c. is desired, it is then necessary to see that the galvanometer deflection between end-points is less than 4 p.c. of the total deflection obtained by cutting off completely the light striking one side of the cell.

If this dilution condition has been complied with, the calibration curve is a straight line. To understand this, we need only make the

assumption, which has been often made before, and is in fact implicit in the calibration of the reversion spectroscope by the wedge trough method, that a partially oxygenated solution of hæmoglobin behaves optically like a mixture of the corresponding quantities of oxy- and reduced hæmoglobin. Let us, for the sake of simplicity, consider the case of a solution 50 p.c. saturated. The absorption curve of such a mixture will lie just half-way between that of the fully oxygenated and that of the fully reduced one given in Fig. 2, and in particular will cut the violet, green and yellow lines of the mercury arc spectrum just half-way between the intercepts of the two extreme curves. The violet light striking one side of the photoelectric cell will then be of an intensity mid-way between that of the two end-point solutions, and the same will be true of the yellow and green light striking the other side of the cell. Since the galvanometer records the algebraic sum of these different effects, its deflection will also be just half-way between its two calibrating end-point positions. Similarly, a solution one-third saturated will produce a galvanometer deflection one-third of the way from the fully reduced to the fully oxygenated positions, and so on.

The argument applies equally well to every reaction which involves any colour change, *i.e.* any alteration in the spectral absorption. In particular, it is applicable to mixtures of reduced hæmoglobin and carboxy-hæmoglobin; and at Dr Roughton's suggestion, I have used these mixtures to test empirically the linearity of the calibration curve, since known mixtures of reduced and carboxy-hæmoglobin are very much simpler to prepare than those of reduced and oxyhæmoglobin. The affinity of carbon monoxide for hæmoglobin is so great that one can make up a solution of saturated carboxyhæmoglobin, and subsequently remove practically all of the excess carbon monoxide without danger of appreciable dissociation. Such a solution was mixed in varying proportions with a solution of hæmoglobin previously reduced by bubbling nitrogen through it and adding a small quantity of sodium hydrosulphite, and its satura-

Actual p.c. COHb	Measured p.c. COHb photo-cell method	Maximum deviation
20	21	4
40	38	4
60	60	3
80	80	8

tion was then measured by the photoelectric cell method. The average of four determinations for each solution are given in the above table, as well as the maximum deviations of individual readings.

By this rough test the method appears accurate to about 4 p.c. for individual readings. The measurements were made under the very unfavourable conditions imposed by an absorption trough consisting of a cylindrical tube less than 2 mm. in internal diameter. This type of trough was dictated by the nature of the kinetic studies for which the method was being developed. With a larger observation vessel, and more light passing through it, a much higher degree of accuracy should be obtainable.

The linear character of the calibration curve is one of the principal advantages of the method. It reduces the labour of calculation and insures uniform accuracy throughout the whole colour range. It is, in this respect, more satisfactory for hæmoglobin reactions than the reversion spectroscope, which has an empirical calibration curve, and which becomes unreliable at both high and low degrees of saturation.

SUMMARY.

A colorimeter using a differential copper copper-oxide photoelectric cell, and colour filters is described. Its advantages are:

(1) Elimination of eye strain and subjective judgments.
(2) Speed and ease of operation.
(3) Linear calibration curve.
(4) Availability for small quantities of material.
(5) Simplicity and low cost.

It has been used to measure the degree of oxygen saturation of dilute hæmoglobin solutions.