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Optical Artifacts in Whole Blood Densitometry

A Review of Undesirable Optical Signals, Nonspecific Effects, Encountered during Measurements of Oxygen Saturation and Dye Dilution Curves by Light Transmission

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Introduction

The intelligent use of any measuring device demands a knowledge of the instrument's capabilities and limitations, and also an understanding of the desirable and undesirable signals one might expect to encounter. Thus, accurate electro-optic sensing and recording of oxygen saturation [26] and indicator dilution curves in whole blood requires extraction of a desired signal from a mixture of desired and undesired optical signals. In addition to specific changes in light absorption caused by the phenomenon under study, there are nonspecific effects due to the presence of erythrocytes. Changes in shape, size and orientation with respect to one another as well as changes in other physical properties cause variations in the reflection and scattering of light. Thus, the red blood cells are responsible for most of the light absorption in whole blood. These nonspecific and hence undesirable effects are due to variations in total red cell concentrations from subject to subject, variations in rate of blood flow, changes in red cell size and shape due to changes in temperature, CO_2 tension and pH, and tonicity of the plasma [21]. In addition, variations in structure, pigmentation and thickness of the ear pinna [25, 26] are also of importance for earpiece oximetry and densitometry.

These physical changes cause changes in light transmission to occur over a wide band width of light[1] wavelength and therefore may cause errors of measurements of blood oxygen saturation and dye dilution curves. These undesirable nonspecific effects are often of the same frequency as the desired signal and cannot be eliminated by frequency discrimination alone. Some methods used to discriminate against these nonspecific effects will also be discussed in this communication.

Light Transmission Defined

A 100 % light transmission through a substance implies that all of the light energy passes through the substance with none of the photons giving energy to the substance. In spectrophotometric studies, transmission of light through a substance is measured relative to some standard, often distilled water, which in itself absorbs varying amounts of light energy at different light wavelengths. To have a *true* 100 % transmission would mean that the standard would not absorb any light, as would be true in a vacuum. The expression '100 % transmission' as used here means only that the solution under spectrophotometric study has the same absorption of light as the standard at a particular wave length. It should be noted that transmission of light through whole blood is a function of both nonspecific effects and desired signal[2].

Definition of 'Monochromatic' and 'Dichromatic' Instruments

Two types of transmission densitometers are in general usage. One type utilizes one wavelength *band*[3] of light and is called a monochromatic densitometer. The other uses two photocell-filter assemblies and is referred

[1] Light is defined, in this instance, to include the short wavelength portion of the infrared as well as visible light.

[2] These effects multiply at a given wavelength and do not add (i. e. if the light transmission through plasma solution with dye is 20 % and the nonspecific effect is 40 % then the total transmission would be 20 % of 40 % = 8 % light transmission).

[3] An instrument's light sensitivity or the light transmission of an optical filter is usually given in terms of one wavelength, although it is to be understood that a band of light wavelengths is involved. The width of

to as a dichromatic instrument. Dichromatic infers two discrete wavelengths but actually two wavelength *bands* of light are usually used.

Effects of Hematocrit

Some of the light impinging on the red blood cell is reflected from the cell at various angles and scattered throughout the plasma. A portion of this scattered light is reflected back toward the light source and another portion is absorbed by the erythrocytes or plasma. The higher the hematocrit or the thicker the blood layer, the more light will be absorbed and/or reflected. The remaining light is transmitted to the detecting photocell (fig. 1). For measurement of oxygen saturation a monochromatic instrument will not only record variations in optical density of whole blood due to oxygen saturation but also changes in optical density due to changes in hematocrit.

Effects of Physical Shape and Agglutination of Red Blood Cells

If the tonicity of the fluid surrounding the cell is decreased the cell will increase its volume [21], swelling until it becomes spherical. In this shape the cell will transmit more light than it will in its normal shape. While some of this increased light transmission may be due to hemolysis, most of it is due to a decrease in reflection and absorption of light by the erythrocytes (fig. 2 b).

In the presence of hypertonic solutions, erythrocytes shrink and their surface becomes irregular. Associated with this decrease in size of the cells, a decrease in light transmission is observed [21], concomitantly with an increase in light reflection (fig. 2 c) [27, 28]. The light sensitive photocell 'sees' less light and photocell output is decreased.

Many colorless solutions affect whole blood optical density. Among the solutions that have been studied are glucose, sodium chloride, potassium chloride, lithium chloride, magnesium sulfate, angiographic contrast media, polybasic polymers and urea [3, 18, 21].

It has been shown by CASTANEDA [3] and NEVO [18] that the addition of polybasic polymers to blood causes the red blood cells to agglutinate. In this study CASTANEDA and colleagues showed that the addition of hexadimethrine bromide (HdBr) to blood causes this effect, and noted that the clumped cells were often trapped in the pulmonary vascular bed. Cell agglutination in a HdBr-blood mixture is a multiple stage process, consisting initially of a phase during which HdBr mixed with and coated the cell surfaces; and a coalescent phase when coated cells collided and polymer bonding of one cell surface to another occurred. The adsorption of such polymers to the red cell surface is a reversible reaction [18]. Therefore continuous formation and breakdown of red cell aggregates probably co-exist. The size of the erythrocyte aggregates is directly related to the molecular weight of the polybasic polymer.

When erythrocytes agglutinate, 'plasma spaces' are created through which light transmission is increased (fig. 2 d). The opposite effect is seen with crenation (fig. 2 c). CASTANEDA *et al.* [3] showed that an intravenous infusion of 20 % NaCl simultaneously caused crenation producing a decrease in light transmission, and agglutination producing an increase in light transmission.

Intravenous administration of urea causes the peculiar effect on light transmission seen in fig. 3. As re-

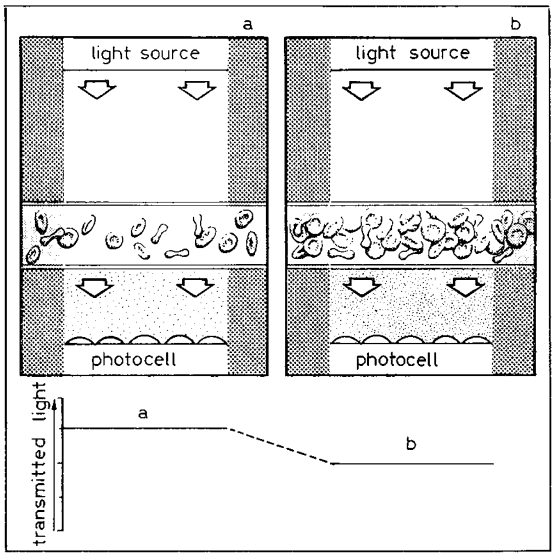
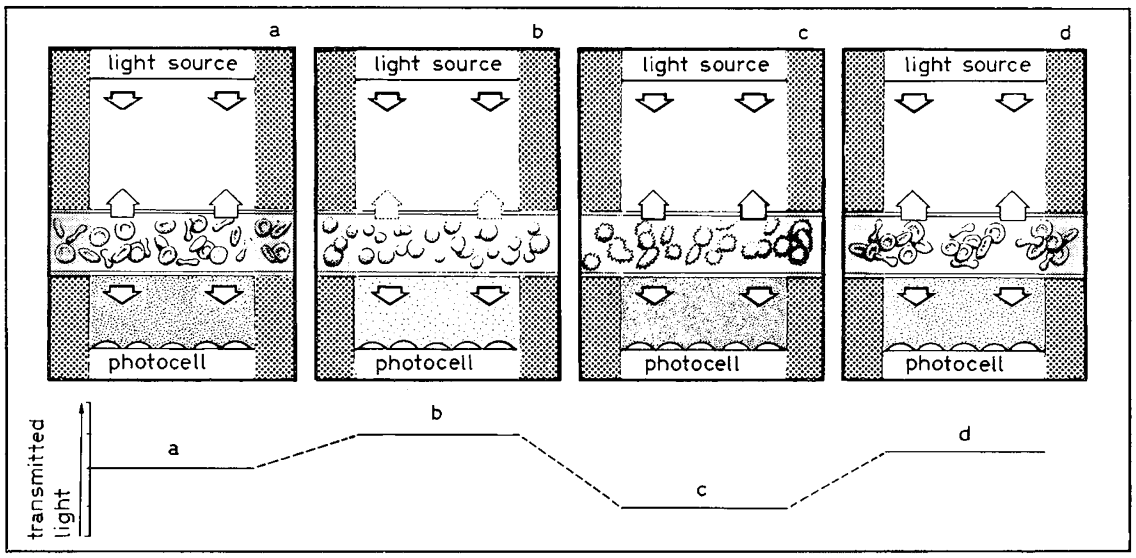


Fig. 1. Diagram showing the relationship between number of red cells per unit volume and light transmission. Part (a) depicts densitometer with 'normal' hematocrit with amount of transmitted light. Part (b) shows a relative reduction of the transmitted light due to an increase in the number of red blood cells in the optical pathway.

this band for an optical interference filter is described by its 'half band width', encompassing the longest and shortest wavelengths for which the filter has 50 % of its peak wavelength sensitivity. For instance, an 800 millimicron interference filter has its peak transmission at 800 millimicrons, but it may have a half band width of 20 millimicrons or more. A dye said to absorb light at 620 millimicrons absorbs some light at wavelengths longer and shorter than 620 millimicrons.



corded by the monochromatic densitometer (marked M in fig. 3), an initial increase in light transmission is observed; this is followed by a decrease. The dichromatic densitometer (line marked D in fig. 3) although possessing the same sensitivity to dye as the monochromatic instrument, illustrates compensation for this nonspecific effect. This instrument is virtually insensitive to this change in optical density. PINTER and others observed that intravenous infusions of 20–30 % urea in a solution of 0.9 % saline and 5 % dextrose produces hemolysis in dog and man [1, 12, 20]. They suggested that as the erythrocytes become overloaded

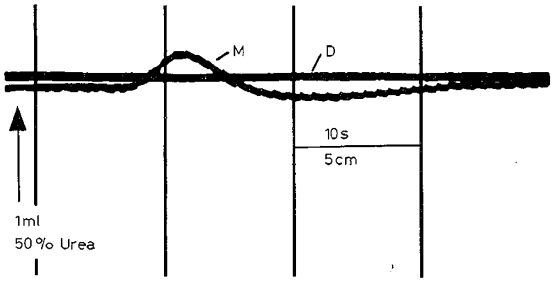


Fig. 3. Effect of 50 % urea on light transmission through whole blood. Response of monochromatic (curve marked 'M') and dichromatic densitometers to an injection of 1 ml of 50 % urea and blood sampled at femoral artery. Sensitivities of the two instruments to Cardio-Green® dye are approximately equal but monochromatic device shows a high sensitivity to a change in optical density due to small injection of 50 % urea (reproduced from SUTTERER, W. F. and WOOD, E. H.: IRE Trans. Biomed. Electronics; vol. BME-9 (April 1962) [23]).

Fig. 2. Diagram to depict the effect of red blood cell shape and agglutination on light transmission. White arrows pointing upwards illustrate relative degree of reflected light from blood cells. The degree of reflected light is shown by the weight of the line outlining arrows. A heavy outline of arrow represents greater magnitude of reflected light.

Part (a) illustrates a 'control' situation with a given hematocrit and normal shape and aggregation of red blood cells and showing an arbitrary magnitude of transmitted light. In part (b) the cells are spherocytes and the reflected and absorbed light decreases while the transmitted light increases. The effect of crenation (c) depicting an increase in light reflection and a decrease in light transmission due to increase in surface area of red cell. The effect of agglutination on light transmission is shown in part (d), illustrating a relative increase in light transmission due to increased plasma spaces.

Fig. 5. Comparison of femoral artery dilution curves of indocyanine green dye recorded by monochromatic and dichromatic densitometers. Calibration of the two instruments to indocyanine green is plotted on ordinate. Slight difference in contour of the two curves is due to difference in linearity of calibration curves of the instruments. Note large biphasic deflection recorded by monochromatic instrument at cessation of flow as compared with virtually no sensitivity of the dichromatic densitometers to same phenomenon. Note also small pulsatile output of dichromatic instrument (reproduced from SUTTERER, W. F. and WOOD, E. H. IRE Trans. Biomed. Electronics; vol. BME-9 (April 1962) [23]).

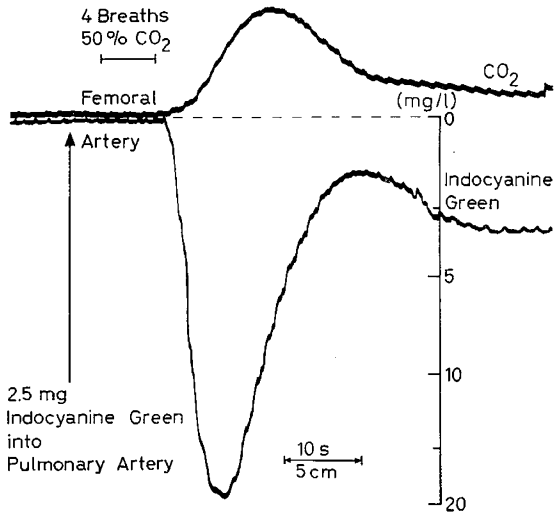


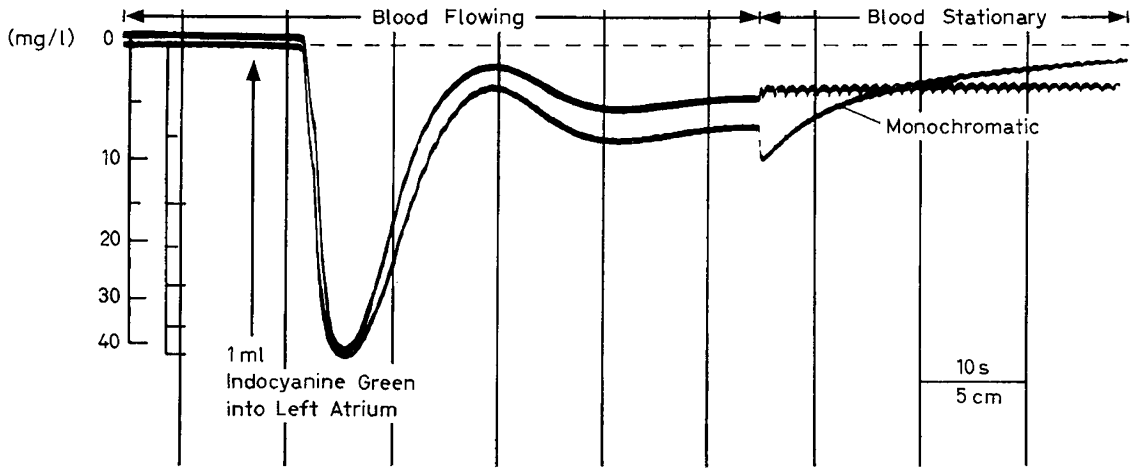
Fig. 4. Densitometric recording made at peak wavelength of 800 millimicrons by sampling canine blood from femoral artery showing effect of sudden ventilation with four breaths of 50 % CO_2 in O_2 instead of 100 % O_2. The upward deflection of monochromatic densitometer produced by breathing 50 % CO_2 in O_2 is compared with that resulting from injection of 2.5 mg of indocyanine green into pulmonary artery of same animal (reproduced from SINCLAIR, J.D.; SUTTERER, W.F.; FOX, I.J. and WOOD, E.H.: J.appl. Physiol. *16:* 669 (1961) [21]).

with urea they take up excessive water and undergo osmotic hemolysis.

Breathing excessive amounts of carbon dioxide produces an increase of light transmission through blood [21]. SINCLAIR *et al.* [21] documented this phenomenon in the dog. They utilized a monochromatic densitometer with a peak sensitivity at 800 millimicrons, to measure the effect in femoral artery blood. The experimental animal was suddenly switched from an atmosphere of 100 % oxygen to varying mixtures of carbon dioxide and oxygen. An upward deflection of the recording galvanometer, reflecting an increase of light transmission, is seen in fig. 4. The amplitude of this increase in light transmission was directly related to the amount of carbon dioxide in the breathing mixture. This increase in light transmission disappeared when the dog again breathed 100 % oxygen. In interpreting this data, it should be remembered that these changes in light transmission were sensed by a monochromatic densitometer with a peak light sensitivity at 800 millimicrons, the isosbestic point of oxyhemoglobin and reduced hemoglobin. Therefore, this is a nonspecific change unrelated to arterial oxygen saturation.

Effect of Flow on Light Transmission

Variations in the rate of blood flow cause relatively large changes in light transmission. Fig. 5 compares the responses of a dichromatic densitometer, with a high degree of compensation for these low frequency nonspecific effects, and a monochromatic densitometer with essentially no compensation for a sudden cessation of flow. A sharp decrease in light transmission occurs in the curve recorded by the monochromatic densitometer as the flow suddenly stops whereas there is an asymptotic increase in light transmission when the blood is stationary. Both these phenomena are produced because the orientation of the blood cells in the



lumen is dependent in a non-linear fashion on the rate of flow[4].

The dichromatic densitometer shows better compensation for such flow effects. It is not as sensitive to the slow shift in optical density as is the monochromatic instrument. However, the rapid pulsating fluctuations in optical density seen after cessation of flow indicate that this instrument is not well compensated for nonspecific effects due to waveforms with higher frequencies.

These variations in optical density of the blood due to flow have been studied spectrophotometrically over a wide range of wavelengths from 600 millimicrons to 2 microns. The studies were made using a special cuvette so constructed that a constant rate of blood flow could be maintained. Light transmission was recorded over these wavelengths during constant flow, during cessation of flow, and after the complete stopping of flow. Dependency of optical characteristics of blood on the rate of blood flow was observed for all wavelengths studied [22].

NAKAMURA and AMADA [17] showed that light transmission is also dependent on the type of particles suspended in the fluid. They observed that the dynamic optical characteristics of iodine crystals in solution were the reverse of that seen with erythrocytes.

The Effect of Temperature and pH on Light Transmission Through Whole Blood

JACOBS *et al.* [11] found that temperature had a profound effect on the size of the erythrocytes of many animal species including the dog, ox, sheep, pig, goat, horse and cat. A decrease in temperature increases the cell Volume and also increases light transmission. Eventually, hemolysis occurs and a further increase in light transmission is observed since at this time there are fewer cells to absorb and reflect light. These investigators also found that blood from the above species when kept at 40° C did not hemolyze even when the temperature was maintained for hours. However, as the temperature was decreased toward 0°, hemolysis up to 85 % of the erythrocytes was observed.

In contrast exposure of blood from man, rat or guinea pig to a temperature of 40° C for as little as five minutes seemed to protect the cells from hemolysis even at very low temperatures.

BROWN [2] found that the shape of red blood cells of man is directly correlated with pH. A sudden increase in pH causes the cells to become spherical and hence increases light transmission whereas lowering the pH reverses this phenomenon and consequently decreases light transmission.

Other Effects on Light Transmission

Blood oxygen saturation can be measured and dye dilution curves obtained from the ear pinna by using earpiece oximeters or densitometers. These devices are not only subject to the nonspecific effects previously described but also are affected by the thickness, pigmentation and structure of the ear pinna. WOOD [25] gives an excellent detailed discussion of the problems connected with earpiece oximetry.

Some Optical Design Considerations for Nonspecific Rejection

The characteristics of the photocells, light source, and electronics as well as the design of the cuvette lumen must be taken into consideration in order to achieve optimal light transmission and maximum frequency response [7]. However, as it is the primary purpose of this review to acquaint the reader with a knowledge of nonspecific effects, emphasis will be placed only on the design considerations directly relating to undesired optical signals, namely consideration of proper choice of optical filter wavebands.

Undesired signals exist for the entire spectrum from 600 millimicrons to 2 microns. The amplitude of the oxygenated hemoglobin transmission varies with wavelength from essentially zero at 600 millimicrons to a maximum at 650 millimicrons. At the isosbestic point (800 millimicrons) oxygenated and reduced hemoglobin have the same light transmission. At longer wavelengths, the amplitude of transmitted light is inversely related to oxygen saturation, i.e. an increase in oxygen saturation is accompanied by a decrease in light transmission. Beyond 1.2 microns, reduced and oxygenated blood have essentially the same light transmission.

A wavelength of 650 millimicrons is used to measure oxygen saturation because at or near this wavelength the maximum difference in light transmission between oxygenated and reduced hemoglobin occurs. This wavelength band therefore gives the maximum optical sensitivity to changes in oxygen saturation. Any useful instrument would have an optical filter giving peak light transmission at 650 millimicrons and a photoelectric cell sensitive at the same wavelength band.

Such a photocell-filter assembly is sensitive not only to oxygen saturation but also unfortunately to many undesired optical signals. An ideal oximeter would be sensitive only to oxygen saturation and would discriminate between total hemoglobin and oxyhemoglobin. ZYLSTRA [28] has pointed out that Lambert-Beers Law

[4] The morphology of the shift in optical density recorded by the monochromatic instrument (fig. 5) is also dependent on the shape and depth of the lumen through which the blood flows. Although this is a constant for the individual instrument this can vary for different instruments.

expresses the relation between light extinction, concentration, and layer thickness. If a system contains N dissolved substances, then at least N wavelengths are required to obtain a complete description of this system. Therefore for the measurement of oxygen saturation two wavelength bands are needed, one for oxyhemoglobin and one for reduced hemoglobin. Another wavelength band must be used that is insensitive to oxygen saturation but sensitive to nonspecific effects. The light transmitted through the blood at this wavelength could be used to measure the effects of undesired signals. As the absorption of oxyhemoglobin and reduced hemoglobin are identical at 800 millimicrons, an instrument sensitive to this wavelength is only sensitive to the nonspecific effects. An ideal instrument provides one photocell-filter assembly sensitive both to the desired signal (oxygen saturation) and to the nonspecific effects while the other is sensitive to nonspecific effects alone. Proper processing of the output from the two photocell-filter assemblies permits partial compensation for nonspecific effects.

History of Two Color Devices

As early as 1933, MILLIKAN [14] designed a two color instrument to determine oxygen saturation in very dilute hemoglobin solutions. He later adapted this two color technic to measure oxygen saturation of muscle in the intact dog [15] and in 1942, he reported a two color earpiece oximeter [16]. In 1942, GOLDIE [10] reported that he had devised a way to compensate for blood volume changes in the ear.

The cuvette oximeter developed by WOOD consisted of two light sources, a plastic cuvette through which saline or blood could be drawn, Wratten filters, and iron selenium barrier layer photocells. The output of the photocells was recorded by two sensitive galvanometers using optical (four meter light arms) rather than electronic amplification. The galvanometer deflections were subsequently measured and oxygen saturation calculated [25, 26].

The photocells in the Wood oximeter are spacially arranged in series. TAPLIN later found that greater accuracy could be obtained if the photocells were arranged in a mosaic so that each photocell received light essentially from the entire illuminated area [24]. In modern oximeters and dye sensitive densitometers both photocells look at the same illuminated area (fig. 6).

Other Design Considerations

Leakage of light around the lumen of a cuvette densitometer affects the shape of the calibration curve, and reduces the sensitivity of the instrument to the optical signals resulting from light scattering in the blood. Also, white light leakage around the optical

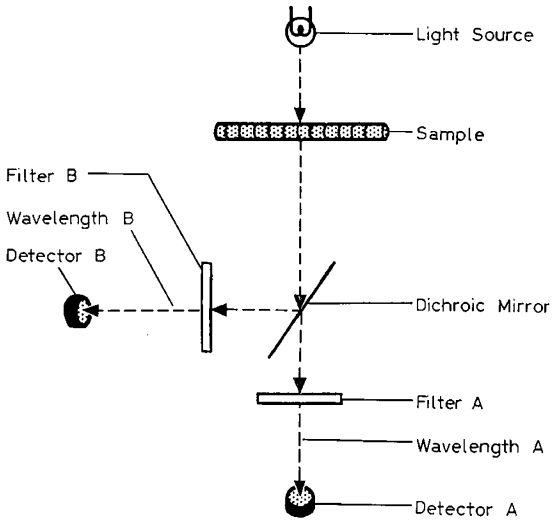


Fig. 6. Diagram of photocell-filter assembly used in dichromatic densitometer to measure transmission of two wavelength bands through the same sample of blood simultaneously. Light passes through blood sample to dichroic mirror fixed in position so that angle of incident light on mirror is 45°. Mirror reflects light in the region of 800 millimicrons; this light then passes through filter B to dye detecting cell (detector B). In this device the light of wavelengths longer and shorter than 800 millimicrons (wavelength A) is transmitted by dichroic mirror through filter A to compensating cell (detector A). Filters A and B were used for a more accurate adjustment of spectral sensitivities of photocell assemblies (reproduced from SUTTERER, W. F. and WOOD, E. H.: IRE Trans. Biomed. Electronics; vol. BME-9, (April 1962) [23]).

filters changes background dye compensation characteristics (fig. 7) [4, 5].

Development of Dye Detectors

Continuous recording of dye dilution curves by an oximeter was reported as early as 1936 by MATTHES [13]. A description of the dye dilution technique for the determination of blood flow [8] and for the diagnosis of cardiovascular defects during cardiac catheterization [9] has been given by Fox and WOOD.

Blue dyes such as Evans Blue (T-1824), the indicators used by early investigators, absorbed light in the red spectrum and therefore the red sensitive cell of the oximeter was used to detect this dye. An infrared sensitive cell in the oximeter compensated for the non-

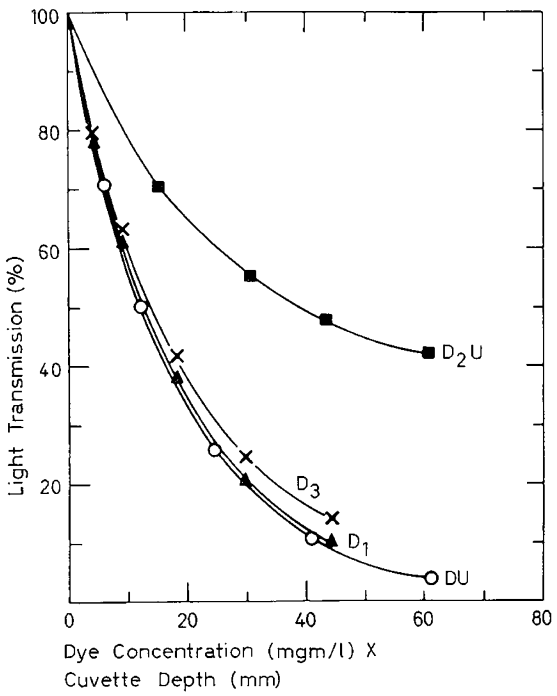


Fig. 7. Calibration curves of four densitometers for indocyanine green in plasma. Densitometers DU, D_1 and D_3 had a narrow wavelength band of incident light (narrow band interference filter). These densitometers have similar calibration curves and differ little from Beers Law. However, densitometer D_2U had a light leak around the interference filter and gave a wide waveband of incident light and a marked deviation from Beers Law results. The deviation from Beers Law for dye in plasma for these four instruments were DU = 1 %, D_1 = 2.6 %, D_3 = 5.9 %, D_2U = 37 %. When these same densitometers were studied for their sensitivities to dye in whole blood, a greater deviation from Beers Law was noted. DU = 6 %, D_1 = 6 %, D_3 = 8 %, and D_2U = 38 % (reproduced from EDWARDS, A.W.T.; ISAACSON, J.; SUTTERER, W.F.; BASSINGTHWAIGHTE, J.B. and WOOD, E.H.: J.appl. Physiol. *18:* 1294 (1963) [5]).

specific effects[5]. Blue dyes could be detected with a dichromatic densitometer (in this case the oximeter) which at least partially compensated for nonspecific effects. However, as these dyes absorbed light in the red spectrum and were detected by the same cell used to detect variations in oxygen saturation, the oximeter

[5] Using the single scale technic described by WOOD (i.e. recording the output difference of the infrared and red cell).

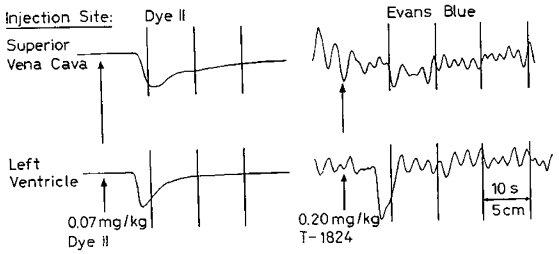


Fig. 8. Comparison of arterial dilution curves recorded following injection of Dye II (an indocyanine dye, 800 millimicrons peak light absorption in blood plasma) and Evans blue into identical sites in circulation of man with atrial and ventricular septal defects and severe pulmonary hypertension. Evans blue curves are distorted by large fluctuation in arterial oxygen saturation caused by variable right-to-left shunt. Dye II curves were recorded within minutes of the former, and are free of distortion caused by large fluctuations of arterial oxygen saturation (reproduced from Fox, I.A. and WOOD, E.H; in GLASSER's Medical physics; vol. 3, pp. 163–178 (1960) [9]).

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could not differentiate between changes in optical density due to the dye and those due to oxygen saturation [8]. In the presence of marked changes in arterial oxygen saturation, such as is seen in cyanotic patients, the recorded dye dilution curves were frequently useless for diagnostic purposes (fig. 8). Thus, for dye dilution recordings, variations in optical density due to changes in oxygen saturation were added to the nonspecific effects and appeared as an undesired signal. A technic or instrument was needed that would be insensitive not only to the nonspecific effects but also to variations in optical density due to changes in oxygen saturation.

FOX and BROOKER developed a new tricarbocyanine dye (Cardio-Green® [6]) with a peak light absorption in blood at 800 millimicrons, the wavelength of light at which oxy-hemoglobin and reduced hemoglobin have the same light absorption [6]. This dye permits the recording of dye dilution curves independently of oxygen saturation. Monochromatic densitometers with peak light sensitivity at 800 millimicrons permit recording of dye dilution curves independently of oxygen saturation (fig. 8, dye II).

[6] Cardio-Green®, Hynson, Westcott & Dunning, Baltimore, Md. 21201.

Cardio-Green® dye is excreted in the bile and has a half life in the cardiovascular system of about ten minutes [6]. If rapid, multiple injections are done, there is an accumulation of dye in the cardiovascular system. This accumulated dye is called the 'background dye' and causes the sensitivity of the instrument to vary with the concentration of background dye due to the nonlinearity of the calibration curve.

The change in sensitivity to the dye due to the nonlinearity of the calibration curve discussed above is beyond the scope of this communication but a detailed analysis and study of this problem has been presented by EDWARDS and colleagues [4, 5].

An Approach to Compensation for Nonspecific Effects with Cardio-Green® Densitometers

Although the development of Cardio-Green® dye and densitometers to sense this dye facilitated recording of dye dilution curves independently of oxygen saturation, the problem of the nonspecific effects now became more acute. This was due both to the increased dynamic response of these systems [7] and to the use of monochromatic densitometers. Some method had to be devised to compensate for the nonspecific effects and still maintain insensitivity to oxygen saturation. A densitometer was needed which was sensitive to Cardio-Green® dye and was insensitive to oxygen saturation and nonspecific effects [23].

Two photocell filter assemblies were designed, one to detect the dye and the other to detect the undesirable nonspecific effects independently of variations in optical densities due to oxygen saturation and to the dye. This has been accomplished [23].

There are many problems associated with the design of instruments which simultaneously sense two wavelength bands of light to compensate for these nonspecific effects.

EDWARDS [5] and NILSSON [19] found that the calibration curve of a monochromatic densitometer utilizing an interference filter with a narrow pass band (half band width of approximately 13 millimicrons) deviates approximately 3 % from Beers Law[7]. A greater deviation from Beers Law results when a filter is used with a wider wave band (fig. 7). The magnitude of the deviation varies with the width of the wave band of incident light. It has also been shown that there will be deviation from Beers Law in the calibration curve of a densitometer due to the presence of red blood cells [5].

[7] Beers Law states $I = I_0 \cdot 10^{-Ecd}$, where I is the transmitted light, I_0 the incident light, E is a constant for the test substance (dye in this case) at a particular wavelength, c is the concentration of a test substance (dye) and d is the logrithmic relationship between concentration of the dye and the transmitted light.

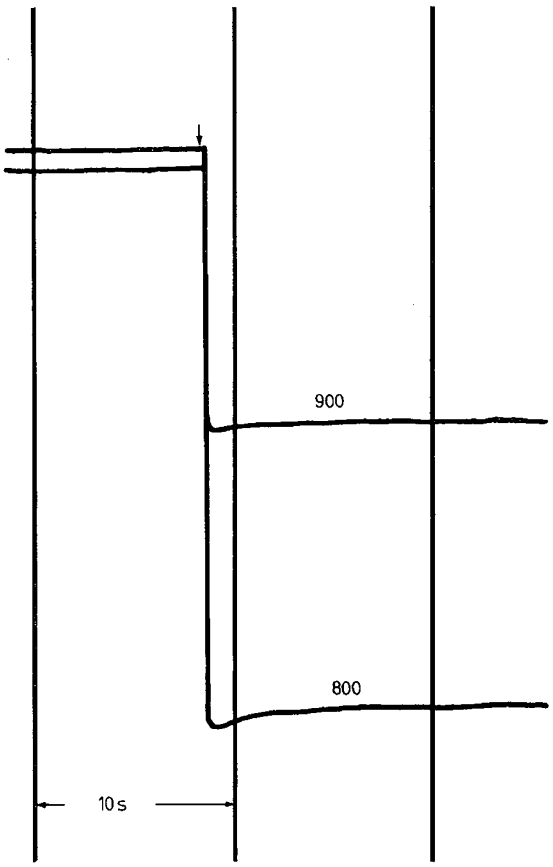

Fig. 9. Effect of sudden unequal decrease in light intensity on two photoconductive cells (discussion in text). Permission for reproduction of these figures has been obtained from the authors and the publishers.

Because of the effect of optical band width on linearity it is important to keep the band width of the two photocell-filter assemblies as similar as possible. A difference in optical band width which is too great produces an instrument in which the two photocell-filter assemblies may have widely divergent nonlinear sensitivities to the nonspecific effects. This complicates the electronic circuits and makes compensation less than perfect or at least more difficult to obtain.

Errors in measurement of the more rapid variations in optical densities can come from the photocells themselves. Under certain conditions a sudden decrease of light intensity can cause an overshoot in a recorded dye dilution curve as illustrated in fig. 9. In this figure the output of two photoconductive cells (recorded by a galvanometer with a natural frequency of 40 cps) filtered at 900 millimicrons and at 800 millimicrons respectively are shown responding to sudden

unequal changes in light intensity. A concentration of 0.1 mg/l of India ink was switched via a 3-way stopcock into the densitometer lumen at the time indicated by arrow. Note that each of these cells exhibit an overshoot to a degree dependent on the light intensity[8]. In addition, the two photocell-filter assemblies might at times be operating under markedly different light intensities as for instance during a dye dilution curve or at low oxygen saturation. Under these conditions, added artifacts in the recorded curve would appear. Fortunately this artifact occurs infrequently, since the variation in the in vivo dye dilution or oxygen saturation is often very slow. If rapid variations are being recorded this effect can become apparent and is sometimes seen during dye sensitivity calibrations.

Recently fiber optics oximeters and densitometers which utilize light reflection technics have been developed. These devices use two or three bundles of incoherent fiber optics to pass incident light through a tube (catheter or needle) into a vein or an artery and pass the reflected (scattered) light from the blood to the photodetectors. Such instruments give promise that a continual record of variation of blood oxygen concentration or dye dilution curves can be recorded without drawing blood from the patient. However, these devices are also subject to inaccuracies resulting from the nonspecific effects and therefore require a minimum of two electro-optical systems. The relatively high cost of such instruments and the need for exacting electro-optical design to permit effective nonspecific rejection constitute major disadvantages to these instruments. The fiber optics systems, unlike the conventional transmission devices, offer no control over undesired effects effects produced by variations in blood flow.

Summary

Undesirable variations (nonspecific effects) of optical density of whole blood during transmission densitometry, occur over a wide light spectrum. These undesirable signals if ignored decrease the accuracy of dye dilution curves and oxygen saturation determinations. Nonspecific effects are caused by the presence of erythrocytes, changes in cell shape and size, and spacial arrangement and flow of erythrocytes. These effects occur, as a result of variations in flow rate, following injection of certain fluids, after breathing CO_2, and as a consequence of changes in temperature and pH. The frequency components of the undesired signals and the desired signals can be identical. Therefore, frequency discrimination alone cannot be used.

According to Lambert-Beers Law, the accurate measurement of N dissolved substances in a liquid, requires sensing at N wavelengths. Thus at least two wavelengths are needed for the accurate measurement of oxygen saturation and also for dye dilution curves.

A number of physical problems complicate perfect compensation for nonspecific effects. The linearity of the calibration curve of these instruments is related to the band width of the optical filter. Two photocell-filter assemblies in a single densitometer may have nonidentical calibration curves. These difficulties can be overcome with proper instrumentation. However, the undesirable characteristics of certain types of photocells are more difficult to control.

Ideally the measurement of physiological variables such as blood pressure, blood flow and oxygen saturation of hemoglobin would be accomplished by sensors on the body surface and obviate the need for puncture of an artery or vein. Although it functions imperfectly, the earpiece oximeter is a device which measures some of these variables in this manner. It is conceivable that improvement can be accomplished, by selection of photocells with appropriate electro-optical characteristics and careful consideration of the optical filters used.

The ideal blood densitometer would provide complete compensation for all undesired optical signals regardless of frequency and independent of the optical density of the desired signal.

[8] This phenomenon is well known but complicated and is thought to be due to the variations in energy levels to which the electron and holes are subjected during excitation, recombination and trapping and is dependent on the energy of the incident photon (i.e. its wavelength).

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