MATHEMATICAL MODEL BASED ON SPECTROSCOPY MEASUREMENT AS: A NEW TECHNIQUES OF CARBOXY-HEMOGLOBIN DETERMINATION IN BLOOD

Awatif R. AL-Dailme, Assit. Lect.*, Ali H. M. Al-hashimi ,prof*
Ghania S. Ghadban, Lect.*

ABSTRACT

Simple accurate, non distractive and quick method of detreming the concentration of carboxy-hemoglobin (COHb) in blood has been develop from mathematical model. The method uses two chosen wave length by any spectrophotometer and measure the optical density with other parameter derived from the mathematical model.

INTRODUCTION

Serious carbon monoxide poisoning leading to death is not difficult to recognize(1,2,3), but the lesser degree which show themselves in shortness of breath and in headache are more difficult, and an accurate method of measuring the percentage of carboxy-hemoglobin in the blood have we suggested. The method suggested has to measure the density of the solution at two wave length; these are best chose to be need result are to be obtained,

1. when the density is measured, the zero error must be determined at the same time and subtracted.
2. To a 1 cm cell the concentration of the blood should be 1%.

Several methods for the determination of COHb blood on spectrophotometeric and multi component spectrophotometeric analysis has been described(4-9)). Most of these methods based on using simple hemolysate without any efficient clearing procedure(10). Therefore, these methods suffered from some sources of errors that were not easily overcome, such as turbidity of Hb hemolysate caused by erythrocytes also plasma-leukocytes, plasma paraproteins, plasma- lipid aggregates, interference rises from plasma pigments like bilirubin, carotence or diagnostic days like Evans blue or cardio green(10). Therefore the aim of this paper was to develop anew techniques based on new mathematical model of multi component spectrophotometrically determine COHb with more accurate results for the inactive Hb-derivatives and active component in COHb form.

THEORY

If a solution cantinas two substance with different absorption spectra and if both obey Beer' s Low(11,12), the concentration of both may be determine, provided both are available. Suppose that the density at wave length λ of a substance A, when present as a 1% solution, is dA, while at wave length λ⁻ the density is dĀ.

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*Department of Physiology, College of Medicine, University of Basrah

Then if the concentration of A is m% the densities will be m.dA and m.dĀ [d = c.1.E] more precisely. If the density of a 1 cm length of a solution containing 1% of the substance is known and is written E ; and the length L of a solution containing c % percent, is found to have a density d then the concentration c can be calculated from the equation $d = c.1.E_{1cm}1\%$

The densities of substance B, whose concentration (n) percent will similarly be n.dB at wavelength λ and ndB¯ at wavelength λ^-. But in absorption the densities are additive, so that the observed densities D and D¯ will be give by the equations:

D = m.dA + n.dB

D¯ = m.dA¯ + n.dB¯

And these can be solved for the concentrations "m" and "n" since all the other quantities are known the solution is :

m = [(D/dB) - (D¯/bB¯)] / [(dA/dB) - (dA¯/dB¯)]

n = [(D/dA) - (D¯/dA¯)] / [(dB/dA) - (dB¯/dA¯)]

In theory these equation serve for all purposes, in practice the fact that they depends on the relative densities of two different solutions makes them unreliable; fortunately they can be transformed, so that all measurements can be made with standardized solution f the two pure bodies, and this last measurement is the relative density R of the unknown mixture at the two chosen wave lengths; thus R is defined as (D/D¯).Further introduce four new relative density; called p,q,r & s and defined by the equation. Will simplified the transformation to the practical reality

p = dA/dA¯   r = dB/dA

q = dB/dB¯   s = dB¯/Ā

these only three are independent for **p.r = q.s p & q** may be determine by measurement on the two pure bodies so that the concentration of the solution used need not be known; but both **r** and **s** compare one pure body with the other, so

that the concentrations in the third experiment must be accurately known(13,14,15).

If now the percentage of A in the mixture of A and B is written p , so that

p = m / m+n¯

an algebraic transformation(16,17) show that:

$$p = \frac{s(R - q)}{s(R - q) + (p - R)}$$

Thus, once **p,q.r** and **s** have been determined at leisure on the pure solution , **p** is a function of R only. If the method is to be used widely, **p** may be plotted as function of R.

Determination of characteristic constant **p.q.r & s :**

In order to obtain the characteristic constants **(p,q,s and r)** of the equation quoted above, a series of 20 experiment was carried out for oxyhemoglobin and carboxy-hemoglobin of samples at concentration of 1% for two chosen wavelength as 5770 and 5600 A° and found :

$$p = \frac{\text{density of CO} - \text{Hb at 5770 A}^\circ}{\text{density of CO} - \text{Hb at 5600 A}^\circ} = 0.868 \pm (*)$$

$$q = \frac{\text{density of O2} - \text{Hb at 5770 A}^\circ}{\text{density of O2} - \text{Hb at 5600 A}^\circ} = 1.66 \pm (*)$$

$$s = \frac{\text{density of CO} - \text{Hb at 5600 A}^\circ}{\text{density of O2} - \text{Hb at 5600 A}^\circ} = 1.322 \pm (*)$$

So that

*Represent SD which are too small to be considered

Substituting these value in the equation

$$p = \frac{s(R-q)}{[R(s-1)+(p-q.s)]}$$

$$p - \frac{1.322(R-1.660)}{(0.322\,R-1.126)}$$

The density ration R at the two chosen wavelength has to be measured on each sample, and the percentage of carboxy-hemoglobin **p**. will be found. For one sample the following neadings obtained table(1):

Where

$$R = \frac{\text{optical density of blood at 5770A°}}{\text{optical density of blood at 5600A°}}$$

Wave-length	Reading		Mean Density	Mean zero	Corrected Density
	Density	Zero			
5770	1.56, 1.57, 1.58	0.04, 0.05, 0.06	1.570	0.050	1.52
5600	1.05, 1.07, 1.03	0.06, 0.07, 0.06	1.057	0.063	0.994

Table(1)

Thus the Ratio R of the density at 5770 and 5600 A° is 1.529 and the percentage of carboxy-hemoglobin is 27.33%.

$$r = \frac{q.s}{p} = 2.529$$

MATERIAL & METHODS

Oxy-hemoglobin

Using a suitable syringe, a m1 of blood withdrawn from a vein and diluted to 100 m1 with distilled water to which a few milliliters of ammonia have been added. The dilution takes the blood, setting free the hemoglobin from the red corpuscles, while the ammonia prevents the proteins coagulating. Most of the hemoglobin obtained in this way will be present as oxy-hemoglobin. To obtain 100 percent conversion, should be sucked through the solution in sunlight. Oxy-hemoglobin

exhibits strong absorption bands, one at 5770 A° and the other at 5600 A°.

Carboxy-hemoglobin

To prepare carboxy-hemoglobin, bubble coal gas for a few minutes through some lakes blood in the dark. The absorption spectrum exhibits two band like those of oxy-hemoglobin, but the bands are displaced these centers at 5600 A° and 7750 A° with modification(18).
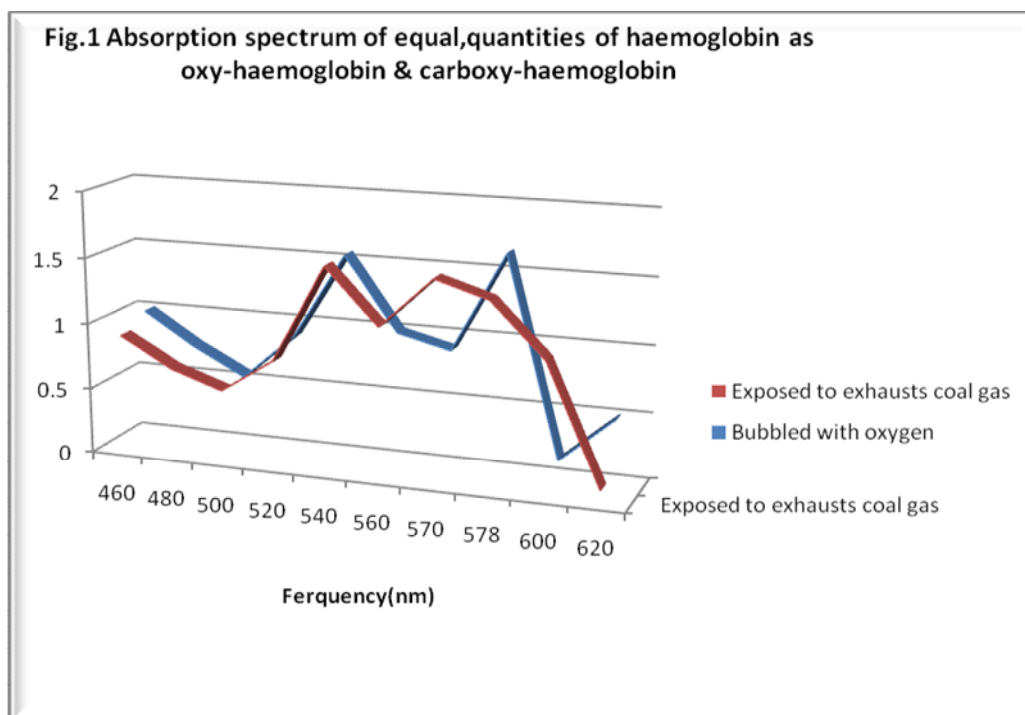
Absorption spectrum of equal, quantities of hemoglobin as oxy-hemoglobin and as carboxy-hemoglobin in two chosen wavelength represent in fig(1) and the two chosen wave length have good regression in linearity fig(2).

Table(3)

λAº	Oxy-Hb Blood bubbled with oxygen Ab%	Carboxy-Hb smoked blood exposed to exhausts coal gas Ab%
4600	1	0.9
4800	0.75	0.68
5000	0.55	0.55
5200	0.90	0.81
5400	1.55	1.55
5600	1	1.14
5700	0.9	1.52
5780	1.65	1.4
6000	0.15	1
6200	0.5	0.1

Table(3) show the absorption at different wavelength.

Fig.1 Absorption spectrum of equal,quantities of haemoglobin as oxy-haemoglobin & carboxy-haemoglobin

LINEARITY:

To verify the concentration of COHb was linear, at various concentration. Using a sample of blood from not known to have had contact with any source of carbon monoxide, a master dilution blood was made. One half of this diluted sample was converted to COHb by bubbling carbon monoxide through the solution for 20 meliutes followed by nitrogen gas for 30 minutes, to remove excess dissolved carbon monoxide. The remainder of the diluted sample had oxygen gas bubbled through for 20 minutes to remove any COHb, followed by nitrogen as to remove excess dissolved oxygen. The oxygenated sample was then dispensed accurately in known quantities into sodium dithionite, the reducing agent. The COHb solution was then added accurately in small known amounts. These were well mixed before being real as above. Results shown in Fig(1) which shown concentration of COHb between O and 93.6%. the correlation between theoretical and observed values is (r=0.99). similar experiments was carried out using sample of blood with a COHb concentration between (0-20)% in 1% steps. The linearity of this was (r=0.99).
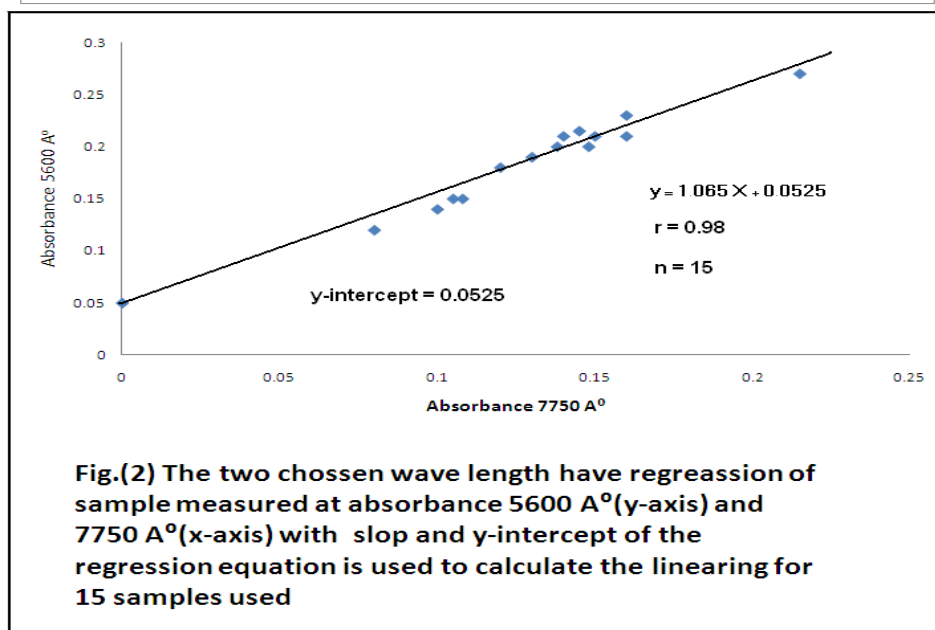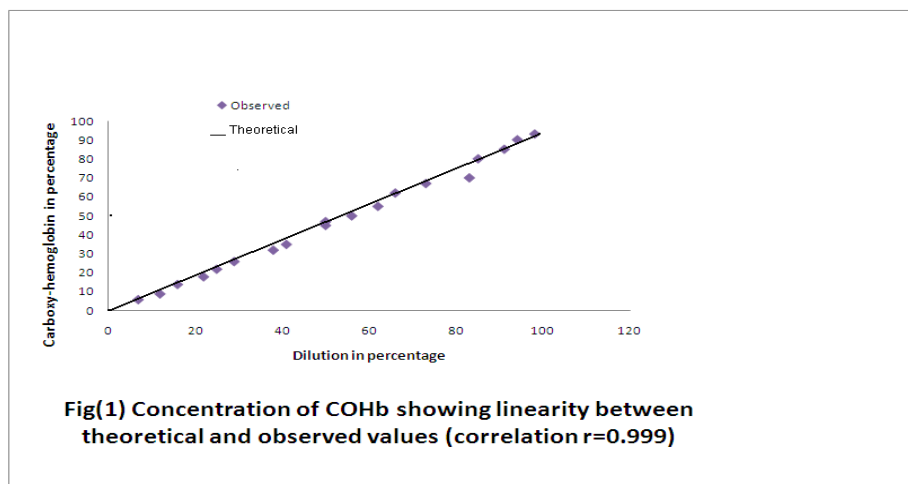
Fig(1) Concentration of COHb showing linearity between theoretical and observed values (correlation r=0.999)

Fig.(2) The two chossen wave length have regreassion of sample measured at absorbance 5600 A°(y-axis) and 7750 A°(x-axis) with slop and y-intercept of the regression equation is used to calculate the linearing for 15 samples used

REPRODUCIBILITY:

Test an reproducibility of the method between reading of the some subsample of the same dilution and dilutions of the same blood were carried out at various concentrations of COHb. The study was carried out at two different chosen was length.

Twenty sample of blood were each diluted (1/100) with 0.4% w/v ammonium hydroxide to give a final volume of 10 ml. each dilution was mixed thoroughly and half of each sample was placed in 10 mg /ml solution of the reducing agent sodium dithionite. Carbon monoxide was bubbled through the remainder for 3 minutes. Each of these samples was then read 3 times at two chosen waves length. The results were analyzed statistically as in table (2)

Reproducibility	λ 5600A°	λ 5770 A°
Between reading	0.2190	0.0563
Between subsamples	0.2091	0.2319
Between dilution	0.2030	0.2211
4.1% COHb	0.1990	0.3351
20.1% COHb	0.1911	0.6006

Table(2)

Analysis of variance of results showing reproducibility between reading, subsamples and dilutions for 20 paired reading at random two chosen wavelength. The variance 0.335/(SD=0.0579) for 4.1 COHb and 0.6006 (SD=0.0775) for 20.1 % COHb.

Variance in the subsamples is due to sampling error which may due to very slight differences in the amount of sodium dithionite used for reducing hemoglobin. Dilution error was not statistically significant.

DISCUSSION

We believe that the mathematical model described above is good, sensitive, non distractive and cheep to be used as quick method to measurement of carboxy-hemoglobin and it is applicable to any mixture of two compounds with an isobathic point.

The advantages of this method are accuracy and speed. Our results show an accuracy of $\pm$ 0.5% COHb over the whole rang.

The accuracy depends on the care taken in setting up the instrument and taking the readings. With the technique described one can distinguish without ambiguity between carboxy- hemoglobin of (0-28)% and this sensitivity is enough for may purpose.

The success of this techniques leads as to suggest that spectrophotometery may be an extremely useful tool in the estimation of COHb (carboxy-hemoglobin) and possibly of other constituents of blood.

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النموذج الرياضي المعتمد على القياس الطيفي
(تقنية جديدة لتحديد نسبة الكربون في الهيموكلوبين الدم)

م.م. عواطف راضي الديلمي*، أ.د. على حسين الهاشمي*، م. غنية سالم غضبان*

الخلاصة

الموديل الرياضي المعتمد على تحليل البيانات باستخدام التحليل الطيفي قد اقترحت طريقة جديدة لتقدير النسبة المئوية للتسمم بأول اوكسيد الكاربون في عينات من الدم الطريقة سهلة وموضوعية.

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* كلية الطب/فرع الفسلجة، جامعة البصرة ، العراق