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Detection the Ratio of Bilirubin in Human Body Using Laser Technology

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Abstract: In this work, an analytical study to detect the ratio of bilirubin in human body by determining the level of it in blood using a 532nm laser light transported through some samples of bilirubin has been presented. A second harmonic generation of Nd:YAG laser with measured maximum output power 52.5 mW was used to determine the concentration of total bilirubin in blood. Initially, the cuvette was filled with a sample of standard bilirubin level and then it is filled by a sample of blood containing unknown levels of bilirubin. The absorption factor was calculated for four samples of adults' blood and five samples of babies' blood, and the scattering factor was neglected for each sample. The unknown concentration of total bilirubin was determined and the transmitted power through these samples of blood was measured. In this work, a good matching was obtained between the results of the concentrations of bilirubin in blood using laser technique and the results of the classical medical procedure of measuring the bilirubin concentrations. Therefore, the jaundice in human was detected.

I. INTRODUCTION

Jaundice is a yellowish staining of the skin, sclera and mucous membranes by deposition of bilirubin (a yellow orange bile pigment) in these tissues. Jaundice was once called the "morbus regius" (the regal disease) in the belief that only the touch of king could cure it. Jaundice indicates excessive levels of conjugated or unconjugated bilirubin in the blood and it is clinically apparent when the bilirubin level exceeds 2mg/dl (34.2 µmol/L) [1].

In fair-skinned patients, jaundice is most notice able on face, trunk, and sclerae; in dark-skinned patients, its noticeable on the hard palate, sclerae, and conjunctivae-pseudo jaundice may be found in black patients with pigmented sclera, from carotinemia, uremia (a sallow yellowish pallor), and quinacrine (a yellow-green color) [2]. Causes of jaundice can be classified into Pre-hepatic, hepatic or post hepatic [2].Tissue deposition of bilirubin occurs only in the presence of serum hyperbilirubinemia and is a sign of either liver disease or, less often, hemolytic disorder. Another sensitive indicator of increased serum bilirubin is darkening of the urine, which is due to the renal excretion of conjugated bilirubin. Patients often describe their urine as tea or cola colored [3].

Jaundice can be classified into two groups; the first group is the physiological jaundice and the second group is the pathological jaundice of neonates. The causes of jaundice can be grouped into the following categories in Fig.1 [3]

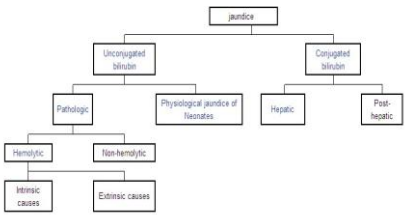

Fig.1 Causes of jaundice

II. BILIRUBIN

Bilirubin (formerly referred to as hematoidin) is the yellow breakdown product of normal heme catabolism. It is excreted in bile and urine, and elevated levels may indicate certain diseases. It is responsible for the yellow color of bruises, urine, and the yellow discoloration in jaundice [4].

Bilirubin consists of open chain of four pyrrole-like rings (tetrapyrrole). In heme, by contrast, these four rings are connected into a larger ring called a porphyrin ring as shown in Fig.2 and the molecular formula of bilirubin is C33H36N4O6 [4].

Bilirubin is created by the activity of biliverdin reductase on biliverdin, a green tetrapyrrolic bile pigment which is also a product of heme catabolism [5, 6].

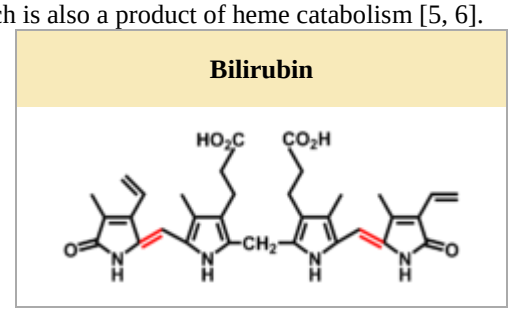

Fig.2 The atomic structure of bilirubin

III. UNCONJUGATED BILIRUBIN

Erythrocytes (red blood cells) generated in the bone marrow are disposed of in the spleen when they get old or damaged. This released hemoglobin, which is broken down to heme as the globin parts. The heme is then turned into unconjugated bilirubin in the reticuloendothelial cells of the spleen, which is not solube in water. It is then bound to albumin and sent to the liver [5,6].

IV. CONJUGATED BILIRUBIN

In the liver it's conjugated with glucuronic acid by the enzyme glucuronyltransferase, making it soluble in water. Much of it goes into the bile and thus out into the small intestine. Some of the conjugated bilirubin remains in the large intestine and is metabolized by colonic

bacteria to urobilinogen,whichisfurther metabolized to stercobilinogen, and finally oxidized to stercobilin, which it gives feces its brawn color [5, 6].

There are many methods for determination the concentration of bilirubin such as [6]:

A. Determination of the concentration of bilirubin in serum by:

- Rapid micro-method employing photoelectric colorimeter.
- Rapid micro-method employing color standards.

B. Determination of free bilirubin and it binding capacity by HAS using a microfluidic chip-capillary electrophoresis device with multi-segment circular-ferrofuild-driven micro mixing injection.

C. Measurements the concentration of bilirubin by using laser technology, this method depend on the laser beam attenuation and it's considered in this work.

V. ATTENUATION OF LIGHT

The beam attenuation coefficient α(λ) is used to characterize the optical transmission properties of matter , it's a measure of decay of the unscattered light and its given by the equation (1) [7]

$$P_1(\lambda) = P_o(\lambda) e^{-\alpha(\lambda)L} \quad \text{Eq.(1)}$$

Where $P_1(\lambda)$ is the measured beam radiant power,

$P_o(\lambda)$ is the initial beam radiant power, and

L is the optical path length.

The beam attenuation coefficient is the sum of the absorption coefficient a(λ) and the scattering coefficient s(λ) which its define by [7]:

$$\alpha(\lambda) = a(\lambda) + s(\lambda) \quad \text{Eq.(2)}$$

The scattering phenomenon is negligible because of the dependence of scattering on wavelength. With a given size of particles, long waves would be expected to be less effectively scattered than short ones, because the particles present obstructions to the waves which are smaller compared with the wavelength for long waves than short ones [8]. Then

$$\alpha(\lambda) = a(\lambda) \quad \text{Eq.(3)}$$

When a beam of light is passed through matter in the solid, liquid or gaseous state, its propagation is affected in two important ways. In the first way, the intensity will always decrease to a greater or less extent as the light penetrates farther into the medium. In the second way, the velocity will be less in the medium than in free space. The loss of intensity is chiefly due to absorption. Then absorption is a way of interaction of the electromagnetic radiation with matter, energy absorbed and transformed to other type, the absorption coefficient ($\alpha(\lambda)$ cm^{-1}) is a property of matter [8].

The observed power is converted to a specific absorption coefficient by using the following formula [7]

$$\alpha = \frac{-\ln[(P/A)/(P_o/A_o)]}{L} \quad \text{Eq.(4)}$$

where P is the measured beam power after sample (mW),
Po is the incident beam power before sample (mW),
A is the spot area of beam after sample,
Ao is the spot area of beam before sample, and
L is the length of the cuvette (cm).

Resulting from short distance between the container of sample (cuvette) and power meter, then A = Ao. Therefore,

$$\alpha = \frac{-\ln[P/P_o]}{L} \quad \text{Eq.(5)}$$

The transmitted beam is define by [7]

$$T(\lambda) = \frac{P(\lambda)}{P_o(\lambda)} \quad \text{Eq.(6)}$$

and the absorption coefficient becomes as:

$$\alpha = \frac{1}{L} \ln \frac{1}{T(\lambda)} \quad \text{Eq.(7)}$$

VI. EXPERIMENTAL SET UP

The essential components in the setup includes: light source (green laser), a precision glass cuvette as the blood container, and a power meter as shown in Fig. 3

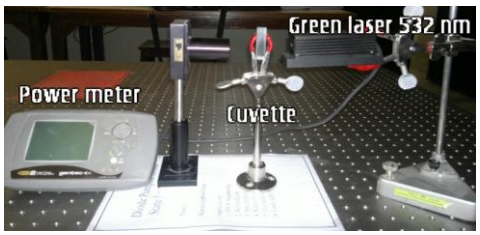

Fig.3 Experimental setup

An Nd: YAG laser (second harmonic generation) with a wavelength of ≈532 nm and the measured output power was about ≈ 52.5 mW as the light source. When a green laser light passed through some samples of blood container the concentration of bilirubin in blood was determined. Initially, the container was filled by a sample of blood containing a standard bilirubin level and then by sample of blood containing unknown levels of bilirubin. For each case, the output power would be measured and calculated by using equations (6) and (7).Its attenuation which was occurred where laser light passing through the sample of blood.

Spectrophotometer was used to measure the absorption coefficient of human blood as a function of wavelength when the cuvette is filled with a sample contains a standard bilirubin level and the result as shown in Fig 4.

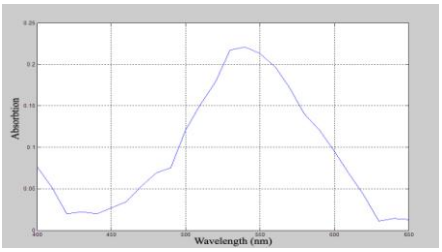

Fig.4 Absorption curve of human blood as a function of wavelength

From Fig.4 the maximum absorption coefficient occurs at range 530-540 nm which has value closely to absorption occur at 532 nm. Therefore a second harmonic generation Nd: YAG laser of 532nm was used.

The samples of blood are dissolved with reagent material because of the normal blood is heavy and the laser cannot be able to pass through it, these reagent material cause an amplification of bilirubin molecules

resulting in dominancy of bilirubin molecules on other molecules substances involved in human blood.

The sample work solution by mix 20R1 (sulfanilic acid 30mmol/l, hydrochloric 150mmol/l, dimethylsufoxide 7mmol/l) volume with 1R3 (sodium nitrite 20mmol/l) volume and R4 represent the standard concentration of bilirubin which its equal 83 µmol/l, then added to the sample. The color of the work solution change to the violet which represent the (test) and when the reagent R1was added alone to the same sample then the solution color became yellow which represent the (blank). These results of work solution as shown in Fig.5

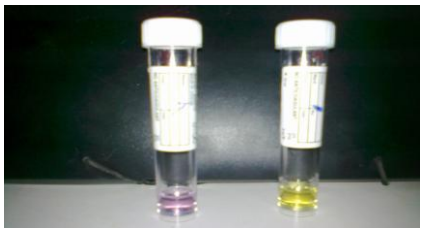

Fig.5 Preparation of sample for measurement

VII. Calculations And Measurements Results

In this work, the concentrations of bilirubin of human blood for all samples were measured by two methods; the first one is the medical laboratory measurements and the other one is the optical activity method using SHG Nd:YAG laser of measured power about 52.5mW.

The concentration of total bilirubin of human blood can be calculated using the below empirical equation:

$$Concentration = \frac{\alpha_{Sample}}{\alpha_{S\tan dard}} \times C_{S\tan dard} \qquad \text{Eq.(8)}$$

Where

$\alpha_{Standard}$ is the measured absorption coefficient of standard sample = 0.434 cm-1, C Standard is the standard concentration of total bilirubin of human blood = 83mg/dl.

The range of the total bilirubin concentrations of blood is about (0.2 -1) mg/dl. Four samples of adults and five samples of babies were taken in this work.

The experiment recording reading of power meter and the medical tested of the concentration of adults samples are shown in table I and figures 6a and 6b using equation (8).

TABLE I

ILLUSTRATE RECORDING POWER AS A FUNCTION OF THE CONCENTRATION OF BLOOD SAMPLES FOR ADULTS

Absorbed power (mw)	Transmitted Power (mw)	Concentration (mg/dl) of samples for adults		Error rate %
		Experimental measurement	Medical laboratory measurement	Average error rate ≅ 1.5%
14.15	38.35	3.533	3.589	1.5
19.75	32.75	5.308	5.384	1.4
28.02	24.48	8.132	8.256	1.5
3.98	48.52	0.886	0.9	1.5

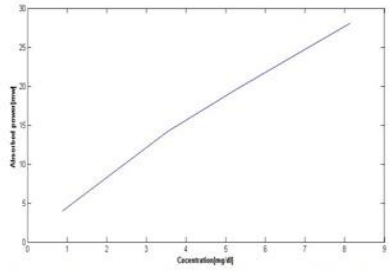

Fig.6a Absorbed power as a function of concentrations (for adults)

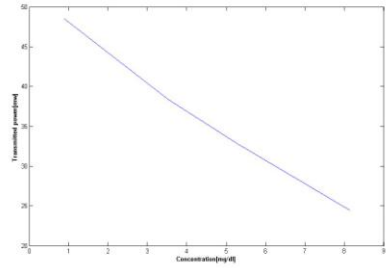

Fig.6b Transmitted power as a function of the concentration (for adult)

From table I, the error rate between experiment results and medical tested of blood samples was about 1.5% and it is acceptable value.

For sample of babies, the error rate was about 2% and the measurement results are shown in table 2 and figures 7a and 7b using equation (8).

TABLE II

ILLUSTRATE RECORDING POWER AS A FUNCTION OF THE CONCENTRATION OF BLOOD SAMPLES FOR BABIES.

Absorbed power (mw)	Transmitted Power (mw)	Concentration (mg/dl) of samples for Babies		Error rate %
		Experimental measurement	Medical laboratory measurement	Average error rate ≅ 2%
21.37	31.13	5.879	5.983	1.7
24.76	27.8	7.152	7.323	0.2
41.6	10.9	17.685	17.973	1.6
27.7	24.8	8.436	8.56	1.4
29.4	23.1	9.235	9.4	1.7

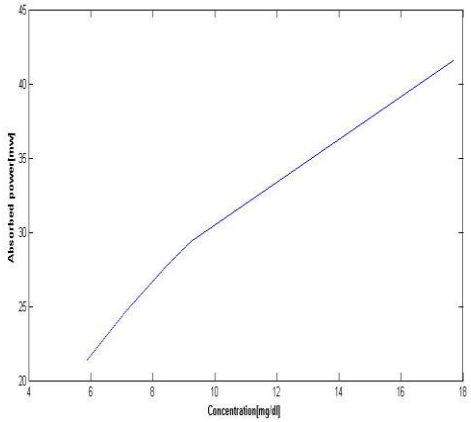

Fig. 7a Absorbed power as a function of the Concentration (for Babies)

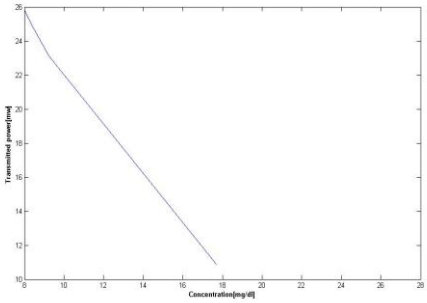

Fig.7b Transmitted power as a function of the Concentration (for Babies)

Figures (8 a, b) show a transmitted power for adults and babies samples as a function of the concentrations of bilirubin measured by two methods

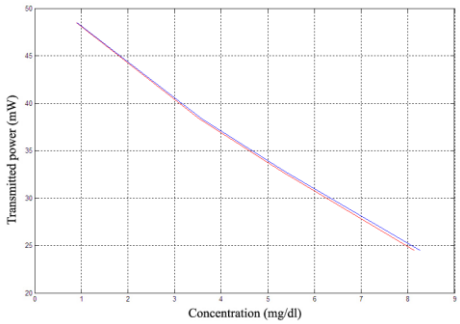

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Fig.8a Variations of concentration of bilirubin measured by medical laboratory and concentration measured experimentally (for Adults)

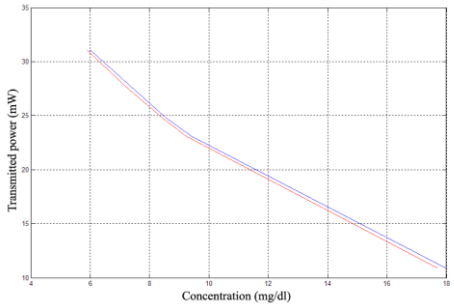

Fig. 8b Variations of concentration of bilirubin measured by medical laboratory and concentration measured experimentally (for Babies)

VIII. CONCLUSIONS

1- A good matching occurs between the experimental measurements and medical laboratory tested of a human blood samples contained bilirubin.

2- The results demonstrate the ability of the power meter to be accurately measure bilirubin concentrations in blood. This method represents an important step toward the development of a noninvasive bilirubin sensor that may eventually be capable of detecting bilirubin levels in the blood.

3- The error (2%) result from the existence of human blood several type of molecules substances, imperfect environment of experimental work, the variation of wavelength for laser used and the peak absorption wavelength and the very small variation of both spot area of laser beam before and after the cuvette which assumed to be equal.

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