

Determination of total protein in human blood by laser**Sahra Salem Jassem and Abdul Rahman Rashid Mohammed****Determination of total protein in human blood by laser****Sahra Salem Jassem¹ and Abdul Rahman Rashid Mohammed²**^{1,2}Applied Science-University of Technology²Abdulrahman2001@yahoo.com

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Abstract

The capabilities of laser analysis of total protein (TP) were studied. Semiconductor laser diode of 5-mW maximum power, (532) nm wavelength and (4) nm bandwidth is used as laser system (LS) to analyze and find the concentration of total protein (TP) in human blood serum. All the absorption spectra of TP were measured with a UV-VIS spectrophotometer. The spectral plot showed that the maximum absorbance of (TP) is at the range (520-580nm) and the peak at (546) nm. The (LS) measurements included the study of absorbance (A) of (TP) as a function of cuvet thickness (t), transmittance (T) as a function of (t) and (A) as a function of laser power. To ascertain our calculations, the results have been compared with the results of spectrophotometer. The (RSD %) values are about (0.67-17.18).

Keywords: Total proteins, Spectrophotometer, Colorimetric method, Laser.**تحليل وتعيين البروتين الكلي (PT) في دم الانسان بواسطة الليزر**ساهرة سالم جاسم¹ و عبدالرحمن رشيد محمد²^{1,2}قسم العلوم التطبيقية - الجامعة التكنولوجية**الخلاصة**

في هذا البحث تم دراسة إمكانية استخدام منظومة ليزيرية لغرض تحليل البروتين الكلي (TP) في مصل الدم لجسم الانسان. تم استخدام ليزر الدايدود شبه الموصل بقدرة عظمى (5mW) وبطول موجي 532 nm وبعرض حزمة 4 nm لإجراء القياسات. جميع أطيف الامتصاص للبروتين الكلي تم حسابها بواسطة المطياف. اظهر المخطط الطيفي أن اعظم امتصاصية للبروتين تقع في المدى (520-580) nm وان قمة الامتصاصية تقع عند الطول الموجي 546 nm. قياسات

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المنظومة الليزرية اشتملت على دراسة الامتصاصية كدالة لسمك النموذج والنفاذية كدالة للسمك والامتصاصية كدالة لقدرة الليزر. لتأكيد وضبط الحسابات تم مقارنة النتائج المستحصلة مع نتائج المطياف أظهرت القياسات توافقاً جيداً وبانحراف معياري نسبي يتراوح بين (0.67-17.18).

الكلمات المفتاحية: البروتين الكلي، المطياف، الطريقة اللونية، الليزر.

Introduction

Laser analysis of blood component is an alternative method with desired properties of laser beam as in monochromatic, coherent...etc. Determination of total protein (TP) is widely used in several areas such as: clinical analysis, food, science, food technology, biochemistry, physiology, protein chemistry, medical researchers, ecology, as well as in much other areas [1][2]. Also (TP) is the one of the most important of human blood serum analysis, a small change in TP can considerably affect the activity of human body. However, in rare occasions, the changed protein leads to a better function in the body, commonly known as evolution. And in some unfortunate cases, a changed protein leads to a disease, such as Alzheimer's disease [3]. Its level falls through kidney in several renal and severe liver diseases [4]. Proteins also are utilized in membranes, such as glycoproteins. When broken down into amino acids, they are used as precursors to nucleic acid and vitamins [5]. Hormones and enzymes are also formed from amino acids in which they help regulate metabolism, support the immune system and other body functions. Finally, protein is needed to form blood cells [6][7]. Many laboratories currently employ spectrophotometer to analyze TP in spite of existence of several methods, using different analysis tools (color photometry, chromatography Polarography ,etc.), developed for the determination of (TP), but the UV-Visible spectrophotometric methods are the common [8][9]. A number of methods have been devised to measure protein concentration, which are based on UV-visible spectroscopy. These methods use either the natural ability of proteins to absorb (or scatter) light in the UV-visible region of the electromagnetic spectrum, or they chemically or physically modify proteins to make them absorb (or scatter) light in this region. The basic principle behind each of these tests is similar. First of all a calibration curve of absorbance (or turbidity) versus protein concentration is prepared using a series of protein solutions of known concentration. The absorbance (or turbidity) of the solution being analyzed is then measured at the same wavelength, and its protein concentration determined from the calibration curve [10]. We can use laser in analysis and comparative studies among these methods are carried out [11][12]. We found that a low-cost and fast analysis can be done using laser diode system instead of spectrophotometer (SP) system.

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There are a wide variety of different instrumental methods available for determining the total protein. These can be divided into three different categories according to their physicochemical principles: (i) measurement of bulk physical properties, (ii) measurement of adsorption of radiation, and (iii) measurement of scattering of radiation. Each instrumental method has its own advantages and disadvantages, and range of sample to which it can be applied [10].

1-Measurement of Bulk Physical Properties

Density: The density of a protein is greater than that of most other sample components, and so there is an increase in density of a sample as its protein content increases. Thus the protein content of sample can be determined by measuring their density.

Refractive index: The refractive index of an aqueous solution increases as the protein concentration increases and therefore RI measurements can be used to determine the protein content [10].

2-Measurement of Adsorption of Radiation

UV-visible: The concentration of proteins can be determined by measuring the absorbance of ultraviolet-visible radiation.

Infrared: Infrared techniques can be used to determine the concentration of proteins in samples. Proteins absorb IR naturally due to characteristic vibrations (stretching and bending) of certain chemical groups along the polypeptide backbone. Measurements of the absorbance of radiation at certain wavelengths can thus be used to quantify the concentration of protein in the sample. IR is particularly useful for rapid on-line analysis of protein content. It also requires little sample preparation and is non-destructive. Its major disadvantages are its high initial cost and the need for extensive calibration [10].

Nuclear Magnetic Resonance: NMR spectroscopy can be used to determine the total protein concentration of sample. The protein content is determined by measuring the area under a peak in an NMR chemical shift spectra that corresponds to the protein fraction.

3-Measurement of Scattering of Radiation

Light scattering: The concentration of protein aggregates in aqueous solution can be determined using light scattering techniques because the turbidity of a solution is directly proportional to the concentration of aggregates present.

Ultrasonic scattering: The concentration of protein aggregates can also be determined using ultrasonic scattering techniques because the ultrasonic velocity and absorption of ultrasound are

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related to the concentration of protein aggregates present. A number of these instrumental methods have major advantages over the other techniques mentioned above because they are non destructive, require little or no sample preparation, and measurements are rapid and precise. A major disadvantage of the techniques which rely on measurements of the bulk physical properties of samples are that a calibration curve must be prepared between the physical property of interest and the total protein content, and this may depend on the type of protein present and the samples matrix it is contained within. In addition, the techniques based on measurements of bulk physicochemical properties can only be used to analyze foods with relatively simple compositions. In samples that contain many different components whose concentration may vary, it is difficult to disentangle the contribution that the protein makes to the overall measurement from that of the other components [10].

Theory

One of the most important applications of the interaction between electromagnetic radiation and matter is the determination of the concentration of certain components in material. This application relies on there being a relationship between the amount of a monochromatic light beam with intensity I_0 absorbed by a material and the concentration of the components present. The intensity (I_s) of light beam exiting a solution is less than the intensity entering the solution (I_0), (Fig.1) Because solute molecules absorb some of the intensity. The amount of intensity absorbed is usually expressed in terms of either the *transmittance* or the *absorbance*. The transmittance is simply the ratio of the exiting and incoming radiation: $T = I_s/I_0$. Also the transmittance through a reference cell is (I_r/I_0); the transmittance for a compound in solution is then defined as (I_s/I_r). and is often expressed as a percentage $\%T = (I_s/I_r) \times 100$. Unfortunately, T or $\%T$ is not proportional to the concentration of the absorbing species and so another parameter, known as the absorbance A , has been defined which is proportional to the concentration:

$$A = \log(1/T) = -\log T = 2 - \log(\%T) \quad (1)$$

When we hold the concentration constant and double the inside diameter of the cell, the effect on absorbance is the same as doubling the concentration, In dilute solutions the absorbance is proportional to the concentration of the absorbing species, which is extremely convenient for quantitative analysis of concentration. The relationship between the absorbance of a solution and its concentration is known as Bouguer's Law or Lambert's law, which can be written as:

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$$I_s = I_o \exp(-\alpha ct) \quad (2)$$

Where (α) is the absorption coefficient and (t) is the light path or cuvet thickness. The overall equation relating these variables may express as [8]:

$$A = \varepsilon ct \quad (3)$$

Where ε is a proportionality constant defined as a (ε) absorptivity, t in cm, and c is the concentration. This equation is called Beer's law and forms the basis of quantitative analysis by adsorption photometry. When $t=1$ and c is expressed in mole per liter, the symbol ε , which is called molar absorptivity is substituted for the constant ε . For $t=1$ cm and $c=1\text{g/ml}$, $A=\varepsilon$. This constant often written as $A_{1\text{cm},1\%}$, and we called as specific absorption coefficient.

Experimental

All the optical absorption spectra of the TP sample were measured with a CECIL, Model C.7200UV-vis spectrophotometer. Round cuvetts, used usually in visible range, are cleaned by copious rinsing with tap water and distilled water. Then both round and square cuvetts may be cleaned in mild detergent or soaked in mixture of concentrated HCl-water-ethanol (1:3:4). A good practice is to fill all such cuvetts with distilled water and measure the absorbance for each against a reference (blank) over the wavelength to be used. This should be essentially zero. Correctness of photometric measurements for narrow bandwidth instruments using spectrophotometer may be verified as described by Rand [13]. The direct proportionality between absorbance and concentration must be established experimentally for a given instrument under specified condition. Frequently there is a relationship up to a certain concentration or absorbance. We say then that the solution obeys Beer's law up to this point. Within this limitation, a calibration constant or factor may be derived and used to calculate the concentration of an unknown solution by comparison to a standard. From equation (2) for two samples:

$$C_{sa} = (A_{sa} / A_{st}) C_{st} \quad (4)$$

Where (st.) and (sa.) represent standard and unknown sample, respectively. Solving for the concentration of unknown, we obtain [12]

$$C_{sa} = A_{sa} (C_{st} / A_{st}) \quad \text{or} \\ C_{sa} = A_{sa} (C_{st} / A_{st}) = A_{sa} \times F \quad (5)$$

Where $F = C_{st} / A_{st}$. Relative standard deviation (RSD %), shown in equation (6), is used to statistically compare the observed results.

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$$R.S.D = \left| \frac{x_i - \bar{x}}{\bar{x}} \right| \times 100\% \quad (6)$$

Where $\bar{x}$ is the value of spectrophotometer and x_i is the value of (LS) [14].

Spectral absorbance is measured for serum samples under test exposed to a monochromatic light for wavelength 450nm to 650nm. The spectrophotometer (SP) was type (CECIL, Model C.7200). Our source is laser diode type. Its wavelength is 532nm; maximum output power is 5mW and class is (IIIB laser product, 21CFR) from Transverse Industry Company. The laser bandwidth ranges about (524-546nm) as experimentally justified using a monochromator type (PENPHAN-M800). The major component of our (LS) is shown schematically in Figure (2). In order to obtain a variety of laser output power a simple variable series resistance ($R_1=0-10$ ohm) circuit is used here as shown in fig (3). Also figure (4) shows the detector, absorbance/percent transmittance meter and parallel variable resistance (R_2) circuit, to normalize the measurements of (LS), where $R_2= (0-5)$ kilo ohm. Table (1) represents the procedure of preparation for (TP), prepared by BioMerieux Company [15][16].

Table (1): preparation of (TP)

Procedure		BioMerieux Co.	
Wavelength		545 nm	
Solution	Blank	Standard(St.)	Sample (Sa.)
Standard(St.)	-	20 μ l	-
Sample (Sa.)	-	-	20 μ l
Dematerialized water	20 μ l	-	-
Working solution	1ml	1ml	1ml
Mix. measure after 5 minutes			
Calculation:			
Sample Concentration (C_{Sa})=($A_{Sample}/A_{Standard}$) * Standard concentration (C_{St})			
Standard Concentration (C_{St})=100g/l			

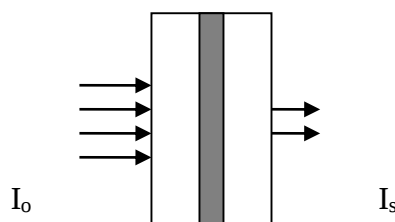


Fig (1) Transmittance through a sample and reference cell

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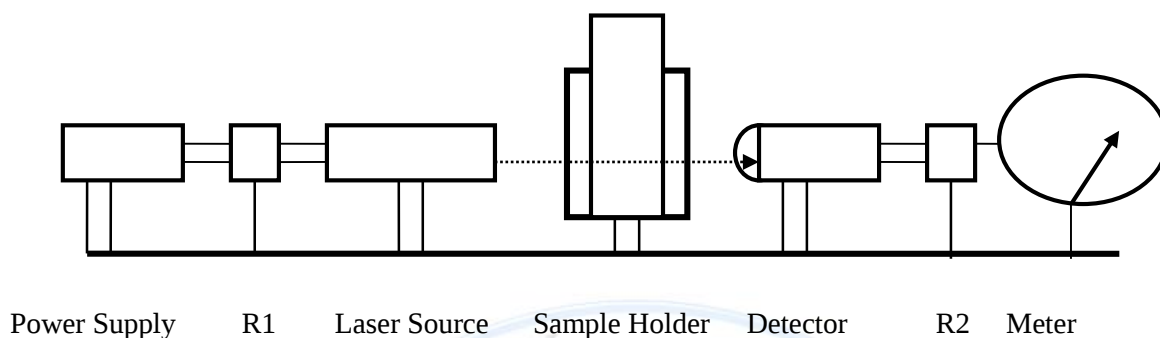


Fig (2): (LS) components to analyze (TP)

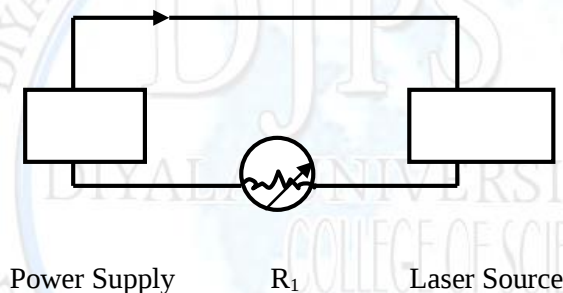


Fig (3): Simple electrical series resistance circuit for controlling laser power.

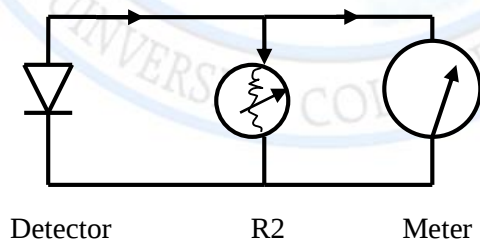


Fig (4): Detector circuit for (LS) normalize

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Figure (5) shows the band width of the laser diode. The maximum irradiant power is approximately at wavelength 532nm. The spectral wavelength ranges about (530-534) nm and the width opposite to half maximum value is about 4nm. This bandwidth is compromise with maximum absorbance of (TP). Figure (6) show the spectral responsivity (SR) of silicon photo detector (DRS-500). It's clear from the figure, there are two regions, and the first one (corresponding to the green spectral region) shows increase in (SR) with wavelength of laser. The smaller (SR) at long wavelength (second region) is attributed to the carriers generation deep in the silicon bulk [17]. Figure (7) exhibits absorption spectrum of (TP), the maximum absorbance lies in the wavelength range (520-580)nm and the peak at 546nm. The region which is convenient to accomplish analysis must satisfy several conditions, these conditions are: it is flat, lies at the peak of maximum absorbance and not overlaps with other compounds [18]. The absorbance of (TP) as a function of curvet thickness (t) can be shown in figure (8). This figure coincides with Lamberts law. Consequently, we can find any unknown concentration of a certain thickness. Figure (9) shows the transmittance of total protein as a function of cuvette thickness. The plot shows an inversely exponential relation which also coincide with Lamberts' law [13]. Figure (10) shows that the absorbance of (TP) is independent of laser power. Consequently, it is possible to use any power to get this analysis. In table (2), three kinds of controllers H, N and L referred to High, Normal, and Low; respectively were used. Several samples were prepared for each sort of age and sex. The Table shows the results of absorbance, concentration and relative standard deviation (RSD) % for each sample using (SP) and (LS). The table exhibits approximate results using those two systems. The values of (R.S.D) indicate that the laser results are more accurate than spectrophotometer results. Sample (4), measured by spectrophotometer, has a high (RSD) because of stray radiations. It can be shown that the values measured with laser system are always less than those of standards; so we can correct the results use a correction factor, this advantage can not be used in (sp) system. Also the values of (R.S.D) are ranging about (0.67-17.18) which obviously within the acceptable medical tolerance. Fig (11) and (12) shows the agreement between (LS) and (SP) of results. Here we used arbitrary samples for different ages and breeding. It is obvious that there is an agreement between values using those two methods. Also there are some errors because of the low monochromaticity and the high divergence of spectrophotometric light beam compared with laser beam.

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Table (2): Absorbance and concentration values for different samples of total protein

Samples	Spectrophotometer(SP)		Laser System(LS)		(RSD)%
	Absorbance	Concentration mg/100ml	Absorbance	Concentration mg/100ml	
St.	0.39	-	0.37	-	
L	0.12	30.78	0.12	32.43	5.36
N	0.27	69.23	0.22	59.46	14.11
H	0.19	48.72	0.15	40.54	16.79
Sa.1	0.52	133.32	0.49	132.42	0.67
Sa.2	0.11	28.20	0.10	27.02	3.29
Sa.3	0.33	84.61	0.30	81.08	4.18
Sa.4	0.16	41	0.16	43.24	5.46
Sa.5	0.21	53.84	0.19	51.35	4.60
Sa.6	0.28	71.8	0.22	59.46	17.18
Sa.7	0.36	115.38	0.44	118.92	3.06
Sa.8	0.38	92.30	0.32	86.48	6.30
Sa.9	0.55	141	0.45	121.62	13.74
Sa.10	0.25	64.1	0.24	64.86	1.18
L=35.10 , N=62.60 , H=43.49 , St.=100g/l BioMerieux Co.					

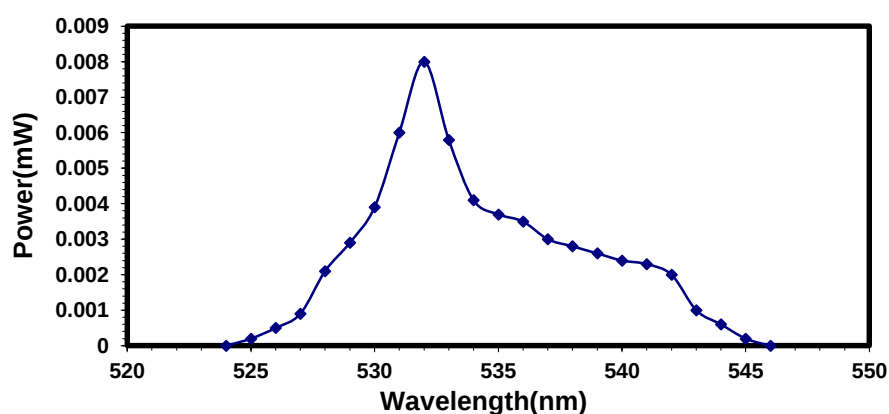


Fig (5): laser diode power as a function of wavelength.

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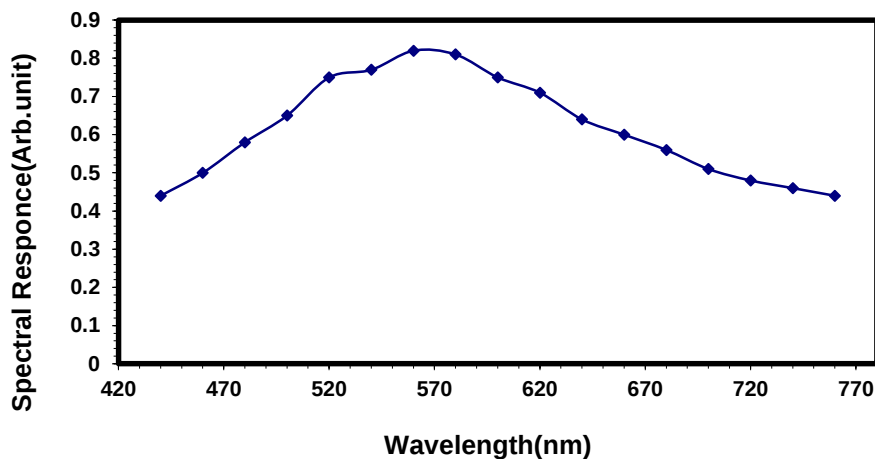


Fig (6): Spectral response as a function of wavelength.

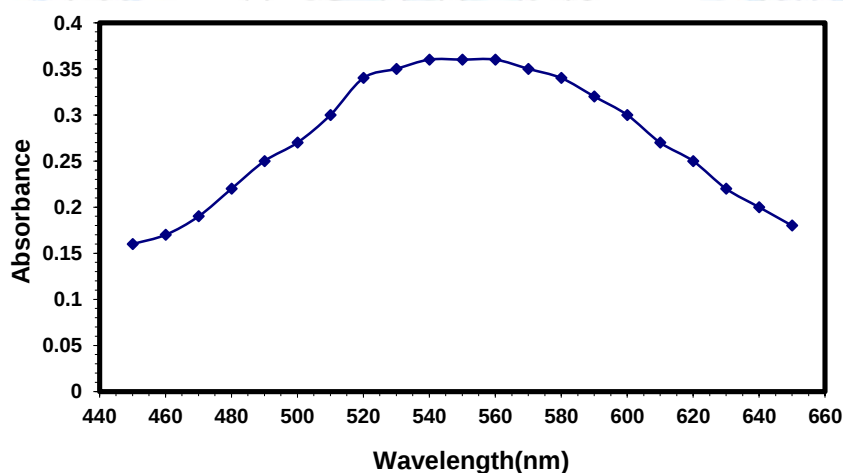


Fig (7): The (TP) absorbance as a function of wavelength.

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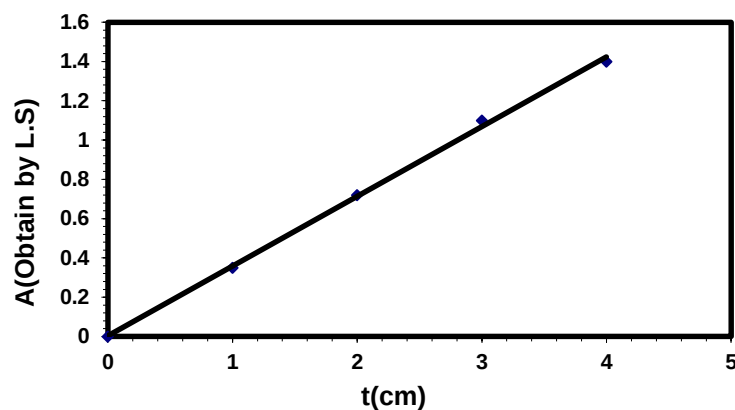


Fig (8): (TP) absorbance for laser as a function of cuvet thickness.

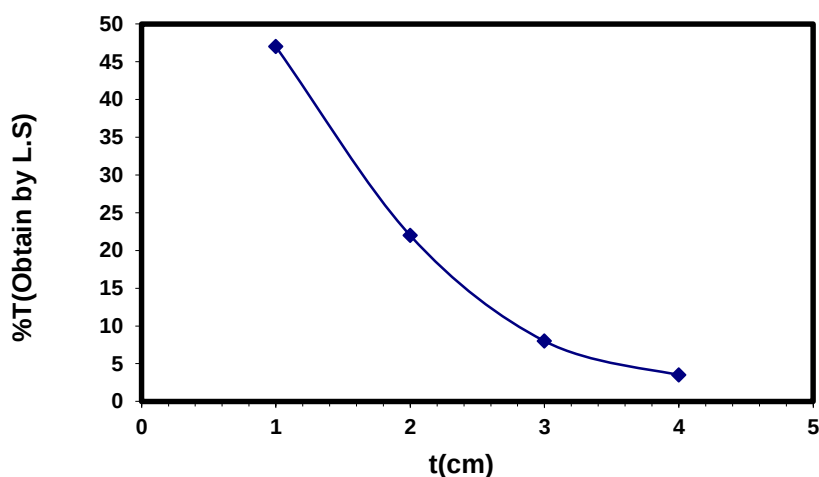


Fig (9): Total protein transmittance for laser as a function of sample thickness.

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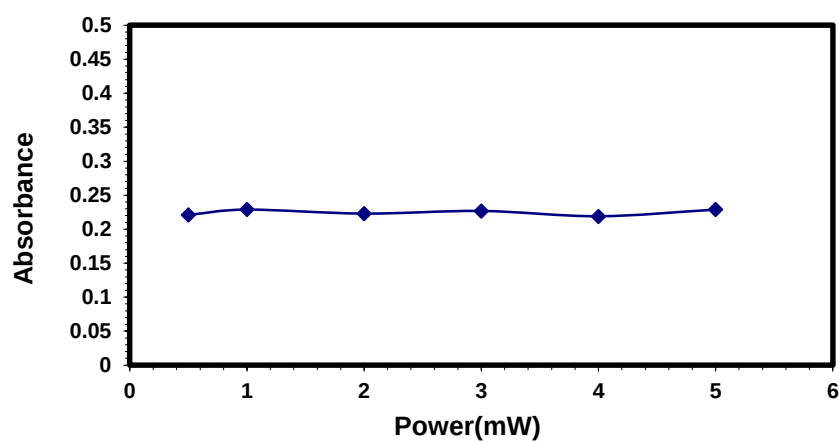


Fig (10): TP absorbance versus laser power.

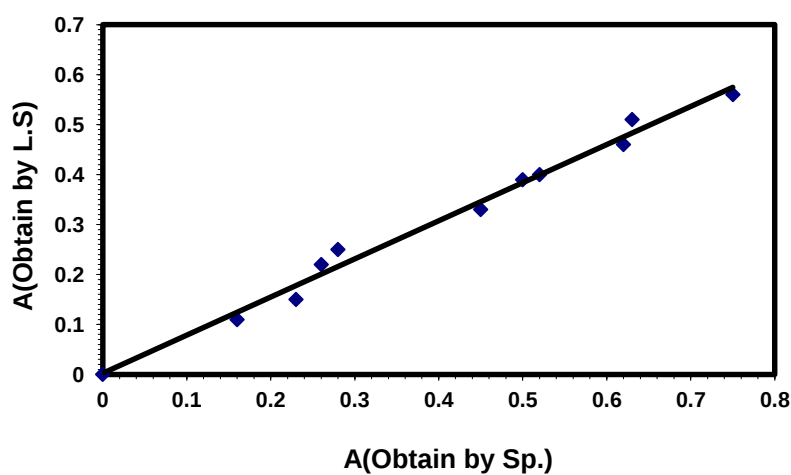


Fig (11): Absorbance values using (SP) and (LS) methods

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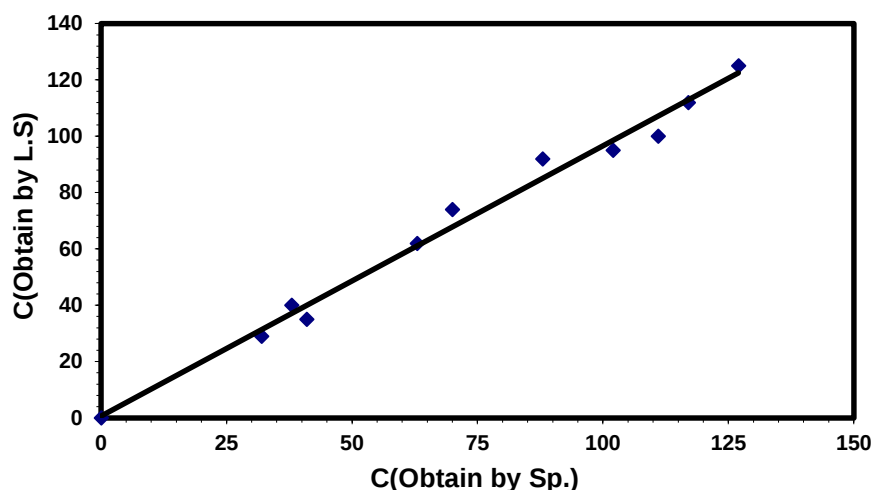


Fig (12): Concentration values using (SP) and (LS) methods

Conclusions

The possibility of using laser diode to accomplish accurate and rapid analysis is presented in this work. The (LS) is highly efficient and easily to hold in addition to variety of powers which can be used to operate the diode. Also it can be easily handled, fast and clean analysis, it have not mirrors or filters so it cost rather low expenses. Since the divergence of laser beam is very low, it is possible to analyze very fine samples of bloods. All results are good agreement with conventional double beam spectrophotometer.

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