Spectral Characteristics of Cholesterol in Blood by Using Simple Semiconductor Laser

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Abstract

In the present work, the possibility of using laser to analyze and find the concentration of total cholesterol (TC) in serum. Semiconductor laser diode of 5mW maximum power, 532nm wavelength and 4nm bandwidth is used to accomplish assay measurements. The spectral plot showed that the maximum absorbance of cholesterol is at the range (470-540nm) and the peak of spectral absorbance centers at 500nm.The laser system measurements include the study of absorbance as a function of both laser power and sample thickness, as well as the transmittance as a function of Laser power. To ascertain our calculations, the results have been compared with the results of spectrophotometer. The RSD values between them are about (1.7-9.8).

Keywords: Total cholesterol, spectrophotometer, color metric method.

استخدام منظومة ليزرية في تحليل وحساب تركيز الكوليستترول في مصل الدم

الخلاصة

في هذا البحث, تم دراسة إمكانية استخدام منظومة ليزرية لغرض تحليل وحساب تركيز الكوليسترول الكلي في مصل الدم لجسم الإنسان. تم استخدام ليزر الدايود شبه الموصل بقدرة عظمى 5 mw وبطول موجي 532 nm وبعرض حزمة 4 nm لإجراء القياسات. اظهر المخطط الطيفي أن اعظم امتصاصية الكوليسترول تقع في المدى 470-540 nm وان قمة الامتصاصية تقع عند الطول الموجي 500 nm . قياسات المنظومة الليزرية تشتمل على دراسة الامتصاصية والنفاذية كدالة لسمك النموذج وكذلك الامتصاصية كدالة لقدرة الليزر. لتاكيد وضبط الحسابات تم مقارنة النتائج المستحصلة مع نتائج المطياف. أظهرت القياسات توافقا جيدا وبانحراف معياري يتراوح (1.7-9.8).

Introduction

TC assay, associated to assays of others, a lipid in serum is used in the diagnosis of hyperlipidemia. Increased levels are also seen in hepatic and thyroid disorder. So this assay is used in the diagnosis and treatment of atherosclerotic diseases [1][2].

Also determination of (TC) is widely used in several areas such as: clinical analysis, science, food technology, biochemistry, physiology, protein chemistry, medical research, ecology, as well as in much other area [2].

Many laboratories currently employ spectrophotometer to

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analyze (TC) in spite of existence of several methods, using different analysis tools (color photometry, chromatography, polarography, etc.), developed for the determination of (TC), the UV-Visible spectrophotometric methods are the common.

We can use laser in analyses, and comparative studies among these methods that was carried out [3][4] .It is found that a low-cost laser diode system can be used instead of spectrophotometer.

Theory

Consider an incident light beam with intensity I_o passing through a square cell containing a solution of a compound that absorbs light of a certain wavelength.

The intensity of transmitted light beam I_s will be less than Io and we may define the transmittance (T) of light as (I_s/I_o). Also the transmittance through a reference cell is (I_r/I_o); the transmittance for a compound in solution is then defined as (I_s/I_r).

When we hold the concentration constant and double the inside thickness of the cell, the effect on absorbance is the same as doubling the concentration.

It, also follows that absorbance is directly proportional to the light length of the sample. The relationship is often called Beer-Lambert s Law, which can be written as:

$$\boldsymbol{I_s = I_o \exp(-a\,ct)} \quad \ldots\ldots (1)$$

Where (a) is the extinction coefficient and t is the light path (sample thickness) and c is sample concentration.

Experimental Details

Cuvets must be clean and optically clear. Etching or deposits on the surface will obviously affect absorbance values. Round cuvets, used usually in visible range, are cleaned by copious rinsing with tap water and distilled water. Then both round and square cuvets may be cleaned in mild detergent or soaked in mixture of concentrated HC l-water-ethanol (1:3:4). A good practice is to fill all such cuvets 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 spectro photometer may be verified. Relative standard deviation (R.S.D), shown in equation (2), is used to statistically compare the observed results.

$$R.S.D = \left| \frac{x_i - \overline{x_i}}{\overline{x_i}} \right| \times 100\% \quad (2)$$

Where ($\overline{x_i}$) is the value of spectrophotometer and (x_i) is the value of laser system [6].

Spectral absorbance is measured for serum samples under test exposed to a monochromatic light of wavelength 400nm to 600nm .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 monochromater type (PENPHAN-M800). In order to obtain a variety of laser output power, a simple series resistance (R1=0-10 ohm) circuit is used.

Results and Discussion

The maximum irradiant power is approximately at wavelength 532nm. The spectral wavelength ranges about 530-534nm and bond width is about 4nm. This range is coinciding with maximum absorbance of (TC). Figure (1) exhibits absorption spectrum of (TC), the maximum absorbance lies in the wavelength range (470-540nm) and the peak of its (500nm.).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 overlap with other compounds [10]. The absorbance of TC as a function of cell thickness (d) is shown in figure (2). This figure coincides with Lambert low. Consequently, the absorbance can be found for any unknown concentration of a certain thickness. Figure (3) shows the transmittance of TC as a function of cell thickness. The plot shows an inversely exponential relation which also coincide with Lambert's law [13]. Figure (4) shows that the absorbance of TC is independent of laser power. Consequently, it is possible to use any power to get this analysis. Three kinds of controllers were used. These controllers were H, N and L referred to High, Normal, and Low; respectively. Several samples were prepared for each sort of age and sex. Table (2) shows the results of absorbance concentration and relative standard deviation (R.S.D) for each sample using spectrophotometer and laser systems. 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 (H), 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 con not be used in (sp) system. Also the values of (R.S.D) are ranging about (1.7-9.8) which obviously within the acceptable medical tolerance. Fig (5) and (6) shows the agreement between (L.S) and (Sp.) results. Here we used arbitrary samples for different ages. 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 [10].

Conclusions

Using laser diode to accomplish accurate and rapid analysis cholesterol in blood is used. The laser system 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, without using mirrors or filters so it rather cost rather low. The values of (R.S.D) in table (2) varied between (1.7–9.8). This means the result is acceptable in medical measurement.

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Table (1) preparation of (TC)

Procedure: BioMerieux Co.			
Wavelength: 500nm(492-550)nm			
Solution	Blank	Standard (St.)	Sample (Sa.)
Standard(St.)	-	10µl	-
Sample (Sa.)	-	-	10µl
Working solution	1ml	1ml	1ml
Mix. measure after 5 minutes at: 37 °C			
Calculation: Sample Concentration(C_{Sa})=(A_{Sample}/$A_{Standard}$) * Standard concentration(C_{St}) Standard Concentration(C_{St})=200mg/100ml			

Table (2) absorbance and concentration values for different samples (TC)

Samples	Spectrophotometer (Sp.)		Laser System (L.S)		(R.S.D)%
	Absorbance	Concentration Mg/100ml	Absorbance	Concentration Mg/100ml	
St.	0.35	-	0.32	-	
L	0.22	125	0.21	131	4.8
N	0.30	171	0.25	156	8.7
H	0.56	319	0.46	287	9.8
Sa.1	0.15	85	0.14	89	4.7
Sa.2	0.25	142	0.22	137	3.5
Sa.3	0.20	114	0.1	112	1.7
Sa.4	0.28	159	0.27	168	5.6
Sa.5	0.40	228	0.38	237	3.9
Sa.6	0.33	188	0.31	193	2.6
Sa.7	0.57	325	0.54	337	3.7
Sa.8	0.17	97	0.16	100	3.1
Sa.9	0.90	514	0.77	481	6.3
Sa.10	0.44	251	0.42	262	4.4
L=132 mg/100ml , N=164 mg/100ml , H=299 mg/100ml , St.=200 mg/100ml					

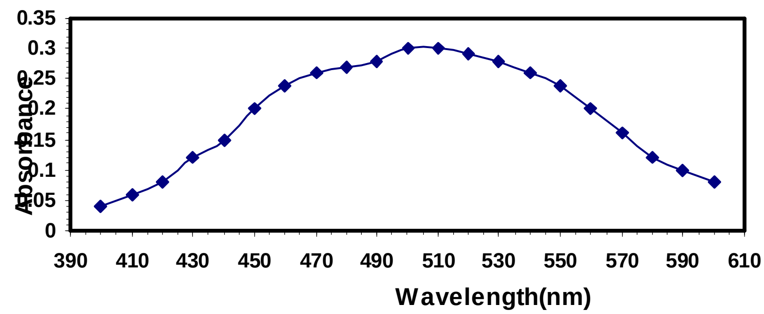


Figure (1) The (TC) absorbance as a function of wavelength

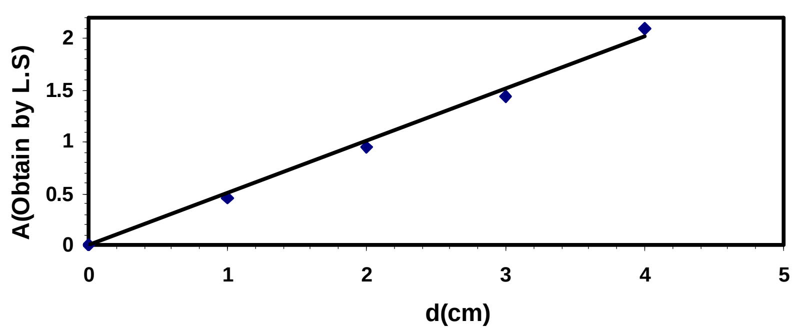
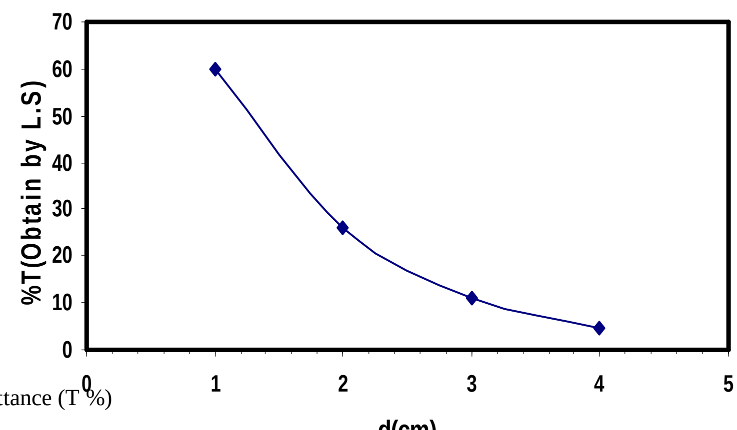


Figure (2) TC absorbance for laser as a function of sample thickness



Transmittance (T %)

Figure (3) TC transmittance for laser as a function of sample thickness

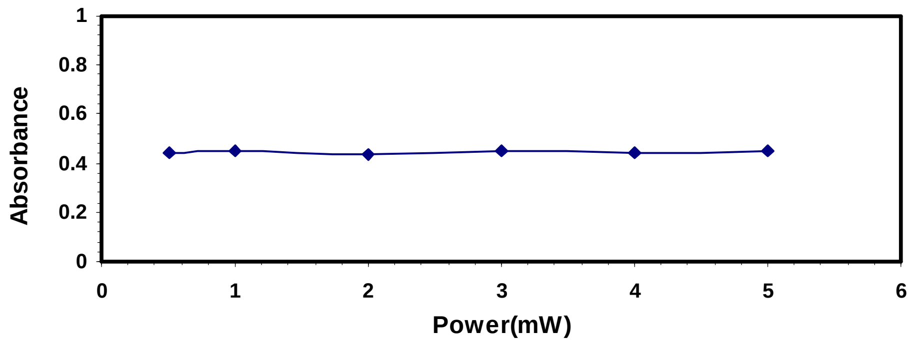


Figure (4) TP absorbance versus laser power

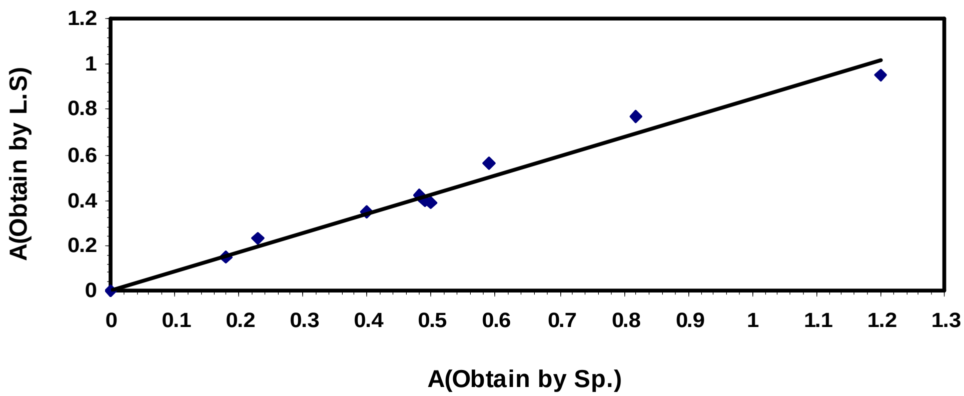


Figure (5) shows the relation between absorbance values (SP) and (LS) methods

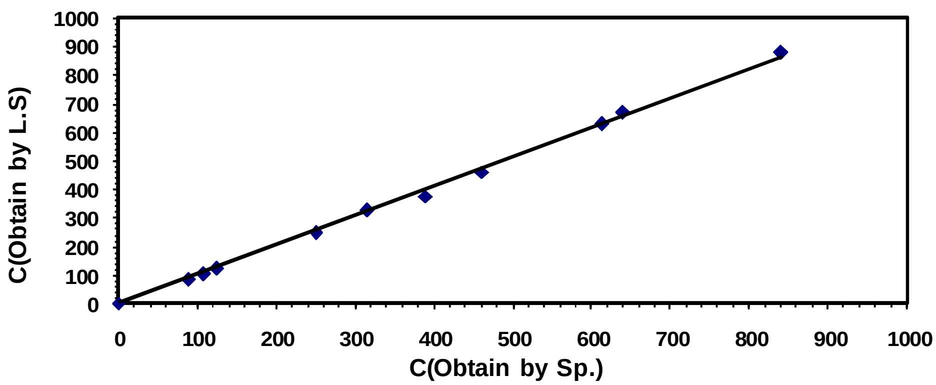


Figure (6) shows the relation of concentration obtained by (SP) and (LS) methods