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Z-SCAN TECHNIQUE FOR MEASUREMENT OF TOTAL CHOLESTEROL AND TRIGLYCERIDES IN BLOOD

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The measurement of cholesterol and triglycerides in blood by Z-scan technique is proposed. The nonlinear refractive index of cholesterol and triglycerides was found to vary linearly with concentration. Hence by calculating the nonlinear refractive index it is possible to measure their concentration in the sample. These measured values are found in equivalence with conventional colorimetric method.

Keywords: Z-scan technique; nonlinear refractive index; cholesterol; triglycerides.

1. Introduction

Cholesterol and triglycerides are two types of fats (lipids) present in the body that can be measured in blood serum and blood plasma. Cholesterol is an essential component of cell membranes, brain and nerve cells, and bile, which helps the body to absorb fats and fat-soluble vitamins. Cholesterol is also used by the adrenal glands to form hormones such as cortisol, by the testicles to form testosterone, and by the ovaries to form estrogen and progesterone. Triglycerides supply energy for the body. Triglycerides either meet immediate energy needs in muscles or they are stored as fat for future energy requirements. Both cholesterol and triglyceride are packaged with proteins and other substances to form particles called lipoproteins. These are the main lipids associated with arteriosclerotic vascular diseases.[1] The estimation of biochemical analytes such as glucose, galactose, uric acid, urea, triglyceride, and cholesterol in biological samples is essential to monitor human health. Among these, estimation of total cholesterol and triglyceride has drawn the maximum attention[2−4] in serum since they are indicators of abnormality in lipid metabolism and its abnormal level is associated with coronary artery disease, diabetes mellitus, hypothyroidism, anemia, and wasting syndromes.[5] Several clinical studies have shown that people with above-normal triglyceride levels (greater than or equal to 200 mg/dl) have an increased risk of heart disease. People with diabetes or who are obese are also likely to have high triglycerides.[6]

Many techniques have been developed and reported in the literature for the determination of cholesterol[7−11] and triglyceride.[12−18] Among them, enzymatic method is standard and most widely used for colorimetric method in clinical laboratory. The corresponding enzymes react with serum cholesterol and triglycerides and form hydrogen peroxide to produce colored complex on the further reaction. The intensity of color is directly proportional to the cholesterol and triglyceride concentration present in the sample.

Optical nonlinearity has been reported for LDL-cholesterol.[19,20] Some more reports are on characterization of lipids in body fluid,[21,22] study

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of the nonlinear refraction of vitreous humor in human and rabbit,[23] and determination of nonlinear refractive index of retinal derivatives.[24] In this investigation, we present one more reliable method for the determination of cholesterol and triglycerides, which is based on the measurement of nonlinear refractive index by Z-scan technique. We have already reported the possibility of determination of glucose by Z-scan technique.[25] This report is the continuation of our previous work.

The single beam Z-scan analysis, which was developed by Mansoor Sheik Bahae *et al.*,[26] is a simple and effective tool for determining nonlinear optical properties of materials.[27−30] This approach has been nowadays widely used in optical characterization of different materials.

Nonlinear refractive index is proportional to the real part of the third-order susceptibility $\mathrm{Re}[\chi^{(3)}]$. Basically, the Z-scan method consists of translating a nonlinear sample through the focal plane of a tightly focused Gaussian laser beam and monitoring the changes in the far field intensity pattern. For a purely refractive nonlinearity, the light field induces an intensity dependent nonlinear phase and, as consequence of the transverse Gaussian intensity profile, the sample presents a lens-like behavior. The induced self-phase modulation has the tendency of defocusing or re-collimating the incident beam, depending on its Z position with respect to the focal plane. By monitoring the transmittance change through a small circular aperture placed at the far field position, it is possible to determine the nonlinear refractive index. In the present study, we have measured cholesterol and triglycerides level in blood by calculating the nonlinear refractive index (n_2) value using a single beam Z-scan method.

2. Methodology

2.1. *Sample preparation for cholesterol*

One milliliter of reagent was added to 10 μl of the prepared serum (cholesterol based on CHOD/POD — a kit supplied by M/S. Excel Diagnostics Pvt. Ltd, Hyderabad, India). The solution was allowed to mix well and incubated at 37°C for 5 min. The solution turned to pinkish red color. The principle involved in this reaction is represented as

$$\text{cholesterol ester} + H_2O \xrightarrow{\text{CE}} \text{cholesterol} + \text{fatty acids},$$

$$\text{cholesterol} + O_2 \xrightarrow{\text{CO}} \text{cholesten-3-one} + H_2O_2,$$

$$H_2O_2 + \text{phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + H_2O.$$

Cholesterol esters are hydrolyzed to free cholesterol by cholesterol ester hydrolase (CE). The free cholesterol produced is oxidized by cholesterol oxidase (CO) to cholesten-3-one with the simultaneous production of hydrogen peroxide, which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase (POD) to yield Quinoneimine dye with maximum absorption at 500 nm.

2.2. *Sample preparation for triglycerides*

One milliliter of reagent was added to 10 μl of the prepared serum (triglycerides based on GPO/POD — a kit supplied by Merck, Mumbai, India). The solution was allowed to mix well and incubated at 37°C for 5 min. The solution turned to brownish red color. The principle involved in this reaction is represented as

$$\text{triglycerides} \xrightarrow{\text{lipase}} \text{glycerol} + \text{fatty acids},$$

$$\text{glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{glycerol-3-phosphate} + \text{ADP},$$

$$\text{glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetonephosphate} + H_2O_2,$$

$$2H_2O_2 + \text{aminoantipyrine} + \text{4-chlorophenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + HCl + 4H_2O$$

Triglycerides are hydrolyzed by lipase to glycerol and free fatty acids. Glycerol is phosphorilated by ATP in the presence of glycerolkinase (GK) to glycerol-3-phosphate (G-3-P) which is oxidized by the enzyme glycerol-3-phosphate oxidase (G-P-O) producing hydrogen peroxide. Hydrogen peroxide so formed reacts with 4-aminoantipyrine and 4-chlorophenol in the presence of enzyme POD to produce Quinoneimine dye compound.

2.3. *Nonlinear refractive index*

The Z-scan experiments were performed using a 532 nm Nd:YAG (SHG) CW laser beam (COHERENT — Compass 215M-50 diode-pumped laser)

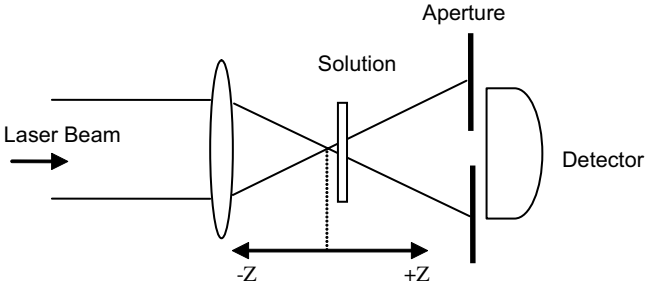


Fig. 1. Experimental setup for Z-scan technique.

focused by a lens of 35 mm focal length. The experimental setup is shown in Fig. 1. A typical closed-aperture Z-scan curve for the standard cholesterol and triglycerides solution at incident intensity $I_o = 7.824\,\mathrm{kW/cm^2}$, is shown in Fig. 2. This normalized transmittance curve is characterized by a pre-focal peak followed by a post-focal valley. This implies that the nonlinear refractive index of standard cholesterol, standard triglycerides, and serum sample is negative ($n_2 < 0$). The defocusing effect shown in Z-scan curve can be attributed to a thermal nonlinearity resulting from absorption of radiation at 532 nm. Localized absorption of a tightly focused beam propagating through an absorbing sample medium produces a spatial distribution of temperature in the sample solution and consequently, a spatial variation of the refractive index, that acts as a thermal lens resulting in phase distortion of the propagating beam.

The nonlinear refractive index (n_2) is calculated using the standard relations[26]

$$\Delta T_{p-v} = 0.406(1-S)^{0.25}\ |\Delta\Phi_o|, \qquad (1)$$

where ΔT_{p-v} can be defined as the difference between the normalized peak and valley transmittances ($T_p - T_v$), $|\Delta\Phi_o|$ is the on-axis phase shift at the focus.

The linear transmittance of the aperture is given by

$$S = 1 - \exp\left(\frac{-2r_a^2}{w_a^2}\right), \qquad (2)$$

where r_a is the radius of the aperture and w_a is the beam radius at the aperture.

$$n_2 \approx \frac{\Delta\Phi_o}{kI_oL_{\mathrm{eff}}}, \qquad (3)$$

where n_2 is the nonlinear refractive index, k is the wave number ($k = \frac{2\pi}{\lambda}$) and

$$L_{\mathrm{eff}} = \frac{1-e^{-\alpha L}}{\alpha}.$$

$I_o = \frac{2P}{\pi w_o^2}$ is defined as the peak intensity within the sample at the focus. L is the thickness of the sample, α is the linear absorption coefficient.

Another experiment was performed with a conventional colorimetric method following the standard procedure of Allain *et al.*[31] and Gowan *et al.*[32] for cholesterol and triglycerides samples, respectively. This involves measurement of optical density variation with respect to concentration. These results are compared with the results calculated with the present Z-scan technique.

2.4. *Statistical analysis*

The error involved in the measurements was determined by t-test, $P < 0.01$. These statistical analysis was conducted using SPSS commercial statistical

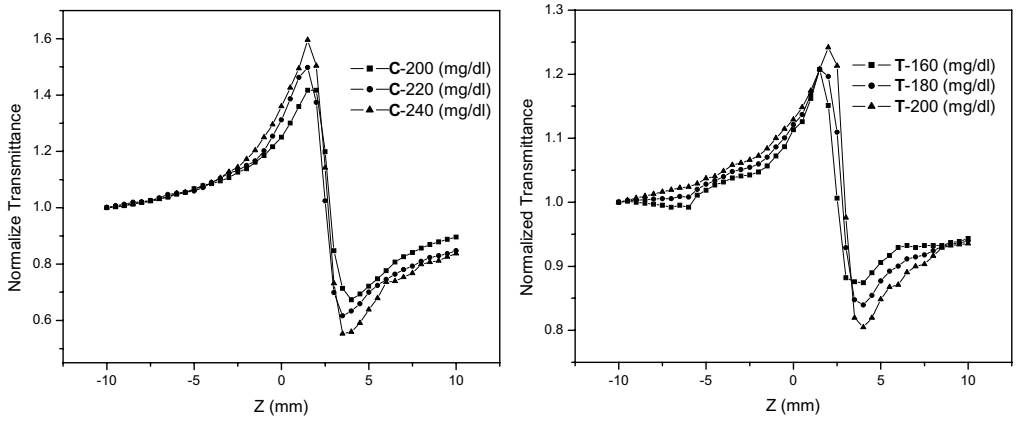


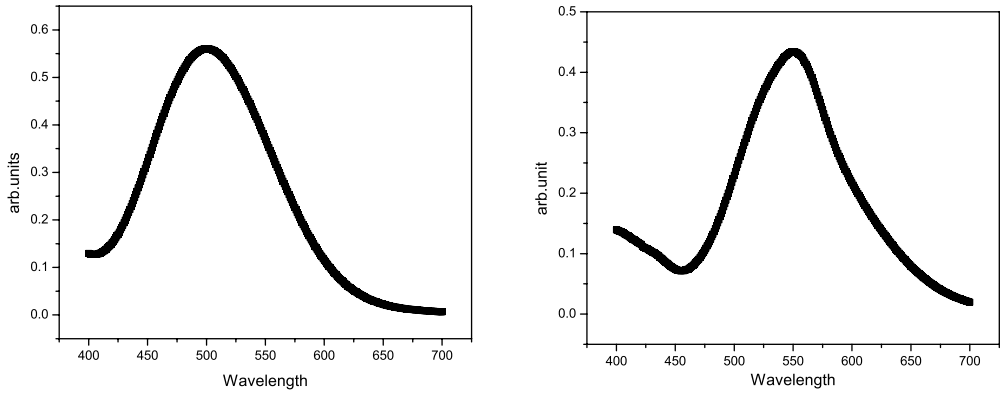
Fig. 2. Z-scan data of the standard cholesterol (C) and triglycerides (T).

package (SPSS, version 10.0 for windows, SPSS Inc., Chicago, USA).

3. Results and Discussion

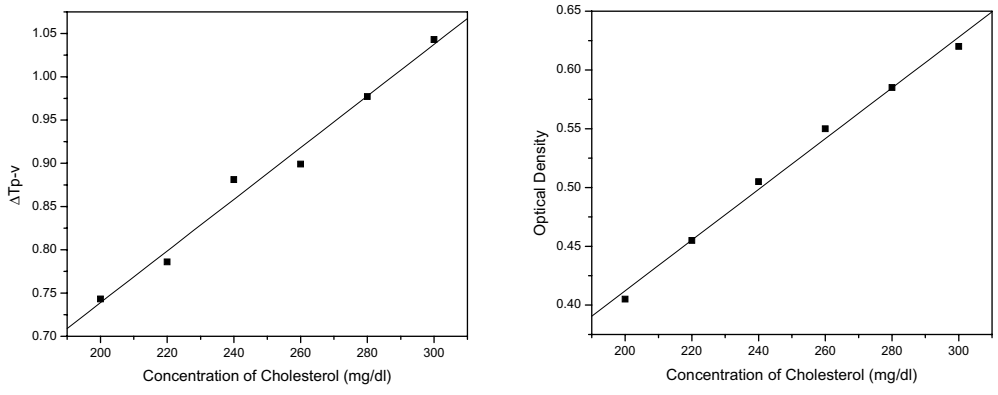
The absorption spectra measured using UV-vis spectrophotometer (Perkin Elmer-Lambda35) are found to be broad banded for both cholesterol and triglycerides. The absorption peaks have occurred at 500 nm for cholesterol and 550 nm for triglycerides, as depicted in Fig. 3. Both have exhibited good absorption at 532 nm. Hence for further study 532 nm laser was used.

The result of typical Z-scan normalized transmittance measurement for cholesterol and triglycerides is shown in Fig. 2. As the concentration of the standard cholesterol and triglycerides increases, the normalized transmittance peak increases whereas the valley decreases, respectively. The graph in Figs. 4(a) and 4(d) shows that the ΔT_{p-v} value linearly increases with concentration of standard cholesterol and triglycerides solution. Similarly in Figs. 4(b), 4(e) and 4(c), 4(f), optical density and refractive index value linearly increase with concentration of standard cholesterol and triglycerides solution. The experiments were repeated five times and the mean value of the nonlinear refractive index (n_2) was calculated from the normalized transmittance values. This calculated value was assumed to be the standard for measurement of unknown cholesterol and triglycerides content present in blood sample. This can be arrived by plotting a linear graph of cholesterol and triglycerides concentration vs nonlinear refractive index. The nonlinear refractive index value was first measured against the reagent blank solution. The calibration was made with the conventional colorimetric method and the results are tabulated in Table 1 for cholesterol and Table 2 for triglycerides. The desirable cholesterol level in blood is < 200 mg/dl, the border line



(a) (b)

Fig. 3. UV–vis spectrum of standard (a) cholesterol and (b) triglycerides at 200 mg/dl with 1 ml reagent.



(a) (b)

Fig. 4. Linear variation of (a) T_{p-v}, (b) optical density, and (c) nonlinear refractive index (n_2) with concentration of cholesterol and linear variation of (d) T_{p-v}, (e) optical density, and (f) nonlinear refractive index (n_2) with concentration of triglycerides.

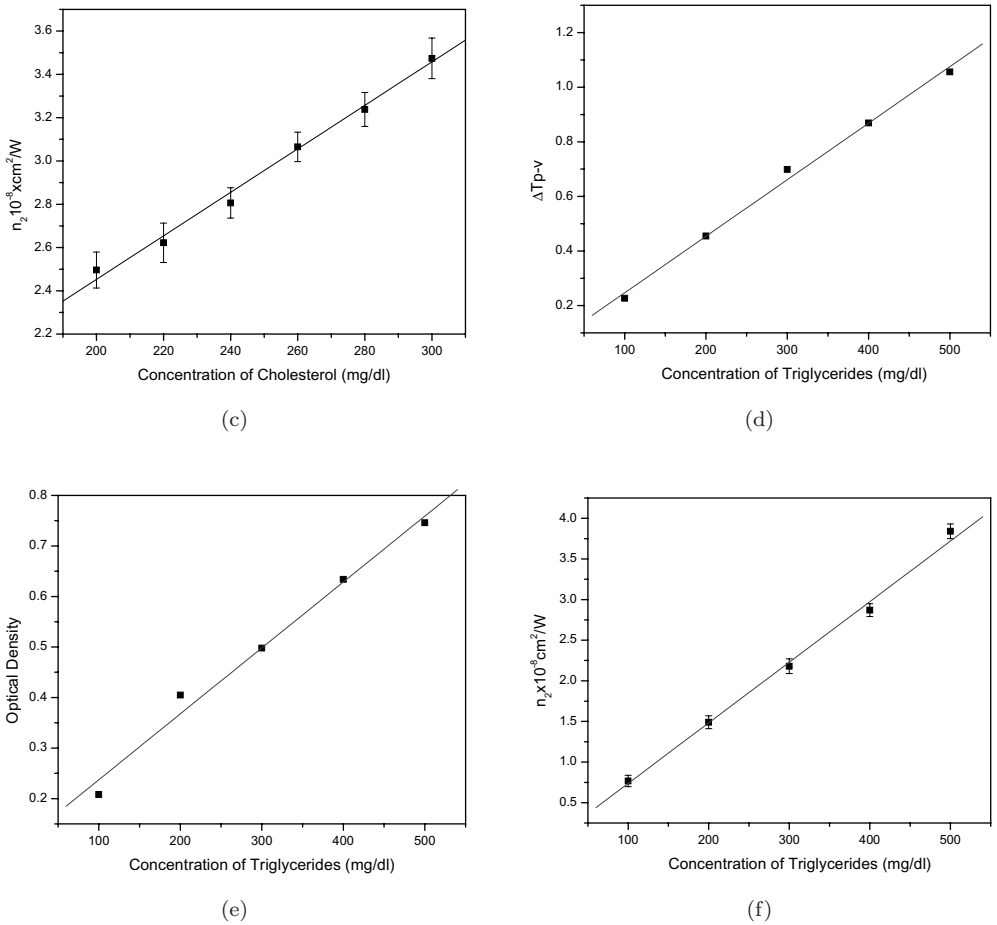


Fig. 4. (*Continued*)

Table 1. Nonlinear refractive index (n_2) values for standard cholesterol.

Standard cholesterol concentration (mg/dl)	Nonlinear refractive index $n_2 \times 10^{-8}$ (cm^2/W)
200	2.49 ± 0.08
220	2.62 ± 0.09
240	2.80 ± 0.07
260	3.06 ± 0.06
280	3.23 ± 0.07
300	3.47 ± 0.09

Table 2. Nonlinear refractive index (n_2) values for standard triglycerides.

Standard triglycerides concentration (mg/dl)	Nonlinear refractive index $n_2 \times 10^{-8}$ (cm^2/W)
100	0.76 ± 0.07
200	1.49 ± 0.08
300	2.18 ± 0.09
400	2.87 ± 0.08
500	3.84 ± 0.09

risk of cholesterol level is 200–240 mg/dl, and high risk of cholesterol level is > 240 mg/dl. The desirable triglycerides level in blood is < 200 mg/dl, the border line risk of triglycerides level is 200–400 mg/dl, and high risk of triglycerides level is > 400 mg/dl.

For estimating the cholesterol and triglycerides levels, one need not plot full Z-scan curve every time. Once, experimental setup explained above is established, one needs to note down peak and valley values of the transmittance curve translating the sample holder continuously along Z-axis. The difference in these two values $T_p - T_v$, $|\Delta\Phi_o|$ when substituted in Eq. (3) yields the nonlinear refractive index value.

Hence from the results we incur that the values of $n_2 < 2.496 \pm 0.083 \times 10^{-8}$ cm^2/W are desirable level of cholesterol, n_2 values in the range between 2.496 ± 0.083 and $2.806 \pm 0.070 \times 10^{-8}$ cm^2/W can be considered as border line risk of cholesterol level, and $n_2 > 2.806 \pm 0.070 \times 10^{-8}$ cm^2/W are known to be the high risk of cholesterol level in blood.

Table 3. Comparative analysis of serum total cholesterol using colorimetric method and Z-scan method.

Sample collection	Cholesterol level	Concentration of cholesterol (mg/dl)	
		Colorimetric method	Z-scan method
Random	Border line Risk	207.19	209.89
Random	High Risk	246.27	243.96
Random	Desirable	188.56	189.37
Fasting	Border line Risk	228.31	225.82
Fasting	Desirable	183.14	185.75
Fasting	Desirable	194.19	195.46

Each value is the mean of five individual observation. The P value (t-test value) is less than 0.01 at 1% significance level.

Table 4. Comparative analysis of serum triglycerides using colorimetric method and Z-scan method.

Sample collection	Triglycerides level	Concentration of triglycerides (mg/dl)	
		Colorimetric method	Z-scan method
Random	Border line Risk	228.12	224.95
Random	Border line Risk	212.21	208.89
Random	Desirable	165.38	167.76
Fasting	Border line Risk	267.85	267.17
Fasting	Desirable	158.54	161.74
Fasting	Desirable	187.13	190.69

Each value is the mean of five individual observation. The P value (t-test value) is less than 0.01 at 1% significance level.

Similarly, the values of $n_2 < 1.491 \pm 0.082 \times 10^{-8}\,\mathrm{cm}^2/\mathrm{W}$ are desirable level of triglycerides, n_2 values in the range between 1.491 ± 0.082 and $2.872 \pm 0.089\,\mathrm{cm}^2/\mathrm{W}$ are considered to be the border line risk of triglycerides level, and $n_2 > 2.872 \pm 0.089\,\mathrm{cm}^2/\mathrm{W}$ are known to be the high risk level of triglycerides in blood.

Many trials were performed to measure the cholesterol level with our proposed method. The blood samples were collected from six volunteers. In this three samples were collected randomly and another three were collected in fasting state. We could see that the results arrived at are in good agreement with those of the conventional colorimetric method for cholesterol as shown in Table 3 and Table 4 for triglycerides. Hence we could clearly ascertain that the proposed method is on par with the conventional colorimetric method.

4. Conclusion

The Z-scan measurements indicate that cholesterol and triglycerides in standard sample and blood sample exhibit nonlinear optical properties. We have measured the nonlinear refractive index values for cholesterol and triglycerides present in the serum sample by Z-scan method with 532 nm Nd:YAG CW laser. Comparative analysis of these values with the one obtained by conventional colorimetric method shows that they are in good agreement. Hence, apart from existing techniques, Z-scan technique can also be used for the measurement of cholesterol and triglycerides in blood.

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