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Z-Scan technique: To measure the total protein and albumin in blood

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ABSTRACT

Z-scan technique is an effective tool for measuring the optical nonlinearity of the materials. By using this technique the measurement was made for total protein and albumin. The nonlinear refractive index of the total protein and albumin were found to vary linearly with concentration. Hence by calculating the nonlinear refractive index it is possible to measure their concentration in the sample. The values measured thus are found in equivalence with conventional colorimetric method.

Keywords: Z-scan Technique; Nonlinear Refractive Index; Total Protein; Albumin

1. INTRODUCTION

Protein is an essential nutrient made up of building-block chemicals called amino acids. Protein provides energy and is needed for the body to make new cells, to maintain and rebuild muscles, to carry other nutrients, to act as messengers in the body, and to support the immune system. A total serum protein test measures the total amount of protein in the blood. It also measures the amounts of two major groups of proteins in the blood: albumin and globulin.

Albumin is made mainly in the liver. It helps keep to the blood from leaking out of blood vessels. Albumin also helps to carry some medicines and other substances through the blood and is important for tissue growth and healing.

Globulin is made up of different proteins called alpha, beta, and gamma types. Some globulins are made by the liver, while others are made by the immune system. Certain globulins bind with hemoglobin. Other globulins transport metals such as iron in the blood and help fight infection.

Low total protein levels can suggest a liver disorder, a kidney disorder, or a disorder in which protein is not digested or absorbed properly. Low levels may be seen in severe malnutrition and with conditions that cause malabsorption, such as Celiac disease or inflammatory bowel disease (IBD). High total protein levels may be seen with chronic inflammation or infections such as viral hepatitis or HIV. They may be caused by bone marrow disorders such as multiple myeloma.

Measurements of protein may reflect liver disease, nutritional state, kidney disease and others. A decreased value of total protein may indicate liver or kidney disease. If levels of albumin are low, there is a possibility of primary liver disease, kidney disease, tissue damage or inflammation, and malnutrition [1,2]. In chronic liver diseases like “cirrhosis” or “nephrotic syndrome” the level is decreased. Poor nutrition or protein catabolism may cause “hypoalbuminaemia”. Measurement of serum-total protein is useful in conditions relating to changes in plasma or fluid volumes, such as shock and dehydration. In these conditions concentration of serum-total protein is elevated indicating hemoconcentration. Haemodilution is reflected as relative hypoproteinemia, which occurs with water intoxication or salt retention syndrome, during massive intravenous infusions.

The most widely accepted assays so far for proteins are the Biuret [3], Lowry [4], Bradford [5,6], Bromophenol Blue [7] and Bromocresol Green[8] methods. In this Biuret reaction is highly susceptible to interference by non-protein substances [9,10,11,12]. The bromocresol green method for determination of serum albumin is the most specific and sensitive of the dye binding techniques [13]. The glyoxylic acid method measures tryptophan content which represents 8-10% albumin and 90-91% globulin. Since the bromocresol green method is specific and simple, it is the method of choice for albumin determination [14].

The Z-scan technique was extended to study the optical nonlinearity has been reported for LDL-Cholesterol [15,16]. Some more reports are on characterization of lipids in body fluid [17,18], study of the nonlinear refraction of vitreous humor in human and rabbit [19], determination of nonlinear refractive index of retinal derivatives [20]. In this present investigation total protein and albumin are subjected to the Z-scan technique to cal-

culate the nonlinear refractive index (n_2). Already work has been done on measurement of glucose [21], total cholesterol and triglycerides [22].

The single beam Z-scan analysis, which was developed by Mansoor Sheik Bahae *et al.* [23], is a simple and effective tool for determining nonlinear optical properties of materials [24,25,26,27]. This approach has been now a day widely used in optical characterization of different materials. Nonlinear refractive index is proportional to the real part of the third-order susceptibility Re[$\chi(3)$]. Basically, the Z-scan method consists in 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 recollimating 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 total protein and albumin levels in blood by calculating the nonlinear refractive index (n_2) value using a single beam Z-scan method.

2. METHODOLOGY

2.1. Preparation of Total Protein Sample

For sample preparation (Total Protein-Biuret method - a kit supplied by Transasia Bio-medicals Ltd, Baddi, Himachal Pradesh, India) was used. To 20 microliter of the serum one milliliter of total protein reagent was added and incubated for 10 minutes at 37 ^{o}C. The principles involved for this reaction is that the peptide bonds of protein react with copper II ions in alkaline solution to form blue-violet complex (Biuret reaction). Each copper ion complexes with 5 or 6 peptide bonds. Tartrate is added as a stabilizer whilst Iodide is used to prevent auto-reduction of the alkaline copper complex. The color formed is proportional to the protein concentration.

2.2. Preparation of Albumin Sample

For sample preparation (Albumin-BCG method - a kit supplied by Transasia Bio-medicals Ltd, Baddi, Himachal Pradesh, India) was used. To 10 microliter of the serum one milliliter of albumin reagent was added and incubated for 1 minute at 37^{o}C. The principle involved in this reaction is that the albumin binds with Bromocresol green (BCG) at pH 4.2 causing a shift in absorbance of the yellow BCG dye. The Blue green color formed is proportional to the concentration of albumin.

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) and He-Ne laser beam (RESEARCH ELECTRO OPTICS–30995 cylindrical helium-neon laser) focused by a lens of 35 mm focal length. The experimental set up is shown in **Figure 1**.

A typical closed-aperture Z-scan curve for the standard total protein solution at incident intensity Io = 7.824 kW/cm^2. Likewise the Z-scan curve for standard albumin solution at incident intensity Io = 1.758 kW/cm^2. This normalized transmittance curves are characterized by a pre-focal peak followed by a post-focal valley. This implies that the nonlinear refractive indices of total protein, albumins are 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 and 633 nm respectively. 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 [18].

$$\Delta T_{p-v} = 0.406\,(1 - S)^{0.25}\,\left|\Delta\Phi_0\right| \qquad (1)$$

Where ΔT_{p-v} can be defined as the difference between the normalized peak and valley transmittances $(T_p - T_v)$, $\left|\Delta\Phi_0\right|$ is the on-axis phase shift at the focus.

The linear transmittance of the aperture is given by

$$S = 1 - exp\,(-2r_a^2 / w_a^2) \qquad (2)$$

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

$$n_2 \approx \frac{\Delta\Phi_0}{kI_0L_{\text{eff}}} \qquad (3)$$

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

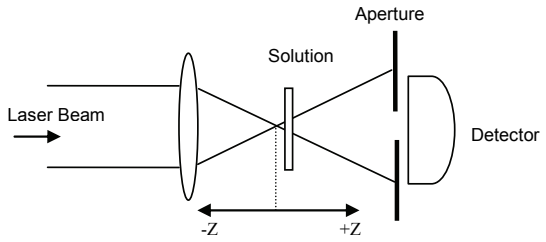

Figure 1. Experimental setup for Z-scan technique.

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

$I_0 = \frac{2P}{\pi w_0^2}$ 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.

An additional experiment was performed with a conventional colorimetric method following the standard procedure of A. G. Gornall *et al.* [3] and R. L. Rodkly *et al.* [8] for total protein and albumin samples respectively. This involves measurement of optical density variation with respect to concentration. These results are compared with the results calculated with the 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 package (SPSS, version 10.0 for windows, SPSS Inc., Chicago, U.S.A).

3. RESULTS AND DISCUSSION

3.1. Measurement of Absorbance Spectra

The absorption spectra were measured using UV-Vis spectrophotometer (SHIMADZU- UV-2401PC), and the spectra for both total protein and albumin were found to be broad banded as depicted in **Figure 2**. Hence for further study 532 nm Nd:YAG laser for total protein and 633 nm He-Ne laser for albumin were used.

3.2. Measurement of Nonlinear Refractive Indices

The results of typical Z-scan normalized transmittance measurement for total protein and albumin are shown in **Figure 3**. As the concentration of the total protein and albumin increases, the normalized transmittance peak increases whereas the valley decreases respectively. The graph in **Figure 4 (a)** and **(b)** shows that the ΔTp-v value linearly increases with concentration of standard total protein and albumin solutions. Similarly in **Figure 4 (c)** and **(d)** refractive index value linearly increases with concentration of standard total protein and albumin solutions.

In addition experiment based on optical density is

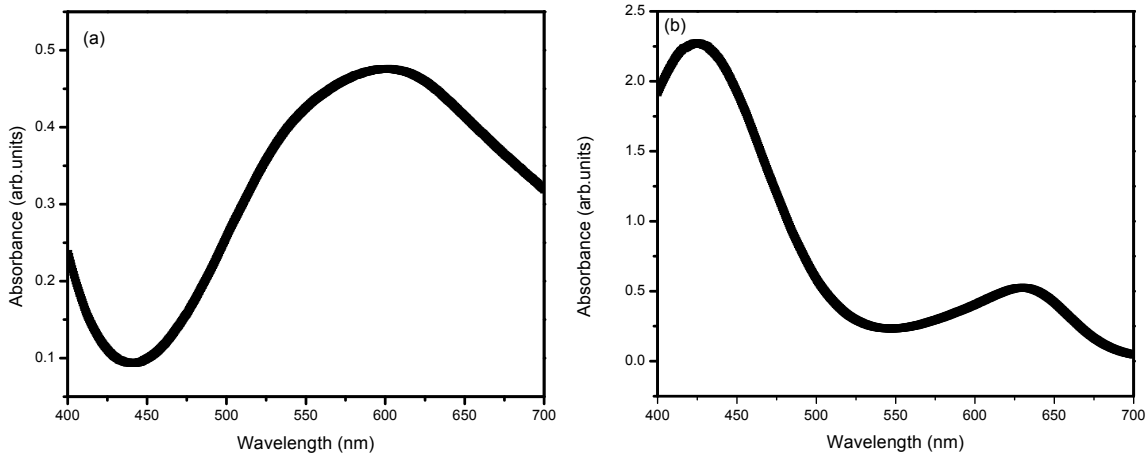

Figure 2. UV-Vis Spectra of standard (a) total protein (b) albumin with reagent.

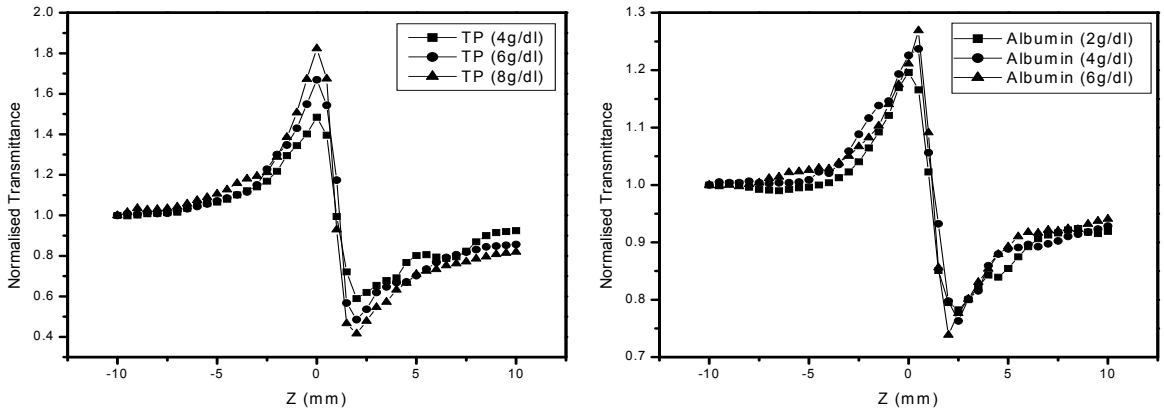

Figure 3. Z-scan data of the standard total protein (TP) and albumin.

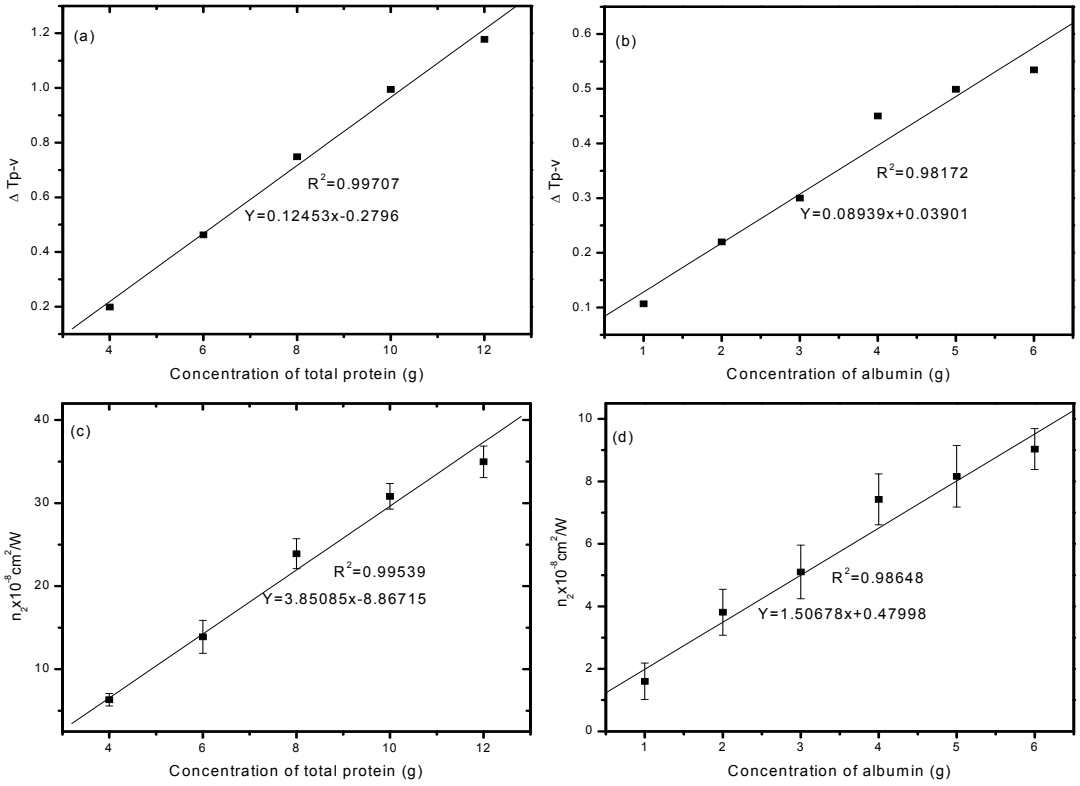

Figure 4. Linear variation of T p-v and nonlinear refractive index (n_2) with concentration of total protein (a,c) and albumin (b,d) by Z-scan method.

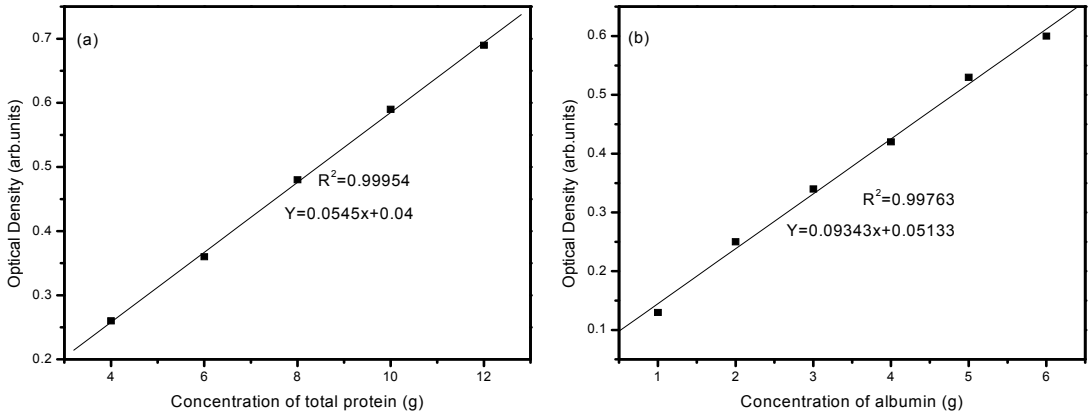

Figure 5. Linear variation of optical density of total protein (a) and albumin (b) by colorimetric method.

given in **Figure 5 (a)** and **(b)**. 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 total protein and albumin content present in blood sample. This can be arrived by plotting a linear graph of total protein and albumin 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 colori-metric method and the results are tabulated in **Table 1** for total protein and in **Table 3** for albumin. The normal level of total protein in serum is in the range of 6–8.3 g/dl, and serum albumin normal level is in the range of 3.2–5 g/dl.

For estimating the total protein and albumin 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 continu-

ously along Z-axis. The difference in these two values Tp–Tv, $|\Delta\Phi_0|$ when substituted in Equation (3) yields the nonlinear refractive index value.

Consequently by the results of Z-scan method, we infer that the n_2 value is to be in the range of 13.90 ± 1.98 to 23.01 × 10^{-8} cm^2/W for normal level of total protein in serum. Likewise, n_2 value for normal level of albumin in serum is to be in the range of 5.26 to 8.16 ± 0.98 × 10^{-8} cm^2/W.

3.3. Valuation with Conventional Method

Many trials were performed to measure the total protein and albumin level with Z-scan method. The blood samples were collected from five volunteers. We could see that the results arrived are in good agreement with those of the conventional colorimetric method for total protein as shown in **Table 2** and for albumin **Table 4**. Hence we could clearly ascertain that the Z-scan method is on par with the conventional colorimetric method. By calculating the total protein and albumin values we can also calculate the globulin level in serum. (Globulin = Total Protein–Albumin) is tabulated in **Table 5**.

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

Standard total protein Concentration (g/dl)	Nonlinear refractive index $n_2 \times 10^{-8}$ (cm^2/W)
4	06.32 ± 0.74
6	13.90 ± 1.98
8	23.91 ± 1.79
10	30.81 ± 1.53
12	34.97 ± 1.89

Table 2. Comparative analysis of serum total protein measurement using colorimetric method and Z-scan method.

Total Protein level	Concentration of total protein (g/dl)	
	Colorimetric method	*Z-scan method*
Normal	6.33	6.22
Normal	6.83	6.90
Normal	6.50	6.54
Normal	7.83	7.79
Normal	7.33	7.26

Table 3. Nonlinear refractive index (n_2) values for standard albumin.

Standard albumin concentration (g/dl)	Nonlinear refractive index $n_2 \times 10^{-8}$ (cm^2/W)
1	1.60 ± 0.58
2	3.81 ± 0.73
3	5.10 ± 0.85
4	7.42 ± 0.81
5	8.16 ± 0.98
6	9.03 ± 0.65

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

Albumin level	Concentration of albumin (g/dl)	
	Colorimetric method	*Z-scan method*
Normal	3.42	3.49
Normal	3.85	3.78
Normal	3.68	3.75
Normal	4.20	4.13
Normal	4.02	4.08

Table 5. Globulin concentration calculated from colorimetric method and Z-scan method.

Concentration of globulin (g/dl)	
Colorimetric method	*Z-scan method*
2.91	2.73
2.98	3.12
2.82	2.79
3.63	3.66
3.31	3.18

4. CONCLUSIONS

The Z-scan measurements indicate that the total protein's and albumin's standard sample and serum sample exhibit nonlinear optical properties. We have measured the nonlinear refractive index values for total protein and albumin present in the serum sample by Z-scan method with 532 nm Nd:YAG CW laser and 633 nm He-Ne laser respectively. 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 bioanalytes in serum.

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