

Study of immunoglobulin 'G' with ultra violet spectroscopy in Duchene muscular dystrophy and Alzheimer's disease

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ABSTRACT

In the present paper, we have studied human IgG in Duchene Muscular Dystrophy (DMD) using ultra violet spectroscopy. A comparison with normal controls is also made. We have found three types of bands in the spectra of IgG of DMD patients and normal healthy controls. First and second band regions cover the ultraviolet behavior of proteins but third band does not show absorbance of proteins because the absorbance intensity greater than 310 nm. Third band does not contain protein without tryptophan. We have to concentrate on the region of two bands below 300nm. We have first region in the range 200 nm to 227.38 nm in DMD patients and 200nm to 229.60 nm in normal controls. Second region starts from 244.40nm to 297.70nm in patients and 244.40 nm to 297.70 nm in controls only. Third band did not show any protein absorbance in all the cases of study. It has been seen that the variation in AD samples is on the decreasing pattern in comparison with healthy controls.

Keywords : Immunoglobulin G (IgG), Duchene muscular dystrophy (DMD), Epilepsy (E), Normal (N) , Ultra violet (UV) and Alzheimer's disease (AD).

1. INTRODUCTION

One of the most important and exciting advances in modern biochemistry has been the application of spectroscopic method, which measure the emission and absorption of energy of different frequencies by molecules and atoms. Spectroscopic studies of proteins, nucleic acids and other biomolecules are providing many new insights into the structure and dynamic process in these molecules.

Ultra violet visible (UV-Vis) spectroscopy is a different tool of spectroscopy. It is used in the solid state to prove the formation of the complexes [1]. If there is any shifting of the bands can indicate the hydrogen bonding with in the complexes. This technique is used to identify the

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charge transfer reaction. It is very important to note that the results of ultra violet spectroscopy are of relatively little importance if not studied infrared spectroscopy along with Nuclear magnetic resonance techniques. On the combined factual view of all the three branches of spectroscopy a valid structural proposal can be made. This is the main reason why the other sections of the work focus on both IR and NMR along with other useful structural determination techniques.

Molecules have the ability to absorb ultraviolet or visible light. Absorption corresponds to excitation of outer electrons in the molecules. These transitions of electrons are important to understand. If a molecule absorbs energy an electron may get promotion from the highest occupied molecular orbital to the lowest unoccupied molecular orbital. The energy differences between electronic levels in most of the molecules vary from 125 to 650 KJ/mole [2].

It has been established that occupied molecular orbitals with the lowest energy are the σ orbitals. If the energy is slightly higher than π orbitals come into play a role. Non-bonding orbitals are at still higher energy levels. The highest energy orbitals belong to π^* and σ^* . These are called unoccupied or anti bonding orbitals. The schematic diagram to represent the electronic transition of the energy levels is given in the Fig. 1.

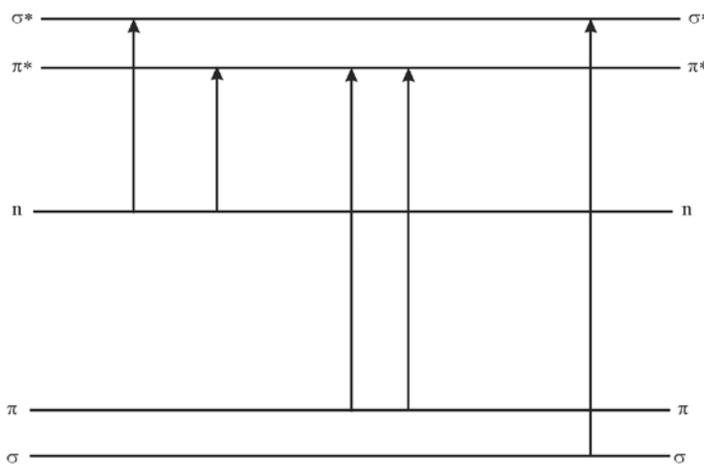


Figure 1: Electronic energy levels and transition

Molecular absorption in the ultraviolet and visible region of the spectrum is dependent on the electronic structure of the molecule. Absorption of energy is quantized. Due to this fact the elevation of electrons from orbitals in the ground state to higher energy orbitals is an excited state. Ultraviolet spectroscopy is limited to conjugated systems for the most part of the electromagnetic spectrum. Ultraviolet spectroscopy provides the facility of recognition of very complexities in molecules. A large portion of a complex molecule may be transparent in the ultraviolet light. A spectrum obtained is similar to that of a much simpler molecule.

An ultraviolet visible spectrum is a graph of light absorbance verses wavelength in a range of ultraviolet or visible regions. Such type of a spectrum can be produced by a more sophisticated spectrophotometer. Data can be collected one wavelength at a time by simple instruments.

The wavelengths of absorption peaks can be correlated with the types of bands in a given molecule. The wavelength is valuable in determining the functional groups within a molecule. Ultraviolet and visible absorption is not a specific test for any given compound. The nature of the solvent, the pH of solution, temperature, high electrolyte concentrations, and the presence of interfering substance can influences the absorption spectra of compounds. The variations can be made in width of the slit for band within the spectrophotometer.

Ultraviolet visible spectroscopy is routinely used in the quantitative determination of solutions of transition metal ions and highly conjugated organic compounds.

Solutions of transition metal ions can be coloured. These can absorb visible light. The 'd' electrons within the metal atoms can be excited from one electronic state to another state. The colour of metal ion solutions is strongly affected by the presence of other species. These may be certain anions or ligands. Organic compounds with a high degree of conjugation also absorb light in the ultraviolet or visible regions of electromagnetic spectrum. Solvents for these determinations are generally water; it can be used for water soluble compounds. Ethanol can be used for organic soluble compounds. The radiations which move with the speed of light are called "electromagnetic radiations". Radiation vibrates perpendicular to the direction of propagation with wave motion.

It is broken completely into the several portions called as electromagnetic (EM) spectrum. Different regions of this spectrum provide different kinds of information as a result of interactions. Light may be considered to have both waved and particle character shown in Fig. 2.

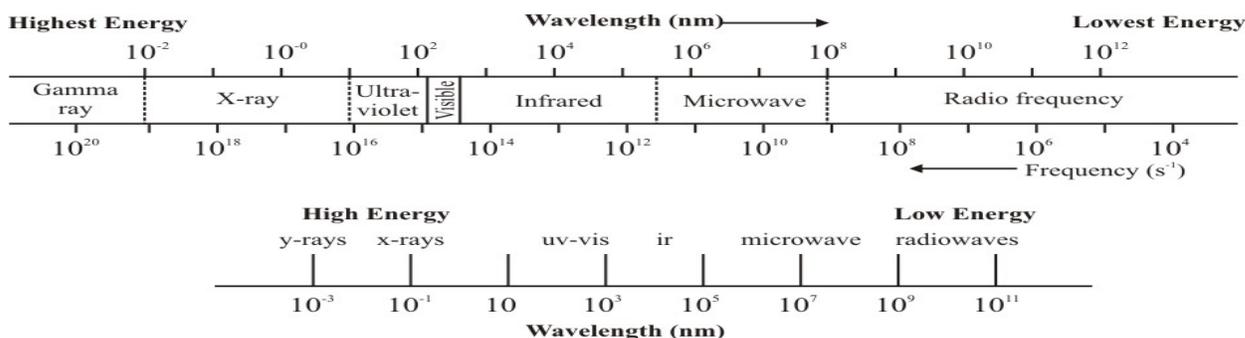


Figure 2: Energy spectrum of different wavelengths to show the different regions.

The part of the e.m. radiation spectrum with the visible light is most familiar to us. This is the small portion of all the possible types. Molecular absorption spectroscopy in the ultraviolet and visible is concerned with the measured absorption of radiation in its passage through a solid

liquid or a gas. The wave length region is used for this type of spectroscopy is from 190 nm to 1000 nm. The absorbing medium is kept at room temperature. Ultraviolet and visible spectroscopy is used to measure the multiple bonds or aromatic conjugation within the molecule. UV-Vis region is subdivided further as vacuum UV (100 nm to 200 nm), near UV (200 nm to 400 nm) and visible region (400 nm to 750 nm). Vacuum UV is called because molecules of air absorb radiations in this region. Molecule absorption spectra are more meaningfully considered as a function of wave number (Cm^{-1}). We use wave length (nm) to measure the quantity of absorption.

UV-Vis spectroscopy involves the absorption of UV and visible light by the molecule causing the promotion of an electron from ground electronic state to an excited electronic state of the transition. Absorption of this high energy light causes electronic excitation. The easily accessible part of the region is from 200 nm to 800 nm. This shows absorption only if conjugated π -electron systems are present. Ultra violet radiation having wavelength less than 200 nm is more difficult to handle. The energies are sufficient to promote or excite a molecular electron to a higher energy orbital. Ultra-violet region is only a small part of the electromagnetic spectrum and it extends from 190 nm to 300 nm. We are interested in the spectrum, which obtained when ultra-violet light passes through medium and not in the source spectrum. The interaction of ultraviolet radiations with matter may result either in the emission or in the absorption spectrum. UV radiations excite the transparent substance from the lower to higher energy state via electronic transitions. The transitions induced by ultra violet are not common to all electronic structures; in fact these take place in conjugated systems. We are in a position to recognize the characteristic groups because the UV spectra are highly sensitive to environmental factors in biological macromolecules. As the energies involved in electronic spectra are large, these are associated with changes in rotational and vibrational states, which blur the observed spectrum, rendering it characterless in liquids. However, even this highly characteristic of a particular molecular group lies both in its frequency and intensity.

The spectra have the absorption peak position and the intensity of the peak. We can express the peak position in wave length (nm), frequency (Hz) or wave number (Cm^{-1}).

The intensity is dependent on the interaction probability and hence the dipole moment of transition. This parameter depends on the electronic charge distribution in transition. Low probability transitions are forbidden.

The polarity of the excited state also affects the intensity. Light absorption laws in transparent medium were formulated by Lambert [3] and the intensity of transmitted light was given by

$$I = I_0 e^{-\alpha l} \quad (1)$$

where I_0 is the intensity of the incident light, the characteristic coefficient of absorption for the medium and ℓ is thickness of the absorbing medium. We can calculate the Bunson-Rosioe extinction coefficient (K) by the formula

$$K = \left[\log_{10} \left(\frac{I_0}{I} \right) \right] \frac{1}{\ell}, \quad (2)$$

$$K\ell = \log_{10} \left(\frac{I_0}{I} \right),$$

$$(\log_e 10)(K\ell) = \left[\log_{10} \left(\frac{I_0}{I} \right) \right] (\log_e 10),$$

$$2.3026 K\ell = \log_e \left(\frac{I_0}{I} \right),$$

or
$$e^{2.3026 K\ell} = \frac{I_0}{I},$$

$$I = I_0 e^{-2.3026 KI} \quad (3)$$

Comparing (1) and (3), we get

$$\alpha = 2.3026 K$$

The intensity of transmitted light is given by Lambert – Beer Law [3],

$$\frac{I}{I_0} = e^{-KC\ell} \quad (4)$$

or
$$KC\ell = \log_e \left(\frac{I_0}{I} \right) = A, \quad (5)$$

The absorbing molecules having concentration C. K is constant characteristic of the solute and ℓ is the solution path length. A is known as absorbance or absorptivity.

When the constituents of the absorbing molecule are unknown, intensity is expressed as the absorption by the solution of 1 gm/100 ml concentration and 1 centimeter path length of the sample ($E_{1cm}^{1\%}$ or $A_{1cm}^{1\%}$)

Thus
$$\left(E_{1cm}^{1\%} = \frac{A}{C\ell} \right) \quad (6)$$

The corresponding energy is given below

$$E\left(\frac{\text{kcal}}{\text{mol}}\right) = N h \nu = \frac{28.587}{\lambda(\text{nm}) \times 10^3} \quad (7)$$

The total energy of molecule is given by

$$E = E_e + E_v + E_r, \quad (8)$$

where E_e is the electronic energy, E_v the vibrational energy and E_r the rotational energy.

Transitions of electrons take place from filled molecular orbits in ground state involving bonding (σ and π) or non bonding (n) orbits (electrons), to higher energy involving the anti-bonding σ^* or π^* orbitals. These remain empty in an unexcited state. It is customary to discuss and to describe the intensities of absorption bands in terms of molar absorptivities ϵ_{max} (molar extinction coefficients). The coefficients can be obtained from the determination of the wavelength of maximum absorption. Accurate measurement of the absorbance at this one wavelength can provide these coefficients. ϵ_{max} is not directly related to any quantity.

Changes in the energies accompany the changes in electronic energy and give rise to the familiar band spectra. A single electronic transition comprises many individual lines (bands), and the quantity, i.e., total energy transferred should be sum of the contributions from all these lines (bands).

The quantity of interest in ultraviolet spectroscopy is not ϵ_{max} but it is the integrated (absolute) intensity (I). This is equal to the area under the absorption curve. Ultraviolet spectra rarely contain isolated bands, and we must extrapolate the band to the abscissa in arbitrary manner.

Now, experimental integrated intensity is mathematically defined by the integral

$$I = \int \epsilon \, d\nu \quad (9)$$

In UV region, strongly overlapping bands are frequently encountered. Evaluation of the integrated intensity of such bands requires analysis into separate bands.

Calculation of Absorption Intensity

Calculation of absolute intensity of an absorption (or emission) bands is not difficult. Mulliken [4] has shown probabilities of emission (A) and absorption (B) between two electronic states from initial (i) to final (f) by the relation

$$A_{if} = (64 \pi^4 \nu^3 e^2 / 3h) G_f D_{if} \quad (10)$$

$$B_{if} = (8 \pi^3 e^2 / 3h^2 c) G_f D_{if} \quad (11)$$

where, e is the electronic charge of electron, h is Planck's constant, c is the velocity of light, ν is the frequency of emission, G_f is the statistical weight of the final state, D_{if} is the dipole strength.

B_{if} can be transformed into a measure of intensity, the oscillator strength f , which is given by

$$f = \left(\frac{8\pi^2 mc}{3h} \right) G_f \nu D_{if} = 1.096 \times 10^{11} G_f \nu D_{if} \quad (12)$$

and is related to the absolute intensity

$$f = 0.102(mc^2/N\pi e^2) \int \epsilon d\nu = 4.315 \times 10^{-9} \int \epsilon d\nu \quad (13)$$

where m is the mass of the electron, N is Avogadro's number, ϵ is the molar absorption coefficient, G_f is considered as unity.

The dipole strength (D) is proportional to the intensity and is given by the relation

$$I^2 \propto D^2 = \int \psi_i M \psi_f d\tau \quad (14)$$

ψ_i and ψ_f are the total wave functions of the initial and final states of the molecule respectively, $d\tau$ represents the product of the volume elements in the coordinates of all the nuclei and electrons, \bar{M} is called dipole moment vector.

According to quantum mechanics, desired average can be obtained as

$$\int \psi_i \sum e r \psi_i dT = (\psi_i M \psi_f dT) \quad (15)$$

where, $\bar{M} = \sum e \bar{r}$ is called the dipole moment vector.

Now, \bar{M} in 3-Cartesian coordinates axes is given by the relation as described below

$$M^2 = M_x^2 + M_y^2 + M_z^2 \quad (16)$$

D^2 can be given by the following relation

$$D^2 = \int \psi_i M \psi_f dT \quad (17)$$

where ψ_i and ψ_f are the total electronic wave functions of the initial and final states, $d\tau$ is the product of the volume elements in terms of the coordinates of all the electrons.

If the spectral shape of a ligand spectrum does not change but the magnitude changes in such a way that it is proportional to the concentration of bound ligand, then the spectral data can be utilized to estimate the equilibrium binding constant, K . The ligands are binding in one binding mode or in constant proportions in more than one mode.

If we consider the equilibrium



where L_b is a bound ligand and L_f is a free ligand, S_f is a free site.

If we treat a macromolecule, which is a series of binding sites of n residues in size, then the total site concentration is given by the following relation

$$\frac{[M]}{n} = S_{tot} \quad (19)$$

Here, $[M]$ is the concentration of the macromolecule residue.

It has been reported that for proteins and sometimes preferable to take as concentration of molecules rather than residues.

$$S_{tot} = n'[M] \quad (20)$$

Here, n' is the number of binding sites per protein.

We have the calculation for k which is given below

$$k = \frac{nC_b}{C_f[M]} \quad (21)$$

Ultraviolet spectroscopy can be used for multi component analysis of mixture consisting of n absorbing analytes is possible provided the Beer-Lambert Law is valid and holds, by measuring the absorbance at k suitable wavelengths with $k > n$, For each wavelength λ_a . This 'a' can have the values 1, 2, $k - 1$, k .

Total absorbance is given by the relation

$$A(\lambda_a) = b \sum_{i=1}^n \epsilon_i(\lambda_a) C_i \quad (22)$$

where $\epsilon_i(\lambda_a)$ is the molar (decadic) absorption coefficient of analyte i at the wavelength λ .

C_i is its molar concentration.

The ratio $A(\lambda_a)/b$ is called linear decadic absorption coefficient. This quantity is also called linear absorbance.

The concentrations of the n analytes may then be calculated from k simultaneously equations.

The use of derivative spectroscopy decreases the signal to noise ratio. It can improve the detection of a sharp band in a broad back ground, or a narrow shoulder on a broad band. Gradual changes in spectral back ground or source flux will be less pronounced in derivative spectroscopy.

Derivative spectra are generally obtained by digital differentiation or by wavelength modulation of the radiation entering the sample cell. The wavelength modulation interval has to be much less than the band width of any absorption band in the spectrum.

The derivative spectrum in terms of wave number can be calculated from the spectrum in terms of wavelength by

$$\frac{dA(\bar{\nu})}{d\bar{\nu}} = (-\lambda^2) \frac{dA(\lambda)}{d\lambda} \quad (23)$$

$$\frac{d^2A(\bar{\nu})}{d\bar{\nu}^2} = (\lambda^4) \frac{d^2A(\lambda)}{d\lambda^2} + 2\lambda^3 \frac{dA(\lambda)}{d\lambda} \quad (24)$$

The first and second derivatives of the Beer' - Lambert Law are. If $\frac{d\phi_0}{d\bar{\nu}} = 0$ then we can write these derivatives as

$$\frac{d\phi_t}{d\bar{\nu}} = -2.303 c b \frac{d\varepsilon}{d\bar{\nu}} \quad (25)$$

$$\frac{d^2\phi_t}{d\bar{\nu}^2} = \left[2.303 c b \frac{d\varepsilon}{d\bar{\nu}} \right]^2 - 2.303 c b \frac{d^2\varepsilon}{d\bar{\nu}^2} \quad (26)$$

Second derivative spectra are predominantly used in quantitative analysis.

Molecular absorption spectroscopy in the ultra violet and visible is concerned with the measured absorption of radiation in its passage through a gas, liquid or a solid. Ultra violet spectroscopy allows for the determination of the concentration of protein in a sample as the absorbance at 280 nm and it is directly proportional to the concentration of protein. The protein sample has tryptophan or tyrosine content, which absorb at 280 nm. Determination of protein concentration can be done by the absorbance at 205 nm. Peptide bonds are analyzed directly by the using a method of Scoopes [5].

Jagger [6] has reported that the important molecular components of proteins that are expected to absorb ultra violet are the aromatic amino acids and peptide bond. Tryptophan and tyrosine are the major components, which absorb ultra violet above 250 nm. Phenylalanine, cystine are also important components because most of the other amino acids do not absorb in this region. These two components may be major absorbers in a protein with low tryptophan – tyrosine content. Disulfide bonds are very crucial structures. Their presence increases the UV liability of proteins. Peptide bond (– CONH –) has some double bond character. It is a relatively weak absorber and is only important below 240 nm. A peptide bond for every amino acid residue in a protein makes its contribution to protein absorption below 240 nm.

There is an interaction in a protein among amino acids. The total absorbancy of a protein solution is quite similar to the sum of the absorbencies of solutions of the component amino acids. Proteins usually exhibit an absorption peak around 280 nm. Scopes, R. K. [7] has measured proteins at 205 nm and given a formula regarding the average absorbance due to tryptophan and tyrosine at 205 nm is given here. as

$$\left[3.6 + \left(\frac{4.6 - 3.6}{4} \right) \right] \times A_{280} = 3.85 \times A_{280} \quad (27)$$

We can write an expression

$$\epsilon_{205}^{1\text{mg/ml}} = C + 3.85 \times \epsilon_{280}^{1\text{mg/ml}} \quad (28)$$

C is the extinction coefficient due to the polypeptide chain in its native configuration, plus contributions from phenylalanine, histidine and other residue side chains but excluding tyrosine and tryptophan.

For a protein solution of unknown concentrations, values of the absorbance. A_{280} and A_{205} are noted. The expression $\epsilon_{205}^{1\text{mg/ml}}$ can be replaced by $\epsilon_{205}^{1\text{mg/ml}} \times A_{280} / A_{250}$.

We can write two possibilities of approxmamation as

$$\epsilon_{205}^{1\text{mg/ml}} = 31 \quad (29)$$

$$\epsilon_{205}^{1\text{mg/ml}} = C + 120 \times (A_{280} / A_{250}) \quad (30)$$

and if we rearrange eqⁿ (28) as

$$\epsilon_{205}^{1\text{mg/ml}} = \frac{C}{1 - 3.85 \times \left(\frac{A_{280}}{A_{205}} \right)} \quad (31)$$

If we substitute $C = 27.0$

We can have the best values as

$$\epsilon_{205}^{1\text{mg/ml}} = 27.0 + 120 \times \left(\frac{A_{280}}{A_{205}} \right) \quad (32)$$

$$\epsilon_{205}^{1\text{mg/ml}} = \frac{27.0}{1 - 3.85 \times \left(\frac{A_{280}}{A_{205}} \right)} \quad (33)$$

Perkampus [8] has provided some of the features of bond analysis with the Gaussian and Lorentzian functions. The bands to be separated in most of the cases can be approximated by Gaussian curves and the extinction coefficient or absorbance can be written here as.

$$A = A_{\text{max}} \cdot e^{-\left\{ B (\bar{\nu}_0 - \bar{\nu})^2 \right\}} \quad (34)$$

$\bar{\nu}_0$ is the wave number at the band maximum.

The parameter B is connected to the full width at half the maximum intensity of the band by the following eqⁿ.

$$B = \frac{4 \log_2}{\Delta \bar{\nu}_{1/2}^2} \quad (35)$$

Eqⁿs (34) and (35) can be written as

$$\left[\log \left(\frac{A_{\text{max}}}{A} \right) \right]^{1/2} = (B\bar{\nu})^{1/2} - (B\bar{\nu}_0)^{1/2} \quad (36)$$

Eqⁿ (36) is the basis for a linear regression for fitting the theoretical to experimental curve.

2. REVIEW OF THE LITERATURE

The application of UV absorption spectroscopic technique for the examination of the concentration of protein molecules has undergone significant change during the past couple of years. Changes in conformation with altered temperature or with the addition of organic molecules to an aqueous solvent medium, are usually associated with positional shifts and

intensity changes in ultra-violet absorption bands. These variations can be explained on the basis of the energy level transitions and empirical correlations with information obtained by other methods.

Proteins interact with ultra violet light with a consequent absorption of energy giving rise to absorption spectrum [8]. Not all molecules are found to exhibit this absorption. This phenomenon is confined to the molecules with double bonds and longer wavelength to conjugated bonds. The energy absorbed by the molecule causes electronic transition from ground state to one of the molecular excited states involving an electron a π orbital or a non bonding p-type orbital into a higher anti-bonding π^* -orbital. Thus the transition between the ground state of entire system and the excited state of the chromosphere proves quite helpful in understanding the structure of proteins. The absorption spectrum in ultra violet region is influenced by a number of factors including the state of bonding and nature of the bonds involved. The shift in the position of absorption maxima and change in the area under the absorption curve help in the recognition of a characteristic group or its environments. The excitation of electrons in the frequency above $50,000 \text{ cm}^{-1}$ has been used to determine the conformation of peptide chains which has characteristic absorption maxima at 185 nm.

Many properties of proteins including their primary structure, modification of sequence of amino acids in polypeptide chains and covalently attached prosthetic groups influencing the nature and function of proteins have been studied in the past by using this technique.

This study also took into consideration the question of inter atomic distances and the bond angle from the shift in absorption peaks on their intensities. The plasma proteins in general give absorption bands between 200 nm to 400 nm. Aromatic proteins absorb in the range 260 nm to 280 nm. The 280 nm absorption is due to tryptophan and tyrosine residues in the aromatic proteins. Phenylalanine absorbs at slightly lower wave length $\sim 255 \text{ nm}$. The free amino acid contents of plasma show concentration of phenylalanine as 0.95 mg/100ml, tyrosine as 0.91 mg/100 ml and tryptophan as 0.98 mg/ 100 ml of plasma.

Most of the previous investigations of ultra-violet spectra of protein measured in the region ranges from 250 nm to 300 nm. In this region absorption is due almost entirely to the aromatic side chains of tryptophan, tyrosine and phenylalanine. The stronger absorption bands of these side chains between 190 nm to 240 nm, not studied rigorously till now. One can attribute this to technical problems and still more because of the overlapping absorption in this region from histidine , methionine, cystine and ionized cystine and the long wave length end of the absorption due to the peptide bond itself.

Absorption bands located below 200 nm have been of great interest. Absorption band of the peptide linkage is centered near 190 nm. The transition from a helix to a random coil is associated with significant increase of ultra violet absorption in this region. The work of

Rosenheck and Doty [9] has given importance to the absorption studies in the ultra violet region. The main factor for this lies in the strong connection between the ultra violet absorption bands and the optical rotatory dispersion (ORD) changes associated with the helix coil transition. The intermediate region of 200 nm to 250 nm has been studied in some of earlier publications [10, 11]. Difference spectra in neutral and acid solutions of several proteins exhibit a significant line near 235 nm. This peak is higher compared to the signals due to tyrosine residues near 278 nm to 287 nm or those due to tryptophan at slightly larger wave lengths.

So far as the structure of native globular proteins is concerned, the amino acids side chains may emerge on the outer side towards the solvent from the main peptide chain or may be folded into the interior of the molecule and enclosed by surrounding groups of the protein itself. Native globular proteins are immunoglobulins such as IgG, IgA, IgM, IgD and IgE. Intermediate situations may also present. An amino acid side chain is likely to be partly covered near the surface of the molecule but flexible enough to appear out occasionally and have interactions with the surrounding solvent. Some proteins are also likely to have water molecules trapped in the interior, so that internal groups may still have association with water.

There is a possibility of some sort of fissures or crevices at the surface of the protein molecule. Small solvent molecules can go through these fissures. The amino acids composition of globulin in the decreasing order or proportion is given below:

- | | | | |
|---------------|---------------|-------------------|-------------------|
| (1) Leucine | (2) Valine | (3) Aspartic acid | (4) Alanine |
| (5) Lysine | (6) Histidine | (7) Phenylalanine | (8) Glutamic acid |
| (9) Theonine | (10) Proline | (11) Glycine | (12) Serine |
| (13) Tyrosine | (14) Arginine | (15) Tryptophan | (16) Methionine |
| (17) Cysteine | | | |

Jiang *et. al.* [12] studied this spectroscopy technique for the protein back bone transitions in aqueous solution. They have made some calculations to simulate and backbone transitions of protein. One can find such type of transitions in the region 180nm to 220 nm. These findings are very sensitive and provide a probe for secondary structure of proteins. They have characterized fine structure of UV spectra accurately for the identification of secondary structure of proteins.

Amoldus *et. al.* [13] have studied the thermal stability of immunoglobulin and measured the protein concentration at 280 nm. The study of the structure of immunoglobulins in detail reveals that these proteins are composed of four polypeptide chains which are connected by disulphide bonds and non-covalent forces. The four polypeptide chains are grouped together in different fragments, two identical F_{ab} segments and one F_c segment forming a Y shaped conformation. The antigen binding sites are located on the far ends of the F_{ab} segments. The F_{ab}

segments are linked to the F_c by the hinge region, which varies in length and flexibility in the different antibody classes and isotypes.

Motrescu *et. al.* [14] have studied spectrophotometric analysis of the blood plasma for different mammals to study and establish a correlation between protein concentration and their absorbance in the ultraviolet region. Protein absorbance band at 280 nm determines the characteristic spectrum of the blood plasma and the absorption maximum strongly depends on the blood plasma protein concentration.

Kanagathara, N. *et. al.* [15] have applied UV-visible spectral study on normal blood samples and found that there is a linear relationship between the protein content and the maximum absorption in the ultraviolet region. A band at 280 nm helps in determining the characteristic spectrum of the blood plasma. They have also reported that the absorption spectrum of the diseased samples of blood show same changes from normal blood sample. It may help us and treated as an evidence for the manifestation of the disease.

Gunasekaran *et. al.* [16] have also studied the ultraviolet spectroscopy on normal and jaundice blood samples and reported their findings as proteins in the sera absorb strongly at 280 nm. It is due to amino acids like tyrosine and tryptophan. The amide backbone of the proteins in the blood shows a band at 210 nm [14].

Yvette *et. al.* [17] have used this technique to characterize and differentiate the types of blood. Yvette *et. al.* [18] have also used the spectroscopy for the quantitative investigation of platelet quality. The optical properties for isolated platelets, platelet rich plasma and leukopleted platelet rich plasma have also been determined.

Akihisa [19] used this technique for the characterization of red blood cells. A Successful simulation of experimental red cell spectra, which contain various amounts of hemoglobin, were also carried out. A quantitative interpretation of the red blood cell spectra was also achieved in context of corpuscular hemoglobin concentration, corpuscular volume, and cell count.

A very important class of proteins that conform to a common submit structures are immunoglobulins. These molecules have domains which are structurally independent, compact globular regions consisting of continuous stretches of the polypeptide chain of hundred amino acids long, with a characteristic fold. This fold contains two β -sheets and essentially no α -helices. These globular protein structures have a unique feature regarding non-polar residues which are sequestered into a core. They avoid contact with water at this stage. Immunoglobulins or antibodies show a very strong structure function relation in the different domains. Due to this property these molecules have an excellent system for various diagnostic tests. The domains of the antibodies with a high specificity to bind the analyte assure that these immunoglobulins can be used for a reliable and fast determination of low concentrations of

analyte. Other domains of immunoglobulin promote protein binding to a surface in the proper orientation. Its binding sites are easily accessible to the antigen.

Welhelm *et. al.* [20] have studied ultraviolet absorption spectra of blood serum and certain amino acids and reported that the absorption band around 280 nm of blood serum was due to the presence of proteins. They were mainly the tyrosine and tryptophan constituents of the proteins are responsible for a nice and clear band. A mixture of tyrosine, tryptophan, phenylalanine, cystine, glycine, leucine and glutamic acid in proportion indicated by analysis of blood serum (albumin and globulin) gives the ultraviolet visible spectra.

Malvin [21] has studied ultraviolet absorption spectra of certain physiological fluids, such as human bile, saliva, pericardial fluid, uric acid, urine, albumin, pseudo globulin euglobulin and O-O blood serum and plotted some graphs, which are valuable to the clinician and researchers. Edward *et. al.* [22] have studied the middle ultraviolet spectra of proteins and found that major absorption band of the aromatic compound was due to a $\pi \rightarrow \pi^*$ transition. Minor transition was involved in tyrosine spectrum.

Alastair *et. al.* [23] have determined protein by UV absorption and found that absorption of radiation in the near UV by proteins depends on the tyrosine and tryptophan content and to a very small extent on the amount of phenylalanine and disulfide bands. They have also reported that the extinction of nucleic acid in the 280 nm region may be as much as ten times that of protein at their same wavelength. Nucleic acid in small percentage can influence the absorption.

Yong *et. al.* [24] have studied the effect of ultraviolet irradiation on molecular properties and immunoglobulin production regular activity of β -lactoglobulin and found that ultraviolet irradiation is effective for alteration of molecular properties and anti-genicities of β -lactoglobulin. Low allergenic foods may be prepared with the help of such types of treatment.

Henry *et. al.* [26] have studied application of ultraviolet absorption spectroscopy to the analysis of biopharmaceuticals. They have suggested that the non-destructive technique depends on contributions from ultraviolet absorption of aromatic amino acids, cystine and light scattering. This technique can be used for determination of concentration and detection of impurities as well as assessment of aggregation state.

Mc Crathy *et. al.* [27] have studied this spectroscopy on IgG aggregate formation and found that few changes were observed in treated IgG. These changes remained un-aggregated but so many significant size dependent changes were also seen in aggregated IgG.

Edelhoch [28] has studied spectroscopic determination of tryptophan and tyrosine in proteins and presented a rapid method for the determination of tryptophan in proteins and it was based on absorbance measurements at 288 nm and 280 nm. of the protein.

Mansor *et. al.* [29] have studied FTIR and UV-Vis study of chemically engineered biomaterial surfaces for protein immobilization. They suggested that proteins have a very particular chain configurations and conformations that promote high levels of specificity during chemical interactions. They have also studied the phenomenon of protein immobilization onto biomaterial with chemically engineered surface. They have succeeded to put forward the views such that all the surface modeled material has shown acute hypoglycemic peak response associated with the insulin bioactivity.

Mirian *et. al.* [30] have studied ultraviolet spectroscopy of hemes in electron carrier C-type cytochromes and its model compounds and found that some of the compounds have shown their spectra at different wavelength such as cytochrome C at 406 and 530 nm. Cytochromes C-557 at 409, 525 nm, Cytochrome C has shown two peaks at 408 and 530 nm. MP-9 at 395, 507 and 622 nm. Hemin has shown two peaks at 383 and 613 nm. These compounds were tested at 7.2 pH.

Sanii *et. al.* [31] have studied UV-Visible spectroscopy of the native membrane and the structure of retinal and found those two bands for protein bacteriorhodospin at 280 nm and at 570 nm. The 280 nm absorption band is due to the $\pi \rightarrow \pi^*$ transition of the tyrosine and tryptophan amino acid.

3. MATERIAL AND METHODS

The blood samples of Alzheimer's disease patients were collected from the Department of Neurology, Safdarjang Hospital, New Delhi. Twenty milliliters freshly drawn blood from each patient was collected in clean and dry test tube without any anti-coagulant. The test tube was kept for 45 minutes at room temperature ($22 \pm 2^\circ\text{C}$) for the formation of clot. Sera of different patients were separated by centrifugation at 1500 r.p.m. upto 15 minutes and were collected in screw capped test tubes. IgG sample were prepared on protein A-Sepharose method [32].

The IgG binding properties of protein A, make affinity chromatography with protein A-sepharose CL-4B a very simple method for preparing IgG. 1.5 g protein-A sepharose CL-4B was swollen in 10 ml phosphate buffered saline (PBS) for 1 hour at room temperature and then packed into a small chromatography column. 10 ml human serum was diluted with an equal volume of PBS. The serum was filtered through the column at a flow rate of 30 ml/h. Washing was done through unbound protein with PBS. Until no more protein left the column (the protein was monitored with a UV flow cell).

The bound IgG was eluted with glycine-HCL buffer having a pH value of 2.8. The pH of purified IgG solution was titrated to near neutrality with NaOH and dialyzed against PBS. The column was regenerated by washing with 2 column bed volume of PBS. The column was stored at 4°C .

The protein A content of the swollen gel is 2 mg/ml and the binding capacity for human IgG is approximately 25 mg/ml of packed gel. As binding of protein A to IgG involves tyrosine residues on the protein A glycosyl tyrosine (0.1 M in 2 NaCl) can be used to elude the IgG rather than the glycine – HCl buffer.

The ultra-violet spectra of the IgG extracted from normal person and Alzheimer's disease patients were recorded on Shimadzu UV visible recording Spectrophotometer UV-260. Normal saline was used as a reference. Block diagram of ultraviolet spectrophotometer is shown in Fig. 3

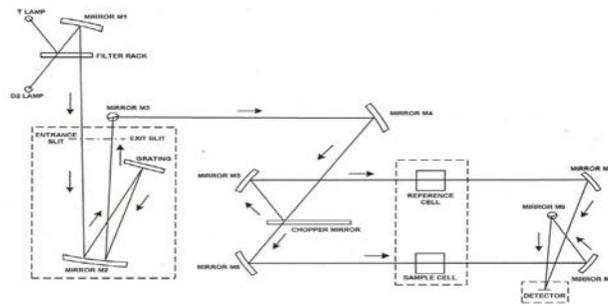


Figure 3: Block diagram of ultraviolet spectrophotometer

4. RESULTS

Ultraviolet absorption spectra of Alzheimer's disease patients were recorded and compared with normal healthy controls. We observe peaks at different wavelength in all samples. A comparative detail of the work is given in Table-1. Typical ultra violet spectra of DMD , AD and control person are given in Fig.4 to Fig.6.

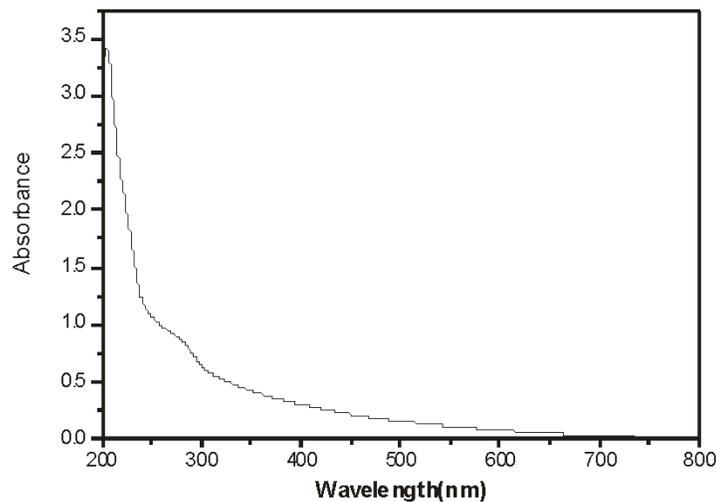


Figure 4 : Ultra Violet spectrum of Normal child

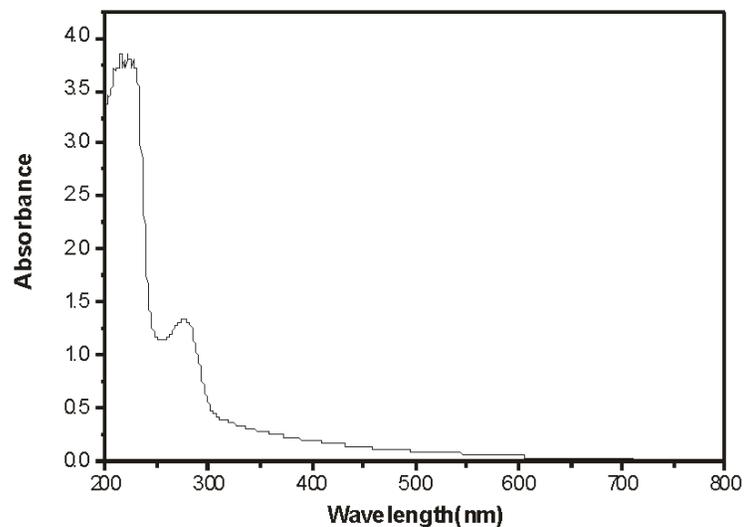


Figure 5 : Ultra Violet spectrum of DMD Child

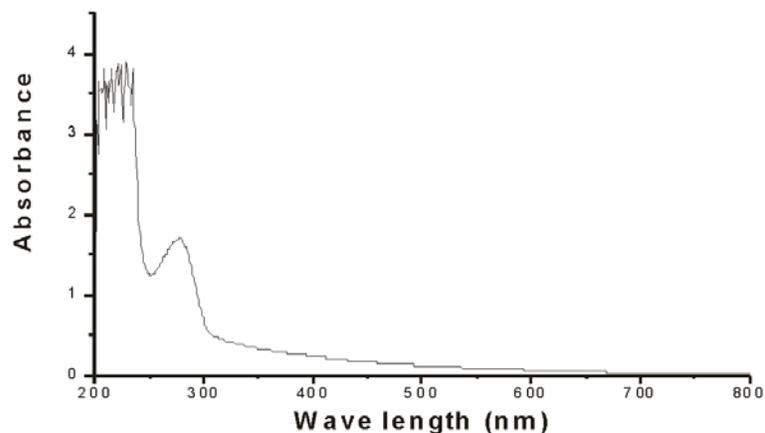


Figure 6 : Ultra Violet spectrum of Alzheimer's diseased person

Table. 1: Experimental findings on the immunoglobulin G molecule of Alzheimer's disease patients and healthy controls and compared with epileptic samples.

E-Epilepsy N-Normals and GME-Grand Mal Epilepsy, AD- Alzheimer's disease, NAD Controls for Alzheimer's. DMD- Duchenne Muscular Dystrophy , NDMD -Controls for DMD

S.No.	Type sample	of	Peak 1 nm.	Peak 2 nm.	Peak 3 nm.	References
1	N		205.80	243.08	299.96	Kumar [33]
2	N		208.32	232.97	275.95	
3	N		205.6	260.6	278.6	
4	N		206.6	260.4	277.8	
5	N		207.2	243.2	279.2	
6	N		208.1	244.1	280.1	
7	N		206.0	244.1	281.0	

8	N	207.8	246.8	280.8		
9	N	203.6	243.2	276.6		
10	N	207.2	241.4	284.6		
11	N	205.4	244.1	282.8		
12	N	207.4	230.44	278.48		
13	GME	205.8	260.8	278.8		
14	GME	205.8	268.4	283.4		
15	GME	205.2	259.8	278.6		
16	GME	205.2	260.8	278.4		
17	GME	206.6	268.6	278.6		
18	GME	205.8	260.4	278.6		
19	GME	205.0	268.8	277.4		
20	GME	205.8	260.8	278.8		
21	GME	206.0	268.8	278.4		
22	GME	205.4	261.0	278.6		
23	GME	206.2	261.2	278.6		
24	GME	205.6	261.0	278.4		
25	GME	205.8	259.9	278.6		
26	E	207.71	237.41	279.13		
27	E	206.45	231.73	280.39		
28	E	202.45	231.73	280.03		
29	E	202.65	232.99	280.03		
30	E	202.65	234.25	281.02		
31	E	203.20	231.71	280.37		
32	E	200.01	231.61	278.38		Kumar [33]
33	E	202.20	234.24	279.11		
34	E	202.40	235.50	279.74		
35	E	203.17	235.40	279.01		
36	E	201.27	235.40	279.01		
37	E	202.65	238.68	279.01		
38	E	202.65	238.05	279.01		
39	AD	232.92	276.65	335.05	Present work	
40	AD	229.20	269.35	305.58		
41	AD	229.20	270.00	298.55		
42	AD	225.55	267.16	305.85		
43	AD	228.47	269.35	313.15		
44	AD	229.20	267.16	313.15		
45	AD	225.55	273.00	308.77		
46	AD	235.77	269.35	298.55		
47	AD	222.63	244.53	274.46		
48	AD	225.55	268.62	301.47		
49	AD	221.90	278.89	296.36		

50	AD	224.82	262.05	300.01	Present work
51	NAD	230.66	270.08	306.58	
52	NAD	228.47	267.89	303.66	
53	NAD	235.77	268.62	318.99	
54	NAD	230.66	267.16	311.69	
55	NAD	228.47	270.00	297.82	
56	NAD	221.17	260.59	301.47	
57	NAD	224.82	271.54	297.82	
58	NAD	228.47	272.27	305.12	
59	NAD	235.77	267.16	301.47	
60	NAD	228.47	272.27	303.66	
61	DMD	222.22	275.85	311.92	
62	DMD	222.20	277.70	303.60	
63	DMD	220.35	273.63	311.00	
64	DMD	204.14	255.50	296.20	
65	DMD	211.10	259.20	307.30	
66	DMD	212.58	264.38	308.78	
67	DMD	227.38	281.40	325.80	
68	DMD	200.00	244.40	286.95	
69	N DMD	225.90	285.10	314.70	
70	N DMD	225.90	297.70	307.30	
71	N DMD	229.60	287.69	329.50	
72	N DMD	200.00	245.51	302.86	
73	N DMD	200.00	245.51	303.60	
74	N DMD	200.00	249.58	296.20	
75	N DMD	200.00	244.40	299.90	
76	N DMD	221.83	281.40	318.40	
77	N DMD	200.00	246.25	351.70	
78	N DMD	220.35	279.55	309.15	

We have found three types of bands in the spectra of IgG of DMD patients and normal healthy controls. First and second band regions cover the ultraviolet behavior of proteins but third band does not show absorbance of proteins because the absorbance intensity greater than 310 nm. Third band does not contain protein without tryptophan. We have to concentrate on the region of two bands below 300nm. We have first region in the range 200 nm. to 227.38 nm. in DMD patients and 200nm. to 229.60 nm. in normal controls .Second region starts from 244.40nm. to 297.70nm.in patients and 244.40 nm. to 297.70 nm . in controls only. Third band did not show any protein absorbance in all the cases of study. We have found 1st band ranges from (221.17 nm⁻¹ to 235.77 nm⁻¹) in all the cases of AD and healthy subjects.

The second band ranges from (244.53 nm⁻¹ to 278.89 nm⁻¹) in AD and normal and available at (260.59 nm⁻¹ to 272.27 nm⁻¹). There is a variation between these two categories for this band region. There is a shift in the lower side of the band in AD cases. On the other hand a shift in the right side of the band is also observed.

The third band was found in the range from (274.46 nm⁻¹ to 313.55 nm⁻¹) in AD samples while in NAD it starts from 297.52 nm⁻¹ to 318.99 nm⁻¹. It has been seen that the variation in AD samples is on the decreasing pattern in comparison with healthy controls.

The band near at 275 nm is due to tyrosine amino acid residues of protein. It has been established that this band corresponds to a $\pi \rightarrow \pi^*$ transition. The increase in absorptivity and the long wave shift of the spectrum of tyrosine with ionization of the phenolic hydrogen provides the basis for studying the hydrogen ion equilibria of tyrosyl groups in proteins.

A band at 258 nm is associated with phenylalanine amino acid residues of protein. The low intensity absorption peak centered slightly below 260 nm corresponds to a forbidden $\pi \rightarrow \pi^*$ transition. Vibrational fine structure is found in the region. Aside from increased blurring of the fine structure on passing into successively more polar media, change of solvent effects little change in the general size, shape and location of the absorption envelope. Forbidden transition occurs due to coupling of the electronic transition with different molecular vibrations in a way which removes the symmetry of the barrier.

The band of phenylalanine is weak and it is obscured in proteins by much stronger tyrosine and tryptophan absorptions. Phenylalanine is occasionally visualized in protein spectra as ripples or we may say fine structure in the region 250 nm – 270 nm. These ripples can be amplified by the difference spectral method.

It is very important to point out that the absorptivity of phenylalanine is very small around 260 nm. It is the strongest absorbers in the region of the peptide absorption.

The spectrum of tryptophan appears in the range 270 nm to 290 nm. The absorptivity of tryptophan is greater than unionized tyrosine and about twice as great as ionized tyrosine.

5. DISCUSSION

The ultraviolet spectra of proteins have been made the subject of study. It has been well established that the spectra of amino acids show proteins have a high intensity absorption band in the neighborhood area of 190 nm. A similar band is found in simple peptides, with absorptivity increasing with increasing chain length in oligopeptides. The band at 190 nm is not available with aliphatic amino acids.

It has been seen in several proteins the molar absorptivity per peptide bond at 205 nm falls in the range 260 nm to 310 nm. This shows that a contribution of seventy percent of total

absorptivity, the peptide absorptivity is conformation dependent. The absorption spectrum of proteins is of great interest and made easy to study.

A detailed study for absorbance on proteins was carried out by some scientist and is given in Table-2 (Rosenheck, K. *et. al.* [9]).

Table. 2: Comparison of different proteins.

S.No.	Type of sample	Peak 1 nm.	Peak 2 nm.	Peak 3 nm.	References
1	Carbonic anhydrase	280.0	-	-	
2	Carboxy peptidase A	278.0	-	-	Neurath [34]
3	Chymotrypsinogen	282.0	-	-	Chervenka [35]
4	α -Lactalbumin	280.0	-	-	
5	β -Lactoglobulin	280.0	-	-	Gorden <i>et. al.</i> [36], Piezt [37]
6	Lysozyme	281.0	-	-	Formageot <i>et. al.</i> [38], Wetlauffer <i>et. al.</i> [39]
7	Papain	278.0	-	-	Glazer <i>et. al.</i> [40]
8	Ribonuclease	277.5	-	-	Hermans <i>et. al.</i> [41]
9	Bovine serum mercaptalbumin	280.0	-	-	
10	Human serum mercaptalbumin	279.0	-	-	
11	Δ -3-Ketosteroid isomerase	277.0	-	-	Kawahara <i>et.al.</i> [42]
12	Tryptophan	267.2	274.7	281.6	Coulter <i>et. al.</i> [43], William <i>et. al.</i> [44]
			275.0		
13	Tryptophan	269.4	279.4	288.8	
			278.9	287.5	
14	Phenylalanine	271.4	-	-	
		263.3	257.6	251.6	
15	Tryptophan	280.0	-	-	Franz [45]
16	Tyrosine	275.0	-	-	
17	Phenylalanine	258.0	-	-	

There is a broad band with a peak found in the spectrum of protein at 280 nm and a minimum at about 250 nm on the shorter wave length side. This band may be correlated with the presence of tyrosine tryptophan and phenylalanine [46]. The absorption band which is found at wave length above 280 nm is due to non protein occur chromophore. Nucleo proteins

shows maximum in the region of 260 nm. This may be associated with the presence of purine and pyrimidine nuclei. Bovine serum albumin (BSA) shows two characteristics UV bands around 280 nm and 215 nm respectively.

Some of spectra reported for proteins end at 235 nm on the shorter wavelength side of the main 280 nm peak. The absorption curves rise rapidly in this region. Steep rise may provide some information regarding the region of continuous absorption. Specific structure of a protein distinguish it from other compounds in the presence of a large number of peptide bands. UV spectra of anhydrides, esters and fatty acids and acid chloride show a broad band in the region of 200 nm. Ultra violet spectroscopic studies below 240 nm are of interest. Ultra violet spectroscopic studies of amino acids were also carried out by Coulter *et. al.* [43] and they have reported that aliphatic amino acids do not show absorption above 250 nm. Tyrosine tryptophan and phenylalanine show spectrum of absorption in the same spectral region as the proteins. The ultra violet absorption band of proteins has in consequence been attributed to the content of amino acids.

It has been seen that the peptide group of the protein main chain absorb the light energy in the range from 180 nm to 230 nm. Aromatic side chains of tryptophan tyrosine and phenylalanine also absorb light in this region. These residues of proteins may absorb light in the region from 240 nm to 300 nm. Disulfide bond also shows a absorption character near to 260 nm. The aromatic amino acids do not absorb any light above 310 nm. The protein absorbance above 310 nm is zero. The proteins without tryptophan residues do not show any absorption spectrum above 300 nm.

There is a region which starts from 285 nm to 295 nm shows several bands which originate from the nine tyrosine and the single tryptophan residue.

We would like to make a point of inference that at 205 nm the side chains of amino acids make relatively small contribution to the total absorption of proteins and it is absent in all AD and normal subjects.

Absorption coefficient for the peptide bond is of an order of magnitude many times higher than that of amide or carboxylic group. It can be said that the side chain of amino acids.

Our data did not support the work of Scopes [5]. Tyrosine absorbance at 205 nm is a fundamental property of the proteins but we could not get any absorbance at this level. It might be due to the aging factor of the human subjects in our study. The diet has also play an important key role in the metabolism of the body. If we take diet in rich proteins and other compounds then there is a possibility to have such types of absorbance due to tyrosine. It might be the change due to some other factors of the body. Our main aim was to throw light on the molecular level of immunoglobulin G molecule of proteins. We have also succeeded in giving our views related to ultraviolet spectroscopy. Now days ultraviolet spectroscopic studies are not

in bulk but few studies are also available at the present period of research. One research group of this ultraviolet activity is engaged in determining all the biological fluids such as plasma, hemoglobin, CSF, urine etc. to give a definite contribution to the total absorption of proteins. It can be taken into consideration for the study of conformation of structure of proteins [47].

Franz [45] studied the ultra violet spectroscopic behavior of biological molecules and supplied suitable information for the general character of proteins such as detection of conformational changes and ligand binding etc.

On the basis of presence finding we can safely said that the tryptophan content is a good parameter for the conformation of structure of IgG.

6. CONCLUSION

Proteins and nucleic acids are supposed to be linear polymers [48]. It has been seen that in the polymers a limited set of residues are bound together with the help of amide or phosphodiester bonds. We have a situation like carbohydrates bound through the linking options.

The UV absorbance spectrum for a biomolecule is sum of the spectra of component parts. The UV absorbance for nucleic acids is found from 200 nm to 300 nm. This spectrum is due to transitions of the purine and pyrimidine bases.

The backbone starts to contribute at about 190 nm. The region, which is accessible and required below 200 nm. It is found to be dominated by

$A \rightarrow A^*$ transition of the bases. Oxygen absorption interferes with the spectrum. The UV spectra of the bases look like simple bands. Each band is a composite of more than one transition.

The base transitions are disturbed significantly by $\pi \rightarrow \pi$ stacking interactions. The wavelength maxima and transition intensities vary depending on the base sequence and structure.

In the case of peptides and proteins the spectroscopy of amide bands, the side chains and any prosthetic group determines the observed UV-visible absorption spectrum. The intensities and wavelengths for nucleic acids can be disturbed by the local environment of the groups. UV spectra for proteins can be divided into two regions like near and far UV region. The near UV zone is started from 250 nm to 300 nm and is also described as the aromatic region. Transition of disulphide bonds also contribute to the total absorption intensity in the said region. The far UV above 250 nm is directly dominated by the transition of the peptide backbone of the protein, but transitions from some side chains may also contribute to the spectrum below 250 nm.

The aromatic side chain, phenylalanine, tyrosine and tryptophan all have transition in the near UV region.

Tryptophans have most intense transitions. Many proteins have few tryptophans compared with the other aromatic groups. These transitions are not dominated in the near ultraviolet regions.

The peptide chromophore gives transitions in the region from 180 nm to 240 nm. It has non bonding electrons on the oxygen and also on the nitrogen atoms. This chromophore has π -electrons which are delocalized to some extent over the carbon, oxygen and nitrogen atoms. This peptide chromophore has the transitions of σ bonding electrons. The lowest energy transition of the peptide chromophore is a $\pi \rightarrow \pi^*$ transition. This transition is analogous to ketones.

The $\pi \rightarrow \pi^*$ transition is dominated by the carbonyl π -bond. This bond is also affected by the involvement of the nitrogen in the π -orbitals. The electric dipole transition moment is also polarized some where near the line between the oxygen and nitrogen atoms. This transition has a centre at 190 nm. It has been seen that in an α -helix, the coupling of the $\pi \rightarrow \pi^*$ transition moments in each amide chromophore results a component at about 208 nm, which contribute to the characteristic α -helix.

Protein absorbances can be seen as dominated by tryptophan residues and a clear out absorbance found at 280 nm. The other aromatic residues also absorb at 280 nm. This absorbance may be used to give an estimate of protein concentrations. It has been experimentally verified and checked by the researchers that 1 mg cm^{-3} protein solution has an absorbance of 1 absorbance unit in a 1 cm path length cell. This is due to the fact that many proteins have a similar percentage of aromatic amino acid residues.

The Beer-Lambert law for the determination of concentration of nucleic acids and proteins is based on the fact that the samples are hundred percent pure. If nucleic acids are found, the interference of these with the protein concentration determination may appear. They also absorb at 280 nm.

Phenylalanine residues contribute fine structure such as wiggles to the spectrum from 250 nm to 260 nm. The aromatic amino acids do not absorb above 310 nm. Neither proteins nor nucleic acids ultraviolet absorbance at 320 nm. It has been well documented that protein absorbance should be zero at wavelengths greater than 310 nm. Proteins without tryptophan residues do not absorb above 300 nm.

The studies of peptides are suggestive consideration of protein structure. Electrostatic effects are very small. The peptide absorption coefficients for a protein should depend on the amino acid composition. It should be greater than triglycine.

We conclude this method is reliable and efficient to detect the changes at the molecular level. Specific changes could be seen in the structure of protein molecule with the help of detailed theory of ultraviolet spectroscopy. This spectroscopy confirms that the absorbance by proteins above 300 nm is not possible and no protein absorb at this wavelength. We have found the absorbance peaks above this wavelength. Other spectroscopic techniques may help in the study of this absorbance. The ultraviolet spectroscopy is reliable and usable technique and more predictive related to protein determination.

Our study shows a clear cut indication regarding protein absorbance that the proteins are damaged in the region of third band completely. We have to study more to get the cause of the non-availability of absorbance of proteins by other suitable method of spectroscopy.

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