Chapter One

Introduction

1.1 Over view

Glucose concentrations are usually measured in whole blood or plasma. Plasma values are influenced by the concentration of proteins, especially those with large volumes, such as lipoproteins. Blood values additionally depend on the total volume of the various blood cells, which is usually expressed as the hematocrit (Marks, 1996)

Diabetes is a chronic disease characterized by high or low blood glucose levels, which results from the pancreas not working properly and not producing enough insulin or when the body cells do not respond to it in the correct way. Types of diabetes are:

1. Type one diabetes the body does not produce insulin.
2. Type two diabetes the body not producing enough insulin or the cells not using insulin properly

The blood glucose is too high or too low both will cause significant impact on health and the complication of diabetes are very serious, such as liver cirrhosis and neuropathy. (Garcia, 2009).

Also chronic inflammation. Inflammatory markers, such as high white cell count, high fibrinogen, or low albumin (Schmidt,1999). In type two diabetes, fibrinogen production is increased both in the post absorptive state and in response to hyperinsulinemia. In type two diabetic patients, post absorptive albumin synthesis and its response to insulin were normal, where as fibrinogen synthesis was increased, irrespective of metabolic control (Tessari, 2006). Fibrinogen, serum Total protein, of long term type two diabetics was significantly elevated.

Fluorescence spectroscopy technique involves the illumination of tissue with light and the collection of the light returned from the tissue, then

generates a spectrum with intensities over a specified wavelength range (Shahzad, et al., 2010).

LIF is a promising new adjunctive technique for in vivo tissue diagnosis. Due to its many advantages such as safe, non-invasive, high sensitivity, short testing time, low sample consumption and in situ testing, which have been reported in an enormous of LIF application papers, LIF becomes an adequate analytical technique for tumorous diagnosis (Yeh, et al., 2010).

1.2 Research Problem

There is an urgent need to develop technology for continuous in vivo glucose monitoring in plasma protein. This work mainly focuses on the applied of laser induce fluorescence (LIF) on human plasma with different glucose concentrations and its effect on albumin and globulin protein.

1.3 Previous Studies

Minoo Shahani (et,al) ., in 2013 there reported glucose and fluoxetine induce fine structural change in human serum albumin that 175 mg/dL glucose concentration is a threshold of kidney activity for the beginning of excretion of glucose. pH denaturation of Human Serum Albumin(HAS) in absence and presence of different concentrations of glucose is studied and based on the Pace two-state model, the findings are analyzed. In addition, florescence emission data of albumin range in the period of 300-500 nm was depicted. The amounts of free energy change and [D]1/2 parameters of unfolding in correspond to florescence date indicate that glucose induces fine structural change in human serum albumin. Results showed that 175 mg/dL glucose concentration is a

critical point for albumin structural and functional alteration (Minoo.et,al, 2013).

Venkataramana et al. , in 2013 there reported changes of plasma total proteins, albumin and fibrinogen in type two diabetes mellitus- a pilot study. That in type two diabetics' plasma albumin levels were decreased compared to controls and plasma fibrinogen, total protein levels were statistically significantly increased compared to controls (Venkataramana, et al., 2013).

JieYang et al. in 2014 Interaction of hyperoside with human serum albumin and effect of glucose on the binding. The conformation and microenvironment of HSA were changed after hyperoside(Hyp) binding to human serum albumin (HAS). By decreasing and of Hyp to HSA, glucose can elevate blood drug level of Hyp, from which we can deduce that Hyp may have stronger biological activity in diabetics than that in normal people. The binding constants decreased with sugar concentration increasing, which indicate concentration of free Hyp rising that is advantageous to prevent of diabetic cataract (Yang et al., 2014).

Hathama et al. in 2015 there reported influence of diabetes disease on concentration of total protein, albumin and globulins in saliva and serum: A comparative study.

Total protein measurement results revealed presence of highly significant decrease in serum and highly significant increase in saliva samples of patients with both types of the disease. Albumin concentration was found to decrease significantly in sera and saliva samples of patients with both types of diabetic and highly significant increase was observed in globulins concentration in sera and saliva samples of the patients groups in comparison to the control groups (Hathama et al., 2015).

MichelleT.Barati et,al in 2016 there reported influence of acute high glucose on protein abundance changes in murine glomerular mesangial cells that protein expression patterns in immortalized rat mesangial cells altered by 2h high glucose (HG) growth conditions as compared to isoosmotic/normal glucose control (NG∗) conditions. Unique protein expression changes at 2h HG treatment were measured for 51 protein spots. These proteins could be broadly grouped into two categories :(1) proteins involved incell survival/cellsignaling and(2) proteins involved in stress response. Immunoblot experiments for a protein belonging to both categories, prohibitin (PHB), supported a trend for increased total expression as well as significant increases in an acidic PHB isoform. .Additional studies confirmed the regulation of proteasomal subunit alpha-type two and the endoplasmic reticulum chaperone and oxidoreductasePDI (protein disulfide isomerase), suggesting altered ER protein folding capacity and proteasomal function in response to acute HG.We conclude that short term high glucose induces subtle changes in protein abundances suggesting posttranslational modifications and regulation of pathways involved in proteostasis (MichelleT etal.,2016).

1.4 Objectives of Dissertation

This work aims to:

- Study the effect of increasing glucose concentration on blood plasma proteins by using laser induced fluorescence (LIF).
- Carry out the emission of the blood plasma proteins samples.
- Analyze the emission spectra to the two proteins albumin and globulin.

- Determine the effect of increasing glucose concentration on albumin and globulin.

1.5 Research Methodology

To achieve the objectives of this dissertation twelve blood plasma samples will be brought from healthy and diabetic patient.

Plasma will be separated using centrifuge then plasma will be excited by nitrogen laser to carry out the fluorescence spectra of the samples; these spectra will be analyzed using origin program then a comparisons will be done.

1.6 Dissertation Layout

This dissertation is consist of four chapters, chapter one Introduction and Previous Studies, chapter two consist of Theoretical Background (Spectroscopy; Blood; plasma blood; Glucose), chapter three consist of Experimental Part (The materials; devices and method), chapter four consist of Results, Discussion results, Conclusions, Recommendations and finally References.

Chapter Two

Theoretical Background

2.1 Spectroscopy

Most of knowledge about the structure of atoms and molecules is based on spectroscopic investigation. Thus spectroscopy has attracted much attention to researchers in almost all fields.

Spectroscopy is the study of experimentally obtained spectra. In general, these spectra may be of two general types, absorption and emission spectra.

In the earliest stages of laser development, appreciation of the potential for collecting spectra with unprecedented resolution was significantly tempered by many real concerns about the practical difficulties. The general consensus was that the associated optical technology was fraught with operational difficulties and required exceptionally painstaking experimentation (Andrews, 2012). Types of spectroscopy (Hollas, 2002) are:

2.1.1 Rotational Spectroscopy

The transitions between diatomic and linear polyatomic molecules levels which are observed in rotational spectroscopy. Of prime importance are the selection rules. These determine which transitions are allowed and which are forbidden. The most important contribution to the relative intensity of a transition is from the relative population of the initial state.

In highly symmetrical molecules, such as homonuclear diatomics, nuclei

With non-zero nuclear spin also have an important effect on transition intensities.

2.1.2 Vibration spectroscopy

Vibrational spectroscopy, whether infrared or Raman, can be divided into two general types. When vibrational spectra of diatomic and small polyatomic molecules are obtained at high resolution in the gas phase, rotational fine structure may be resolved and interpreted to give important structural information, namely bond lengths and bond angles.
On the other hand, it is much more difficult to resolve the rotational fine structure for large molecules, and to obtain detailed structural information. In addition, it may be impracticable to get the molecule into the gas phase with sufficiently high vapour pressure for a spectrum to be obtained. For these molecules, vibrational spectroscopy is commonly used as an analytical tool. For example, it may be used to identify a molecule, or to confirm that a particular group has been substituted as a result of a chemical reaction. Then, it is much more convenient for the sample to be in the form of a pure liquid, a solution or, for an infrared spectrum, a compressed powdered solid in a spectroscopically transparent material.

2.1.3 Electronic Spectroscopy

Electronic spectroscopy is employed as an analytical tool, particularly for large molecules. As in analytical vibrational spectroscopy, such electronic spectra are usually obtained in the liquid phase. However, the unique character of a vibrational spectrum, allowing the identification of a molecule, or a group within a molecule, is not so apparent in an electronic spectrum. On the other hand, the application of the Beer-Lambert law, to an electronic spectrum allows the measurement of concentration of a molecule whose molar absorption coefficient is known.

2.1.4 Laser Spectroscopy

The word laser is an acronym for Light Amplification by Stimulated Emission of Radiation. The laser makes use of processes that increase or amplify light signals after those signals have been generated by other means. These processes include (1) stimulated emission, a natural effect that was deduced by consideration relating to thermodynamic equilibrium, and (2) optical feedback (present in most lasers) that is usually provided by mirror (William, 2004).

The properties of the laser beam (Svelto, 2010) are:

1. Monochromaticity.

2. Coherence (spatial and temporal).

3. Directionality.

4. Brightness.

5. Short Pulse Duration.

In laser spectroscopy, chemists train a laser beam on a sample, yielding a characteristic light source that can be analyzed by a spectrometer. But laser spectroscopy falls into several different schools, depending on what kind of laser chemists favor and which aspect of an atom's excited response they study.

2.1.4.1 Laser Emission Spectroscopy

Let us suppose that the atom is found initially in level 2 and that an electromagnetic wave of frequency $\nu = \nu_0$ (i.e. equal to that of the spontaneously emitted wave) is incident on the material .Figure (2.1). Since this wave has the same frequency as the atomic frequency, there is a finite probability that this wave will force the atom to undergo the

transition 2 to 1. In this case the energy difference E_2 - E_1 is delivered in the form of an electromagnetic wave that adds to the incident one. This is the phenomenon of stimulated emission. There is a fundamental difference between the spontaneous and stimulated emission processes. In the case of spontaneous, the atom emits an electromagnetic wave that has no definite phase relation with that emitted by another atom. Furthermore, the wave can be emitted in any direction .In the case of stimulated emission, since the process is forced by the incident electromagnetic wave, the emission of any atom adds in phase to that of the incoming wave and long the same direction (Svelto, 2010).

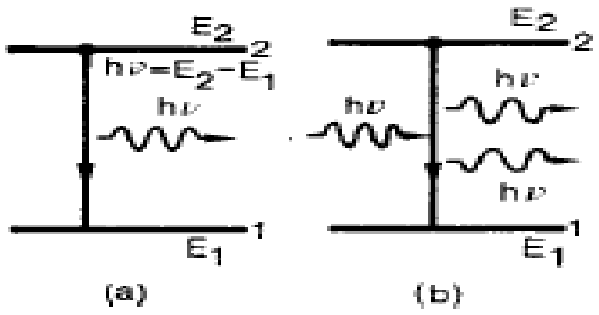

Figure 2.1: (a) Spontaneous emission; (b) Stimulated emission

2.1.4.2 Laser Induced Fluorescence (LIF)

Fluorescence as for atoms, energy diagrams of molecules consist of electronic energy levels. However, molecules can also rotate and vibrate, introducing a splitting of the electronic energy levels into many sub-levels. If the energy of an incoming photon matches the difference between two energy levels in the molecule, the photon can be absorbed. From here different processes can take place as illustrated in Figure (2.2). All these processes result in the molecule releasing its excess energy gained when absorbing the photon.

The molecule is most probable to be located in the ground state, S_0at energy equilibrium. After absorption the molecule is excited to

another singlet state, S_n. From here the molecule will directly relax into the lowest vibrational state within this excited electronic state. This non-radiating process is called vibration relaxation (VR). The release of the excess energy is converted into heat.

From the excited state, S_n, the molecule will move to a lower electronic state, S_{n-1}, through a process called internal conversion (IC). The molecule will once again relax to the lowest vibrational level within this state through VR. After several IC and VR processes the molecule will end up in the stateS_1. From here the remaining excess energy of the molecule can be released as a photon when the molecule returns to any vibrational level in the ground state. This process is called fluorescence.

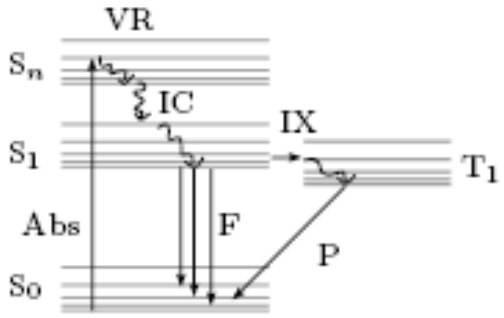

Figure 2.2: Jablonski Diagram

Figure (2.2) shows the energy level diagram where the different processes that can occur after excitation of the molecule are illustrated. S and T denote a single and a triplet state in the molecule, respectively. Abs–absorption, VR–vibrational relaxation, IC–internal conversion, F–fluorescence, IX–intersystem crossing, P–phosphorescence.

The fluorescence photon will have a longer wavelength than the incoming photon, as the molecule looses some energy in the non-radiative relaxation processes. The generated fluorescence contains light of a broad wavelength range and not a sharp peak since the relaxation process can be carried out to any vibrational level in the ground state. A transition of the molecule into a triplet state from a singlet state is also

possible, which is called inter-system crossing (IX). The molecule will then relax to the lowest triplet state, T1, through VR and IC processes. From here the molecule can return to its ground state by emitting a photon. This phenomenon's called phosphorescence. During phosphorescence it takes longer time for the molecule to return to the ground state as compared to the fluorescence process. This is due to the fact that the lifetime of the triplet state is long, because the transition from a triplet to a singlet state is forbidden as a pure dipole-transition. There is yet another relaxation path where the absorbed energy is transferred to and exciting a neighboring molecule. (Svensson, 2007).

Laser induced fluorescence (LIF) spectroscopy is method developed for tissue diagnostics. An important application is to find tumors and delineate its borders. The method is non-invasive and can be performed in real-time. The method makes it possible to investigate inner hollow organs by using optical fibers compatible with endoscopes. The tissue is irradiated with a laser of specific wave length that induces fluorescence from the present fluorophores (Svensson, 2007). For example basal cell carcinomas (BCCS), are well suited for fluorescence detection systems (Klinteberg, 1999). Sometimes it can be difficult to visualize the borders of tumors with the naked eye, and in those cases fluorescence imagine can provide additional information (Fischer, 2001). Also fluorescence imaging is used is in tumor detection in lungs and larynx, often referred to as light-induced fluorescence endoscopy (LIFE) (Kusunoki, 2000). To visualize these kinds of tissues, the imaging modality needs to be compatible with endoscopes used to reach the organ (Svensson, 2007).

LIF has another large application in spectroscopy (Demtroder, 2013):

1. LIF serves as a sensitive monitor for the absorption of laser photons in fluorescence excitation spectroscopy.

2. Its well suited to gain information on molecular states if the fluorescence spectrum excited by a laser on a selected absorption transition is dispersed by monochromator. The fluorescence spectrum emitted from a selectively populated rovibronic level ($v_k^{'}$,$J_k^{'}$) consists of all allowed transitions to lower level ($v_m^{''}$,$J_m^{''}$) . Figure (2.3).The wave number differences of the fluorescence lines immediately yield the term differences of these terminating levels ($v_m^{''}$,$J_m^{''}$).

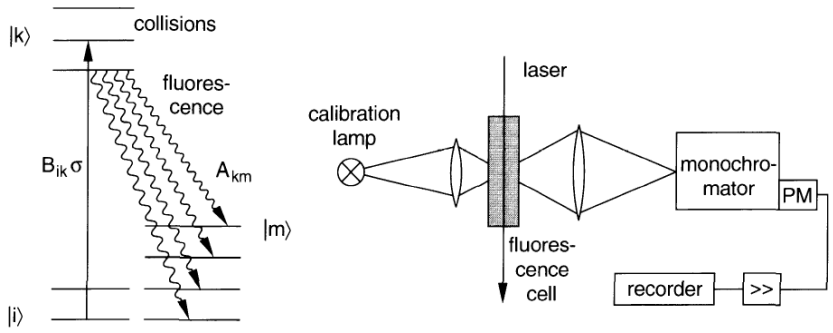

Figure 2.3: Laser-induce fluorescence (a) level scheme and (b) experimental arrangement for measuring LIF spectra.

3. Spectroscopic study of collisions process. If the excited molecule is transferred by inelastic collisions from the level (v_k' , J_k') into other rovibronic levels, the fluorescence spectrum emitted from these collisionally populated levels gives quantitative information on the collision cross section.

2.1.4.3 Laser Absorption Spectroscopy

During absorption, the intensity of an incident electromagnetic wave is attenuate in passing through a medium. The absorbance of a

medium is defined as the ratio of absorbed and induce intensities. Absorption is due to partial conversion of light energy into heat motion or certain vibrations of molecules of absorbing material.

Assume that atom is initially lying in level 1 Figure (2.4). If this is the ground level, the atom will remain in this level unless some external stimulus is applied to it. We shall assume, then, that an electromagnetic wave of frequency $\nu = \nu_0$ is incident on the material. In this case there is a finite probability that the atom will be raised to level 2. The energy difference E_2 - E_1 required by the atom to undergo the transition is obtained from the energy of the incident electromagnetic wave .This is the absorption process (svelto, 2010).

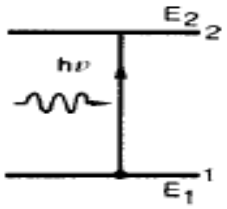

Figure 2.4: Absorption

2.2 Blood

Blood is a fluid tissue, which circulates through every organ of human body and participates in all functional activities of the body.

Blood is pumped around the body by the heart. The major vessels that take blood away from the heart called arteries. The major vessels that take blood back to the heart are called veins. Between the two net works are many tiny blood vessels called capillaries. The composition of the three types of vessel varies slightly.

It contains plasma and several types of cells termed as formed elements. Blood has about 55% of plasma which contains a large number

of proteins, glucose used in cellular metabolism, amino acids, vitamins, lipids, hormones etc; And many more in organic substances (Donovan, 1969).

The types of formed element figure (2.5) (Frederic, 2005) are:

1. Red blood cells (RBCs) or erythrocytes: transport oxygen.
2. White blood cells (WBCs) or leukocytes: part of the immune system.
3. Platelets: cell fragments involved in clotting.

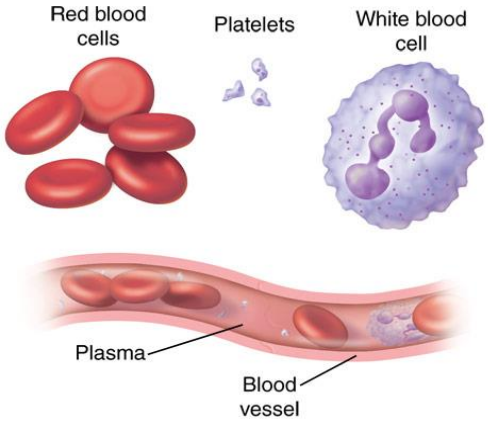

Figure 2.5: Blood compound

2.2.1 Blood plasma

Is a yellowish colored liquid component of blood that normally holds the blood cells in whole blood in suspension; this makes plasma the extracellular matrix of blood cells. It makes up about 55% of the body's total blood volume (Dennis , 1999) figure (2.6) .

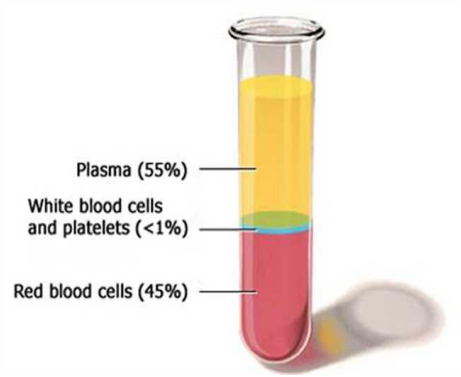

Figure 2.6: Plasma compound

It is the intravascular fluid part of extracellular fluid (all body fluid outside cells). It is mostly water (up to 95% by volume), and contains dissolved proteins (6–8%) (I.e. serum albumins, globulins, and fibrinogen).

2.2.1.1 Albumin

In control group of subject mean value of albumin was found to be 4.24 gm% which significantly decrease in pretreated (zero days) T.B. patients to 3.58 + 0.572. Thus mean decrease in albumin in T.B. patients with respect to control in 16%. The values slightly increases on treatment with T.B. drugs and it reaches to 3.82 + 0.39 on 15th day post treatment, the magnitude of increase being 10% as compared to control.

Afterward the post treatment values significantly increases from 1 month, 2 months, 3 months and 4 months upto 6 months as 4.55 + 0.75, 4.52 + 0.58, 4.32 + 0.72, 4.52 + 0.85 and 4.35 + 0.48 respectively. The percent increase being 7.5%, 6.3% and 2.3% respectively with respect to control. On 6th month the value was only 2.3% high with respect to control. (HKHAN,2012). Figure (2.7) shows albumins structure.

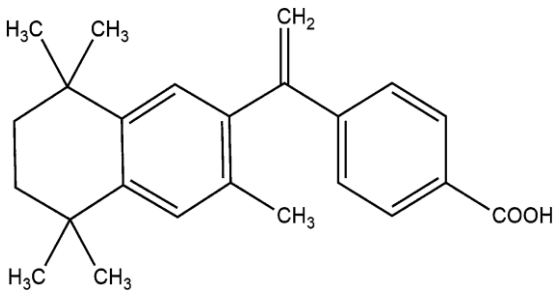

Figure 2.7: Albumins structure

2.2.1.2 Globulins

By electrophoresis plasma globulins are separated into α1, α2,β and γ-globulins are synthesized in liver, whereas ¥-globulins are formed in the cells of reticulo-endothelial system. The average normal serum globulin (total) concentration is 2.5 gm / 100 ml (Howe method) or 3.53 gm/100 ml by electrophoresis. Figure (2.8) shows globulins structure.

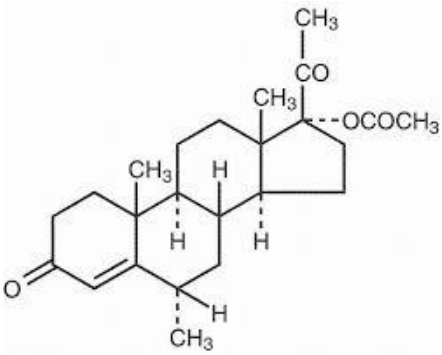

Figure 2.8: Globulins structure

I. α-1 Globulin

The mean value of α-1 protein in control group was found to be 0.38 gm/dl which decreases to 0.24 + 0.078 in pulmonary tuberculosis patients (Pretreated group). After post treatment, values decreases from 15 days, 1 month. 2 months and 3 months as 0.22 + 0.048, 0.14 + 0.053, 0.21 + 0.069 and 0.15 + 0.07 corresponding to 47.8%, 44.8%, 45.2% and 61.4% respectivelyAfter post treatment values further increases on 4 month and 6 month as 0.61 + 1.10 and 0.62 + 0.29 respectively. The value of 6 month post treatment period in significantly higher as compared to control group.

II. α-2 Globulin

The healthy subjects of control group showed mean value of α-2 globulin as 0.62 gm/dl which slightly increases in pretreated pulmonary T.B. group. The value being 0.64 + 0.65.

15 days after treatment of anti T.B. drugs, the value further increases to 0.78 + 0.33 which is higher as compared to control group of patients. After treating for 1 month and 2 month, the value in patients become 0.75 + 0.16 and 0.70 + 0.21 respectively. The lowest value was observed on 3 month of 0.57 + 0.28 which significantly increases on 4 and 6 month as 0.67 + 0.32 and 0.87 + 0.13 respectively.

III. β-1 and β-2 Globulins

Similar pattern has been observed for β-1 and β -2 globulins. In control group of healthy subjects, the mean value for β-1 globulin was 0.28 g/dl and for β-2 0.68 g/dl. In pulmonary T.B. patients before treatment, β-1 value increase to 0.60 + 0.55 whereas ß-1 was slightly decreases in pulmonary T.B., the value being 0.40 + 0.41.

After post treatment of 15 days, 1 month and 2 month ß-1 value decreases to 0.11 + 0.30, 0.14 + 0.29 and 0.13 + 0.22 as compared to control group, while ß-2 globulin decreases slightly after 15 days upto six month as compared to control group.

Increase in β-1 value has been observed in 3 month post treatment, the magnitude being 0.78 + 1.77, while on4 month and 6 month post treated period it decreases and come towards normal.

IV. β - Globulins

In control group of healthy subjects mean value was 0.76 g/dl which was greater as compared to pretreated T.B. patients, value being 0.24 + 0.43 g/dl.

From 15 days to 1 month, 2 months, 3 months and 4 months post treatment period a significant gradual decrease in ß-globulin was observed. On sixth month post treatment period ß-globulin value was high as compared to control group.

V. γ-Globulin

In control group of healthy subjects mean value of γglobulin was found to be 1.06 mg/dl which significantly increases in pretreated T.B. patient to 2.74 + 0.43 which was 74% higher than control. The value decreases on treatment with T.B. drugs and it reaches to 2.34 + 1.11 on 15 days. The

magnitude of increase being 20% higher as compared to control. After one month treatment of T.B. drugs till six months significantly gradual decrease in γ-globulin observed which reaches to normal level on six month post treatment(HKHAN,2012).

2.2.1.3 Fibrinogen

It is a fibrous protein with a molecular weight of 340,000. It has 6 polypeptide chains which are held together by disulphide linkages. Fibrinogen plays an important role in clothing of blood where it is converted to fibrin by thrombin.

In addition to the above mentioned proteins, the plasma contains a number of enzymes such as acid phosphatase and alkaline phosphatase which have great diagnostic value. Figure (2.9) shows fibrinogen structure.

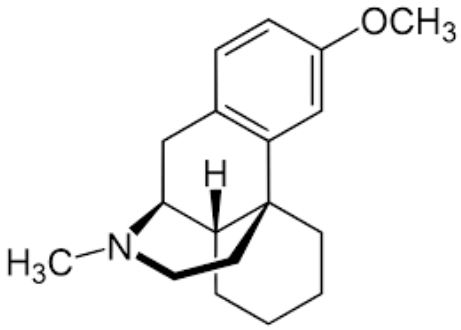

Figure 2.9: Fibrinogen structure

2.2.2 Glucose

Glucose is an obligate metabolic fuel for some tissue (e.g. erythrocytes) and preferred fuel (particularly in the short term) for many others (e.g. central nervous system). In the body there are three sources: dietary carbohydrate; gluconeogenesis (e.g. from lactate) and hepatic gluconeogenesis. Glycogen is glucose polymer and the principle storage

form. Glycogen is also stored in skeletal muscle but the glucose derived from it is not released into the circulation.

Glucose is a simple sugar with a molecular formula $C_6 H_{12} O_6$,which means that is a molecule that is made of six carbon atoms, twelve hydrogen atoms, and six oxygen atoms. Figure (2.10)

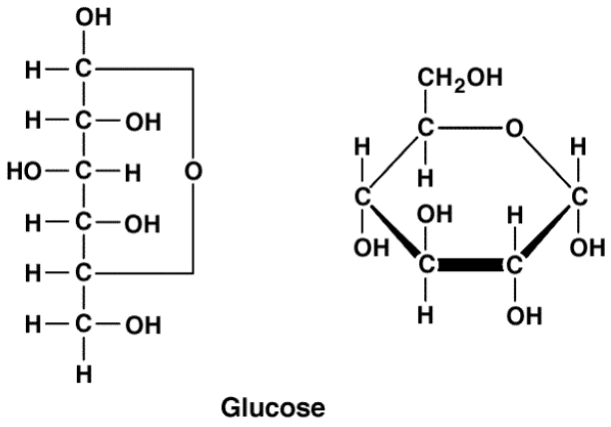

Figure 2.10: Glucose complex

2.2.3 Blood Glucose

Glucose is the major carbohydrate found in blood and a chief source of energy in human body. The blood glucose levels are perfectly maintained under the influence of hormones like insulin and glucagon. However, the hormonal imbalance sometimes may result in abnormalities of glucose metabolism and result in diseased condition (Tomita, 2002).

This blood glucose regulation process can be explained in the following steps:

1. After the glucose has been absorbed from the food eaten, it gets released in the bloodstream. High blood glucose levels triggers the pancreas to produce insulin. Insulin enables the muscle cells to take glucose as their source of energy and to form a type of molecule called

glycogen that works as secondary energy storage in the case of low levels of glucose. In the liver cells, insulin instigates the conversion of glucose into glycogen and fat. In the fat cells of the adipose tissue, insulin also promotes the conversion of glucose into more fat and the uptake of glucose.

2. The pancreas will continue to release insulin and liver and fat cells continue to use glucose till the drop of concentration of glucose is below a threshold; in that case, glucagon will be released instead of insulin.

3. When glucagon reaches the liver cells, it initiates the conversion of glycogen into glucose, and fat into fatty acids, which many body cells can use as energy after the glucagon, enables them to. The cells will continue to burn fat from the adipose tissue as an energy source, and follow with the protein of the muscles, until the levels of glucose increase again by the digestion of food, and that terminates the cycle (Yanez, 2013).

Most people with diabetes should try to keep their blood glucose levels as close as possible to the level of someone who doesn't have diabetes .This normal target range are about 70-130.

If your blood glucose levels stay above 180 for 1to2 hours they may too high. High blood glucose, also called hyperglycemia**,** means you don’t have enough insulin in your body (type two diabetes).

If your blood glucose levels drop below 70, you have low blood glucose, also called hypoglycemia.

Taking control of your diabetes can help you feel better and stay healthy. Research shows that keeping your blood glucose (blood sugar) close to normal reduces your chance of having eye, kidney, and nerve problems. To control your diabetes, you need to know your blood glucose number and your target goal (NIDDK, 2013).

Chapter Three

Experimental Part

3.1 Materials

The samples were collected by specialized technicians and were ordered based on their concentrations with a known glucose concentration in their blood. See figure (3.1).

Figure 3.1: Blood Plasma samples

Table 3.1 Samples with their concentrations

Samples	concentration(Mg /Dsl)
Sample 1	92
Sample 2	103
Sample 3	110
Sample 4	127
Sample 5	150
Sample 6	211
Sample 7	225
Sample 8	250
Sample 9	264

Samples	concentration(Mg /Dsl)
Sample 10	336
Sample 11	537
Sample 12	545

3.2 Devices

3.2.1 Centerifuge

Hettich Centerfige Machine EBA 20S.

Figure 3.2: Centerifuge

3.2.2 Nitrogen (N_2) Laser

Nitrogen laser is an example of molecular laser, using transitions between vibrionic state. This laser oscillates at wavelength of 337 nm, in ultraviolet region, and belong to a categroy of 'self – terminating' laser. Figure (3.3) .

Figure 3.3: The Nitrogen laser(N_2) at Alneelain university

3.3 Method

Twelfth blood samples (2-3 ml) were used in this work. Four of them were collected from healthy persons while the other eight samples were collected from diabetic persons. The plasma was separated from the blood by the centrifuge; about 2ml of plasma was taken for photoluminescence. Nitrogen (N_2) laser with wavelength of 337.3 nm, power 0.04 mW, and periodic time of 100 msec. When N_2 laser excited the samples; it absorbed the laser then it emitt the flourecese. The flourecense was colected using the flourecense spectrometer. Figure (3.4). The result depited in chapter four.

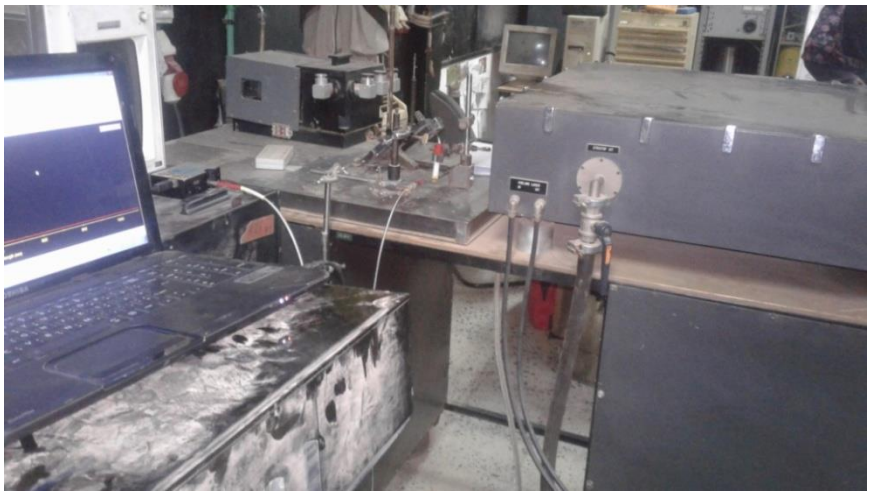

Figure 3.4: Experiment setup

Chapter Four

Results and Discussion

4.1 Results

4.1.1 The emission spectra of the plasma samples:

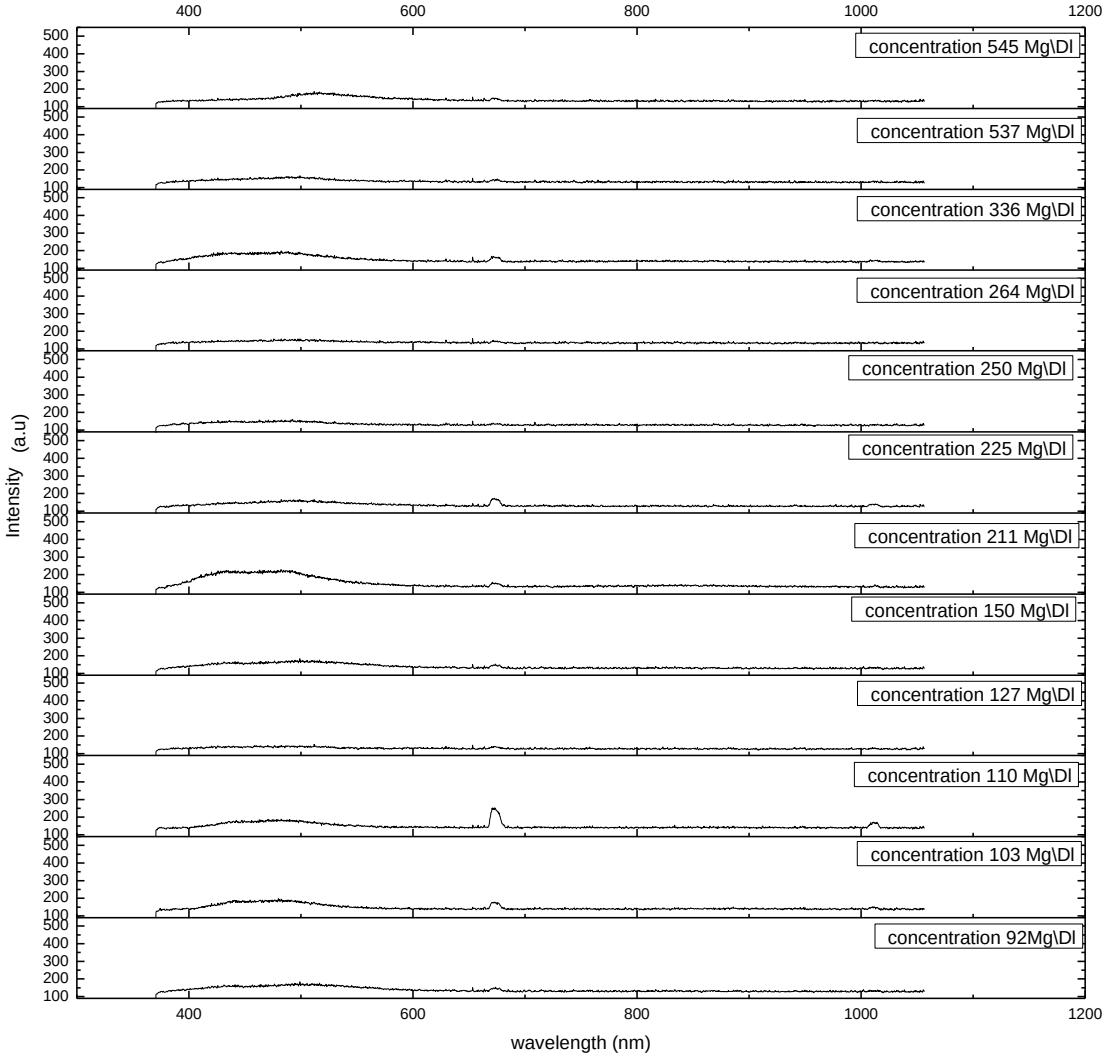

Figure 4.1: Emission spectra of the twelve samples.

Table 4.1 Glucose concentration and intensity of albumin and globulin.

Glucose concentration (Mg /Dl)	Albumens intensity (a.u)	Globulins intensity (a.u)
92	4886.5	4878.8
103	4412.5	4397.2
110	3957.6	3946.1
127	3529.5	3525.6
150	3047.8	3043.9
211	2535.5	2535.5
225	2145.6	2143.1
250	1638.1	1679.2
264	1232.02	1224.3
336	737.06	727.4
537	314.57	303.1
545	144.15	159.4

4.2 Analysis and discussions

The relations between the concentration of glucose and parameters in table 4.1 was studied and discussed below:

4.2.1 The relation between glucose concentration and albumin.

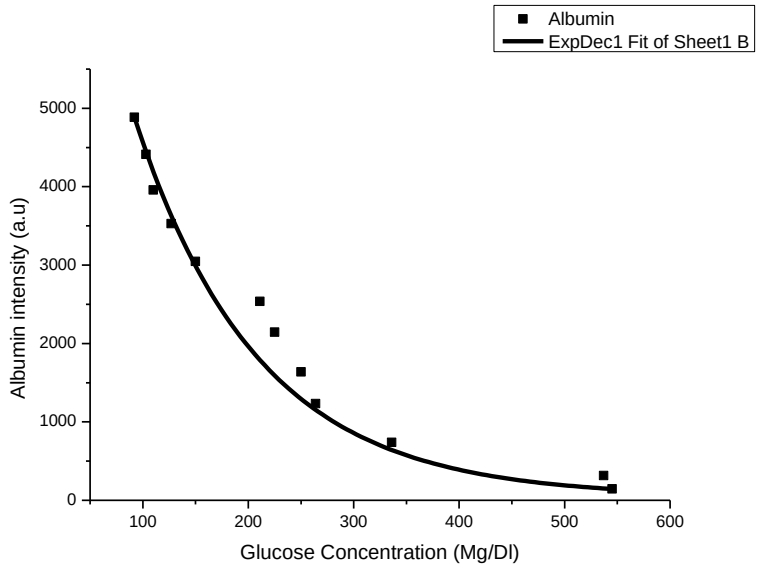

Figure 4.2: Glucose concentration and albumin.

Protein denaturation study is an important method for characterization of protein structure and function. This process is concerned grossly by environmental factors such as temperature, chemical components, etc. (Peters , 1995). Albumin is one of the most important proteins of body due to the variety of functions such as ligand binding capacity, hormones and a wide variety of drugs (Nicholson, J.P, 2000). Glucose concentration is constant in blood in normal condition but it can be changed via hypoglycemia and hyperglycemia (as diabetic c), During insulin deficiency, there is significant decrease in fractional synthetic rate of albumin and concomitant significant increase in fibrinogen. These data indicate a differential effect of insulin deficiency on the fractional synthetic rate of two hepatically synthesized plasma proteins (Peters, 1995). (Laurel et al., 1972) observed significant decrease in albumin in diabetes mellitus may be due to insulin deficiency and significant decrease in the fractional synthetic rate of albumin.

In this study at low glucose concentration the intensity of albumin levels were very high above (3000)(a.u); Probably, the occurrence of negligible alteration in HSA structure and function in the presence of glucose concentration below 211 Mg/DL may indicated to changing biological environment of protein. At high glucose concentration (211-545) Mg/DL (diabetics) the intensity of albumin levels decreased to (144) (a.u) .This decrease may be indicated to decrease of insulin in the body or another effect and in accordance with the above studies.

4.2.2 The relation between glucose concentration and globulins.

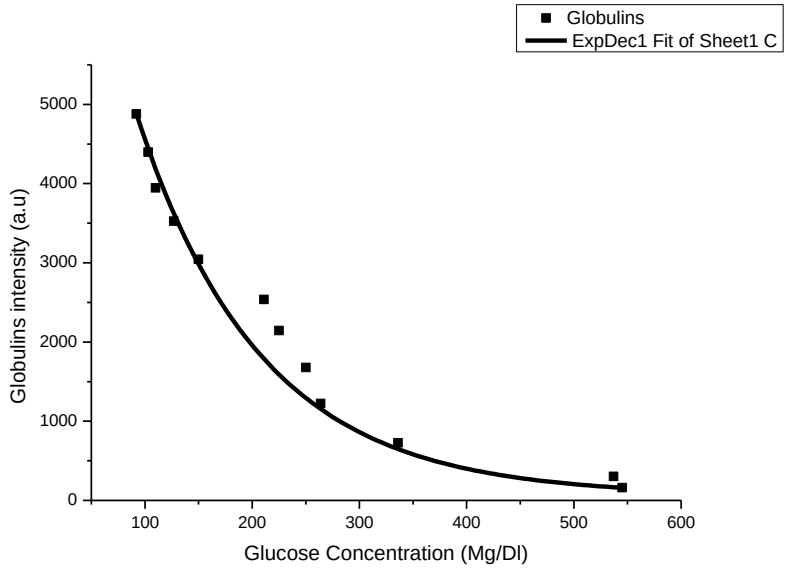

Figure 4.3: Glucose concentration and globulins

Globulins are a group of proteins in your blood. They are made in your liver by your immune system. Globulins play an important role in liver function, blood clotting, and fighting infection. Low globulin levels can be a sign of liver or kidney disease. High levels may indicate infection, inflammatory disease or immune disorders. High globulin levels may also indicate certain types of cancer, such as multiple myeloma, Hodgkin's disease, or malignant lymphoma. However, abnormal results may be due to certain medications, dehydration, or other factors.

In this study at low glucose concentration the intensity of globulins levels were very high above (3000) (a.u) ; Probably, the occurrence of negligible alteration in human serum globulins(HSG) structure and function in the presence of glucose concentration below 211 Mg/DL may indicated to changing biological environment of protein .At high glucose concentration(211-545) Mg/DL (diabetics) the intensity of globulins levels decreased to (144) (a.u) .This decrease may be indicated to decrease of insulin in the body ; liver or kidney disease.

4.3 Conclusions

This study explain the effect of increasing glucose concentration on the human plasma protein albumin and globulin when it excited by nitrogen laser.

The results explained when glucose concentration was increased the intensity of (albumin levels, globulin levels) decreased, and it's agree with some studies; at low glucose concentration the intensity of globulins and albumin levels were very high above (3000) (a.u); and function in the presence of glucose concentration below 211 Mg/DL At high glucose concentration from (211 - 545) Mg/DL (diabetics) the intensity of globulin and albumin levels were decreased to (144) (a.u).

The Low albumin levels may be indicated to low levels insulin or some diseases like (malnutrition; liver disease; et.al); low globulin levels may be indicated to (insulin resistance; liver or kidney disease; et.al E).

One of the weakness of this report is that cannot determine which of several potential underlying mechanisms might underpin the association of globulin with type two diabetic.

4.4 Recommendations

From this research following idea can be done:

Studying the causes of increased or decreased the levels of albumin and globulin in human plasma with increased glucose concentrations also can calculate the ratio of albumin / globulin to determine the critical point of the increasing glucose concentration.

In future studies the following idea can be done: studied the effect of increasing glucose concentration with human serum albumin; globulin and fibrinogen by another devices like Raman spectroscopy; also can influence of diabetes disease on concentration of total protein, albumin and globulins in liquid samples like tears; saliva and serum: A comparative study.

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