Chapter One

Introduction

1.1 Preface

Light may interact with matter in many different ways and its response can yield useful information about properties of the material. A good feature of light in non-invasive probing of the material when it used at low intensities. This important when deals with human tissue.

Determination of blood glucose levels is very important to know the physiological condition of the Human beings as the hormonal imbalance may cause abnormalities in glucose metabolism (M.Srikanth, 2004).

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. Thus, the detection of blood glucose levels can provide a basic understanding of the malfunctioning of the tissues and body (M.Srikanth, 2004). The estimation of glucose levels in blood is being done so far by the colorimetric and titrimetric methods; however these were involved with huge cost and time (M. Srikanth, 2004).

Diabetes has become a common disease in modern society. The blood glucose is too high or too low both will cause a significant impact on health. The complications of diabetes are very serious such as liver cirrhosis and neuropathy. Diabetes is a disease difficult to cure, only through diet or insulin injections to control blood glucose. It is necessary to know their blood glucose real time and accurately for the patients (Garcia-Compean, 2009).

Photo medicine is the study and subsequent treatment of diseases in the body by exposing the body to light. This can include diagnostic and therapeutic

applications such as using light for the detection and curing of disease, or phototherapy.

Simple clinical chemistry be used to measure the concentration of various components in samples such as blood or serum. For many clinical tests, the time it takes to perform an assay is critical. Producing accurate and reliable results is important when making measurements (Garcia-Compean, 2009).

1.2 Literature Review

M.Srikanth, G.Venkateswara Rao and K.R.S.Sambasiva Rao in 2004, studied in the protocol for the modified method of glucose estimation such that the glucose reagent supplied along with the kit aliquoted "300 µl" into 3 different flat bottom micro wells of 96 well plate and the wells were marked as blank, test and standard . An aliquot of 3 µl of distilled water was added to the well marked as blank, later aliquots of 3 µl of sample and 3 µl of glucose standard (supplied with kit) were dispensed into the test and standard wells respectively. The absorbance of the test was measured at 492 nm against a reagent blank and the concentration of glucose was calculated using standard reference. The results obtained through this procedure were compared with the normal colorimetric method using the same kit.

Kanagathara N, M Thirunavukkarasu, Esther Jeyanthi C,P. Shenbagarajan in 2011 used Forier Transform Infra Red (FTIR) and UV-Visible spectral to study the normal blood samples. They collected blood samples of healthy subjects from healthy volunteers of different age groups and each blood sample was allowed to coagulate naturally without adding any anticoagulant agents for about half an hour. The serum was separated from every sample and centrifuged at a speed of 1200 rpm in REMI electric centrifuge. The serum was subjected to both FTIR and UV-Vis spectroscopic techniques. Their results were to spectroscopic technique has become a powerful and analytical tool for the qualitative and quantitative analysis of pharmaceutical and biological materials

Akesh Govada, Ch Renumadhavi and K B Ramesh in 2014, their study was to Measure Non Invasive Blood Glucose in Indian. India is one of the most diabetic populated countries in the world. Today the technologies available in the market are invasive methods. Since invasive methods cause pain, time consuming, expensive and there is a potential risk of infectious diseases like Hepatitis & HIV spreading and continuous monitoring is therefore not possible.They used NIR spectroscopy for determining the blood glucose concentration non-invasively has been demonstrated by many groups and much progress has been made in the past few years. Optical sensors can be used to measure the NIR spectra of the human finger. A NIR spectrometer with a fiber optic accessory can be used for the non-invasive measurement of blood glucose.

Their proposed system has been equipped optical sensor, signal conditioning, ADC, microcontroller and LCD display. NIR signals are passed through the fingertip with and without blocking the blood flow. The received reflected signal is amplified and filtered, using a precision amplifier and a low pass filter. So for this Signal conditioning section is used which consists of a preamplifier and a low pass filter. An ADC buffer is used to perform the ADC conversion of the received signal from signal conditioning section. The microcontroller unit used to convert the values into corresponding blood glucose value, which is then displayed on LCD.

Their aim was to provide an innovative idea to solve the existing problems, which patients are facing with the current glucose meter technique. This can be used for continuous monitoring of glucose in home by the patients which are low cost and high accuracy.

D. X. Guo, Y. Z. Shang, R. Peng, S. S. Yong, X. A. Wang in 2015 used a new non invasive blood glucose monitoring method based on four near infrared spectrums and double artificial neural network analysis. They choose four near infrared wavelengths, 820 nm, 875 nm, 945 nm, 1050 nm, as transmission

spectrums, and capture four fingers transmission PPG signals simultaneously. The wavelet transform algorithm is used to remove baseline drift, smooth signals and extract eight eigenvalues of each PPG signal. The eigenvalues are the input parameters of double artificial neural network analysis model. Double artificial neural network regression combines the classification recognition algorithm with prediction algorithm to improve the accuracy of measurement. Experiments show that the root mean square error of the prediction is between 0.97 mg/dL - 6.69 mg/dL, the average of root mean square error is 3.80 mg/dL. They use the wavelengths of 820 nm, 875 nm, 945 nm and 1050 nm as near-infrared light as transmitted wavelengths, gather four PPG signals of fingertips, and design a set of non-invasive blood glucose estimating system according to Lambert Beer's law. Double artificial neural network algorithms are used to build model with personal PPG signals. The average root mean square error is 3.80 mg/dL.

V. H. GHADAGE1, G. R. KULKARNI, B. N. ZAWARE in 2015 their work was collecting blood samples from normal human subjects under standard laboratory conditions. Blood samples were irradiated by He-Ne laser (Wavelength λ = 632.8 nm, Power P = 3mW). The FTIR spectra for non-irradiated normal blood samples are compared with the FTIR spectra of irradiated blood samples. Significant changes are observed between the various bonds from the FTIR transmission spectra between C=O (Amide I), C-O (Anhydrides), N=O (Nitro), C-N (Amines) and C-H (Alkenes) . The significant results are obtained when He-Ne laser irradiation is incident on whole blood for 30 and 40 minutes. The transmittance decreases due to denaturation of proteins.

1.3 Research Problem:

Blood glucose determination is one of the most common clinical diagnostic tests. Often, blood is collected in a field station and analysis is carried out in a remote laboratory. Because blood cells can continue to metabolize glucose, the time of determination the blood glucose after drawing the blood is important.

This research focuses on studying the diagnostic of blood glucose by UV-Vis spectroscopy.

1.4 Objective of this Thesis:

The goals of this research are to:

1. Apply UV-Vis spectroscopy on Diabetic blood.
2. Apply UV-Vis spectroscopy on healthy blood.
3. Compare the two spectra of the two groups of samples.

1.5 Thesis Layout:

This thesis is consist of four chapters, chapter one introduction and literature review. Chapter two consists the basic concepts of spectroscopy, applications of UV-Vis spectroscopy and sugar disease. Chapter three consist methodology (materials, device and method). Chapter four consists of results, discussion, conclusion and recommendations.

Chapter Two

Basic Concepts

The aim of this chapter is to present theoretical background of light matter interaction, UV-Visible spectroscopy and blood.

2.1 Light Matter Interaction

When optical radiation interacts with matter, it may be reflected, absorbed, or transmitted (Carey, N. R., 2005).

2.1.1 Absorption

If a light wave of a given frequency strikes a material with electrons having the same vibration frequencies, then those electrons absorb the energy of the light wave and transform it into vibration motion. During its vibration, the electrons interact with neighboring atoms in such a manner as to convert its vibration energy into thermal energy. Subsequently, the light wave with that given frequency is absorbed by the object. It is the transformation of radiant power to another type of energy, usually heat, by interaction with matter. In physics, absorption of electromagnetic radiation is the way in which the energy of a photon is taken up by matter, typically the electrons of an atom (figure 2.1). Thus, the electromagnetic energy is transformed into internal energy of the absorber, for example thermal energy. The reduction in intensity of a light wave propagating through a medium by absorption of a part of its photons is often called attenuation. Usually, the absorption of waves does not depend on their intensity (linear absorption), although in certain conditions (usually, in optics), the medium changes its transparency dependently on the intensity of waves going through, and satiable absorption (or nonlinear absorption) occurs (Carey, N. R., 2005).

The absorbance of an object quantifies how much of the incident light is absorbed by it. This may be related to other properties of the object through the

Beer–Lambert's law. The absorption coefficient determines how far into a material light of a particular wavelength can penetrate before it is absorbed. In a material with a low absorption coefficient, light is only poorly absorbed, and if the material is thin enough, it will appear transparent to that wavelength. The absorption coefficient depends on the material and also on the wavelength of light which is being absorbed. Semiconductor materials have a sharp edge in their absorption coefficient, since light which has energy below the band gap does not have sufficient energy to excite an electron into the conduction band from the valence band (Carey, N. R., 2005).

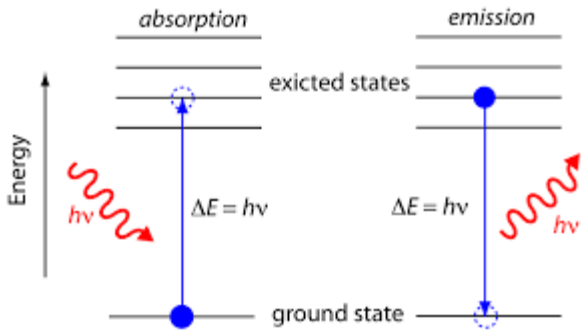

Figure 2.1: Diagram of Specular Absorption (Carey, N. R., 2005).

2.1.2 Reflection

Reflection is the process by which electromagnetic radiation is returned either at the boundary between two media (surface reflection) or at the interior of a medium (volume reflection) .It is the change in direction of a wave front at an interface between two different media so that the wave front returns into the medium from which it originated. Common examples include the reflection of light, sound and water wave (Carey, N. R., 2005).

The law of reflection says that for secular reflection the angle at which the wave is incident on the surface equals the angle at which it is reflected. Mirrors exhibit secular's reflection (figure. (2.2)). Reflection of light is either seculars (mirror-like) or diffuses depending on the nature of the interface. In specula

reflection the phase of the reflected waves depends on the choice of the origin of coordinates.

Diffuse reflection happen when light strikes the surface of a (non-metallic) material it bounces off in all directions due to multiple reflections by the microscopic irregularities inside the material and by its surface, if it is rough. Thus, an 'image' is not formed. This is called diffuse reflection.

The exact form of the reflection depends on the structure of the material. Reflection and transmission of light waves occur because the frequencies of the light waves do not match the natural frequencies of vibration of the objects (Carey, N. R., 2005).

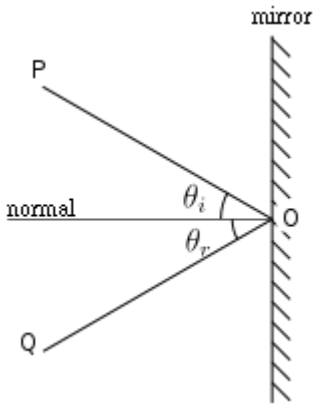

Figure 2.1: Diagram of specular reflection (Carey, N. R., 2005).

2.1.3 Transmission

It is the passage of electromagnetic radiation through a medium .The transmittance of a material is the proportion of the incident (approaching) light that moves all the way through to the other side. For example, let's say you're shining a flashlight on a semi-transparent glass block.

You start off with 100% of your incident light. The first thing that happens is that30% of that light is reflected off the outer surface of the glass. That leaves you with70% to continue through the glass block. Another 50% of the light is absorbed by the molecules inside the glass block itself. That leaves you with

20% that immerges from the opposite side. So you could say that the glass block has a transmittance of 20%.

The transmittance of a material depends on its thickness, but it also depends on the type of 'light' (or electromagnetic waves) you are using. A material might have a different transmittance for visible light than it does for infrared, or x-rays. This is why hospital x-rays go through your skin until they reach the bones, even though visible light does not (Carey, N. R., 2005).

2.1.4 Light Scattering

Light scattering can be thought of as the reflection of a ray from a straight path, for example by irregularities in the propagation medium, particles, or in the interface between two media. Deviations from the law of reflection due to irregularities on a surface are also usually considered to be a form of scattering. Most objects that one sees are visible due to light scattering from their surfaces as in figure 2.3, (Carey, N. R., 2005).

Indeed, scattering of light depends on the wavelength or frequency of the light being scattered. Since visible light has wavelength on the order of a nanometer, objects much smaller than this cannot be seen, even with the aid of a microscope. (Vande Hulst .H.C 1981) (Bohren, 1983).

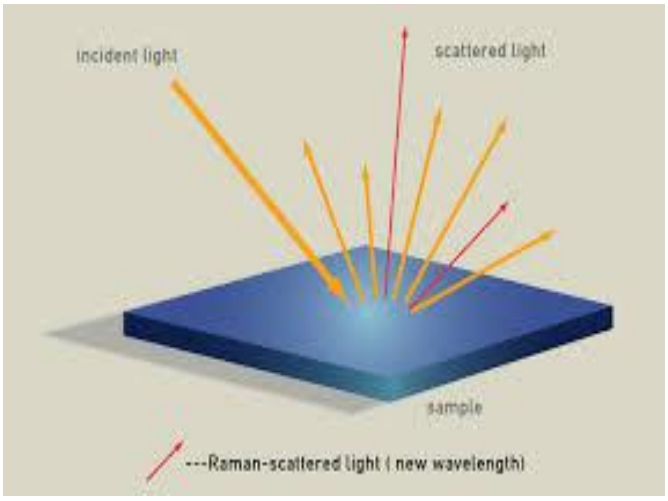

Figure 2.3: Diagram of Specular Scattering

2.2 UV-Visible Spectroscopy

Spectroscopy means study of the interaction between matter and radiated energy and it used to refer to the measurement of radiation intensity as a function of wavelength. Spectroscopy is basically an experimental subject and is concerned with the absorption, emission or scattering of electromagnetic radiation by atoms or molecules (Gigahertz Optics, 2015). Ultraviolet (UV) and visible radiation comprise only a small part of the electromagnetic spectrum, which includes such other forms of radiation as radio, infrared (IR), cosmic, and X rays as in figure 2.5.

UV-Vis spectroscopy is absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared [NIR]) ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state (Gigahertz Optics, 2015).

A hydrogen, deuterium or discharge lamp covers the ultraviolet range, and a tungsten filament (usually tungsten \halogen lamp) covers the visible range. The radiation is separated according to its frequency\wavelength by a diffraction grating followed by a narrow slit. The slit ensures that the radiation is of a very narrow waveband it is monochromatic.

The cells in the spectrometer must be made of pure silica. Detection of the radiation passing through the sample or reference cell can be achieved by either photomultiplier or photo diode, that converts photons of radiation into tiny electrical currents; or semiconducting cell (that emits electrons when radiation is incident on it) followed by an electron multiplier similar to those used in mass

spectrometers. The spectrum is produced by comparing the currents generated by the sample and the reference beams (Quintero-Ramos, 2004).

2.2.1 Basic Components UV-Visible:

1. Light source Sample.
2. Sample cell.
3. Uniform wave length (Monochromator).
4. Scout (detector).

Optical sources are two types of photovoltaic sources: a tungsten bulb (Lamp Tungsten) for the measurement of visible rays (Visible) in the range (350 - 800). The light source is a deuterium bulb (Lamp D2) It is a light bulb Seeing it with the naked eye because it can cause temporary blindness due to the strength of its radiation. This for the measurement of ultraviolet radiation in the range (200 - 350).

The sample cells are either to be made from Glass or quartz (figure. (2.4)), and quartz are best made because the cell is made of glass Among its components is sodium synthesis which is absorbed in the field UV Therefore, it is preferable to use cells made Of quartz these cells are not among the components made by sodium and the prices of cells Quartz between 300 - 1000 SR depending on the quality of the cell and its thickness.

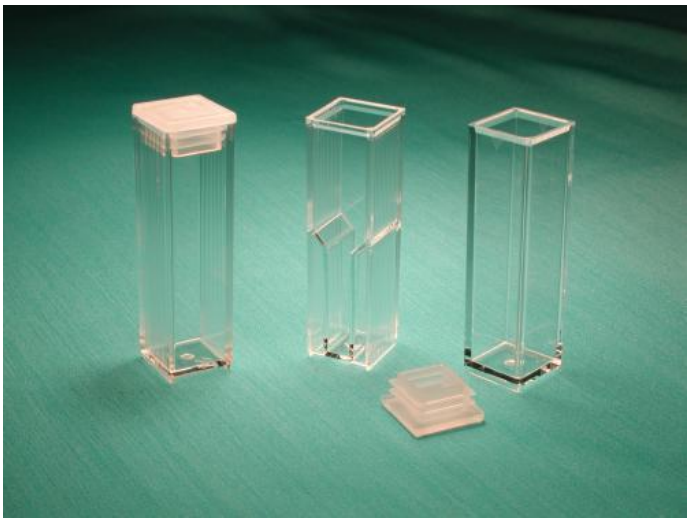

Figure 2.4: Sample Cell (Akihisa Nonoyama,2004).

Uniform wave length (Monochromator) is a glass publication and this publication was used in old machines Currently, in the modern instruments of spectroscopy, there is a so-called reserve and function It scans the sample to determine the wavelength at which the highest catch occurred when it was shed Light whether the light of the tungsten bulb to measure the visible rays or from the deuterium bulb to measure The ultraviolet rays produce a uniform wavelength of many beams of light based Monochromator The reception of the beam whose angle of fall is appropriate on the uniform wavelength and then The wavelength uniformly reverses the ray of radiation on it and directs it to a filter This filter selects the appropriate package very accurately and then continues to move The packet to a reflective mirror sends the fallen optical beam to the sample cell and then To the Scouts (Hiroaki Misawa, 2004).

Scouts (Detector) that shows the amount of light coming out of the sample cell and explains what the quantity is The light outside the sample cell is equal to the amount of light inside the sample if it happens The amount of light inside the sample is equal to the amount of light outside the sample that did not absorb And therefore to get only a straight line does not have any absorption. If the opposite happened the light outside the sample cell is less than the light inside the sample.

Diffraction grating is an optical component with a periodic structure, which splits and diffracts light into several beams travelling in different directions. The emerging coloration is a form of structural coloration. The directions of these beams depend on the spacing of the grating and the wavelength of the light so that the grating acts as the dispersive element. Because of this, gratings are commonly used in monochromators and spectrometers. For practical applications, gratings generally have ridges or *rulings* on their surface rather than dark lines. Such gratings can be either transmissive or reflective. Gratings which modulate the phase rather than the amplitude of the incident light are also produced, frequently using holography.

The principles of diffraction gratings were discovered by James Gregory, about a year after Newton's prism experiments, initially with items such as bird feathers. The first man-made diffraction grating was made around 1785 by Philadelphia inventor David Rittenhouse, who strung hairs between two finely threaded screws. This was similar to notable German physicist Joseph von Fraunhofer's wire diffraction grating in 1821, figure (2.6) shows the basic components UV-Visible spectrometer.

2.2.2 Principle of Ultraviolet-Visible Absorption

Molecule containing π-electrons or non-bonding electrons (n-electron) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals. The more easily excited the electrons (i.e. lower energy gap between the HOMO and LUMO), the longer wavelength of light it can absorb (Akihisa Nonoyama,2004).

2.2.3 Applications of UV-Vis Spectroscopy

UV-VIS spectroscopy is routinely used in analytical chemistry for the quantities determination of different analyses, such a transition metal ions, highly conjugated organic compounds, and biological macromolecules. Spectroscopic analysis is commonly carried out in solutions but solids and gases may also be studied.

Solutions of transition metal ions can be colored (i.e. absorb visible light) because electrons within the metal atoms can be excited from one electronic state to another. The color of metal ion solutions is strongly affected by the presence of other species, such as certain anions or ligands. For instance, the color of a dilute solution of copper sulfate is a very light blue; adding ammonia intensifies the color and changes the wavelength of maximum absorption (λ_{max}).

Organic compounds, especially those with a high degree of conjugation, also absorb light in the UV or visible regions of the electromagnetic spectrum. The solvents for these determinations are often water for water-soluble

compounds, or ethanol for organic-soluble compounds. Solvent polarity and PH can affect the absorption spectrum of organic compounds. Tyrosine, for example increases in absorption maxima and molar extinction coefficient when PH increases from 6 to 13 or when solvent polarity decreases (Skoog, 2007).

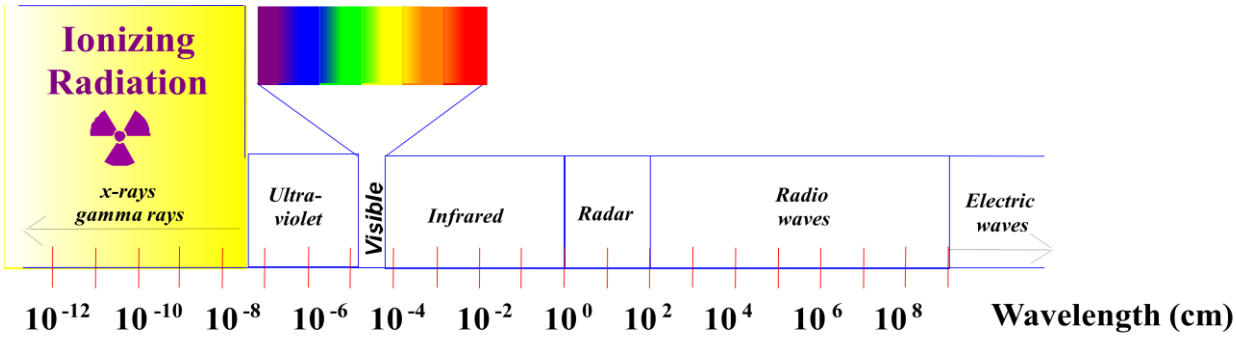

Figure 2.5: Common Ultraviolet Lasers

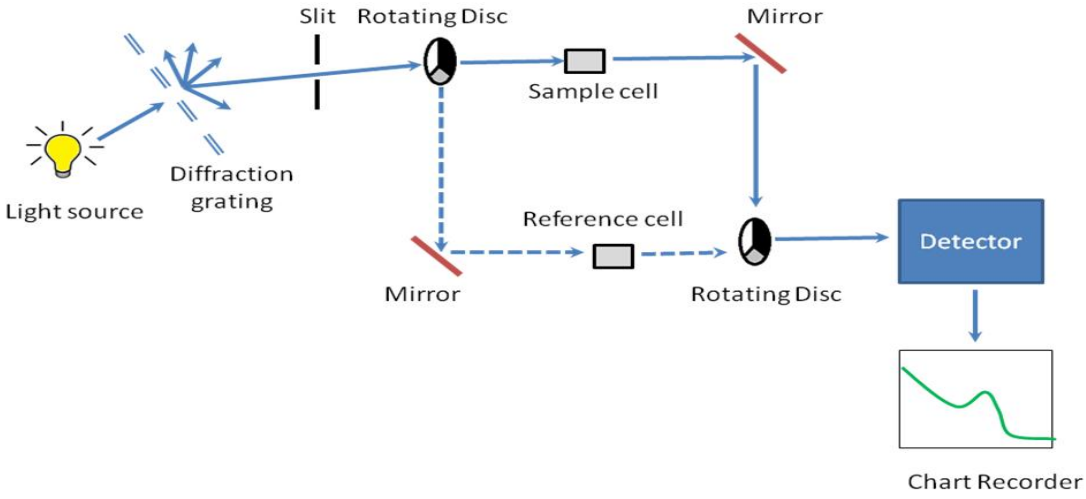

Figure 2.6: Block diagram of UV-Visible Spectrometer (Akihisa Nonoyama,2004).

2.3 Blood

The capabilities of multi wavelength UV-visible spectrophotometer offer the characterization of whole blood. Whole blood is a complex system with the major components being red blood cells (RBC, or erythrocytes), white blood cells (WBC, or leukocytes), platelets, and plasma, each making a contribution to

the whole blood spectrum. Each individual component exhibits unique spectral features based on their physical characteristics that impact their optical behavior. The combination of important parameters (size, shape, chemical composition) that influence the cumulative spectral attributes of the particle is referred to as the joint property distribution (JPD). (Galv, 2004)

2.3.1 Glucose

Glucose is simple sugar with the molecular formula $C_6H_{12}O_6$. Glucose circulates in the blood of animals as blood sugar. It is made during photosynthesis from water and carbon dioxide, using energy from sunlight. It is the most important source of energy for cellular respiration. Glucose is stored as a polymer, in plants as starch and in animals as glycogen (Boerio-Goates, *1991)*.

With 6 carbon atoms, it is classed as a hexose, a subcategory of the monosaccharide. D-Glucose is one of the 16 aldohexose stereoisomers (figure (2.7). The D-isomer, D-glucose, also known as dextrose, occurs widely in nature, but the L-isomer, L-glucose, does not. Glucose can be obtained by hydrolysis of carbohydrates such as milk sugar, cane sugar, maltose, cellulose, glycogen, etc. It is commonly commercially manufactured from cornstarch by hydrolysis via pressurized steaming at controlled pH in a jet followed by further enzymatic depolymerization (Wikipedia, 2015).

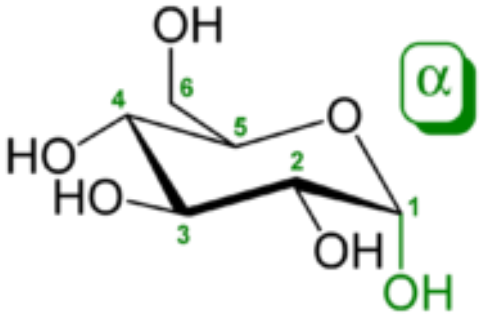

Figure 2.7: α-D-glucopyranose chair form (Maldonado-Garza, H,2009).

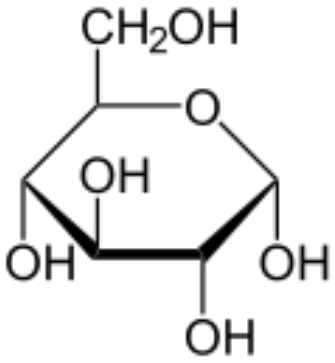

Figure 2.8 Haworth projection of α-D-glucopyranose (Maldonado-Garza, H,2009).

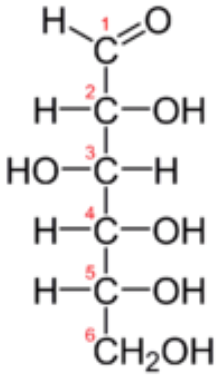

Figure 2.8 Fischer projection of D-glucose (Maldonado-Garza, H,2009).

2.3.2 Blood Glucose

Glucose ($C_6H_{12}O_6$) is a carbohydrate whose most important function is to act as a source of energy for the human body, by being the essential precursor in the synthesis of ATP (adenosine triphosphate). The energy stored in ATP can then be used to drive processes requiring energy, including biosynthesis, and locomotion or transportation of molecules across cell membranes.

According to cellular requirements, glucose can also be used in the creation of proteins, glycogen, and lipids. The blood glucose concentration is very tightly regulated (Miriam Garcia Yanez, 2013).

Human body has two hormones released by pancreas that have opposite effects: insulin and glucagon. Insulin is produced by beta cells of the pancreas

while glucagon is produced by alpha cells. The release of insulin is triggered when high levels of glucose are found in the bloodstream, and glucagon is released with low levels of glucose in the blood.

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 (Miriam Garcia Yanez, 2013).

A blood glucose test measures the amount of a type of sugar, called glucose, in your blood. Glucose comes from carbohydrate foods. It is the main source of energy used by the body. Insulin is a hormone that helps your body use and control the amount of glucose in your blood. Insulin is produced in the pancreas and released into the blood when the amount of glucose in the blood rises.

Normally, your blood glucose levels increase slightly after you eat. This increase causes your pancreas to release insulin so that your blood glucose levels do not get too high. Blood glucose levels that remain high over time can damage your eyes, kidneys, nerves, and blood vessels. Several different types of blood glucose tests are used (accessed 26.iv.2012)

1- Fasting blood sugar (FBS) measures blood glucose after you have not eaten for at least 8 hours. It often is the first test done to check for diabetes.
2- Hour postprandial blood sugar (2-hour PC) measures blood glucose exactly 2 hours after you eat a meal.
3- Random blood sugar (RBS) measures blood glucose regardless of when you last ate. Several random measurements may be taken throughout the day. Random testing is useful because glucose levels in healthy people do not vary widely throughout the day. Blood glucose levels that vary widely may indicate a problem. This test is also called a casual blood glucose test.

Before breakfast blood sugar should be 70-110 mg/dl and after 2 hours blood sugar should be 140 mg/dl or less and at bedtime blood sugar should be 100-140mg/dl, high blood sugar in 200 mg/dl or greater and it is dangerous which can cause problems with your heart, eyes, kidneys and nerves.

A blood sugar of less than 70 is usually a low blood sugar, and also it is dangerous, You could pass out by low blood sugar, Low blood sugar happens to everyone with diabetes from time to time.

2.3.3 Diabetes

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 (Miriam Garcia Yanez, 2013).

There are three types of diabetes:

1- Type 1 diabetes is also known as juvenile diabetes because it is typically diagnosed in children and young adults. In this type of diabetes, the body does not produce insulin. 5% of the population with diabetes has this type of illness.
2- Type 2 diabetes is the result of the body not producing enough insulin or the cells not using insulin properly. This is the most common form of diabetes. 90% of the population with diabetes has this type. Some of the risk factors are physical inactivity, excess body weight, genetics, age greater than 45 years, and ethnicity.
3- Gestational diabetes is high blood glucose levels first diagnosed during pregnancy. This does not mean that the woman will have diabetes after she gives birth or that she had it before she conceived, but it is a risk factor for type 2 diabetes in the future (Miriam Garcia Yanez, 2013).

2.4 Spectroscopy of Blood

Blood is one of the most important biological fluids dealt in clinical

Settings, much is known about its properties as well as methods of its evaluation (Gigahertz, 2004). In terms of optics, Angstrom was the first to use spectroscopic methods to study blood characteristics in 1855, and the spectroscopy of hemoglobin came soon after in 1862. Since That time, advancements in this field of research have brought about various ways of depicting the optical properties of blood components. In terms of optical contributions by cells, erythrocytes which compose approximately 99% of all blood cells, is the major contributor of spectral features. This is further compounded by the fact that the hemoglobin is a strong chromophore. It is not to say, however, that other cells such as leukocytes and platelets are spectrally

invisible. The sensitivity of multiwavelength visible spectroscopy allow for the detection of more subtle changes such as leukocyte depletion (Maldonado-Garza, H,2009).

Changes in spectral features as a result of platelet activation can be detected as well, with calculations reflecting the alterations in the platelet count and size due to aggregation.9 Moreover, the plasma, which is approximately 55% of the total whole blood volume, will contribute absorbance bands in the UV range owing to its protein content (Maldonado-Garza, H,2009).

Chapter Three

Methodology

3.1 Introduction

The aim of this chapter is to present the materials, apparatus and method that used in this work (sample preparation and setup).

3.2 Materials

Samples were collected with specific characteristics from healthy individuals and others diabetics with a known age. The concentration and type of glucose in the blood were also known.

Figure 3.1: Blood Samples.

3.3 Apparatus

3.3.1 UV-VIS 6505 Spectrophotometer

The UV-Vis spectrophotometer was used to measure the absorption of the samples. Using a wavelength in the range between 100-800 nm from ultraviolet to visible. The UV Spectrophotometer that used in this work was micro spectrophotometer's Bogota

6505 contains a quartz cell of thickness of 1 cm as a sample holder.

Figure 3.2: 6505 UV-VI Spectrophotometers.

3.4 Method

Six blood samples were collected from four healthy individuals and three diabetics, then the blood has to dilute with solution because UV-Vis spectrophotometer can give good results when diluted by solution. So we dilute the blood by sodium chloride so that it would not decompose and lose its properties.

Table 3.1 Samples with their Concentration and Age.

Samples	Concentration/mol/Dsl	Age/year
Sample1	100	26
Sample 2	95	39
Sample 3	110	45
Sample 4	145	48
Sample 5	150	65
Sample 6	148	74

Chapter Four

Results and Discussion

4.1 Introduction

This Chapter obtains the results of this work and discusses them. Results include figures and tables.

4.2 The UV-Visible Spectra of the Samples

Figure (4.1) shows the spectra of UV-visible spectrophotometer for sodium chloride (the solution that was used diluted the blood samples).

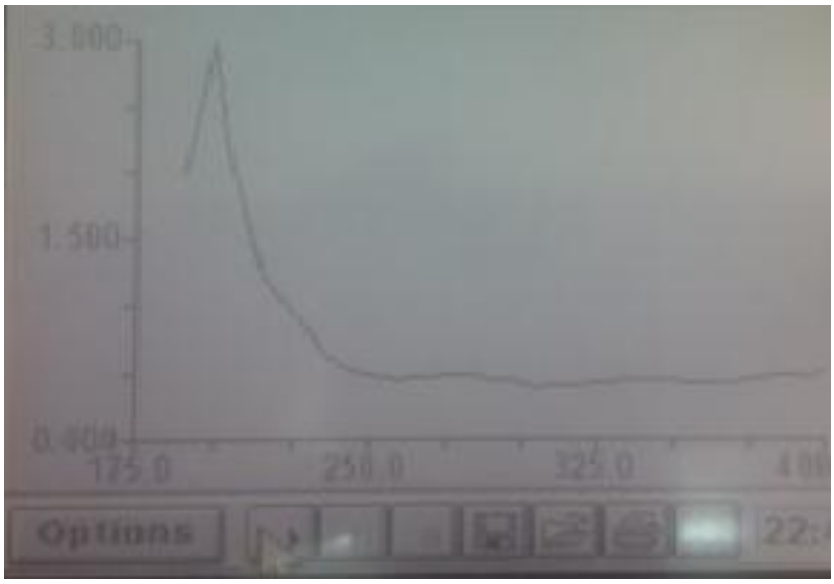

Figure 4.1: Spectrum of Sodium Chloride.

This Figure shows the Spectrum of Sodium Chloride in wavelength range between 175-400 nm. It shows that the absorbance is very high at 200 nm and absorbance is 3.000/au.

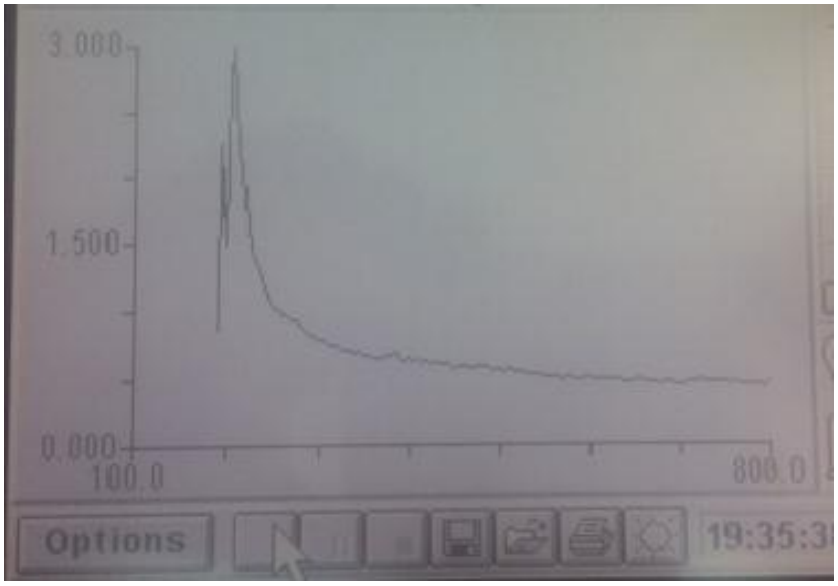

Figure 4.2: Spectrum of Sample (1).

Figures (4.2) shows the spectrum of UV-visible spectrometer for sample (1) (healthy, concentration 100 and age 26 years); which shows a peak in specific wavelength 220 nm. It shows that the high absorbance region in the ultraviolet light with absorbance of 3.000/au.

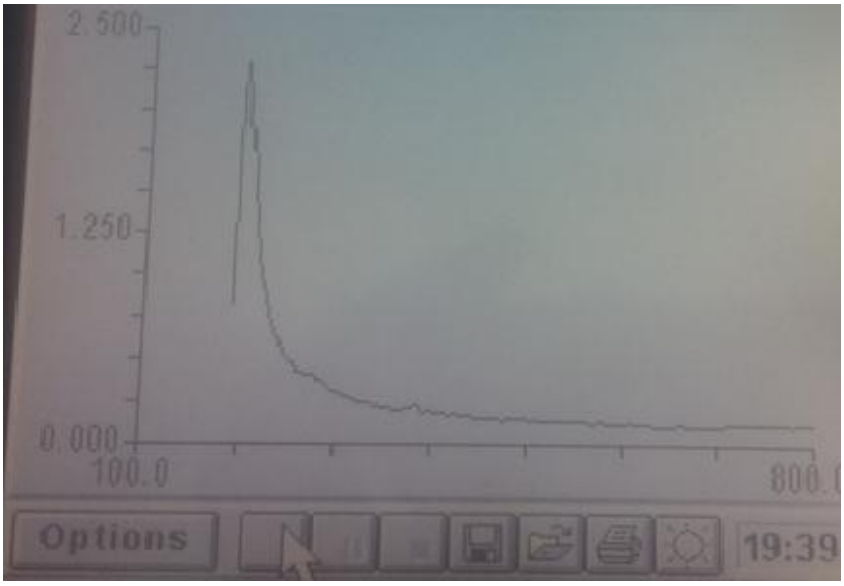

Figure 4.3: Shows the Spectrum of Sample (2).

This is spectrum of UV-visible spectrometer for sample (2) (healthy, concentration 95 and age 39 years), it shows the high absorbance 2.250/au in 220 nm.

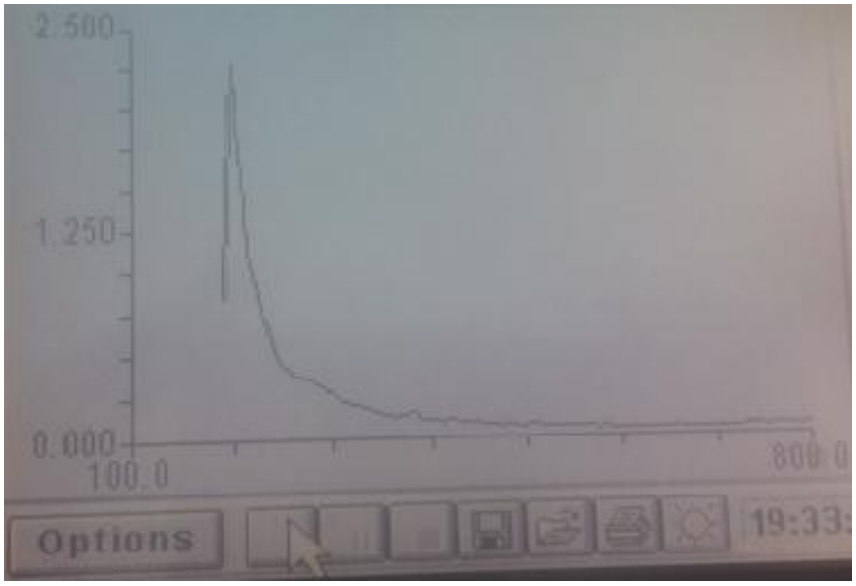

Figure 4.4: Shows the Spectrum of Sample (3).

This spectrum referred to sample (3) (healthy, concentration 110 and age 45 years), It shows that the high absorbance 2.250/au in 220 nm.

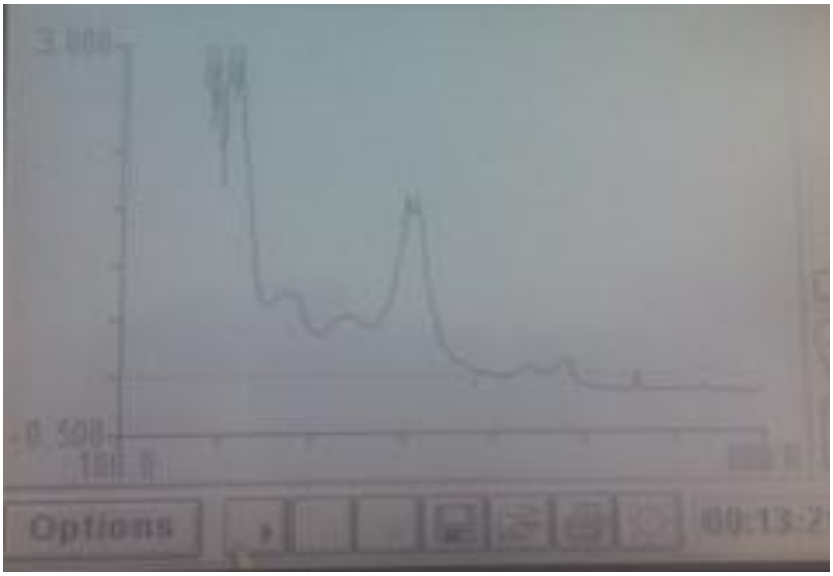

Figure 4.5 Shows the Spectrum of Sample (4).

This spectrum referred to sample (4) (patient, concentration 145 and age 48 years). It shows six peaks around (290, 340, 415, 520, 590 and 760nm) at different heights and the heights peak in 415 nm was in 1.500/au.

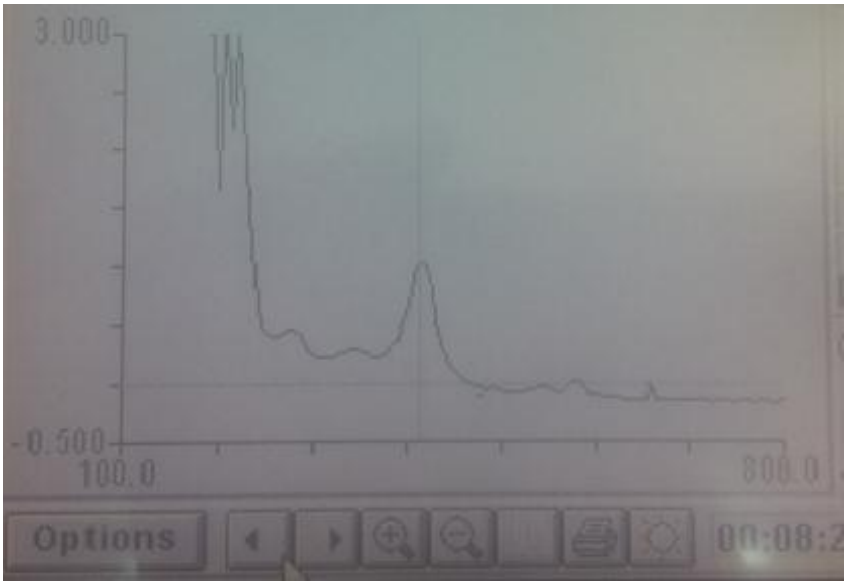

Figure 4.6: Shows the Spectrum of Sample (5).

This spectrum referred to sample (5) (patient, concentration 150 and age 65 years). It shows six peaks around (290, 340, 415, 520, 590 and 760nm) in different wavelengths.

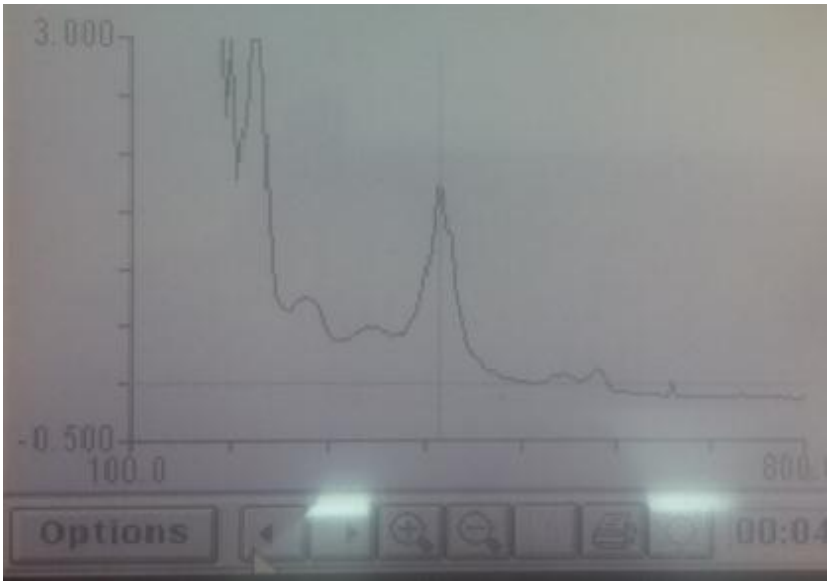

Figure 4.7: Shows the Spectrum of Sample (6).

This spectrum referred to sample (6) (patient, concentration 148 and age 74 years). It shows six peaks around (290, 340, 415, 520, 590 and 760nm) at different wavelengths.

4.3 Conclusion

The results of the UV-Visible spectral analysis of healthy and diabetics' samples are well in accordance. UV-Vis spectroscopy applied on Diabetic blood and on healthy blood; it was found that one peak of the spectra of healthy blood at wavelength 220 nm. The diabetic blood revealed some remarkable differences were elucidating six peaks at different wavelengths around (290, 340, 415, 520, 590 and 760nm). Therefore concluded that there is an observable between the spectra of diabetics and healthy samples. In this study, it has been demonstrated that the study of UV-Vis spectra of serum samples may be used to differentiate between the healthy and diabetic samples.

4.4 Recommendations

The further studies in this research problem could be done using another devices such as FTIR and Raman spectroscopes, also could be done using UV-Vis spectrometer too but for another liquid samples such as tears. Also could be apply UV-Vis spectroscopy on Patients blood and healthy blood to study the Protein molecule or any other molecules and also could be done quantitative study.

4.5 References

Akihisa Nonoyama(2004) Using multiwavelength UV-visible spectroscopy for the characterization of red blood cells: An Investigation of hypochromism**.**

Boerio-Goates, Juliana (1991), "Heat-capacity measurements and thermodynamic functions of crystalline α-D-glucose at temperatures from 10K to 340K", J. Chem. Thermodynam., 23 (5): 403–9.

Carey, N. R., Murphy, S.C., Zadoks, R.N. and Boor, K.J.(2005) Shelf lives of Pasteurized Fluid Milk Products in New York State: a Ten-year Study. Food Protection Trends. 25:No.2:102-113.

GALV-HighBloodSugarLowBloodSugarStudentRevised1104 (2004).

Garcia-Compean, D., Jaquez-Quintana, J.O., Gonzalez-Gonzalez, J.A. and Gigahertz Optics." Reflection, Transmission, and Absorption". [Online] available from: http://light-measurement.com/reflection-absorption/.[Accessed on 2015-08]. http://www.diabetes.org/heart-disease-stroke.jsp

"**Glucose**." The Columbia Encyclopedia, 6th ed.. 2015. Encyclopedia.com. 17 Nov. 2015 http://www.encyclopedia.com

Hiroaki Misawa and SauliusJuodkazis, 2004

Maldonado-Garza, H. (2009) Liver Cirrhosis and Diabetes: Risk Factors, Pathophysiology, Clinical Implications and Management. World Journal of Gastroenterology, 15, 280-288. http://dx.doi.org/10.3748/wjg.15.280

Miriam Garcia Yanez, Glucose Meter Fundamentals and Design, Rev. 1, 01/2013

Mitrović, M., Popović, Đ.S., Naglić, D.T., Paro, J.N., Ilić, T. and Zavišić, B.K. (2014) Markers of Inflammation and Microvascular Complications in Type 1 Diabetes. Central European Journal of Medicine, 9, 748-753. http://dx.doi.org/10.2478/s11536-013-0335-6.

M.Srikanth*, G.Venkateswara Rao and K.R.S.Sambasiva Rao, (2004), Indian Journal of Clinical Biochemistry, 19 (1) 34-35].

Quintero-Ramos, A., J.J. Churey, P. Hartman, J. Barnard, and R.W. Worobo,(2004)" Modeling of Exherichia coli inactivation by UV irradiation at different pH values in apple cider". J. Food Prot. 67:1153-1156.

Skoog, Douglas A., Holler, F. James, Crouch, stanly R, (principle of instrumental analysis (6^{th} ed). Belmont, CA: Thomon Brooks/Cole.pp. 169-173. ISBN 9780495012016.

V. H. GHADAGE, G. R. KULKARNI, B. N. ZAWARE, (2015), He-Ne Laser Irradiation of Blood in vitro and FTIR Spectral Analysis, International Journal of Chemical and Physical Sciences, ISSN:2319-6602, IJCPS Vol. 4 Special Issue ETP – 2015.