CHAPTER ONE

INTRODUCTION

1.1 Background

Laser irradiation has bio stimulation effects in various cell types. There are many applications of He-Ne laser in the medical field; for instance,in blood cell analysis (cytometry), for the diagnostic and treatment. Helium-Neon laser is a type of small gas laser with the typical operational wavelength of 632.8 nm in the red color region of the visible spectrum (Mangi FA et al 2017).

Blood is the fluid that circulates in the heart, arteries, capillaries, and veins of a vertebrate animal carrying nourishment and oxygen to and bringing away waste products from all parts of the body, Blood constitutes 7% of the human body weight and about 5 liters in volume. It is a life-saving fluid in the body and has the most essential function of supplying nutrients and oxygen to all parts of the body It also take away CO2 from the system. Blood contains cellular components (red blood cells (RBCs); white blood cells (WBCs); and platelets) and Plasma, which constitutes 55% of blood fluid, is mostly water (92% by volume) (which contains a host of proteins, hormones, glucose, etc.) **(**Dutta etal 2009 **)**

Laser interacts with tissue by three mechanisms, Photochemical and Biostimulation and photodynamic therapy (PDT), photochemical interactions is, effects of laser on tissues chemically, photodynamic therapy (PDT) and Biostimulation attributed to based on photochemical interactions. Interaction of lasers with biological materials such as blood, skin, and tissues is important to be understood. The study of blood change by spectroscopic techniques like UV/Visible and FTIR Spectroscopy can be used for understanding the biological nature of the disease, and also for the diagnosis of the disease.

Fourier transform spectroscopy is applied to study disease patterns have been identified. Structural and conformational changes of proteins, nucleic acids and lipids have been reported on the basis of FTIR. Complex structure of globular proteins and biomolecules in blood are identified on the basis of FTIR spectra. The absorption spectra of whole blood, erythrocytes, and plasma to study photochemical reactions initiated by exposure of blood in vivo to UV radiation has been reported(Ghadage, V et al 2011)

1.2 Research Problem

The study of the interaction of laser with biomaterials such as blood and the effect of laser on such materials is very important for laser applications. The biostimulation effect of low-power laser irradiation has been noticed and studied for about two decades. Some progress was achieved in treating various pathologic processes. Intravenous low power laser irradiation has been applied clinically to treat various diseases, and the results have been encouraging. In the literature so far, the bio-effects of laser stimulation are evident; however, the mechanism of interaction is not fully understood**.** Laser tissue interactions can be understood by using different spectroscopic techniques and Scanning electron microscope.

The Studies conducted on the effects of low-power laser on human blood by spectroscopy techniques are not enough .It is very important to understand the effect that occurs to take advantage of the treatment. In particular, UV/vis spectrophotometer and Fourier Transform Infra Red Spectra (FTIR) analyze and show clear results of the interactions.

1.3 Previous Studies

Many studies were made for laser tissue and blood interactions. Here one exhibits some attempts. Al Khalid et al. (2009) studied the effects of 160μW Laser on some blood characteristics like osmotic fragility and testing ways. The study showed that fragility changes by time. RBCs hemolysis increases by time of storage. Spectrophotometer was used at 540nm for

identifying absorption of all samples, before and after irradiation. The best result for decreased hemolysis was found when 160µW was used for the duration of 24 hours. Before exposure, the RBCs hemolysis was decreased by 5.5% when used in NaCl solution of 50% density. The results indicated that irradiating RBCs with 160µW leads to decreasing RBCs hemolysis by ½, which means that blood life time, can be increased under the conditions prescribed.

Kanagathara et al. (2010) employed FTIR and UV/Vis spectroscopic technique to study the spectral differences in the serum of normal blood samples. Analysis of the blood sera spectrum for different samples under FTIR and UV/Vis spectroscopic technique showed that there were some differences between each and every spectrum. There will be a linear relationship between the protein content and the maximum absorption spectrum in the UV region. This study was suggests that the spectrophotometric analysis of the blood Serum is a useful tool for determination of diseases in human body.

Vijay H. Ghadage et al. (2011) was used He Ne laser (λ= 632nm, power=2mW) to irradiate human red blood cells absorption spectrum, FTIR and fluorescence spectra of RBC The absorption spectrum of RBC after irradiated to He-Ne laser shows a significant decrease in absorbance. The FTIR spectrum of irradiated RBC clearly shows changes in transmittance. FTIR spectra related to C=O group and percentage of transmittance increases for O-H, C=C, N=O, C-O and C-H group.

Zahra Al Timimi et al. (2011) was investigated the influence of different levels of laser on the damage threshold of blood Cells.Laser diodes were used as a source of radiation in different levels of irradiation protocol. The samples were made to stand for 30 minutes before determining the change in rheological properties of blood cells. Results were established that low level laser therapy when used on human blood in vitro, affects the

rheology of erythrocytes and leucocytes. BSR, aggregability indices of blood It was observed that it changes the erytherocytatory, leucocytatory, .Conclusions Thus it was concluded that low level laser therapy can affect the physical as well as chemical properties of blood cells which is not only helpful in preservation of blood but also in revitalizing the physically and chemically stressed erytherocytatory membranes. It was determined that the laser therapy decreases the viscosity of blood thus increasing the electrophoretic mobility of erythrocytes

Al-khaled et al. (2012) studied blood characteristics like osmotic fragility, Conductivity and Dielectric constant on RBCs after being irradiated by (690μW) of HeNe Laser,. The study showed that fragility changes with time. RBCs hemolysis increases with time of storage. The study also showed that there is no negative effect on the Dielectric constant of the irradiated RBCs by (690μW) of He-Ne Laser. On the contrary; there is a little decreasing of RBCs Dielectric constant's and conductivity's, after irradiation by (690μW) of He-Ne Laser, which means increasing of RBCs resistance. In conclusion; that result gives us a clear indication of very positive and sensitive Laser effect.

Samir Abdel (2012) evaluated the effects of non-coherent light (solar light) at 400 mW and the effects of He-Ne laser at wave length 633 nm at 10 mW, on human blood, on Glycated hemoglobin, G6PD, glucose consumption by blood cells, CBC and osmotic fragility of erythrocytes. found that the non-coherent and coherent light decreased HbA1c, the glucose consumption are increased, osmotic fragility of erythrocytes decreased and some parameters of CBC are very influenced strongly by light irradiation.He-Ne laser and non-coherent light decreases the osmotic fragility of erythrocytes (increases the resistance of erythrocytes to hypotonic solution). He-Ne laser and Non-coherent exposed to blood, improve its rheological properties and reduces the glycated hemoglobin (decreases glycated hemoglobin during incubation

through increasing consumption of glucose). There is no effect in this study on G6PD enzyme.

Ghadage et al. (2015) Blood samples were irradiated in that study by He-Ne laser (Wavelength λ = 632.8 nm, Power = 3mW). The FTIR spectra for irradiated blood samples showed significant changes 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) etc. The significant results were obtained when He-Ne laser irradiation is incident on whole blood for 30 and 40 minutes and the transmittance decreases due to denaturation of proteins.

Al-Khalid Isam Zuhaier (2015) evaluated Shelf-life enhancement of donor blood by He–Ne laser Biostimulation. Shelf life of stored blood in a bank is an important parameter for the effective use of blood drawn from the donors. Several attempts have been made (such as antioxidant treatment and magnetic field intervention) to improve the above lifetime of 42 days. The study showed that biostimulation by a He–Ne laser could enhance the shelf life to 63 days. The results are based on the fragility and conductivity measurement of red blood cells.

Vijaya Ushasree et al. (2016) studied infra red spectroscopy on human blood, reported IR spectroscopic data on human blood and its constituents. IR analysis has been made on whole blood, plasma and serum. The characteristic spectral bands pertaining to fibrinogen, hemoglobin, erythrocyte membrane lipids and other plasma proteins were identified. The result explores the possibility of disease analysis by IR spectroscopy.

Wasil Salih et al. (2017) O-Xp laser 820 nm and HeNe laser 632.8, were Used to irradiation blood samples to check for the change in the blood parameters. The results showed enhance the counts of the human blood components when used He-Ne laser more than O-Xp laser. For instance, the white blood counts of the samples irradiated to He-Ne laser increase of about

60% while just 45% is noticed for the O-Xp irradiated samples. However, the O-Xp increases the human blood absorption of light as checked by UV/VIS spectrometer more than samples He-Ne irradiated. The effects of low power violet laser irradiation on human blood samples some rheological factors such as mean red blood cell volume (MCV) and erythrocyte sedimentation rate (ESR) in vitro examine. Samples were irradiated for 20, 30, 40 or 50 min with a laser of power 10 mW. The measurements were done directly after irradiation by applying westergen method and used a computerized hemtoanalyzer. The RBCs volume and ESR were decreased after irradiation for 40min by 0.44% and 6.7% respectively. It was possible to suggested that laser irradiation could reduction red blood cells volume because of the increased concentrations of free intracellular Ca^{2+}.The result showed that ESR reduction exposed to low power laser was mostly by reason of the effect of laser on composition of the plasma that finally affects in ESR of whole blood.

Mustafa Al Musaw et al. (2017) evaluated the effects of low power violet laser irradiation on some human blood samples rheological factors such as mean red blood cell volume (MCV) and erythrocyte sedimentation rate (ESR) Samples were irradiated for 20, 30, 40 or 50 min with a laser of power 10 mW. Used westergen method to measurements directly after irradiation by acomputerized hemtoanalyzer .The RBCs volume and ESR were decreased after irradiation for 40min by 0.44% and 6.7% respectively. It is possible to suggest that laser irradiation can reduction red blood cells volume because of the increased concentrations of free intracellular Ca^{2+}.The result shows that ESR reduction exposed to low power laser is mostly by reason of the effect of laser on composition of the plasma that finally affects in ESR of whole blood.

Siti Sakinah Mohd Fuad et al. (2018) yellow laser of power density of 450mW/cm² was used to irradiation blood samples with random diseases from 10 minutes to 60 minutes at 10 minutes intervals. The morphology of the red blood cell was also observed for different irradiation time. The result

showed that there was a significant different in the absorption of light with varying laser irradiation time ($p<0.01$). The maximum absorption recorded at 40 minutes of irradiation at 340nm peak. Blood smear of the samples reveals that there were observable changes in the morphology of the red blood cell at 40 minutes and 60 minutes of irradiation.

Ghadage Vijay et al. (2018) studied laser radiation effects on diabetic human blood using FTIR Spectroscopic Techniques. Blood samples were collected from diabetic human subjects blood samples were collected from diabetic human subjects. HeNe (Helium-Neon) laser (wavelength λ=700 nm, Power =5 mW), was used to irradiation Diabetic blood samples. The FTIR spectra of non irradiated diabetic blood samples were compared with the FTIR spectra of irradiated diabetic blood samples. The significant changes were observed between the O-H(Free group),P-H(Phosphine) and C=O (amide group) due to laser radiation on diabetic blood samples for time 10 to 40 min. respectively. The result also showed decreased in percentage oftransmittance for C=O (amide group) for 30 and 40 min, that means diabetic blood samples showed the denaturation of proteins

1.4 Objectives of Thesis

The main objective of the present work is to study the interaction of laser with Whole blood and the effect of laser on it.

The specific objectives are:

1. To study the effects of He-Ne laser radiation ((λ= 632.8 nm, power = 2 mW) on human whole blood in vitro using Fourier transform infrared (FTIR) and ultra violet (UV) spectrophotometer and study change of spectra of whole blood due to laser effect and report the changes in various functional groups.

2. To study the effects of He-Ne laser radiation ((λ= 632.8 nm, power = 1 mW) on human whole blood in vitro using in FTIR and UV/Vis

spectrophotometer , and study change of spectra of whole blood due to laser effect and report the changes in various functional groups.

1.5 Research Methodology

Blood samples were collected from healthy volunteers; 3 ml of each volunteer by medical standard laboratory conditions and blood samples were saved in a tube to prevent from coagulation to (EDTA) and each sample was divided into three samples one sample was the control and other was exposed to the helium-neon laser with different exposure times. Whole blood Samples were irradiated to a Helium-Neon laser beam, continuous operating wave mode, as a radiation source (632.8 nm, Power = 1 mW), and (632.8 nm, Power = 2 mW). Fourier Transform Infra Red Spectra (FTIR) and UV/Vis spectrophotometer SP UV-26 (Sco TECH) were used to study the effect of laser radiation on human blood. The FTIR and UV/Vis spectrum for He-Ne laser irradiated blood serum samples and none irradiated were obtained.

1.6 Thesis Layout

The thesis consists of four chapters. Chapter one is the introduction, while chapter two is concerned with the theoretical background. The materials and methods are in chapter three. Discussion and conclusion are in chapter four.

CHAPTER TWO

BASIC CONCEPTS

2.1 Introduction

Laser plays an important role in modern technology. It widely used in medicine, industry and telecommunication. This chapter is, therefore concerned with the nature of laser, beside some important laser types used in medicine and other applications**.**

2.2 Laser

The word (laser) is the abbreviation of the word, Light Amplification by Stimulated Emission of Radiation. The American physicist Thomas H. Maiman, is who invention laser. It was at the end of the 1950s that obtained laser from of a ruby rod that had been placed along the axis of a helical flash tube.Laser is a device that generates coherent radiation, based on stimulated emission of electromagnetic radiation at frequencies in the infrared, visible or ultraviolet and other regions of the electromagnetic spectrum from receiving energy from a pumping source. The basic phenomenon for laser emission, stimulated emission of radiation by excited atoms, was introduced by Albert Einstein as early as 1917, from thermodynamical speculation on the interaction of a collection of atoms with blackbody radiation. Lasers are different from other light sources by their coherence. Spatial coherence is typically expressed through the output being (Germain Chartier, 1997).

Power Density: The rate of laser energy delivery is called power and is measured in watts. The wattage is equal to the amount of energy, measured in joules, divided by the duration) of exposure, measured

$$\text{Watts (W)} = \text{Joules (J)}/\text{Seconds (S)} \qquad (2.1)$$

An important factor in the effective application of the laser is a concept of power density, or irradiance. Power density is defined as the amount of power that is concentrated into a spot, or watts/cm ^{2}

Power density = Watts/Spot Size (cm^2) (2.2)

Mode of Operation a laser may be a continuous constant-amplitude output (Known as CW or continuous wave); or pulsed, by using the techniques of Q-switching and Mode locking**:**

- In the continuous wave (CW) mode of operation, the output of a Laser is relatively consistent with to time. Required a steady pump source to the population inversion and lasing is continually maintained.
- In the pulsed mode of operation, the output of a laser varies With time, typically taking the form of alternating 'on' and 'off' Periods. In many applications one aims to deposit as much energy as Possible at a given place in as short time as possible (Gupta SK et al 2018)

2.2.1 Properties of Laser:

Laser radiation distinguishes by a high degree of monochromaticity, coherence, directionality and brightness as compared from those ordinary light sources**.**

A. Monochromaticity:

laser radiation has unique property that The Monochromatic o light, results from the circumstance that light oscillation sets in at one resonance frequency of the optical cavity, Since a two-mirror arrangement forms a resonant cavity, oscillation can occur only at the resonance frequencies of this cavity. The latter circumstance leads to an often much narrower laser line width (by as much as 10 orders of magnitude) than the usual line width of the transition and owing to the balance between gain and loss in CW operation the line width ΔvL (Orazio Svelto,2002).

B. Coherence:

Laser radiation is a coherent, that means we can introduce two concepts of coherence, namely, spatial and temporal coherence, that refers to the time period Δt in which the phase undergoes random changes, and the coherence

length is a Measure of the propagation distance over which the beam stays coherence.

c. Directionality:

The directionality of the laser beam propagate in specific direction is due to the fact that the gain medium is placed inside an open optical resonator take mirror direction.

D. Brightness:

The laser system oscillator is emit high optical power in a small solid angle of space is the capability of the brightness of laser and is closely related to the directionality (Orazio Svelto, 2002).

2.2.2 Elements of Laser:

The basic components of the laser are pumping source, an active material and the optical resonator. Each of these components controls the stimulated emission of the laser and need to be understood.

A. Pumping Source:

Pumping source in the laser is giving the act of energy transfer from an external source into the gain medium in a laser system. The energy absorbed in the active medium, producing an excited state in its atoms. Population inversion is achieved when the number of particles in one excited state exceeds the number of particles in the ground state or a lower-energy-level state, in this condition, the mechanism of stimulated emission can take place and the medium can act as a laser or an optical amplifier. The pump energy is usually provided in the form of light or electric current, but more exotic sources have been used, such as chemical or nuclear reaction. (H. Haken.1985)

B. Active material and Laser Gain Medium

The materials that used as the active medium of a laser system it may be Gases, liquid and solids. The origin of laser photons is most often in a

transition between discrete Upper and lower energy states in the medium, regardless of its state of matter.

He-Ne, ruby, CO_2 and dye lasers are familiar examples, but different materials are frequently used: the excimer laser has an unbound lower state, the semiconductor Diode laser depends on the transition between electron bands rather than discrete States and understanding. All these materials provide optical gain in the cavity Quantity that is determined by the length of the optical cavity and a number of reflected passes through the active material. results from stimulated emission of electronic or the molecular transition from higher to lower energy state populated by a pump source For each pass, through the optical cavity, a loss occurs due to the mirrors that are proportional to the gain. When pumping is applied to the active material, the gain increases for each pass through the optical cavity. Population inversion occurs when the gain reaches a Value higher than the loss from reflection (Bernard et al 1954).

C. The optical resonator or optical cavity:

The Optical Resonator is two parallel mirrors placed around the gain medium Cavity designed to internally reflect infrared, visible, ultra -violet. It can contain gases, Liquids or solids which provide feedback of the light. Cavity materials can determine the wavelength of the output. Figure 2.1 shows the components of laser system.

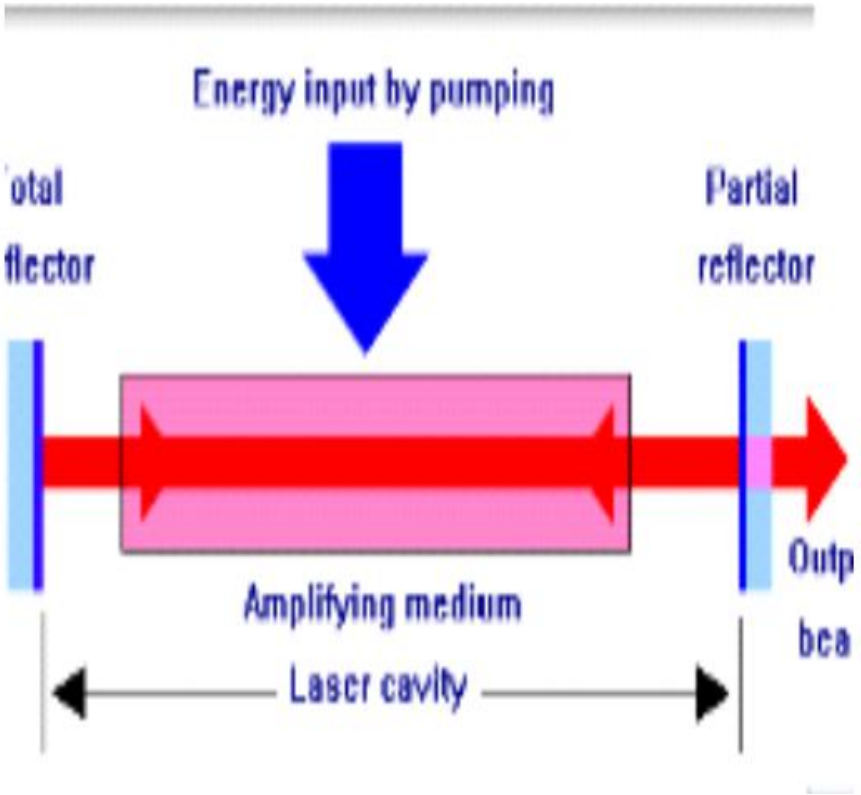

Figure 2.1: Laser system

2.2.3 Laser Types

Laser has various types developed from invention in the laboratory in 1960 so far display a wide range of physical and operating parameters. lasers are characterized According to the physical state of the active material, will call them solid state, liquid, or gas lasers. A rather special case is where the active material consists of free electrons at relativistic velocities passing through a spatially periodic magnetic field free (Electron lasers). If lasers are characterized by the wavelength of emitted radiation, one refers to infrared lasers, visible lasers, ultraviolet (UV) and X-ray lasers (Orazio Svelto, 2002).

A. Gas Laser

A gas laser contains atoms or molecules. Gas lasers in which the laser active medium is pumped by collisions with electrons or atoms and the transitions can be either electronic (He-Ne laser) or ro-vibronic ones (CO_2 laser)(K. Shimoda, 1984).

Active medium are stimulated transitions occur in atoms between electronic states and in molecules between rotational, vibrational, or electronic states. We describe various gas discharge lasers: helium–neon laser; metal vapor laser; argon ion laser; excimer laser; nitrogen laser; CO_2 laser; optically pumped gas lasers (Karl F. Renk, 2011).

The helium–neon (He-Ne) laser:

This was second of lasers, discovered in the early 1960s. Its red output has found uses in everything from holography to bar code scanning. A few years ago it was the most common laser, but it's replaced by semiconductor lasers it's cheaper and become dominant still, it makes an excellent example of how a typical laser operates. The basic laser itself consists of a glass tube filled with helium and neon gases in a ratio of about 10 parts helium to 1 part neon. The internal pressure is low (about 1.8 torr, where 760 torr is 1 atmosphere), allowing a sustained electrical discharge. The electrical energy required is supplied by a high-voltage power supply, which is often encapsulated in a small block of epoxy material. When energized, the tube glows a bright pink color. Most common HeNe lasers have a red output at 632.8 nm; however, the quantum mechanics of the neon atom (which is the active lasing species) also allows lasing transitions in the orange, yellow, and green. The discharge itself takes place between a small anode and a much larger cathode through a tiny capillary tube called a plasma tube. At either end of the laser are cavity mirrors. One mirror is fully reflecting, the other partially reflecting. The tiny portion of light (typically around 1%) that is transmitted through the front mirror is the actual laser beam itself. **(**Mark Csele2004).

The HeNe laser operates in a high-voltage (kV), low-current (mA) glow discharge. Its most familiar output wavelength is 633 nm (red), but HeNe lasers are also available with output at 543 nm (green), 594 nm (yellow), 612

nm (orange), and 1523 nm (near infrared). Output power is low, ranging from a few tenths to tens of milliwatts, depending on the wavelength and size of the laser tube. Figure 2.2: show the typical He-Ne tube and structures.

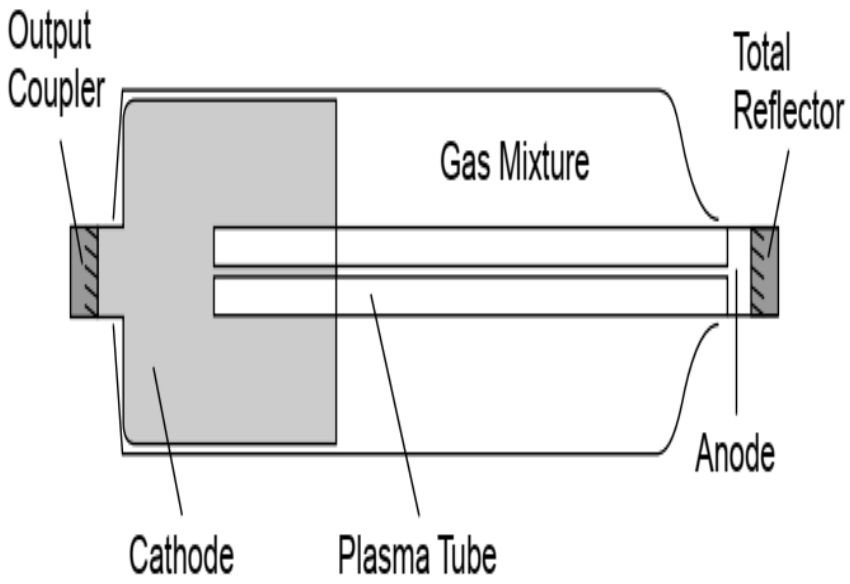

Figure 2.2: Typical He-Ne tube and structures.

B. Solid State Laser

Solid state lasers that use electronic states of impurity ions in dielectric crystals or in glasses — other types of solid state lasers, Namely semiconductor lasers that are based on electrons in energy Bands of semiconductors. It consist the ruby laser, the titanium– sapphire laser, neodymium-doped YAG laser, of other neodymium lasers, and of other YAG lasers. The Ruby laser has red light output with a wavelength of 694 nm, early Ruby laser systems use in retinal surgery, but weren't used widely for Dermatologic work until the development of Q-Switching technology in
The mid 1980's for tattoo treatments. Ruby laser light is strongly absorbed by blue and black pigment, and by melanin in skin and hair.

C. Liquid Lasers

Dye lasers use solutions as active medium organic dye in a liquid solvent such at ethyl or methyl alcohol or water. It is pumping energy optically either by any other laser or by a flash lamp. Dye lasers have very important features offer is tunability. The monochromatic laser output of available can be tuned over abroad Range, from ultraviolet to the near infrared. To introduce a specific dye material, let us consider the dye rhodamine 6G, one of the important laser dye materials. It has several Benzene rings and the chemical formula $C_{26}H_{27}N_2O_3Cl$, with a molecular Weight around 450. It is soluble in methyl and ethyl alcohols and in other Organic solvents.

D. Semiconductor Lasers

Semiconductor lasers consider the very important class of lasers in use today, not only because of the large variety of direct Application, but also because they have found widespread use as pumps for solid-state lasers. Semiconductor lasers require a direct-gap material, so normal elemental semiconductors (e.g., Si or Ge) cannot be used. These lasers will, therefore, be considered at some length. For the active medium, the majority of semiconductor laser materials are based on a combination of elements in the third group of the Periodic Table (such as AI, Ga, in) and the fifth group (such as N, P, As, Sb). For example diode laser, generating optical gain in the recombination of injected holes and electrons (and consequent emission of photons) in a forward-biased semiconductor pn junction (OrazioSvelto, 2002).

2.2.4 Laser Applications

Now a day Lasers has many applications and associated with so much a part of daily life that many people may not realize how ubiquitous they are every home with a CD. The player has a laser; hardware stores are now selling a wide variety of laser levels; many, if not most, computers, printers, and copiers are using laser technology. Laser applications are so numerous

that it would be fruitless to try to list them all; however; one can give some illustrative examples of how lasers are used today. there wide lasers applications in industrial high-power lasers have used for cutting and welding materials by using, ruby laser, argon ion laser , CO_2 laser pulsed Nd:YAG laser.

Today the frames of automobiles are assembled using laser welding robots, complex cardboard boxes are made with laser-cut dies, and lasers are routinely used to engrave numbers and codes on a wide variety of products. Other applications include Communication, holography and super market scanners and information processing, scientific research applications; Lasers are used extensively in the scientific laboratory for a wide variety of spectroscopic and analytic tasks. Examples are Ramman spectroscopy and confocal scanning microscopy.

2.2.4.1 Medical Applications of Lasers:

Laser are used in medical applications early one of the applications of lasers in medicine was photocoagulation, using an argon-ion laser to seal off ruptured blood Vessels on the retina of the eye. Laser radiation passed through the lens and vitreous humor in the eye and focused on the retina, creating scar tissue that effectively sealed the rupture and staunched the bleeding. In ophthalmology, various types of lasers are being applied today for either Diagnostic or therapeutic purposes. In diagnostics, lasers are advantageous. If conventional incoherent light sources fail, one major diagnostic tool is confocal laser microscopy which allows the detection of early stages of retinal Alterations. By this means, retinal detachment and also glaucoma1 can be recognized in time to increase the probability of successful treatment.

The first indications for laser treatment were given by detachments of the Retina. Meanwhile, this kind of surgery has turned into a well-established tool and only represents a minor part of today’s ophthalmic laser procedures.

Others are, for instance, treatment of glaucoma and cataract. l And, recently, refractive corneal surgery has become a major field of research There exist six, retinal tumors (retinoblastoma) (Markolf H. Niemz1996).

- **Laser surgery:**

It uses the cutting power of a laser beam to make bloodless cuts in tissue or remove a surface lesion such as a skin tumor. There are a number of different types of lasers that differ in emitted light wavelengths and power ranges and in their ability to clot, cut, or vaporize tissue when using laser in medicine, potential advantages for both surgeons and patients are provided. These advantages are No-touch technique, Possibility of operating in inaccessible regions, Dry surgical field with sterilization of the operative site, Reduced blood loss, Reduced oedema and pain yielding more rapid recovery of the patient., Limited fibrosis and stenosis, Limited damage of the adjacent tissue (for a few tens of micrometers) – Precision, Reduced postoperative pain and No evidence of causing genetic damage or cancer (Hecht J 2003).

- **Dermatology:**

Laser has successfully used for cauterization and local treatment of skin growths and skin deformities. Laser treatment provides two major advantages over conventional treatment in case of burn injuries and skin grafting. They are the area, which has been treated remains sterile and hence provides an ideal bed for immediate skin grafting and the blood loss is minimal. Laser finds wide application in dermatology especially in Homeostasis, which means stopping of bleeding and Removal of hair, tattoos, warty keratosis, cell carcinomas, freckles, acne and various growths, both benign and malignant. CO2 lasers have been used for treating skin tumors. Another application of laser more specifically photo medicine, is in the surgical treatment of port wine stains (PWS). A port wine stain is a defect in the skin, which is manifested by discolored or blotchy and darkened patches in the skin. The most commonly used laser in the treatment of PWS is the pulsed argon lase

- **Cardiology**:

  One of important area of medical application of lasers is the laser assisted balloon angioplasty, which has become very common in clearing blocked arteries. In balloon angioplasty, a tiny deflated balloon, housed in a thin catheter, is threaded through the artery in the blocked region. When it reaches the right spot the balloon is inflated to open up the vessel by compressing the obstructing plaque against the walls of the artery. This method is less expensive and less prone to complications, as compared to conventional bypass surgery. The disadvantage with balloon angioplasty is that the vessel can close again because there is no permanent removal of the clot. About an estimated 30% case need repetition of the process. This technique is of no use in case of totally blocked arteries.Lasers have helped in overcoming this problem. Lasers can pave theway for the balloon or clear the arteries on their own. In laser-assisted angioplasty, an Ar+ laser delivers the energy through the catheter and disposable balloon. The balloon helps in inflating the site for blood flow cessation and centering the transmitting the laser by optical fibre, which andthe opening the vessel. Taking turns, the laser, and the balloon clear path for each other, via the bloc(K R. Nambiar,et al.2004)

- **Ophthalmology:**

Laser has become a common tool now in photocoagulation such as the one used to reattach a detached retina. For example, the green beam of argon ion laser is focused on a certain point of the retina. The beam penetrates through the lens of the eye and the vitreous chamber without being absorbed. But the beam gets strongly absorbed by the red blood cells of the retina and the resultant thermal effect leads to reattachment of retina. In the past, high powered xenon lamps were also often used to concentrate enough heat in the area of the unattached retina.

- **Photocoagulation:**

The laser produces a pulse of light energy as directed by the surgeon, which is passed to the eye under treatment. The pulse of light is focused by the lens of the patients' eye to produce a minute lesion or coagulation of the tissue of the retina and the choroids (the vascular membrane of the eyeball between the sclera and retina). The retina is so welded to the choroids by a series of these lesions through several repeated pulses from the laser. The amount of energy required for coagulation is different for different patients. The two lasers are commonly used in ophthalmology, are the ruby laser of 0.69 μm and argon ion laser of 488 to 514 nm (Javan A et al 2005), Silfast WT (2004)

2.3 Blood

The blood is a tissue constitute from a suspension of cells in liquid called plasma. Blood accounts for 7% of the human body weight, with an average density of approximately 1060 kg/m^3, very close to pure water's density of 1000 kg/m^3.

Blood is contain of red blood cells, white blood cells, plasma and platelets, a connective tissue, which is very essential for many organisms such as human and animal, and so important to jobs,a transfer of materials (food and oxygen), vitamins and waste (carbon dioxide) and hormones to all tissues and cells of the body and the degree of natural temperature is 37 degrees Celsius. Figure 2.3: shows the components of blood.

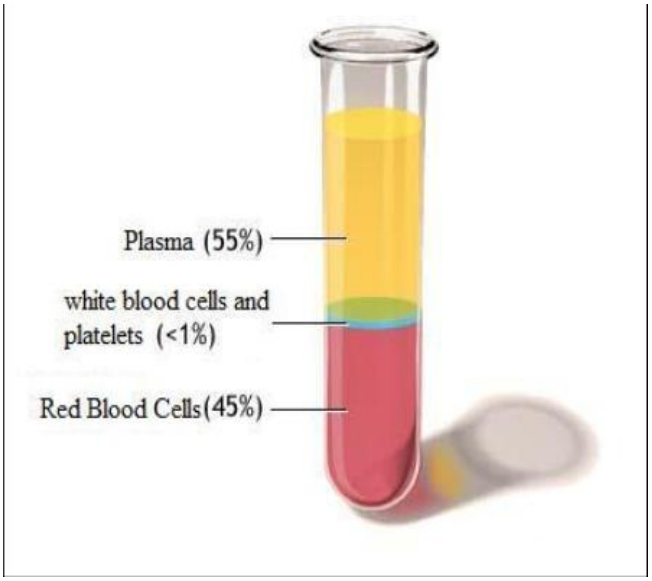

Figure 2.3: the components of blood.

- **Plasma**

Plasma is liquid material in blood, which by itself is straw-yellow in color About 55% of blood is, a fluid . The blood plasma volume totals of 2.7–3.0 liters (2.8–3.2 quarts) in an average human. It is essentially an aqueous solution containing 92% water, 8% blood plasma proteins, and trace amounts of other materials. Plasma circulates dissolved nutrients, such as glucose, amino acids and fatty acids (dissolved in the blood or bound to plasma proteins), and removes waste products, such as carbon dioxide, urea, and lactid acid. Other important components include:

- Serum albumin
- Blood-clotting factors (to facilitate coagulation)
- Immunoglobulins (antibodies)
- lipoprotein particles
- Various other proteins
- Various electrolytes (mainly sodium and chloride)

The term serum refers to plasma from which the clotting proteins have been removed. Most of the proteins remaining are albumin and immunoglobulins

- Narrow range of pH values.

Blood pH is regulated to stay within the narrow range of 7.35 to 7.45, making it slightly basic (Waugh, Anne 2007). Blood that has a pH below 7.35 is too acidic, whereas blood pH above 7.45 is too basic. Blood pH, partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2), and HCO_3- are carefully regulated by a number of homeostatic mechanisms, which exert their influence principally through the respiratory system and the urinary system in order to control the acid - base balance and respiration. An arterial blood gas test will measure these. Plasma also circulates hormones transmitting their messages to various tissues. The list of normal reference ranges for various blood electrolytes is extensive. (lert, Glenn 2012).

- **Red Blood Cells**

Cells are disc shaped concave – sided, and concave surface in order to increase the gas exchange area, its function transport of gases and features, (red blood cells, RBC) can change shape under a given level of applied stress, a flexible membrane cell position to pass even in the narrowest capillaries. Arise from the red marrow in large bones and renewed every 120 days and breaks up in the liver , spleen and go to the bile to participate in contents it has red color to the presence of material hemoglobin consists hemoglobin protein and iron,

See Figure 2.4: show Red Blood cell.

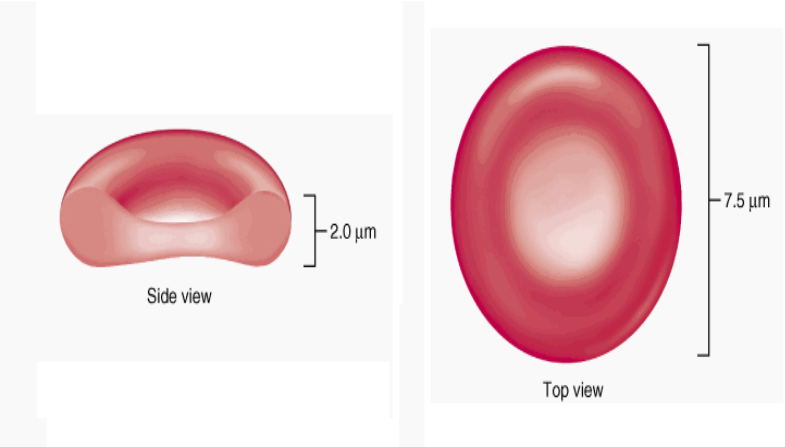

Figure 2.4: Red Blood cell

Shape change of erythrocytes under applied forces (i.e., shear forces in blood flow) is reversible and the biconcave-discoid shape, which is normal for most mammals, maintained after the removal of the deforming forces. In other words, erythrocytes behave like elastic bodies, while they also resist shaping change under deformingforces. This viscoelastic behavior of erythrocytes is determined by the following three properties. Approximation the number of RBC in men's 4 - 5 million in the women's 4 - 4.5 million. Its mission is limited to carrying gas oxygen from the lungs and replace gas carbon dioxide. Build red blood cells are controlled by the kidneys by a hormone called Balaritropuecan and supports the secretion of this hormone on the partial pressure of oxygen in the blood. In the high - altitude partial pressure of oxygen is low so activates the secretion of the hormone Balaritropuecan which raises the red blood cell concentration of the population of the areas Alajabilh.

Red blood cell immature contain a nucleus and mitochondria and Golgi complex and Alraibusomat and grow these cells divided split evenly so as to give the mature red blood cell after losing the nucleus and other organelles to make the largest possible area of the pigment hemoglobin.

- **White cells:**

White blood cells are the cells that provide protection to the body from disease and their number is less than red blood cells as it's between seven hundred and fourteen erythrocytes find white balls and one as she varying sizes and shapes, with one core as it is larger than red blood cells; of between (5000-10000) cell in a cubic millimeter. And it is one of the most important means of defense of the antigens (antibody generators) in the body and their number is increasing at the disease. Figure 2.4 shows the white blood cells Shmukler, Michael (2004)).

There are five types of white blood cells, are: acidic and basic and neutral and lymphatic; and only it was broken by the appearance of the cytoplasm.

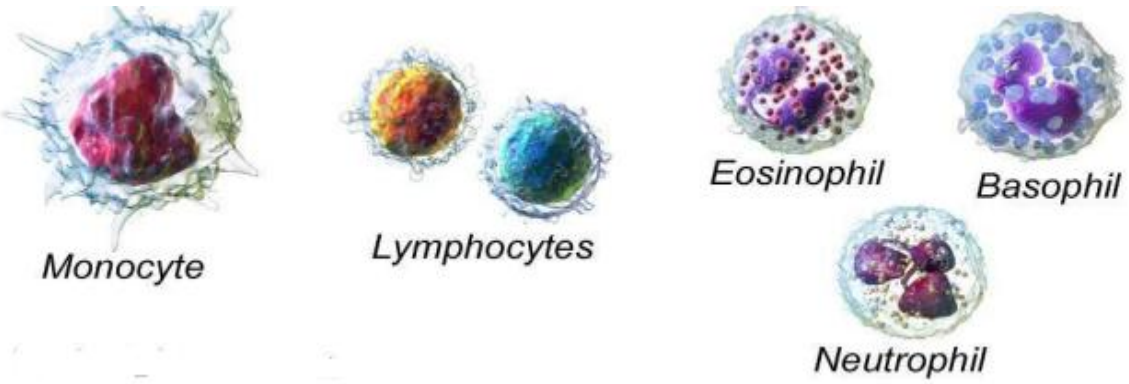

Figure2.5: White Blood Cells

The nucleus to form two groups:

1. Granulocytes cells: These are large and cytoplasm granular and nucleus consisting of several lobes, and these cells vary in acceptable dyes include neutral and acidic and basic.

2. Non - grainy cells: the appearance of the cytoplasm is granular nuclei and is divided into lobes; and includes lymphatic.

- **Platelets:**

Antibodies Sitoblazm found in the blood and broken on contact with the air of a blood clot until the bleeding does not cause damage to not have a specific form of atenslq slide second nature in the blood as long as the constant velocity of blood do not change and are found in a natural person by a quarter of a million per mm 3 primary role is to convert liquid material protein in the blood It Alfbarungan into a solid substance called Alvberen a stiff. Filaments clustered around the surface to prevent cutaneous blood out of the skin. Figure 2.5 shows the platelts.

There is also the question: Why do not the blood clot inside the blood vessels? Answer: because the blood left naturally and also heparin substance secreted by the liver which stops platelets work and learned the platelets break from the liver and spleen every 10 days to regenerate constantly and can say that it is the bodies' phones because it breaks down.

Figure 2.6: Platelets

2.3.1 Blood functions

- **Oxygen transport**: blood carries oxygen from the lungs to the tissues, as well as carbon dioxide generated by the activity of the tissues to the lungs in the air exhaled.

- **Nutrition:** Holds Blood primary nutrients that are absorbed by the intestine to different cells to beused in necessary for the activity of the body energy production.
- **Directed by waste process**: the blood to carry harmful waste remaining as a result of metabolism in the body and through the output devices Kalkly skin Vikhals including the body through urine and sweat.
- **Immune:** contains blood on the white blood cells as it produces antibodies that play a key role in protecting the body and protect him from disease.
- **The water balance of the body** helps the blood in maintaining water balance in the body to carry excess water to output devices so that there is a balance between what we get from the water through food and drink and what we lose through urine and sweat.
- **Regulate body temperature:** the blood absorbs heat from the interior and muscular members and as it moves them to external users, and under the skin the body can get rid of excess heat by radiation and pregnancy and evaporation. Transfer hormone the blood transfer hormones hormones signs of damage to the tissue.

2.3.2 Blood types

It was thought by the early twentieth century that the blood is one and identical among all human kind and often were attempts transfusion of blood from persons proper for patients lead to the death of patients which led to the prevention of blood transfusions for long periods in Europe. And even the world of the Austrian Karl Landsteiner in 1902 when he noticed the death of some patients when blood transfusion, the discovery of the so - called Alantginat in the blood, which is about glycoprotein's present on the surface of the red blood cell, were divided blood groups later into four types are A, B, AB and O, Are determined blood group genetically where there humans are two types of gene type a and type B , and when there are both types a and B with the DNA of this person be coterie blood AB either if you find the gene

an only Vzmrh his blood is and in the same way for the group of blood B but in the absence of either of these Morttin be blood group O (Alberts, Bruce (2012).

A worker Alraisesa RH since there is another kind of glycoproteins (antigen) on the surface of the red blood cell, and named for the monkey Alraisesa it carries this factor, and attached to each of these types positive signal (+) or negative (-) denotes where the signal (+) to the presence of additional protein symbol RH and the reference (-) symbolizes the absence of this protein and blood cliques positive because it is more prevalent genetic characteristics prevalent Alberts, Bruce (2012).

2.3.3 Blood Test and Results

It is a medical procedure indispensable to diagnose the health condition of the human person and the diagnosis of many diseases. You can infer information about an individual's health and habits of one point from their blood as follows:

- **Nutrition:** eat a lot of meat has increased cholesterol but do not be required to have the level of cholesterol in the blood embarrassment. Cholesterol purifies the blood vessel walls of the etiology of calcification and go out to the liver for disposal.
- **Age:** on the outskirts of chromosomes Almokhodh of blood cells exist Taylomirat maintain it. These telomeres length decreases with age (limit multiple cell divisions). It is thus a way to tell how old a person is biological.
- **Viruses:** the screening for anti - virus in the blood, which is formed by the immune system to resist the virus. Many of the tests do not show the presence of viruses in the blood directly.

- **Fitness:** Set the concentration of lactate resulting from the metabolism in the blood can be drawn fitness of a person. Marathon runner, for example, a rate of lactate in the blood is less than that in the non - athletes.
- **Pregnancy:** A few days after the fertilized after the egg begin hormone CG to appear in the blood. It also appears in the urine, but after two weeks. However, in rare instances can be a tumor is causing is the high concentration of this hormone in the blood.
- **Platoon blood:** There are four factions of blood, followed by each individual species of them. Those factions are: A, B, AB, O and distinguish certain types of proteins that cover the red blood cells ; and these proteins are not found in type O blood.
- **Alcohol:** The number of white blood cells is reduced because of it frequent drinking alcohol spoils the blood. As part of the liquor destroy red blood cells and grow in size. And measured those changes by setting the value of MCV.
- **Rheumatology:** when the immune system to resist the healthy cells of the body itself, Know autoimmune. In people who have that property left in their blood against the so- called ANA.
- **Thyroid:** the ability to concentrate may be suffering from poverty in the function of the thyroid gland. People who labor quickly goes down they have In that case. It appears in the blood high proportion of hormone TSH.
- **Cancer**: moving in the circulator y system, tumor caused by dead cells there are ways to be tested in the blood. They can see through genetic Tgieradtha know cancer causing type.
- **Anguish:** the secretion of the body of the hormone ncorticosterone, which can be set in the blood, in the case of stress increases. However, the high value of cortisone may arise from a reduction in blood sugar or a carrying case (Martini, Frederic; et al. 2007).

2.4 Laser Matter Interaction

When light radiation interacts with matter, it may be reflected, absorbed, or transmitted. Figure 2.6 represent the light interaction with matter.

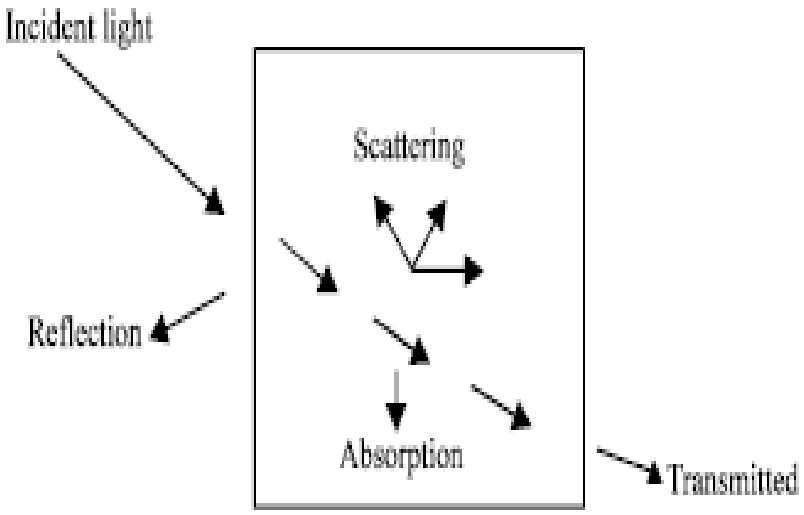

Figure 2.7: laser matter interaction

Reflected, refracted, scattered, absorbed or transmitted when laser light strikes a tissue surface.

1. Reflected
2. Scattered
3. Transmitted
4. Absorbed

2.4.1 Reflection

Reflection of electromagnetic radiation is occurs when the waves encounter a surface or other boundary that does not absorb the either at the boundary between two medium (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 waves. 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.

Reflection of light is either secular (mirror-like) or diffuses depending on the nature of the interface. In Secular reflection the phase of the reflected waves depends on the choice of the origin of coordinates.

Diffuse reflection occurs when light incident the surface of a (nonmetallic) 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 (Lekner, John (1987).

2.4.2 Light Scattering

Scattering occurs when the frequency of electromagnetic wave not matching to particle neutral frequency; Can be thought of as the deflection 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.

The resulting oscillation is determined by vibration. Also, the phase of the forced vibration differs from the incident wave, causing photons to slow down when penetrating into a denser medium. Hence, scattering can be regarded as the basic origin of dispersion. The incident photon energy determined Elastic and inelastic scattering is converted during the process of scattering. Elastic scattering does not change in energy incident and scattered photons have the same energy.

A special kind of elastic scattering is Rayleigh scattering. Its only restriction is that the scattering particles be smaller than the wavelength of incident radiation. In particular, we will find a relationship between scattered intensity and index of refraction, and that scattering is inversely proportional to the fourth power of wavelength. 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 (VandeHulst .H.C 1981).

2.4.3Transmission

when electromagnetic radiation strike to a surface of material pass through it this called transmission. The transmittance of a material is the proportion of the incident (approaching) light that moves all the way through to the other side.

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**.**

2.4.4 Absorption

when light radiation of a given frequency incident on a material with electrons having the same vibration frequencies, then those electrons will absorb the energy of the light wave and transform it into vibration motion. During its vibration, the electrons interact with neighboring atoms this manner convert its vibration energy into thermal energy. Then, 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 method in which the energy of a photon is receives by matter, typically the electrons of

an atom. Thus, the Electromagnetic energy is transformed into internal energy of the absorber, for example thermal energy (VandeHulst .H.C 1981).

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 the Beer–Lambert law.

Lambert's law and Beer's law, and are expressed by

$$I_{(z)} = I_0 \exp(-\alpha z) \quad (2.3)$$

And

$$I_{(z)} = I_0 \exp(-k\, cz)\,, \quad (2.4)$$

where z denotes the optical axis, I(z) is the intensity at a distance z, I0 is the incident intensity, α is the absorption coefficient of the medium, c is the concentration of absorbing agents, and k depends on internal parameters other than concentration (Markolf H. Niemz1996).

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. Each tissue has specific absorption characteristics base on its composition and chromophore content. The principal chromophores present in mammalian tissue are:

- Hemoglobin
- Melanin
- Water
- Protein

Infrared light is absorbed primarily by water, while visible and ultraviolet light are primarily absorbed by hemoglobin and melanin, respectively. As wavelength decreases toward the violet and ultraviolet, scatter or absorption from covalent bands in protein limits penetration depth in the range (Mayers RA 2011).

Figure 2.8: shows Absorption of the Main Chromophere in mammalian tissue are: Hemoglobin , Melanin, Water.

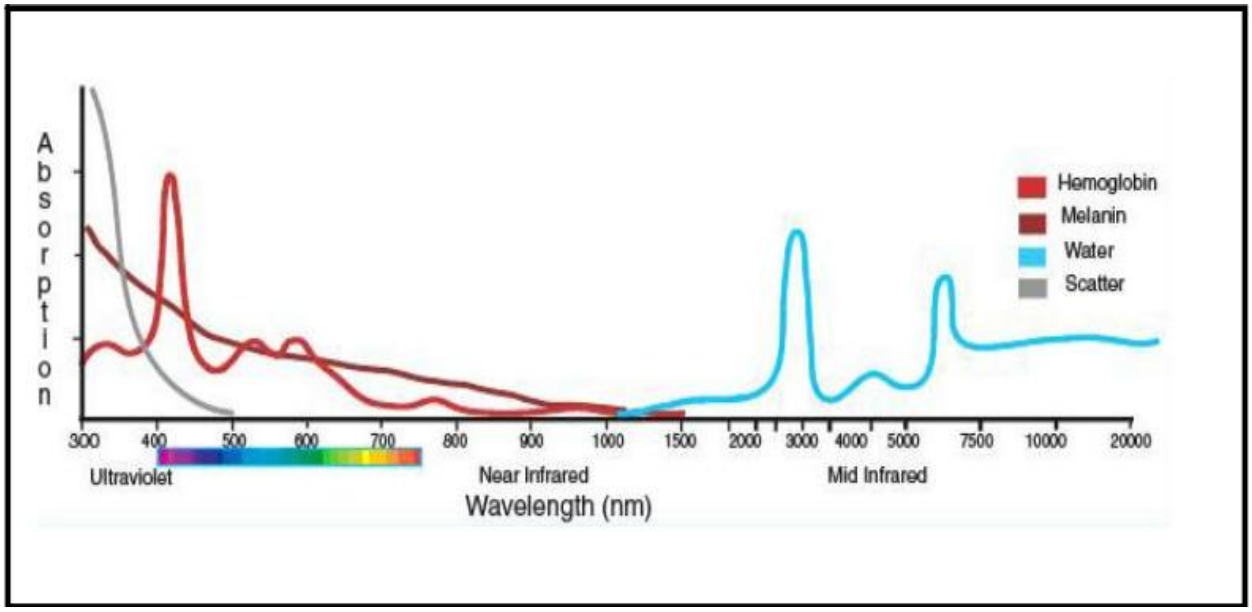

Figure 2.8: Absorption of the Main Chromophere.

The penetration of the laser beam in depth depends upon the laser wavelength, color and the tissue consistency, the beam power, duration of beam exposure, and beam spot size. As the laser beam pass through tissue, it will continue to heat and destroy tissues in deep; If there is concern about accidental damage to adjacent tissues (Kaya Ball). the describe of the main four laser-tissue interactions, which are used in medicine.

In the following the lasers used and the most important medical applications will be presented. The interactions are based on the absorption of radiation by the water contained in the tissues, by hemoglobin in the blood and pigments or chromophore normally (or externally administered) present in some tissues.

- **The photochemical interaction:**

occurs when the energy of photons is greater than that of chemical energy bond, typically greater than about 5 eV; occurs at very low levels of intensity or irradiance (I ~ 1 W/cm2)) and for high exposure times (duration time greater than a second). Structural modifications of the molecules existing occur when the energy absorbed in the tissue is very high it is to achieve an ablation If the energy density deposited is very high (M.J.C. van Gemert ,1989).

The chemical effect of some lasers is produced as the laser energy activates light-sensitive drugs to disrupt and change tissue. This process is used in photodynamic therapy to selectively destroy malignant cells

The energy absorbed in the tissue is used for the production of new substances as a result of chemical reactions triggered by laser radiation. In this case, the light energy is used to excite a particular chromophore (molecules which confer a particular color to a substance and which absorb a specific wavelength) which gives rise to a complex biological process whose final products may have therapeutic relevance: the molecule acts with energy transfer functions, after having undergone a photoexcitation. A chromophore able to produce photoinduced reactions in molecules which themselves do not absorb light in the same spectral region is said photosensitizer. An example is the photodynamic reaction (PDT) (M.J.C. van Gemert, 1989).

- **Photoablation**: Type of laser-tissue interaction Photoablation was first discovered by Srinivasan and Mayne-Banton in the year 1982. They identified it as ablative photodecomposition, meaning that material is decomposed when exposed to high intense laser irradiation. It occurs when the energetic photons of the laser light decompose the molecules by breaking the chemical bonds. In this interaction, photoablation is due to the "volume stress" as a result of bond breaking. Typical threshold values

of this type of interaction are 107-108 W cm^{-2} at laser pulse durations in the nanosecond range (Beesly1978, karu 1999).

- **Photothermal interaction**, approximately 85% of lasers used today produce a thermal effect at the tissue level. These lasers cut, coagulate, vaporize, and ablate tissue from the interaction site where the thermal response originates.

  laser- tissue impact site there is a zone of vaporization. Immediately adjacent to this site is a zone of necrosis caused by the thermal spread. Farther from the impact site is a zone of coagulation as thermal injury decreases see Figure 2.9: Thermal Zones after Laser Impact

  The mechanical effect on tissue is produced by some lasers as the laser beam generates sonic energy that mechanically disrupts tissue. Breaking apart kidney stones in the ureter or disrupting the posterior capsule within the eye are examples of this type of mechanical effect. (Gupta et al,2018).

  Table2. 1 notes the changes that occur as the laser beam is absorbed.

Table 2.1: Tissue changes with temperature increases

Temperature	Visual change	Biological change
37-60° C	No visual change	Warming, welding
60-65° C	Blanching	Coagulation
65-90° C	White/grey	Protein denaturization
90-100° C	Puckering	Drying
100° C	Smoke plume	Vaporization, carbonizatio

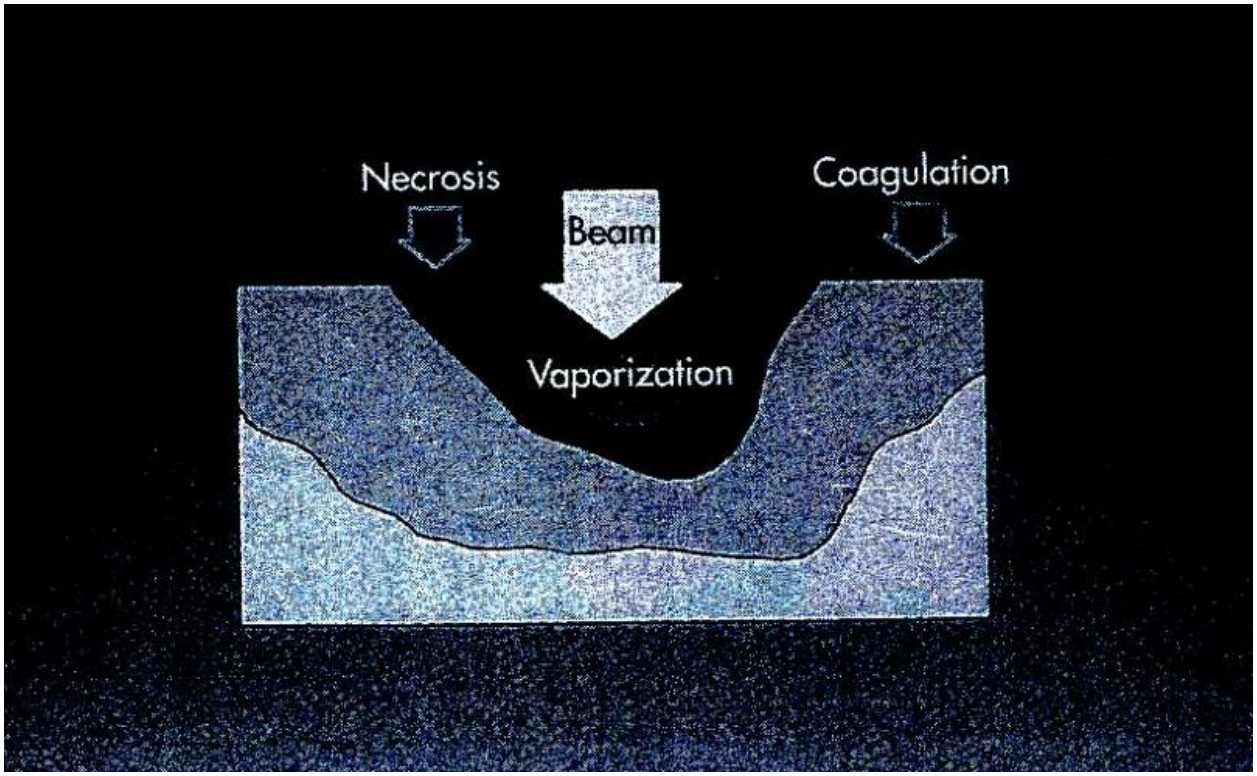

Figure 2.9: Thermal Zones after Laser Impact.

2.5 Spectroscopy

The visible region very small part of electromagnetic waves spectrum, broad consisting of a range from gamma rays to radio waves, Our eye has ability to detect different color in visible range restricted when to study interaction electromagnetic wave with matter we need specialized instrument developed, which are called spectroscopes. Different ranges of electromagnetic spectrum are developed Different regions of spectrum have different instruments associated with them. The change in electronic states is associated with Xrays, UV and Visible region, change in vibration and rotational states can be studied with Infrared measurements and similarly nuclear spins can be studied in radio wave region. Spectroscopic techniques such as UV/Visible, IR spectroscopy, X-ray diffraction, X-ray fluorescence and NMR. it used 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 (Robert Silverstein, M.2006).

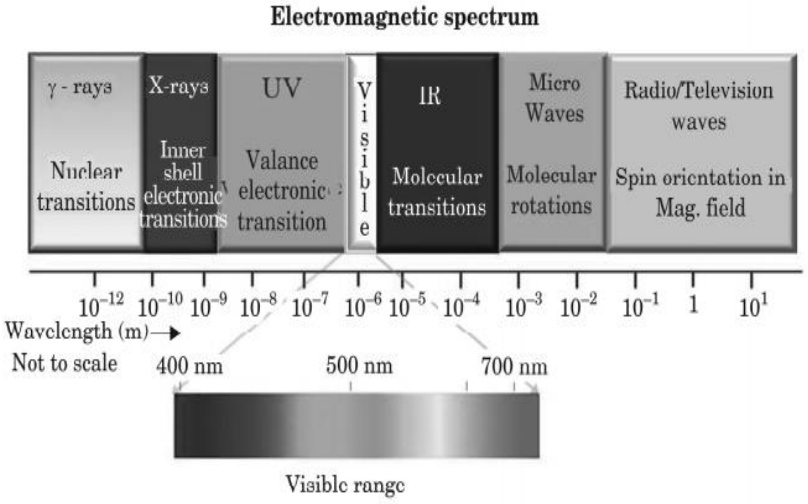

Figure 2.10: Electromagnetic spectrum

The energy of electromagnetic radiation is defined by the following equation

$$E = hv \qquad (2.5)$$

Where E is energy (in joules), h is Planck's constant (6.62 × 10-34 Js), and v is frequency (in Hertz). Electromagnetic radiation can be considered a combination of alternating electric and magnetic fields that travel through space with a wave motion. Because radiation acts as a wave, it can be classified in terms of either wavelength or frequency, which is related by the following equation:

$$v = c/\lambda \qquad (2.6)$$

Where ν is frequency (in Hertz), c is the speed of light (3×10^8 ms-1), and λ is wavelength (in meters). In UV/Visible spectroscopy, wavelength usually is expressed in nanometers (1 nm =10^{-9} m). we find from the above equations that radiation with shorter wavelength has higher energy. In UV/Visible spectroscopy, the low-wavelength UV light has the highest energy. In some cases, this energy is sufficient to cause unwanted photo chemical reactions when measuring sample spectra (remember, it is the UV component of light

that causes sunburn). When light radiation interacts with matter, it may be reflected, absorbed, fluorescence/phosphorescence or transmitted (absorption and transmission), and photochemical reaction (absorbance and bond breaking). when use UV/Visible spectra, we want only absorbance to occur. Because light is a form of energy, absorption of light by matter causes the energy content of the molecules (or atoms) to increase.

The total potential energy of a molecule generally is represented as the sum of its electronic, vibration, and rotational energies:

$$\text{Total} = E_{\text{electronic}} + E_{\text{vibrational}} + E_{\text{rotational}}$$

The molecule has energy amount in each form is not a continuum but a series of discrete levels or states. The differences in the energy among the different states are in the order:

$$E_{\text{electronic}} > E_{\text{vibrational}} > E_{\text{rotational}}$$

UV and visible light photons have enough energy to cause transitions between the different electronic energy levels; this is in some molecules and atoms. The wavelength of light absorbed is that having the energy required to move an electron from a lower energy level to a higher energy level (Daniel Harris et al 1989).

2.5.1 UV/Visible spectrometer:

Is there many molecules and atoms absorb visible, ultraviolet radiations in different frequencies. The absorption of radiation give us the important information related to the identification of atoms and molecules present in the matter, for example, the absorption bands in UV and visible range matches to excitation energy of electrons in the atom. UV/Visible spectrometer refers to 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.

The major components of a spectrophotometer are:

(a) Source: The component used to generate radiation is known as source. Sun is the best example of radiation. In the instrumentation these radiations are generated in a particular band of wavelengths.

(b) Slits: A slit is used to control the light beam and to collimate it. It plays a vital role in controlling unwanted radiations and attaining high resolutions in the spectrometer.

(c) Monochromator: This is used to separate out monochromatic radiation from a bundle of polychromatic radiation. In the visible range, a glass prism is it's the best example as it splits white light into a spectrum. In spectroscopy, the prism is very effectively

(d) Detector: The detector is a device which converts the radiation energy into the electrical signal. It is the intensity of radiation which is subsequently digitally processed and produced at the output of the instrument.

(e) Electronic processing unit: Any modern spectrometer is not complete without electronic control and processing. All the movable parts are controlled by motors managed by computer.

Absorption measures transitions from the ground state to the excited state. This technique is complementary to fluorescence spectroscopy, the fluorescence deals with transitions from the excited state to the ground state.

A hydrogen, deuterium or discharge lamp covers the ultraviolet range, and 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 spectrometer cells made of pure silica. The radiation passing through the sample or reference cell can be Detected either photomultiplier or photo diode,that

converts photons of radiation into tiny electrical currents; or semiconducting cell (that emits electrons when radiation is incident onit) 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 (Michael Hollas, J. 2003). See Figure 2.11: block diagram of UV Visible spectrometer.

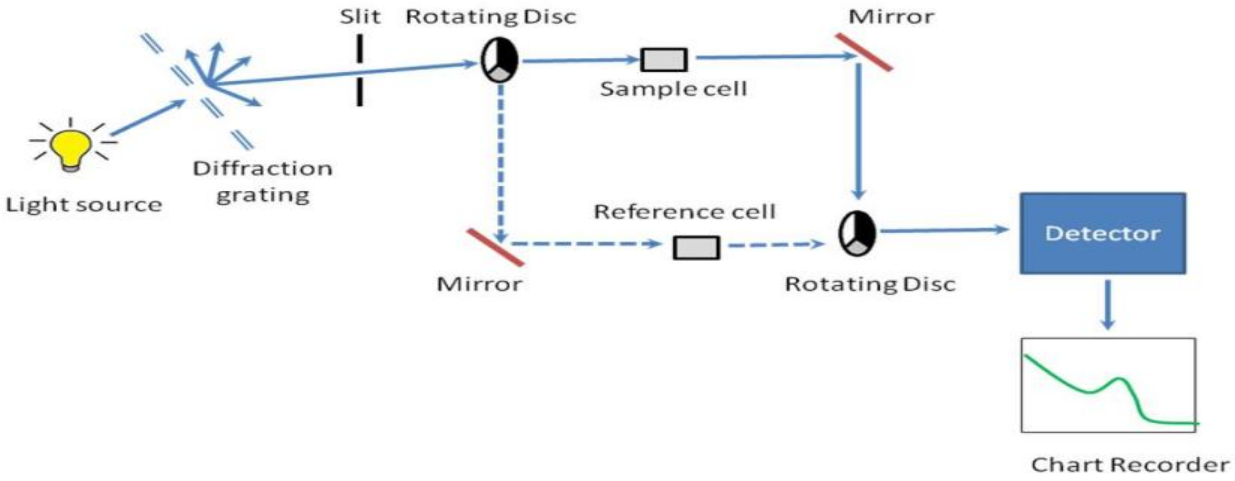

Figure 2.11: block diagram of UV Visible spectrometer

2.5.2 Fourier Transform Infrared (FTIR):

Fourier-transform infrared spectroscopy (FTIR) is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. Infrared light is guided through an interferometer and then through the sample (or vice versa). A moving mirror inside the apparatus alters the distribution of infrared light that passes through the interferometer.

The signal directly recorded, called an "interferogram", represents light output as a function of mirror position. a mathematical process called is required to convert the raw data into the actual spectrum, (the sample's spectrum): Light

output as a function of infrared wavelength (or equivalently, wavenumber). The spectrum is produced by comparing the currents generated by the sample and the reference beams (Brault, James W., 1996).

FTIR has Biological materials application such as used to investigate proteins in hydrophobic membrane environments. Studies show the ability of FTIR to directly determine the polarity at a given site along the backbone of a transmembrane protein (Manor, Joshua et al 2012). See Figure 2.12: Fourier transform infrared spectrometer

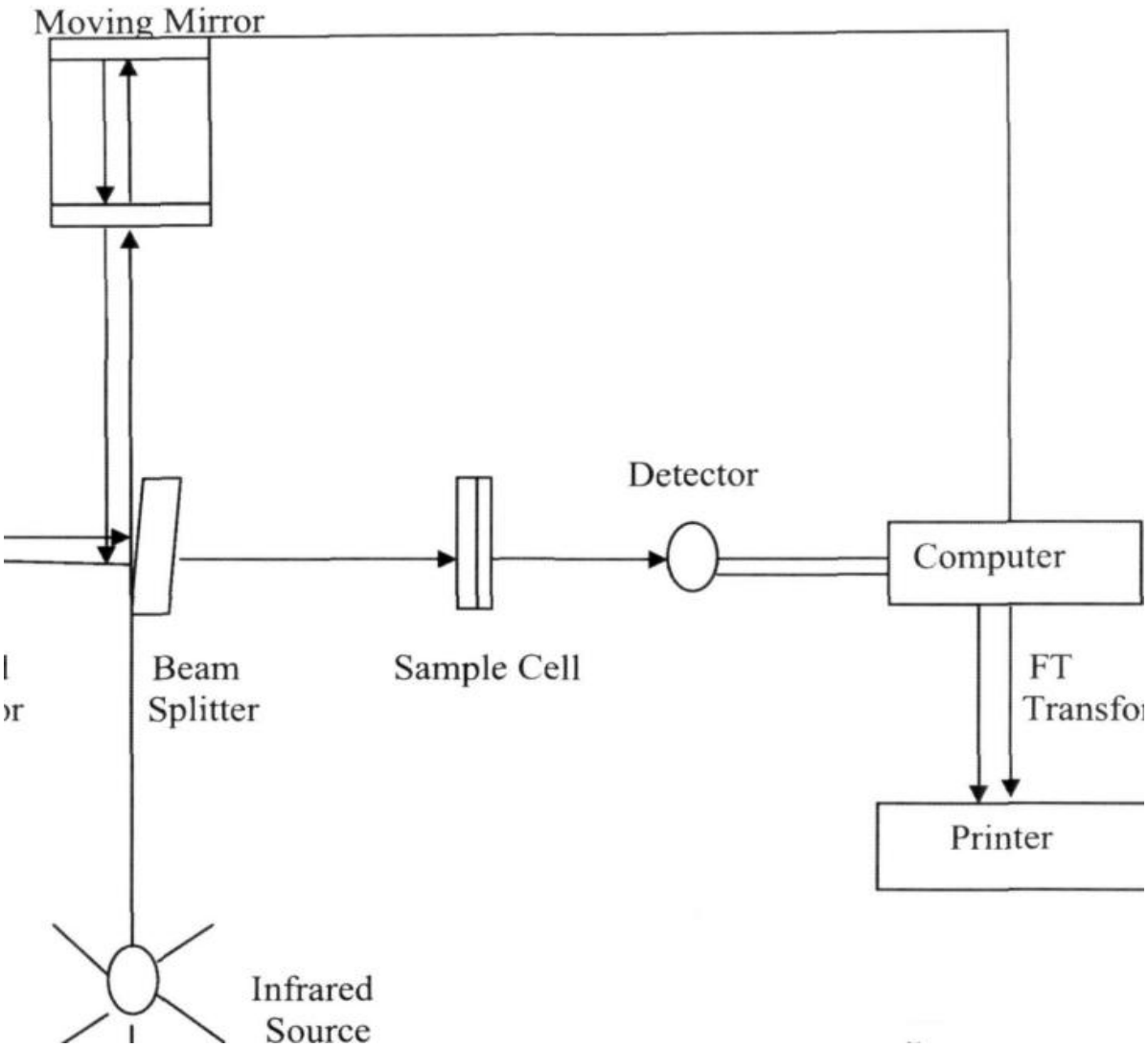

Figure 2.12: Fourier transform infrared spectrometer

scanning monochromatic method is an alternate method for acquiring spectra is the "dispersive" In this approach, to be a sample irradiated sequentially with various single wavelengths. The dispersive method is more common in UV/Vis spectroscopy, but is less practical in the infrared than the FTIR method. One reason that FTIR is favored is called "Fellgett's advantage" or the "multiplex advantage": The information at all frequencies is collected simultaneously, improving both speed and signal-to-noise ratio. Another is called "Jacquinot's Throughput Advantage": A dispersive measurement requires detecting much lower light levels than an FTIR measurement. There are other advantages, as well as some disadvantages, but virtuallyall modern infrared spectrometers are FTIR instruments (Soran Shadman.et al ,2016)

CHAPTER THREE

EXPERIMENTAL PART

3.1 Introduction

This chapter includes the materials used in this work and the following methods (sample preparation and setup) and the procedure.

3.2 Materials

3.2.1 Blood samples

Blood samples were collected from normal human subjects. the samples were irradiated by Helium-Neon laser operating in continuous wave mode, as a radiation source (632.8 nm, 2 mW), for (10, 20, 30, 40 , 50, 60, 70,80, 90 and 100) minutes. The samples of blood were obtained from tine volunteers, and each sample was divided into two samples (1 ml for every one) for irradiation and control.

3.2.2 Instruments

3.2.2.1 Laser Power 1mW

Helium-Neon laser number (08181,93) is founded in optics lablotory . It used in many applications, the specification of laser Helium-Neon laser Wavelength (632.8 nm, 1mW), Output power, 2 Laser Classification, 220V/35VA.made in Germany by PHYWE.

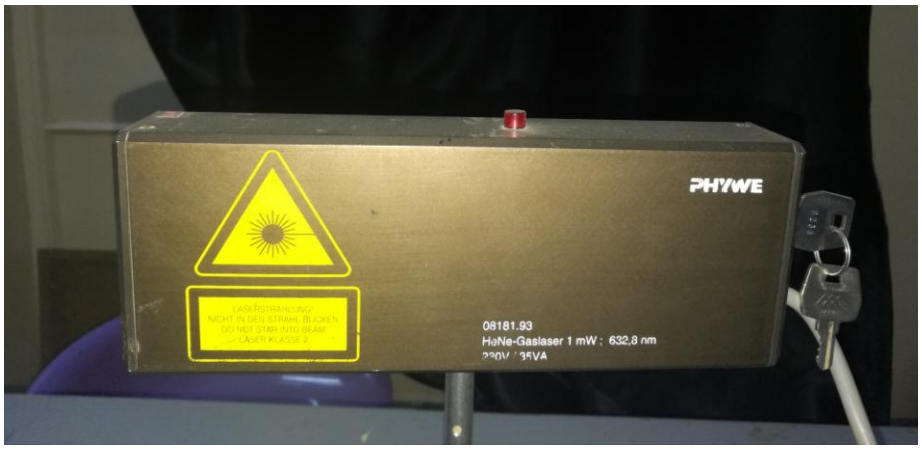

Figure 3.1: He-Ne Laser (632.8 nm, 1 mW)

3.2.2.2 Laser Power 2mW

Helium-Neon laser number (08181, 93) is founded in optics laboratory. It used in many applications, the specification of laser Helium-Neon laser Wavelength (632.8 nm, 2 mW), Output power, 2 Laser Classification. Din: 58126220V/35VA , made in Germany by PHYWE

Figure 3.2: He-Ne Laser (632.8 nm, 2 mW)

3.2.2.3 SP Uv-26 Spectrophotometer

The UV/VIS device was used to measure the absorption of the solution before and after irradiation by UV light. It is covering a wavelength from 190-1100 nm with auto lamp switch from visible to ultraviolet. The UV spectrophotometer used here was supplied from SP UV-26 (Sco TECH); made in Germany, contains a quartz cell of thickness 1 cm as a sample holder.

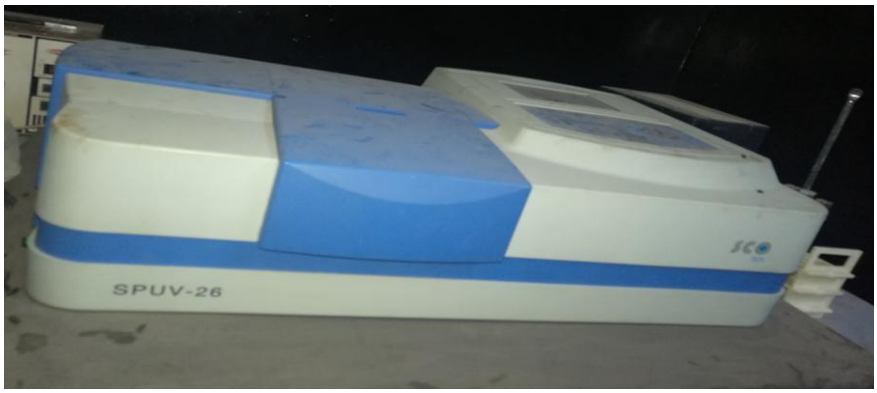

Figure 3.3: SP UV-26 Spectrophotometer

3.2.2.4 FTIR spectrometer:

The Fourier transform infrared spectrometer used Shimadzu Spectrometer made in Japan. This instrument has a thermal source, KBr beam splitter, and DLATGS IR detector. The FTIR spectra of the samples were obtained in the spectral range 4000 to 400cm-1 with scanning speed of 2mm/sec and resolution of 4cm-1. Aphotograph of the FTIR system is shown below.

Figure 3.4: FTIR Spectrophotometer

3.3 Methods

3.3.1 Sample Preparation

Blood samples were taken from healthy volunteers; 3 ml of each volunteer by medical standard laboratory conditions and blood samples were saved in a tube to prevent from coagulation to (EDTA) and each sample was divided into three samples one sample was control and other exposed to the helium-neon laser power 1mW and power 2mW with different exposure times. (see fig.3.5)

Figure 3.5: Blood sample

3.3.2 Sample Exposure to Helium-Neon Laser

Samples were exposed to a Helium-Neon laser beam, operating in continuous wave mode, as a radiation source (632.8 nm, 2 mW) and radiation source (632.8 nm, 1 mW), for (10, 20, 30, 40, 50, 60,70,80,90 and 100) minutes The distance between the laser source and the samples was set to be 10 cm and the diameter of a laser spot was chosen to be 1.5 cm. To studied the effect of

Laser radiation was used UV/Vis spectrophotometer (**SP** UV-26**)** and Fourier Transform Infra Red Spectra (FTIR) was obtained used FTIR spectrophotometer (Shimadzu) for control, and He-Ne laser irradiated blood serum samples.

FTIR spectra of sera samples were recorded in the frequency range 4000 – 450 cm^{-1} on using Shimadzu at Central Laboratory University of Khartoum. IR transparent Thallium Bromide material without the serum was scanned as the background for each spectrum and 16 scans were co-added at a spectral resolution of 1 cm-1. FTIR spectra were obtained by spreading a small volume of serum on a Thallium Bromide plate (IR transparent material) and

allowed to dry for few minutes to remove the water bands. To minimize problems from avoidable baseline shifts, the spectra were baseline corrected and normalized.

UV-Vis spectra. Blood were diluted with normal saline and placed in Kartell disposable polystyrene cuvette of 10 mm path length. The cuvette is placed in SP Uv -Vis spectrophotometer for analysis The spectra were scanned in the region between 300nm to 800nm using Uv-26) at Laser institute laboratory, SUST, Khartoum.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Introduction

This chapter summarizes results obtained during the work. Results include photographs, figures, and tables as shown below. In addition of experimental results, data fitting was also shown, beside discussion and conclusion

4.2 Results

The following tables and figures shows the UV&FTIR spectra for all samples irradiated by different laser powers at different exposure times

4.2.1 UV/Vis spectra results

Table 4.1 and Table 4.2 show the values of UV-vis spectrophotometer Absorbance of normal and irradiated blood samples with (He-Ne) Laser power 2 mW at different exposure times.

Table 4.1: The Absorbance of normal and irradiated power 2 mW samples

Wave length(nm)	Absorbance a.u					
	control	10 min	20 min	30 min	40 min	50 min
340	1.253	1.01	0.933	1.065	1.12	0.868
416	2.604	2.49	2.391	2.501	2.538	2.347
542	0.755	0.633	0.536	0.614	0.699	0.492
576	0.793	0.653	0.547	0.633	0.718	0.525

Table 4.2: The Absorbance of normal and irradiated to power 2 mW samples

Wave length(nm)	**Absorbance (a.u)**					
	control	**60 min**	**70 min**	**80 min**	**90 min**	**100 min**
340	1.253	1.064	0.819	1.285	0.726	1.139
416	2.604	2.491	2.274	2.610	2.001	2.518
542	0.755	0.641	0.473	0.778	0.425	0.715
576	0.793	0.651	0.481	0.800	0.435	0.733

Table 4.3 and Table 4.4 show the values of UV-vis spectrophotometer Absorbance of normal and irradiated blood samples with (He-Ne) Laser power 1 mW at different exposure times.

Table 4.3: The Absorbance of normal and irradiated power 1 mW samples

Wave length(nm)	Absorbance a.u					
	Control	10 min	20 min	30 min	40 min	50 min
340	1.253	1.548	1.035	0.904	1.386	1.081
416	2.604	2.462	2.462	2.387	2.676	2.495
542	0.755	0.632	0.632	0.520	0.866	0.622
576	0.793	0.641	0.641	0.548	0.883	0.651

Table 4.4: The Absorbance of normal and irradiated to power 1 mW samples

Wave length(nm)	Absorbance a.u					
	Control	60 min	70 min	80 min	90 min	100 min
340	1.253	1.073	01.429	0.435	0.960	1.298
416	2.604	2.528	2.699	1.210	2.415	2.603
542	0.755	0.651	0.898	0.275	0.548	0.754
576	0.793	0.679	0.921	0.266	0.576	0.773

Figure 4.1 show The spectra of UV-vis spectrophotometer and relation between Absorbance (a) and wavelength (λ) for whole blood before irradiated to (He-Ne)laser

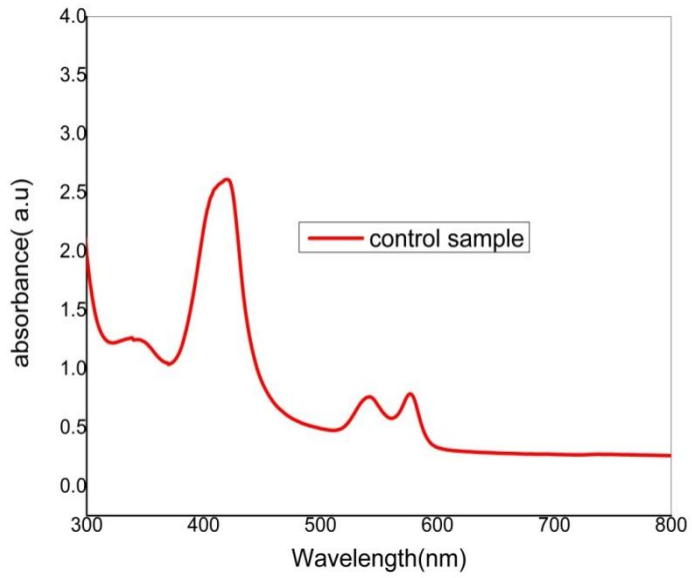

Figure 4.1: The relation between Absorbance (a) and wavelength (λ) for whole blood before irradiated to (He-Ne)laser

Figure 4.2 and Figures 4.3 show The spectra of UV-vis spectrophotometer and relation between Absorbance (a) and wavelength (λ) for whole blood

before and after irradiated to (He-Ne)laser power 2 mW at exposure times from (10 to 100) minutes.

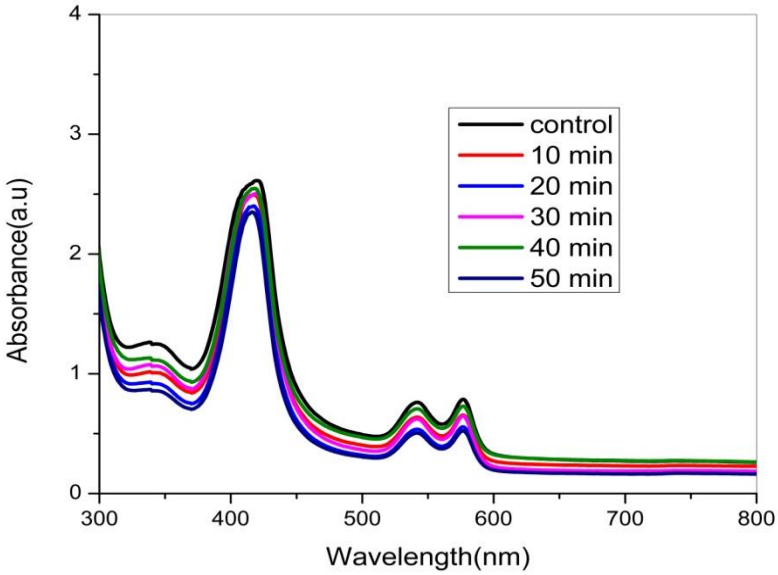

Figure 4.2: Relation between Absorbance (a) and wavelength (λ) for whole blood before and after irradiated to (He-Ne) laser power 2 mW

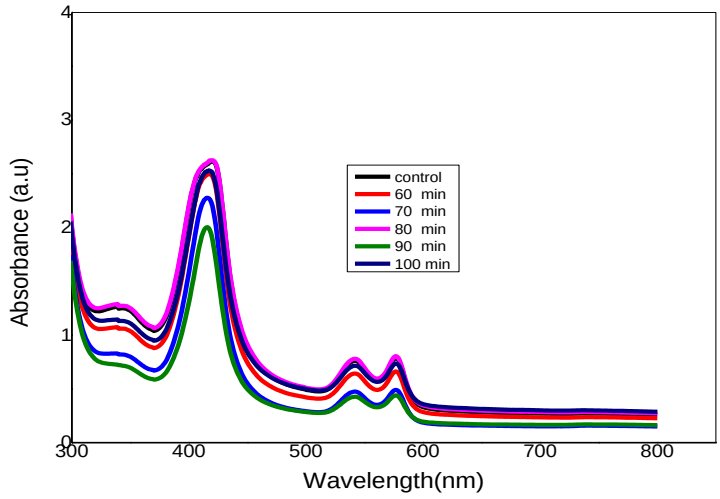

Figure 4.3: Relation between Absorbance (a) and wavelength (λ) for whole blood before and after irradiated to (He-Ne) laser power 2 mW

Figure 4.4 and (4.5) Figure show The spectra of UV-vis spectrophotometer and relation between Absorbance (a) and wavelength (λ) for whole blood

before and after irradiated to (He-Ne)laser power 1 mW at exposure times from (10 to 100) minutes.

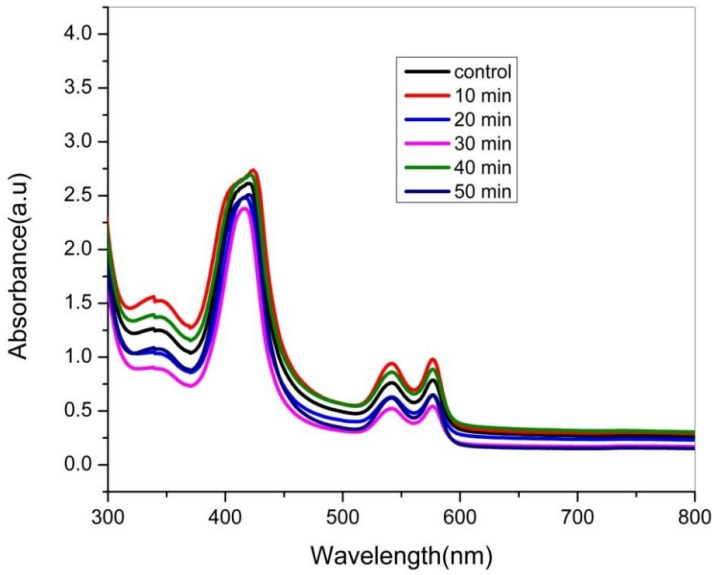

Figure 4.4: The relation between Absorbance (a) and wavelength (λ) for whole blood before and after irradiated to (He-Ne) laser power 1 mW at difference exposure time 10,20,30,40 and 50 minute

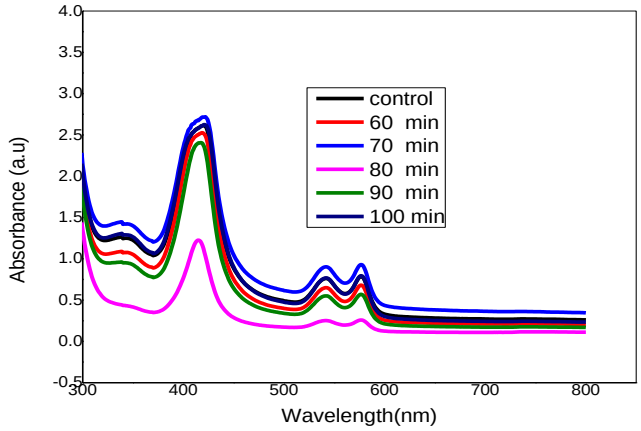

Figure 4.5: The relation between Absorbance (a) and wavelength (λ) for whole blood before and after irradiated to (He-Ne) laser power 1 mW at difference exposure time 10,20,30,40 and 50 minute

Figure 4.6 and Figure 4.7 show Absorption intensity of light at 340nm with different irradiation times for blood samples before and after irradiated to He-Ne laser (λ= 632nm, power=2mW).

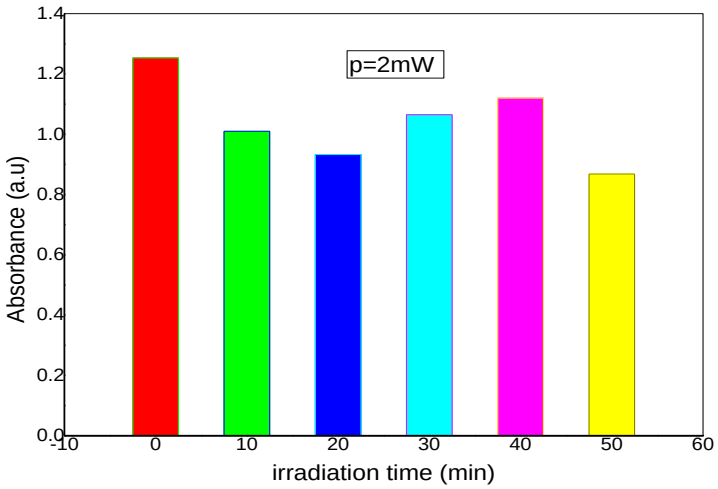

Figure 4.6: Absorption of light at 340nm with different irradiation time

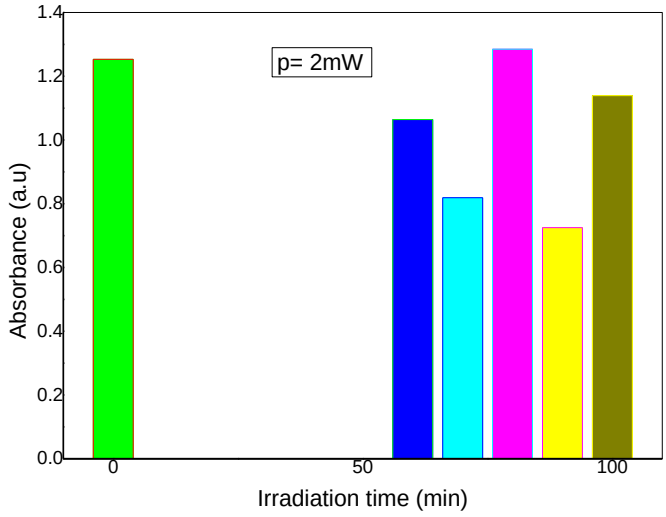

Figure 4.7: Absorption of light at 340nm with different irradiation time

Figure 4.8 and Figure 4.9 show Absorption intensity of light at 340nm with different irradiation times for blood samples before and after irradiated to He-Ne laser (λ= 632nm, power=1mW).

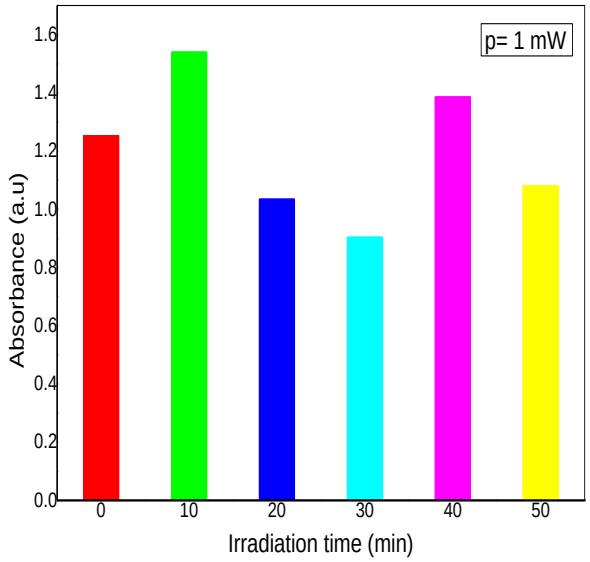

Figure 4.8: Absorption of light at 340nm with different irradiation time

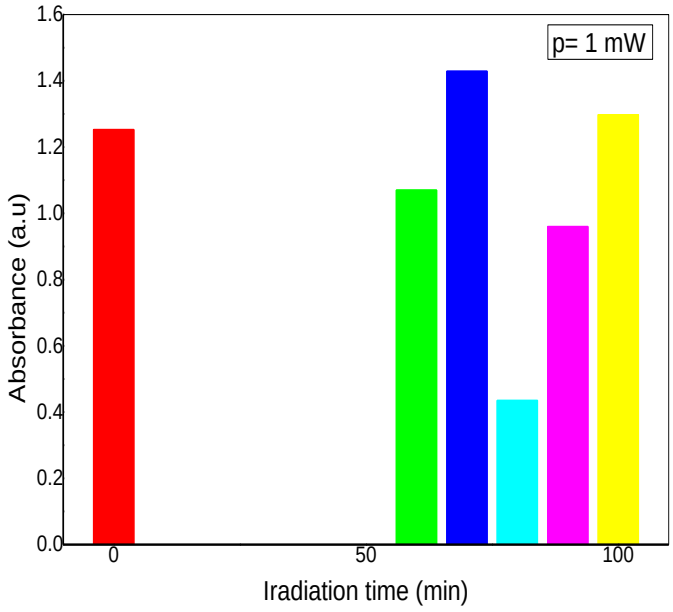

Figure 4.9: Absorption of light at 340nm with different irradiation time

4.2.2 FTIR Spectra results

Table 4.1 show the values FTIR spectral data (wave number, function group and transmission) for normal blood control **.**

Table 4.5: FTIR spectral data (wave number, function group and transmission) for normal blood control

FTIR spectral data for normal blood (control)			
Sr. No	**Wave number** cm^{-1}	**Group**	**% T**
1	3444.63	O-H	0.48
2	1650.95	C=O	1.19
3	1548.73	N=O	6.36
4	1452.30	C-H	14.26
5	1317.29	N-H	15.3
6	1168.78	C-O	17.12

Table 4.6 and Table 4.7 show FTIR spectral data (wave number, function group and transmission) for irradiated blood sample power 2mW at different exposures times

Table 4.6: FTIR spectral data (wave number, function group and transmission) for irradiated blood sample power 2mW

FTIR spectrum of blood irradiated with He-Ne laser power 2mW for duration 10, 20, 30,40 and 50 min				
Sr. No	**Irradiated Time (minute)**	**Wave number CM^{-1}**	**Group**	**T%**
1	10	3396.77	O-H	0.77
2		1650.96	C=O	1.78
3		1545.10	N=O	4.49
4		1450.73	C-H	15.20
5		1312.56	N-H	16.12
6		1161.74	C-O	18.70
7	20	3442.45	O-H	0.65
8		1651.63	C=O	1.68
9		1545.10	N=O	4.68
10		1451.01	C-H	11.43
11		1312.59	N-H	12.58
12		1161.74	C-O	13.76
13	30	3410.57	O-H	4.92
14		1651.63	C=O	6.50
15		1551.23	N=O	12.82

16		1451.01	C-H	22.14
17		1312.59	N-H	24.29
18		1167.96	C-O	26.31
19	40	3304.04	O-H	12.12
20		1645.41	C=O	13.12
21		1545.10	N=O	16.45
22		1447.23	C-H	25.34
23		1312.59	N-H	27.11
24		1161.59	C-O	28.50
25	50	3442.45	O-H	2.49
26		1651.63	C=O	6.46
27		1545.10	N=O	12.54
28		1451.01	C-H	22.28
29		1318.81	N-H	23.44
30		1167.96	C-O	25.5 9

Table 4.7: Show the FTIR spectral data (wave number, function group and transmission) for irradiated blood sample power 2mW

FTIR spectrum of blood irradiated with He-Ne laser power 2mW for duration 60, 70, 80,90 and 100 min				
Sr. No	**Irradiated Time (minute)**	**Wave number CM^{-1}**	**Group**	**T%**
1	60	3416.17	O-H	5.33
2		1644.23	C=O	8.42
3		1546.19	N=O	13.68
4		1454.74	C-H	22.53
5		1316.47	N-H	24.51
6		1166.49	C-O	26.01
7	70	3450.29	O-H	26.93
8		1644.23	C=O	29.71
9		1546.19	N=O	34.42
10		1454.74	C-H	40.57
11		1310.61	N-H	42.42
12		1166.49	C-O	43.35
13	80	3342.45	O-H	12.13
14		1644.23	C=O	14.06

15		1541.07	N=O	16.98
16		1448.89	C-H	25.85
17		1316.47	N-H	27.42
18		1166.49	C-O	29.25
19	90	3381.89	O-H	6.46
20		1650.48	C=O	9.53.
21		1541.07	N=O	14.70
22		1446.89	C-H	22.48
23		1315.61	N-H	31.94
24		1167.96	C-O	23.95
25	100	3386.47	O-H	5.93
26		1655.93	C=O	9.08
27		1546.19	N=O	17.23
28		1460.59	C-H	27.55
29		1315.91	N-H	26.57
30		1161.39	C-O	30.31

Table 4.8 and Table 4.9 show FTIR spectral data (wave number, function group and transmission) for irradiated blood sample power 1mW at different exposures times

Table 4.8: Show the FTIR spectral data (wave number, function group and transmission) for irradiated blood sample power 1mW

FTIR spectrum of blood irradiated with He-Ne laser power 1mW for duration 10, 20, 30,40 and 50 min				
Sr. No	**Irradiated Time (minute)**	**Wave number CM^{-1}**	**Group**	**T%**
1	10	3306.77	O-H	24.79
2		1650.96	C=O	25.63
3		1545.10	N=O	30.12
4		1451.73	C-H	38.87
5		1315.56	N-H	41.11
6		1161.74	C-O	43.24
7	20	3416.04	O-H	24.69
8		1651.63	C=O	26.81
9		1545.10	N=O	30.61
10		1451.01	C-H	38.74
11		1312.59	N-H	40.46
12		1161.74	C-O	42.05
13	30	3442.45	O-H	24.04
14		1651.63	C=O	26.02

15		1545.23	N=O	31.31
16		1451.01	C-H	39.80
17		1312.59	N-H	41.92
18		1167.96	C-O	42.31
19	40	3348.63	O-H	11.90
20		1651.41	C=O	14.21
21		1545.10	N=O	20.40
22		1444.23	C-H	30.48
23		1325.03	N-H	31.94
24		1167.96	C-O	33.94
25	50	33040.4	O-H	22.22
26		1651.63	C=O	24.03
27		1545.10	N=O	27.07
28		1457.01	C-H	34.36
29		1312.59	N-H	35.33
30		1167.96	C-O	36.19

Table 4.9: Show the FTIR spectral data (wave number, function group and transmission) for irradiated blood sample power 1mW

FTIR spectrum of blood irradiated with He-Ne laser power 1mW for duration 60, 70, 80,90 and 100 min				
Sr. No	**Irradiated Time (minute)**	**Wave number CM^{-1}**	**Group**	**T%**
1	60	3416.17	O-H	25.21
2		1650.08	C=O	27.19
3		1546.10	N=O	31.12
4		1448.89	C-H	40.84
5		1310.61	N-H	42.17
6		1161.61	C-O	42.62
7	70	3439.59	O-H	29.00
8		1651.08	C=O	33.93
9		1546.19	N=O	35.15
10		1448.89	C-H	40.45
11		1310.61	N-H	41.76
12		1166.49	C-O	42.83
13	80	3416.17	O-H	0.34
14		1644.23	C=O	1.29
15		1541.07	N=O	4.47

16		1448.89	C-H	11.61
17		1310.61	N-H	13.30
18		1161.37	C-O	14.99
19	90	3411.05	O-H	14.55
20		1650.08	C=O	17.64
21		1546.19	N=O	22.50
22		1448.89	C-H	31.32
23		1310.61	N-H	33.53
24		1161.37	C-O	34.85
25	100	3307.16	O-H	13.21
26		1650.08	C=O	13.44
27		1545.10	N=O	17.85
28		1454.74	C-H	27.90
29		1312.59	N-H	30.22
30		1167.96	C-O	32.19

Figure 4.10 show FTIR spectrum of non irradiated to(He-Ne) laser blood sample .

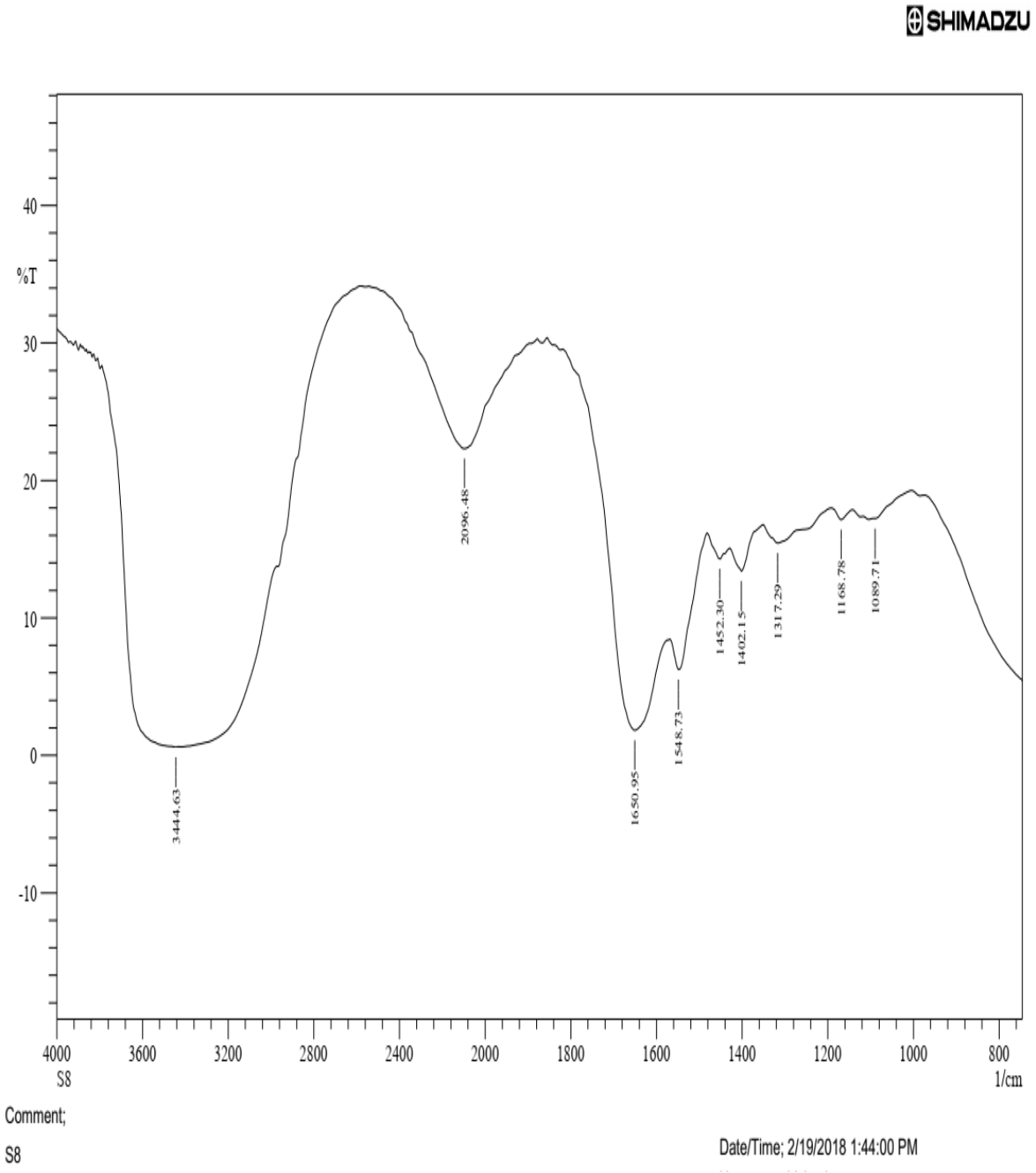

Figure 4.10: FTIR spectrum for normal blood samples (control)

figures 4.11 and figure 4.12 show FTIR spectrum of blood sample irradiated to(He-Ne) laser power 2mW at different exposure times.

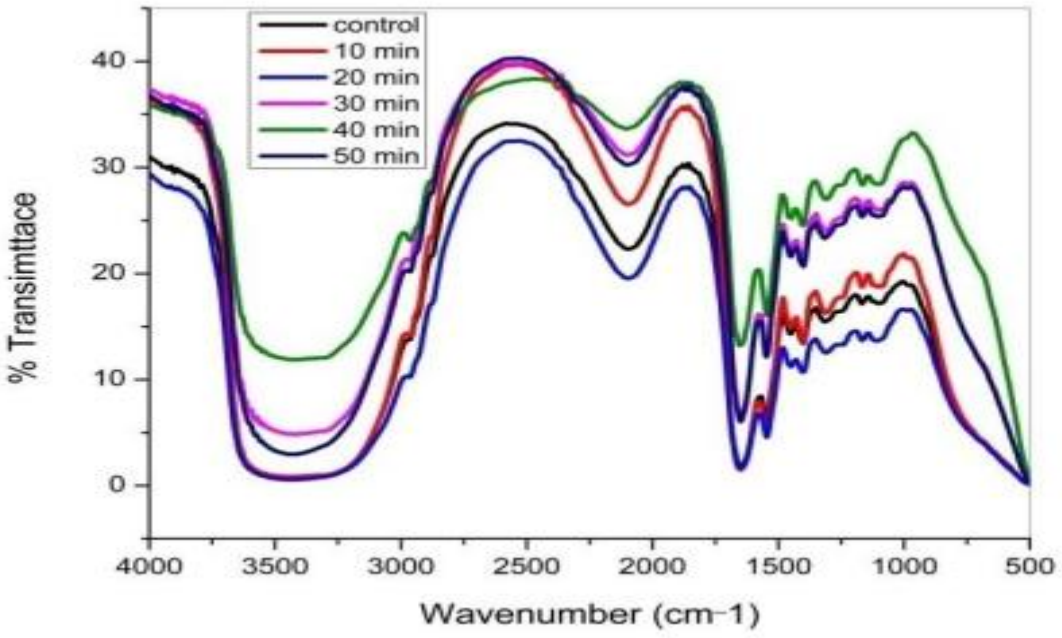

Figure 4.11 FTIR spectrum for before and after irradiated blood to He-Ne laser power 2mW from 10,20,30,40 and50 minute

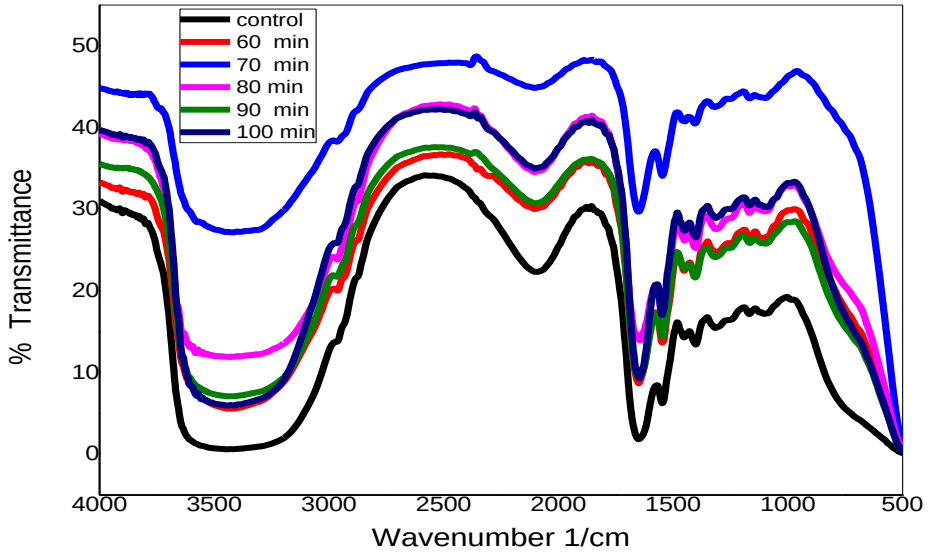

Figure 4.12 FTIR spectrum for before and after irradiated blood to He-Ne laser power 2mW from 60,70,80,90 and100 minutes

Figure 4.13 and figure 4.14 show FTIR spectrum of blood sample irradiated to(He-Ne) laser power 1mW at different exposure times.

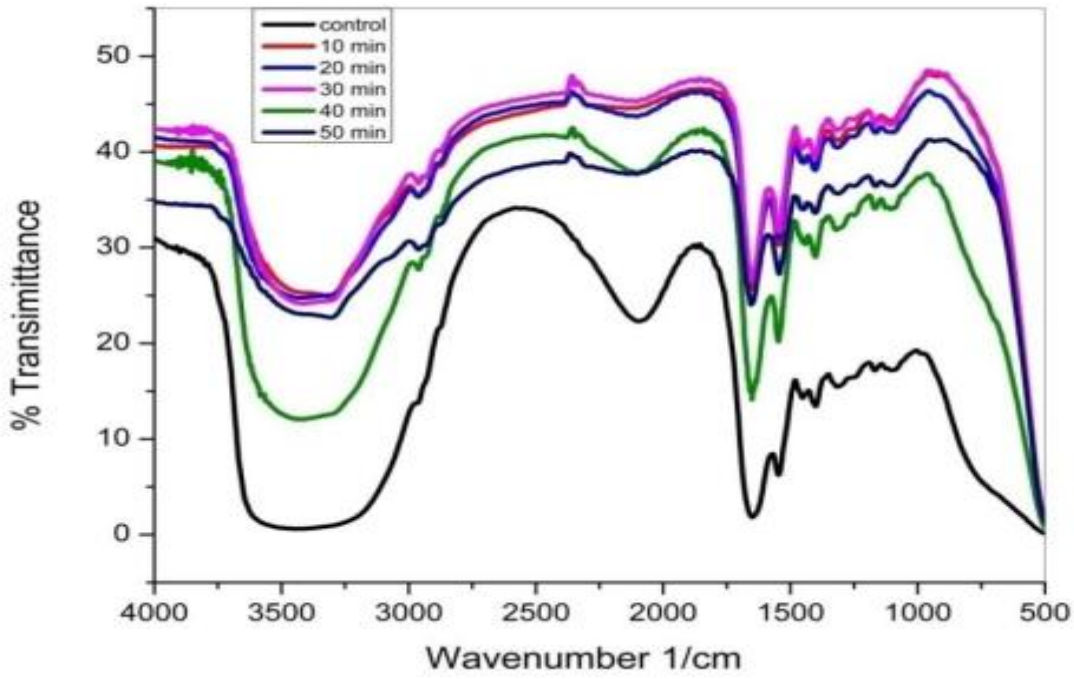

Figure 4.13 FTIR spectrums for before and after irradiated blood to He-Ne laser power 1mW from 10,20,30,40 and50 minute

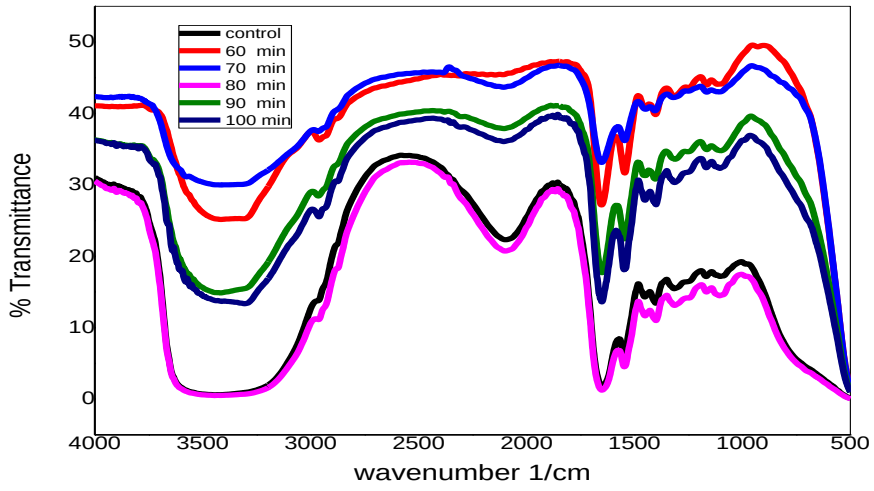

Figure 4.14 FTIR spectrums for before and after irradiated blood to He-Ne laser power 1mW from 60,70,80,90 and100 minute

4.3 Discussions

Fig. 4.1 shows the spectrum of non- irradiated blood sample (control). This spectrum is referred to non- irradiated blood sample which is specified by peaks at (576.0, 542.0, 416.0 and 340.0) nm with intensities 0.793, 0.755, 2.604 and 1.253 respectively. The absorption spectra of the whole blood are in the range 300–800 nm.

Fig. 4.2 and Fig. 4.3 shows absorption peaks with λ_{max}= 340, 416 nm, λ_{max} = 542 and 576nm. Only those Changes in the absorption spectra of the whole blood exposed to the He-Ne laser (λ= 632nm, power=2mW) radiation that was detected for all the samples were studied.

In tables. 3, and 4 different serum samples are analyzed quantitatively by calculating the absorbance among the absorption peaks which show decrease of absorbance, for all irradiated serum sample compared to control serum sample except that sample irradiated to 80 min. These results indicate that there is photodegradation happened to the blood components. This indicated that laser radiation interacts with blood at the molecular level. Hemoglobin is a blood photoreceptor that selectively absorbed visible spectra**.** Absorption intensity slightly decreases for all peaks, due to increasing ligand electronegativity.

Figure 4.6 and Figure 4.7 showed absorption of light at 340 nm with different irradiation time. Results showed the higher absorbance at 80 min and lower absorbance at 90 min.

Fig. 4.4, and Fig. 4.3 contain absorption bands with λ_{max}=, 340, 416 nm, λmax = 542 and 576nm. Only those Changes in the absorption spectra of the whole blood exposed to the He-Ne laser (λ= 632nm, power=1mW) radiation that was detected for all the samples were studied .From tables1&2 we found that different serum samples are analyzed quantitatively by calculating the intensities among the absorption peaks which show that the UV/Vis absorption increases for 10 ,40 and 70 min but it decreases as the

exposure time at 20,30 ,and it continues to decrease at 50,60 ,80 ,90 and 100 minutes, due to increasing ligand electronegativity (Gunasekaran.2008) , where the concentration of absorbing centers is decreasing. Also we found that fluctuation of light absorption which is known as a biphasic response.

The mechanism of LLLT at the cellular level has been associated with the absorption of monochromatic visible and near infrared radiation. Effective tissue penetration is maximized at a specific optical window (Anders JJ,et al.1993) (Yu c et al.2005). Laser affect biological tissues, bio stimulated it. Fluctuation of light absorption illustrates the biphasic dose-response curve. When the blood sample is irradiated, the enzymatic activity of the membrane sodium (Na+) and potassium (K+) ion pump changes in dose and fluence-dependent manner. Consequently, the biological function of the cells is stimulated and increases the light absorption. But a further increase of irradiation time inhibits the enzymatic activities due to the suppression of the Na+ and K(Kujawa J et al.2004).

Figure 4.8 and figure 4.9 showed absorption of light at 340nm with different irradiation time .Results showed the higher absorbance at 70 min and lower absorbance at 80 min show a significant decrease for un known reason. In the FTIR spectra of the whole blood, without irradiation to laser presented in fig.4 10. Table 4.5 shows the groups O-H, C=O, N=O, C – H, NH, and C–O in the region between the wave numbers 4000 to 500 cm-1. In the spectral region 2800–3700 cm-1, the band with λ_{max} = 3444.63 cm-1 is O–H bond peak. Amide-I is mainly associated with C-O, C = O, and C-H stretching vibrations and also related to the backbone conformation. The wave numbers 1650.95 cm-1, 1548.73cm-1 1452.30cm-1 indicate C = O, N=O and C - H peaks respectively. The absorption peak in the 1317.29 cm-1 and 1168.78 cm-1 arises due to the N-H stretching vibrations of the proteins methylene group of the proteins, and gives rise to the existence of glucose due to C-O symmetric stretching, The prominent absorption peak 3444.63 cm-1 is due to

the N – H stretching mode (amide - A) of proteins. The most intense absorption band in proteins is the amide I peak, which is observed at 1650.95 1/cm. Amide I is mainly associated with C=O symmetric stretching and or C-O stretching vibrations. There are another very strong prominent amide absorptions one at 1545 1/ cm due to strong N-H in-plane bending and termed as an Amide II band (Gunasekaran S et al.2008) (Rathore S ,et al.2013).

The whole blood sample is irradiated to He-Ne laser(λ= 632nm, power=2mW) radiation for 10, 20, 30, 40,50,60,70,80,90 min. and 100 min duration respectively, Fig 4.11 to Fig 4.14 .Table 4.6 and table4.7 shows the groups associated with spectral peaks for whole sample irradiated by He-Ne laser (λ= 632nm, power=2mW) radiation for 10 min duration shows an increase in transmittance for all groups except for C-H decreases due to the denaturation of the protein. FTIR spectra of whole blood irradiated with He-Ne laser for 20 minute show decreases in transmission for group, C-H, and N-H, to denaturation of protein i.e. it breaks the polypeptide bonds due to conformational changes of proteins, but in 30,40, 50,60,70,80,90 and 100 minutes show an increase in transmittance for all groups is observed the separate chromophore and auxochrome groups (C=O, N=O, C-H, N-H, C-O, O-H) show also significant changes and indicates a significant increasing in their concentration. Laser irradiation of blood causes changes in absorption band in stretching and bending Vibrations of peptide group.

Fig 4.21 to Fig 4.30 and table 4.8 and table 4.9 show the whole blood sample is irradiated to He-Ne laser (λ= 632nm, power=1mW) radiation for 10, 20, 30 ,40, 50, 60, 70, 80, 90,min. and 100 min duration respectively. The groups associated with spectral peaks whole sample irradiated to He-Ne laser radiation increase in transmittance for all groups except sample irradiated for 80 min show decrease transmittance for groups (, N=O, C-H, N-H, C-O, O-H) but increase for group C=O only is observed, that was the increasing in their concentration. Also significant increasing the transmission indicate that

chromospheres and auxochrome groups (C=O, N=O, C-H, N-H, C-O, O-H) show also significant, Laser irradiation blood.

In comparison with a previous study done by Vijay H. Ghadage, Gauri R. Kulkarni(2011) He Ne laser (λ= 632nm, power=2mW) was used to irradiation human red blood cells and exposure time was10, 20, 30, and 40 minutes. Absorption spectrum, FTIR and fluorescence spectra of RBC and other work done by (V.H. GHADAGE, G.R.KULKARNI, B.N. ZAWARE) (2015) they used He-Ne laser (Wavelength λ= 632.8 nm, Power P = 3mW) and exposure time was10, 20, 30, and 40 minutes. While in this study we used He-Ne laser (λ= 632nm, power=2mW) l (λ= 632nm, power=2mW) radiation for 10, 20, 30, 40, 50, 60, 70, 80, 90, min. and 100 min. In the study conducted (2011), the absorption spectrum of RBC showed a significant decrease in absorbance. The FTIR spectrum RBC changes in transmittance in FTIR spectra related to C=O group and percentage of transmittance increases for O-H, C=C, N=O, C-O and C-H group. In the study conducted (2015) for low doses: (at 10 and 20 min) the FTIR spectra show the groups O -H and C=O, and other groups C-O and C-H were absent.

For high doses: (at 30 and 40 min) show the groups O -H, C = O, C - O, C - H, C - N and N = O. But transmittance decreases for 40 minutes due to denaturation of proteins. While in this study the when used power 2 mW absorption peaks which is show decrease absorbance, except that sample irradiated to 80 min. for low dose FTIR spectra show radiation for 10 min duration shows an increase in transmittance for all groups except for C-H decreases. FTIR spectra of whole blood irradiated for 20 minute show decreases in transmission for group, (C-H, and N-H).

For high dose at (30, 40, 50, 60,70,80,90 and 100) minutes show an increase in transmittance for all groups. When used power 1 mW the result showed absorption decreases for exposure time except at (10, 40 and 70

min). FTIR spectra result showed increase in transmittance for all groups except sample irradiated for 80 min show decrease of transmittance for groups (, N=O, C-H, N-H, C-O, O-H).

4.4 Conclusion

From the obtained results the following conclusion is drawn:

This work had shown that laser radiation interacts with blood at the molecular level. Hemoglobin is a blood photoreceptor that selectively absorbed He-Ne Laser beam with output power 2 mW, (632.8 nm). The absorption of laser beam by blood leads to partial photodissociation. The results showed a decrease in absorbance**,** increase in transmittance of FTIR spectra for all groups observed except for group C-H, and (C-H, N-H) which show decreases in transmission for 10 and 20 minute. For all exposure times irradiation by He-Ne laser (λ= 632nm, power=1mW) of whole blood, the transmittance of C=O, O-H, N=O, C-O & C-H, N-H group transmittance increases show also significant changes and indicates a significant decreasing in their concentration. Results showed that absorption decreases for all exposure times except at (10, 40 and 70 min). In general peptide group's bands show more changes. The secondary structures of blood proteins undergo conformational changes.

4.5 Recommendations

Future study could be done by:

1- Studying low power He-Ne laser effects on human blood by medical test.

2-Selecting different doses of laser used in medical application by changing exposure time, wavelength, power, modes.

3- Studying the possibility of using this low power in the treatment of blood vessels diseases.

REFERENCES

Alaa Alabaid, Wafaa Salah, Bashir Algaily, Wasil H.M. Salih ,2017. Biostimulation Of Human Blood Using He-Ne 632.8 Nm And O-Xp 820 Nm Lasers, Neelain Journal Of Science And Technology. Volume 1, Issue 1, , 54–60.

Al-Khalid Isam Zuhaier Et A l, 2015. Shelf-Life Enhancement Of Donor Blood By He–Ne Laser Biostimulation. Research Communications. Current Science, Vol. 109, NO. 6

Anders, J.J., Borke, R.C., Woolery, S.K. And Van De Merwe, W.P., 1993. Low Power Laser Irradiation Alters The Rate Of Regeneration Of The Rat Facial Nerve. Lasers In Surgery And Medicine, 13(1), Pp.72-82.

Bernard, Jaffe. 1954. A Compact Science Dictionary. Edited By G. E Speck. London.

Beesly, M.J., 1978. Laser And Their Applications. Taylor And Francis Ltd., London.

Alberts, Bruce, 2012. "Table 22-1 Blood Cells". Molecular Biology of the Cell. NCBI Book shelf.

Brault, James W. (1996). "New Approach To High-Precision Fourier Transform Spectrometer Design". Applied Optics. 35 (16): 2891–2896. Bibcode:1996apopt..35.2891B. Doi:10.1364/AO.35.002891. PMID 21085438

Csele, M, 2004. Fundamentals Of Light Sources And Lasers, A Jhon Wiley And Sons. Inc., Publication. New Jersey, 360.

Chung, H , Dai, T., Sharma, S.K, Huang, Y.Y., Carroll, J.D. And Hamblin, M.R., 2012. The Nuts And Bolts Of Low-Level Laser (Light) Therapy. Annals Of Biomedical Engineering, 40(2), Pp.516-533.

E. Z. Al-Khaled; M. Kosa And M. Al-Salhi 2012. Effect Of Electric Field Of Deferent Frequencies; On Rbcs After Being Irradiated By (690μw)Of He-Ne Laser Damascus University Journal of Basis Sciences -Volume 29 - Issue 1

Daniel Harris, C. And Michael Bertolucci, D.1989. Symmetry And Spectroscopy: Anintroduction To Vibrational And Electronic Spectroscopy, Dover Publications Inc, New York

Dutta, A.K, Chandrakar, S. And Dewangan. S, 2009, POPULATION Based Demographic & Hematological Analysis With Special References To The Workers Of Mats University Of Chhattisgarh.

Fuad, S.S.M., Suardi, N. And Mustafa, I.S., 2018, April. In Vitro UV-Visible Spectroscopy Study Of Yellow Laser Irradiation On Human Blood. In Journal Of Physics: Conference Series (Vol. 995, No. 1, P. 012053). IOP Publishing.

Gabriele Boccalini,2013. An Optical Sensor For Online Hematocrit Measurement: Characterization And Fitting Algorithm Development. Magistrale In Ingegneria Biomedica.

Ghadage, V. H And. Kulkarni, G. R" Effects, 2011 Of He-Ne Laser Irradiation On Red Blood Cells In Vitro", Optical Interactions With Tissue And Cells XXII, Proc. Of SPIE, BIOS, San Francisco, California, USA, Vol. 7897, Pp. 789701 - 789710,

Ghadage, V.H., Kulkarni, G.R. And Zaware, B.N., 2015. He-Ne Laser Irradiation Of Blood In Vitro And FTIR Spectral Analysis. Int J Chem Phys Sci, 4, Pp.148-153.

Ghadage Vijay H And Lokare SA, 2018. Study Of Laserradiation Effects On Diabetic Human Blood Using FTIR Spectroscopic Techniques, Int. Res.Journal Of Science & Engineering, , Special Issue A2: 183-186.

Gupta SK, Jangam H, Sharma N 2018 Study And Applications Of Laser Light. Res J Opt Photonics 2:2

Gunasekaran, S. And Uthra, D., 2008. FTIR And UV-Visible Spectral Study On Normal And Jaundice Blood Samples. Asian Journal Of Chemistry, 20(7), P.5695.

Gunasekaran, S. And Uthra, D., 2008. FTIR And UV-Visible Spectral Study On Normal And Jaundice Blood Samples. Asian Journal Of Chemistry, 20(7), P.5695.

Haken. H., 1985 , Light North-Holland, Amsterdam.

Hecht J (2003) Laser Guide, Donnelly And Son Company, Chicago, Illinois, United States.

.I. Z. Al Khalid, M. Kosa And M. Al Salhi, 2010. Decreasing Blood Fragility By Irradiation With He-Ne Laser (160μw) Damascus University Journal of Basis Sciences -Volume 26 - Issue 1

Javan A, Bennett WR, Herriot DR 2005 Population Inversion And Continuous Optical Maser Oscillation In A Gas Discharge Containing A He-Ne Mixture. Phyrev Lett 6: 106-110

Kaya Ball, “Lasers The Perioperative Challenge”, Second Edition,

K R. Nambiar, 2004. “Laser Principle’s Types And Applications”, New Age International P Limited Publishers,

Karu, T., 1999. Primary And Secondary Mechanisms Of Action Of Visible To Near-IR Radiations On Cells. J. Photochem. Photobiol. B, 49: 1-1

Kanagathara. N. Thirunavukkarasu, M, Jeyanthi, E.C. And Shenbagarajan, P., 2011. FTIR And UV-Visible Spectral Study On Normal Blood Samples. IJPBS, 1(2), Pp.74-81.

Lert, Glenn 2012. "Volume Of Blood In A Human". The Physics Factbook. His Students. Archived From The Original On 1 November 2012. Retrieved 2012-11-0

Lekner, John 1987. Theory Of Reflection, Of Electromagnetic And Particle Waves. Springer. ISBN 9789024734184

Lundeberg, T. And Malm, M., 1991. Low-Power Hene Laser Treatment Of Venous Leg Ulcers. Ann Plast Surg, 27(6), Pp.537-9.

Mangi FA, Simons BJ, Jamro DA, Shaikh NA, Mallah GA,2017. Medical Application Of Output Intensity Characterization Of Red He-Ne Laser

Mi, X.Q., Chen, J.Y., Liang, Z.J. And Zhou, L.W., 2004. In Vitro Effects Of Helium-Neon Laser Irradiation On Human Blood: Blood Viscosity And Deformability Of Erythrocytes. Photomedicine And Laser Therapy, 22(6), Pp.477-482.

Manor, Joshua; Feldblum, Esther S.; Arkin, Isaiah T. 2012. "Environment Polarity In Proteins Mapped Noninvasively By FTIR Spectroscopy". The Journal Of Physical Chemistry Letters. 3 (7): 939–944. Doi:10.1021/Jz300150v. PMC 3341589. PMID 22563521.

Martini, Frederic, et al. (2007). Anatomy and Physiology. Rex Bookstore, Inc. p. 643. ISBN 9789712348075.

Mayers RA 2011 Encyclopedia of lasers & optical technology, 1st Edtn, Academic Press, Elsevier, United States.

Michael Hollas, J. 2003. Modern Spectroscopy, 4th Ed., John Wiley & Sons Ltd.

M.J.C. van Gemert and A.J. Welch, (1989) Clinical use of laser-tissue interactions, IEEE Engineering in Medicine and Biology Magazine.

Mustafa S. Al Musawi, M. S. Jafaar, Naser M. Ahmed, B. T. Al-Gailani, And Fatanah M. Suhaimi , 2017. Effects Of Low Power Violet Laser Irradiation On Red Blood Cells Volume And Erythrocyte Sedimentation Rate In Human Blood. American Institute Of Physics.

Nuha Hassan Kinash , 2008. Application Of Laser In Medicine M.Sc Thesis U Of K.

Ogundipe, J.O. And Akinlade, J.A.,2016. haematological parameters of west african dwarf sheep and goat fed a basal diet of cassava peel supplemented with fresh gliricidia sepium leaves.

Orazio Svelto, 2002, Principles Of Lasers, Springer.

Ushasree, U.V., Jaleeli , K.A. And Ahmad, A., A Study On Infrared Spectroscopy Of Human Blood.

Rathore, S. And Ali, B., 2013. Effect Of He-Ne Laser Radiation On Viscometric Behavior Of Human Blood. Journal Of Chemical, Biological And Physical Sciences (JCBPS), 3(3), P.2124.

Robert Silverstein, M. And Francis Webster, X. (2006). Spectrometric Identification Of Organic Compounds 6th Ed., John Wiley & Sons Ltd

Samir Abdel-Wahab Abdel-Atty Saad,2012. Effects Of Coherent And Non-Coherent Light On Glycated Hemoglobin And Some Other Human Blood Components. Cairo University.

Shmukler, Michael (2004). "Density of Blood". The Physics Factbook. Archived from the original on 19 September 2006. Retrieved 4 October 2006

Silfast WT (2004) ,Laser Fundamentals, 2nd Edtn, Cambridge University Press,London.

Shimoda, K. 1984 Introduction To Laser Physics (Springer, Berlin,).

Soran Shadman; Charles Rose; Azer P. Yalin 2016. "Open-path cavity ring-down spectroscopy sensor for atmospheric ammonia". Applied Physics B. 122

The Franklin Institute Inc “Blood – The Human Heart Retrieved 19 March 2009

Waugh, Anne; Grant, Allison 2007. "2". Anatomy And Physiology In Health And. Illness (Tenth Ed.). Churchill Livingstone Elsevier. P. 22.

Yu, C. And Irudayaraj, J., 2005. Spectroscopic Characterization Of Microorganisms By Fourier Transform Infrared Microspectroscopy. Biopolymers: Original Research On Biomolecules, 77(6), Pp.368-377.

Zalesskaya , G.A. And Kalosha , I.I., 2009. Effect Of In Vivo Irradiation Of Blood By Low- Intensity Emission From A He–Ne Laser On Molecular Components Of Blood. Journal Of Applied Spectroscopy, 76(5), P.720.

Zahra Al Timimi, M.S. Jaafar, Mohd Zubir Mat Jafri,2011. Photodynamic Therapy And Green Laser Blood Therapy. Global Journal Of Medical Research Volume 11 Issue 5.

Appendix:

The figures below show the FTIR spectra of irradiation blood severally for all samples.

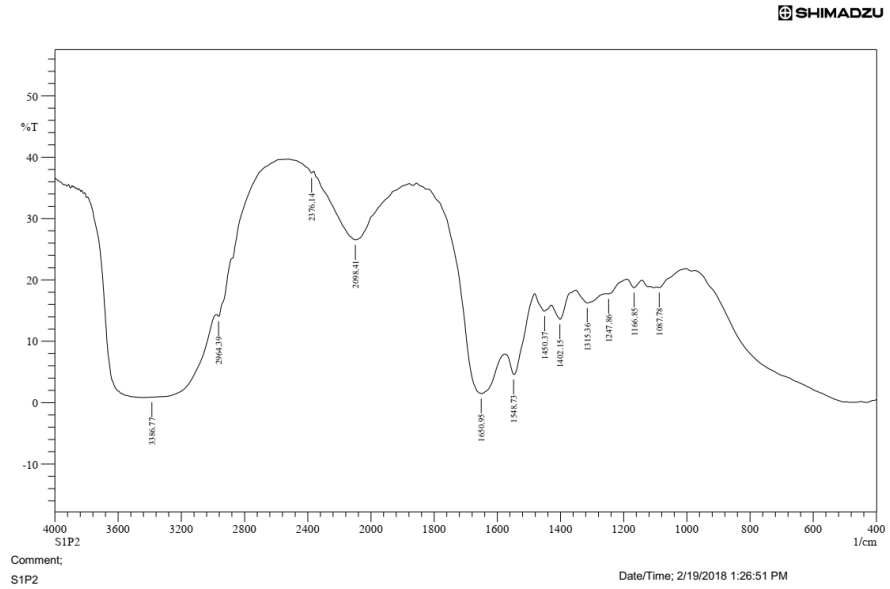

Figure 4.15: FTIR spectrum for normal blood sample irradiated to He-Ne laser power 2mW for 10 min

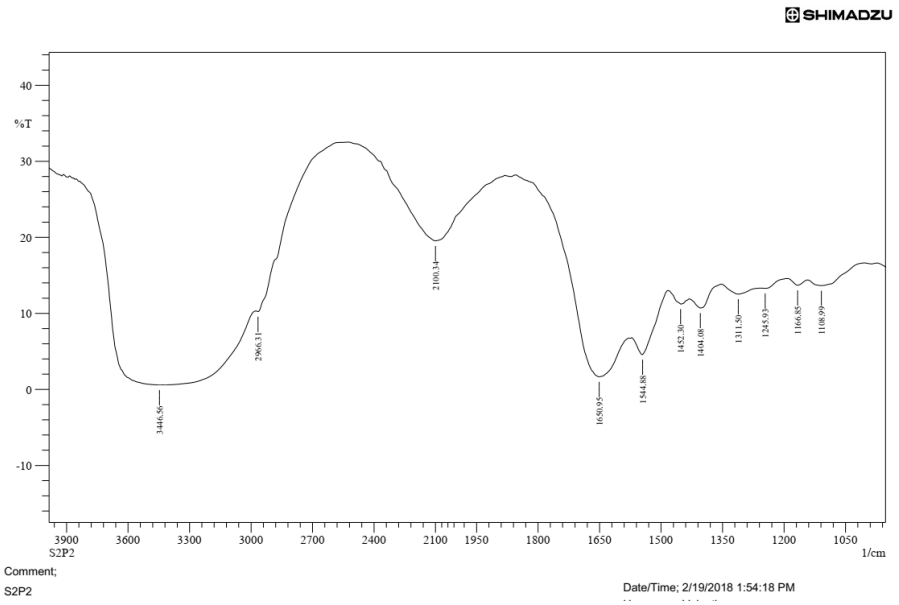

Figure 4.16: FTIR spectrum for normal blood sample irradiated to He Ne laser power 2mW for 20 min

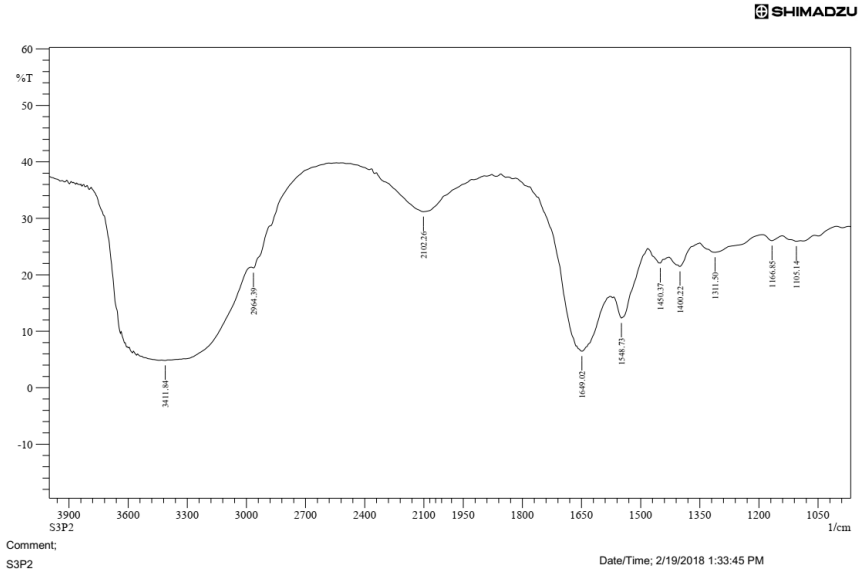

Figure 4.17: FTIR spectrum for normal blood sample irradiated to He-Ne laser power 2mW for 30 min

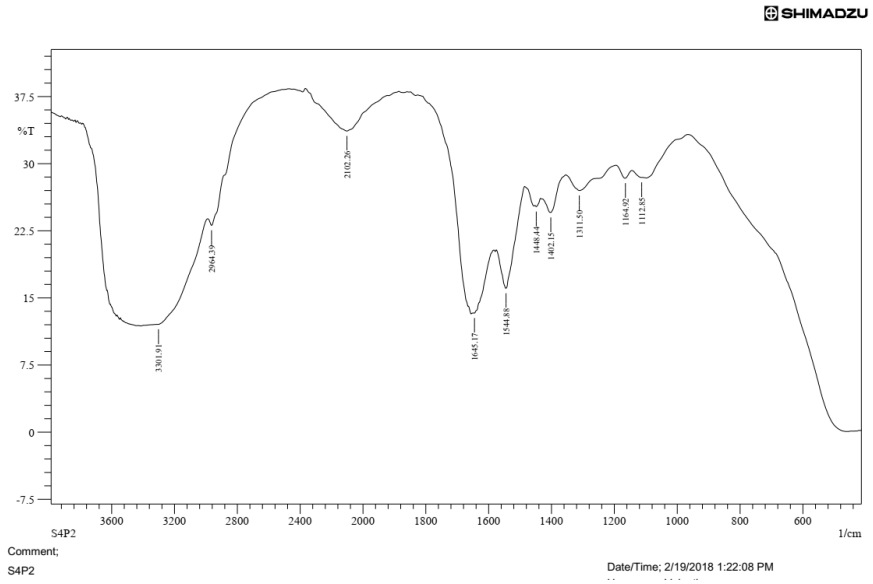

Figure 4.18: FTIR spectrum for normal blood sample irradiated to He-Ne laser power 2mW for 40 min

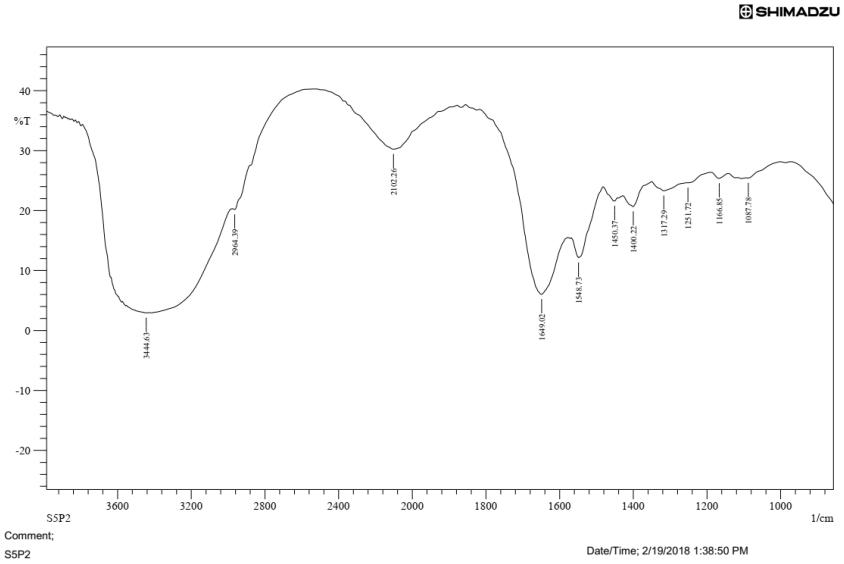

Figure 4.19: FTIR spectrum for normal blood sample irradiated to He-Ne laser power 2mW for 50 min

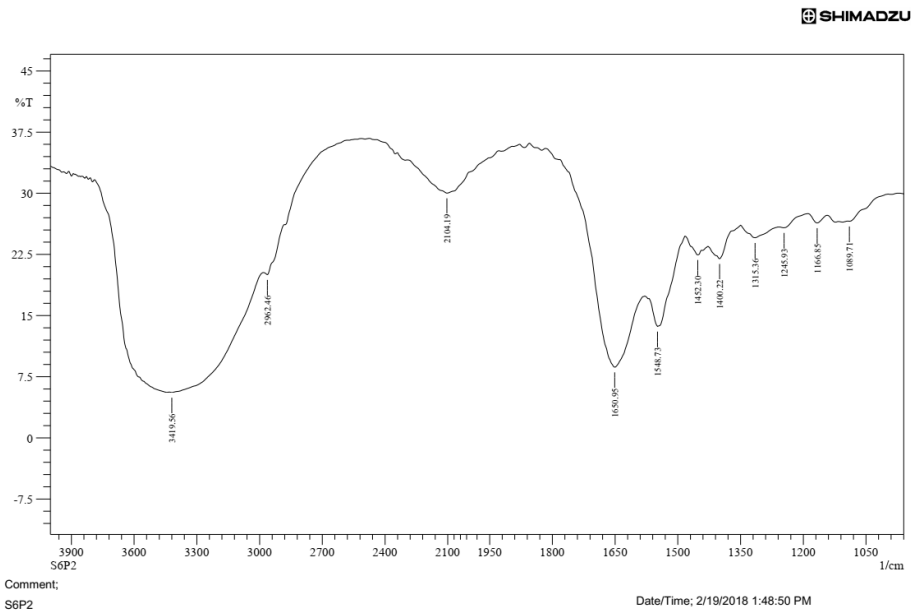

Figure 4.20: FTIR spectrum for normal blood sample irradiated to He-Ne laser power 2mW for 60 min

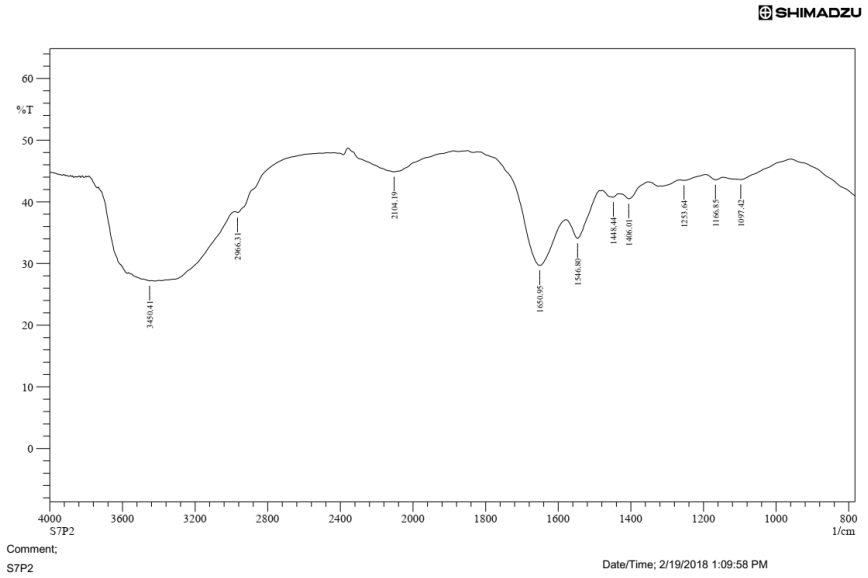

Figure 4.21: FTIR spectrum for normal blood sample irradiated to He-Ne laser power 2mW for 70 min

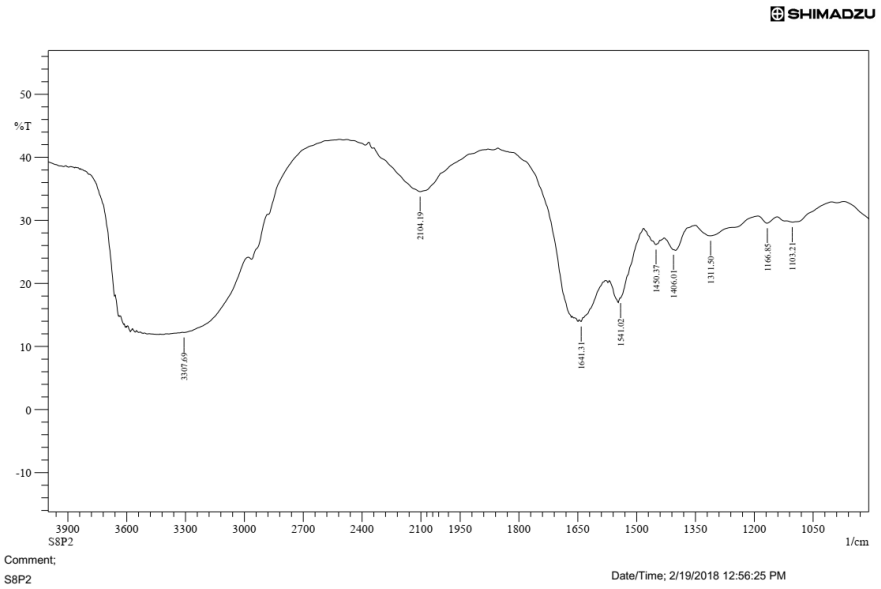

Figure 4.22: FTIR spectrum for normal blood sample irradiated to He-Ne laser power 2mW for 80 min

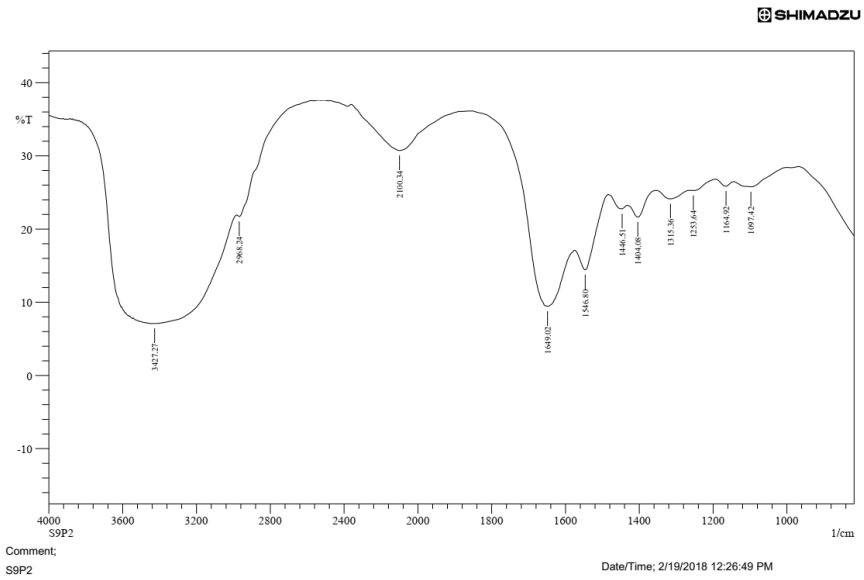

Figure 4.23: FTIR spectrum for normal blood sample irradiated to He-Ne laser power 2mW for 90 min

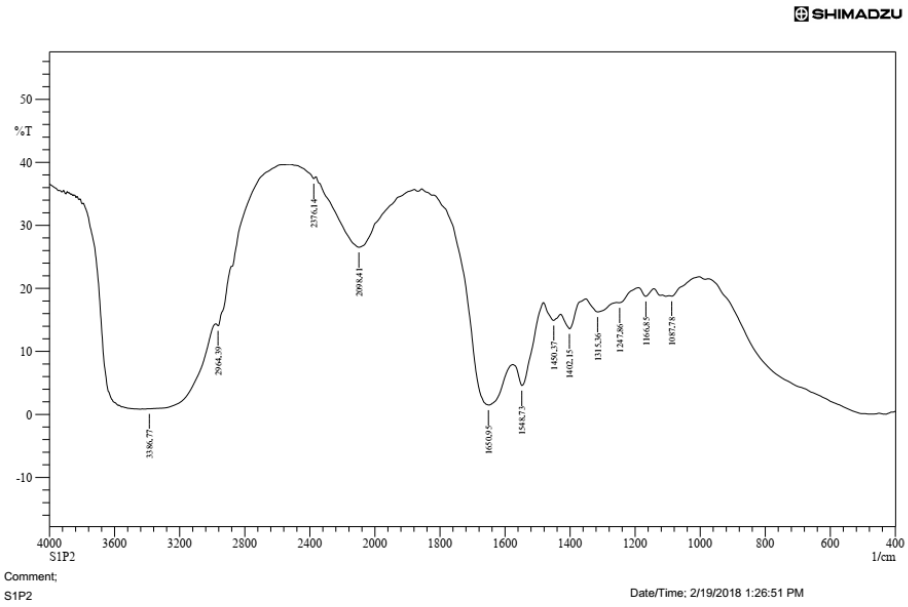

Figure 4.24: FTIR spectrum for normal blood sample irradiated to He-Ne laser power 2mW for 100 min

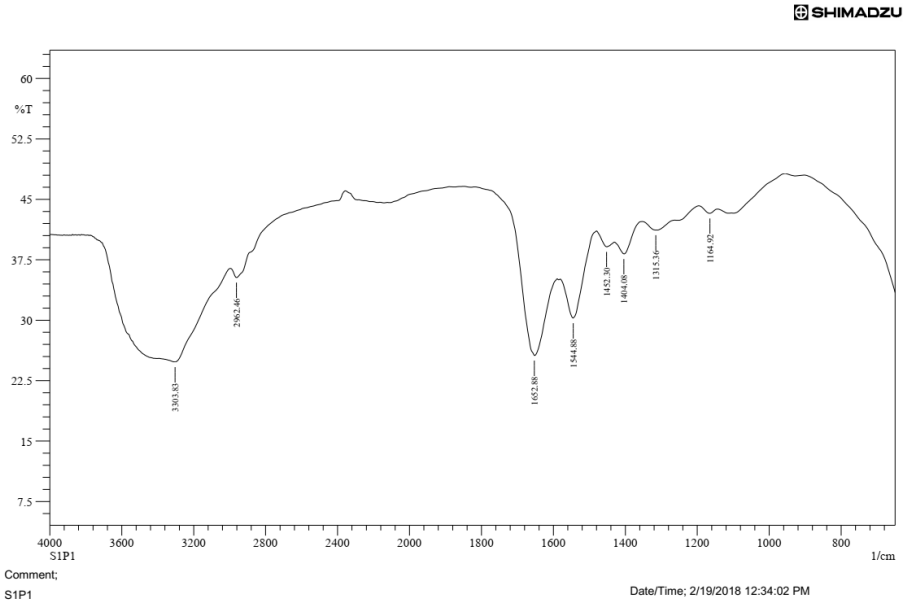

Figure 4.25: FTIR spectrum for normal blood sample irradiated to He-Ne laser power 1mW for 10 min

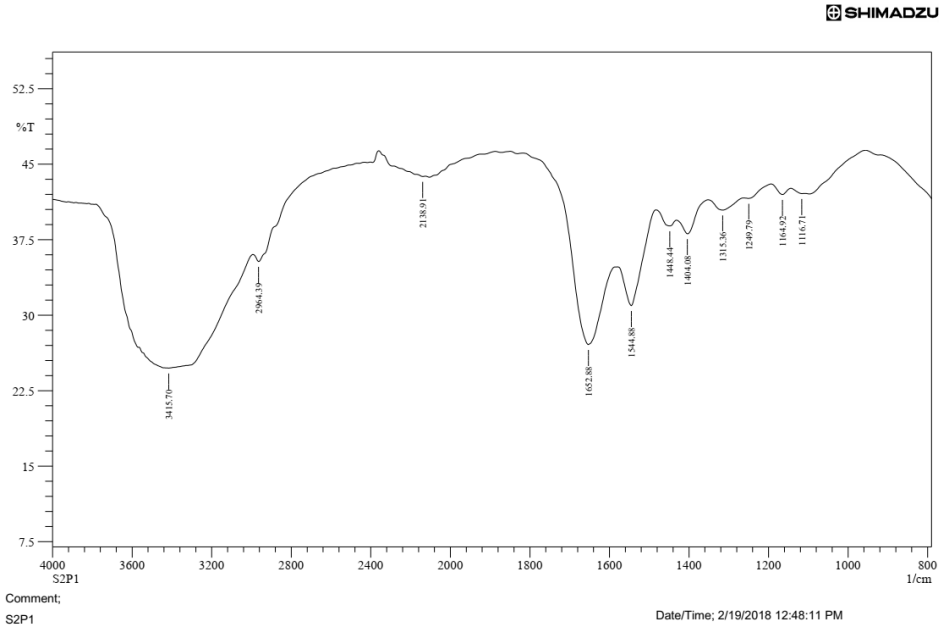

Figure 4.26: FTIR spectrum for normal blood sample irradiated to He-Ne laser power 1mW for 20 min

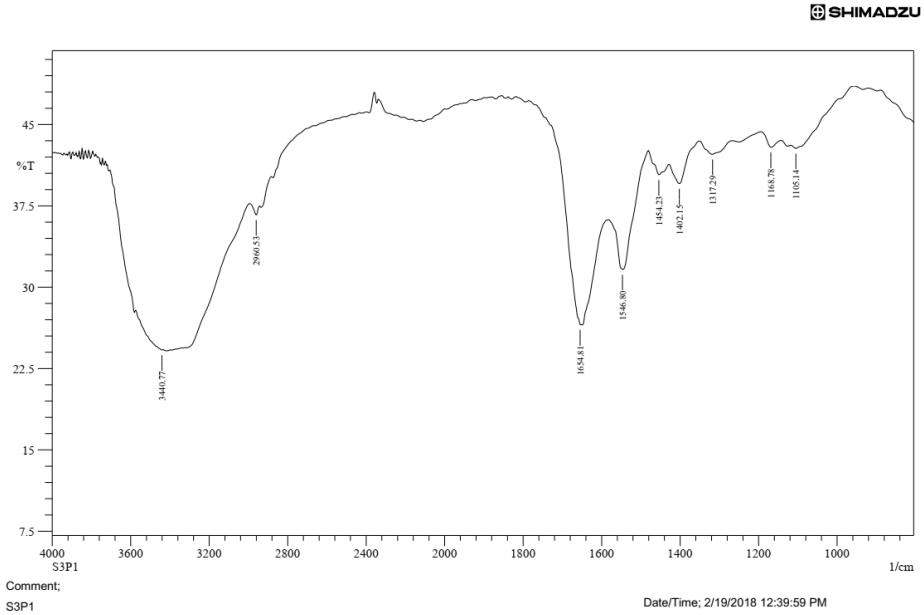

Figure 4.27: FTIR spectrum for normal blood sample irradiated to He-Ne laser power 1mW for 30 min

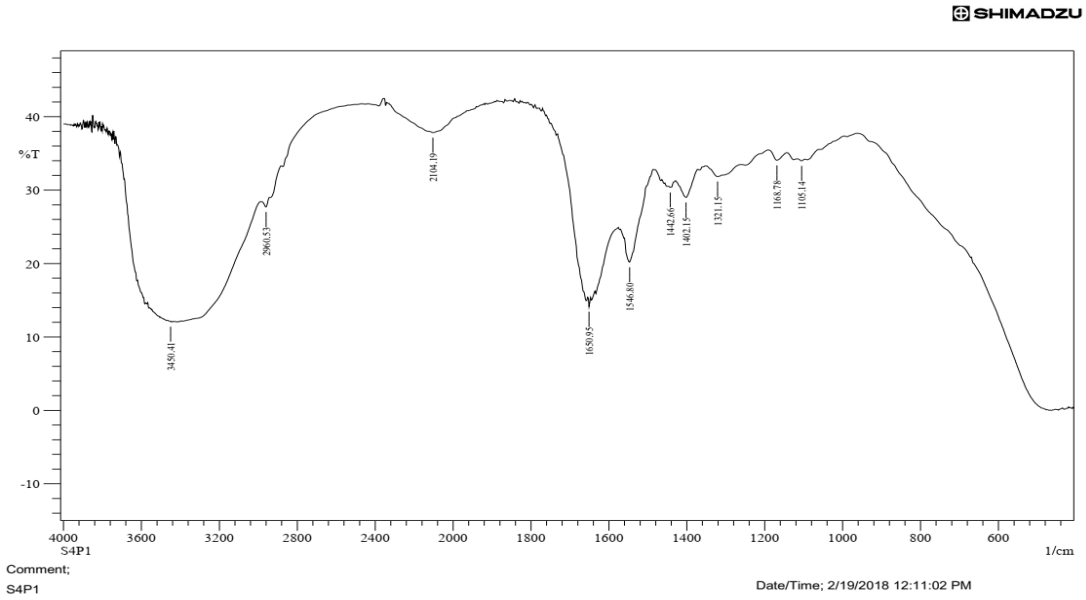

Figure 4.28: FTIR spectrum for normal blood sample irradiated to He-Ne laser power 1mW for 40 min

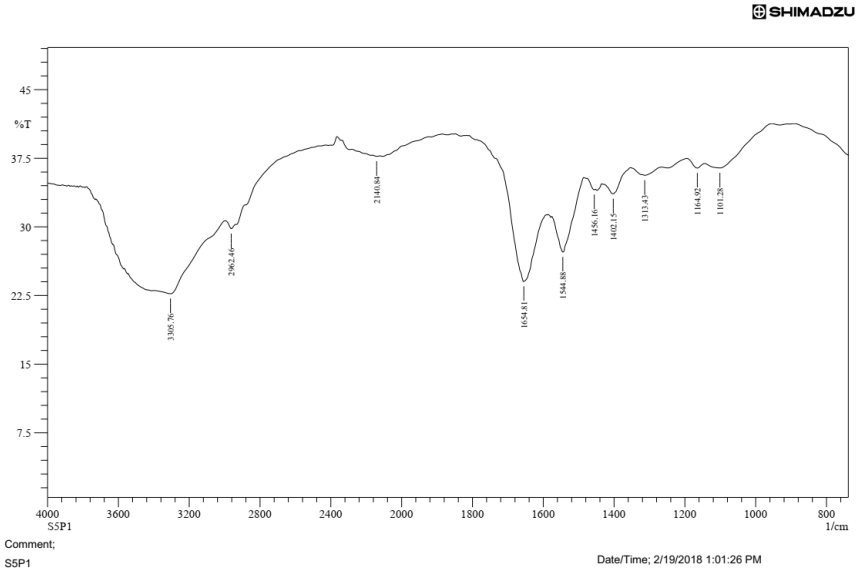

Figure 4.29: FTIR spectrum for normal blood sample irradiated to He-Ne laser power 1mW for 50 min

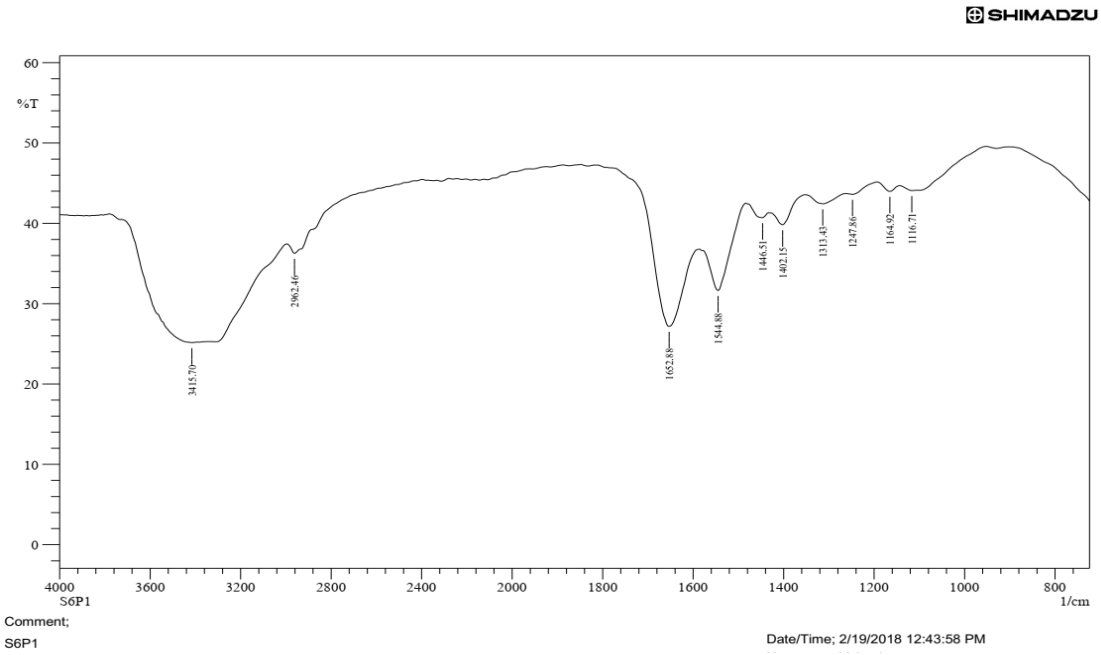

Figure 4.30: FTIR spectrum for normal blood sample irradiated to He-Ne laser power 1mW for 60 min

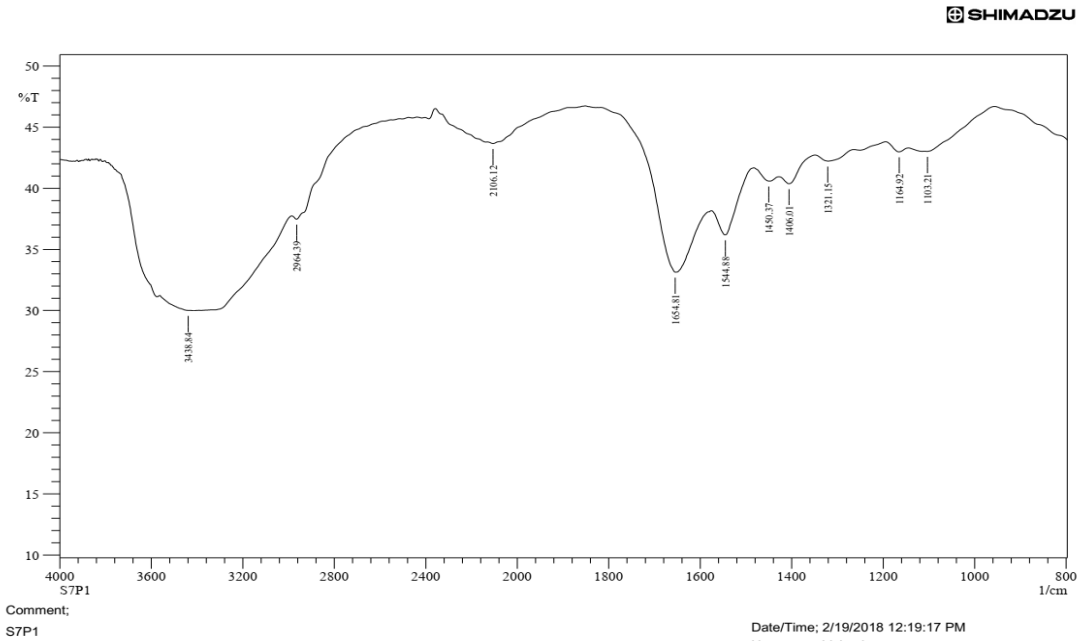

Figure 4.31: FTIR spectrum for normal blood sample irradiated to He-Ne laser power 1mW for 70 min

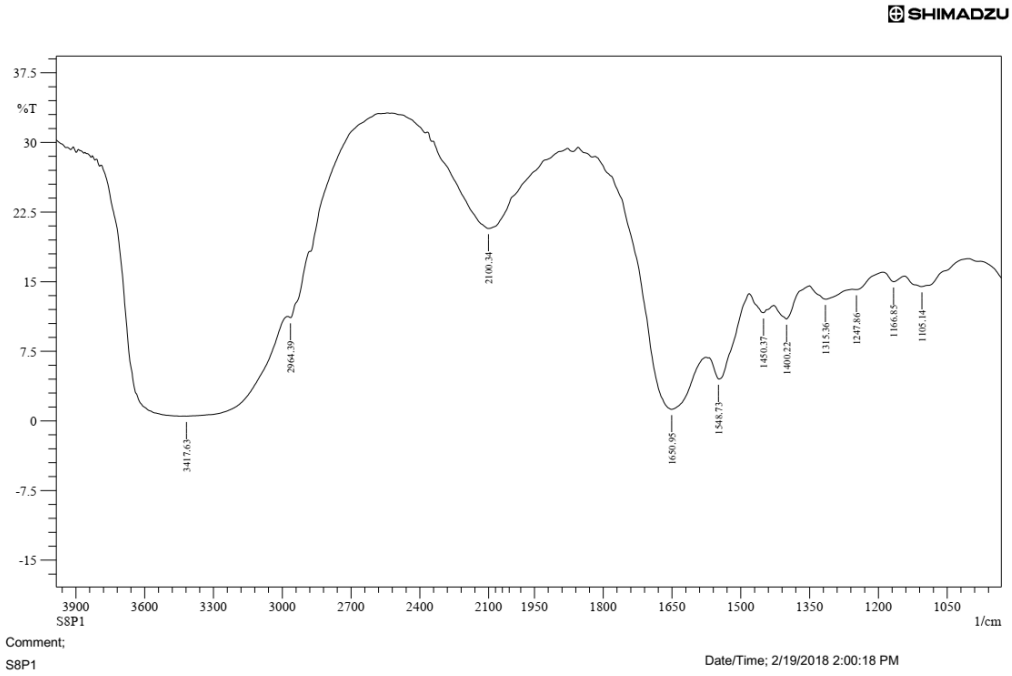

Figure 4.32: FTIR spectrum for normal blood sample irradiated to He-Ne laser power 1mW for 80 min

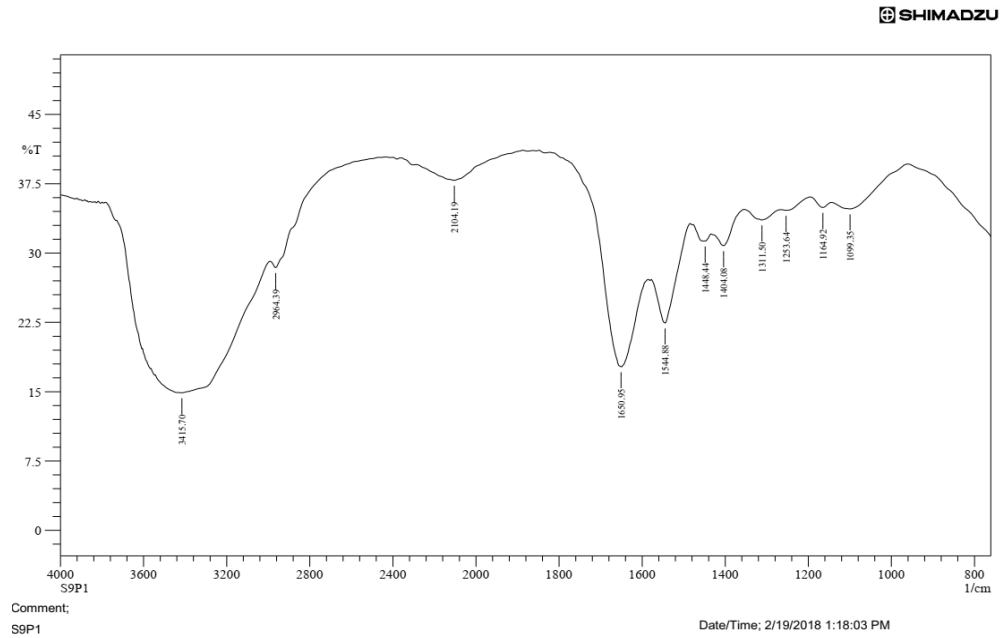

Figure 4.33: FTIR spectrum for normal blood sample irradiated to He-Ne laser power 1mW for 90 min

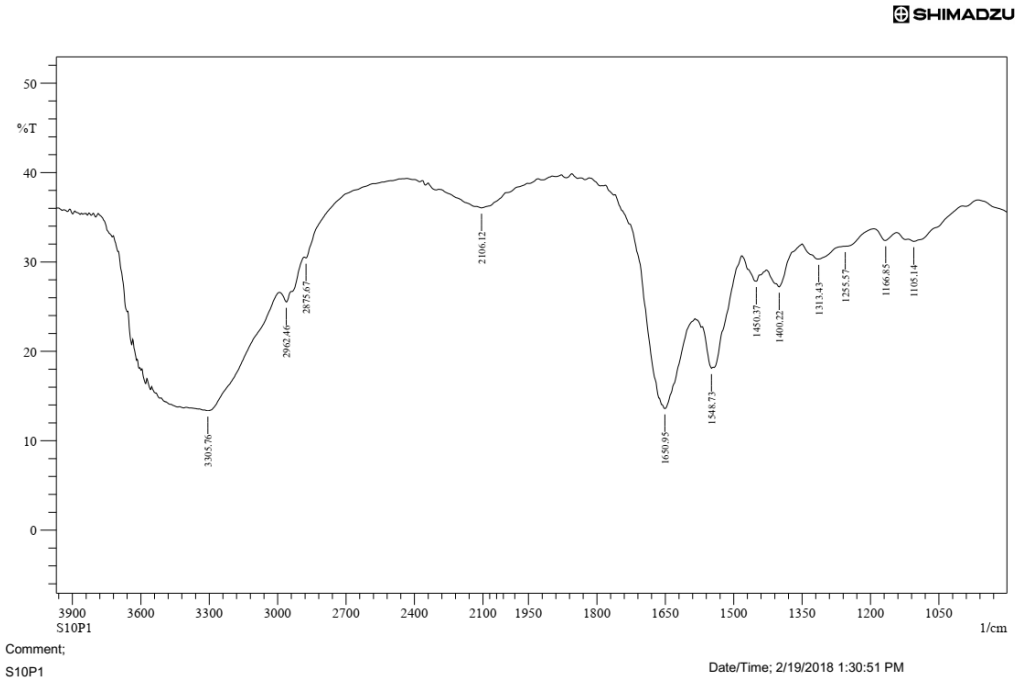

Figure 4.34: FTIR spectrum for normal blood sample irradiated to He-Ne laser power 1mW for 100 min