Chapter one
Introduction and Literature Review

Introduction 1.1

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 removes CO_2 from the system. Blood contains cellular components (red blood cells (RBCs); white blood cells (WBCs); and platelets) and plasma (which contains a host of proteins, hormones, glucose, etc.)

In photochemical interactions, light induces reactions and chemical effects within the tissue. Biostimulation and photodynamic therapy are essentially based on photochemical interactions. 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. In biostimulation methods laser irradiation is used to treat some diseases.

Fourier transform spectroscopy is applied to biofluids and 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.

1.2 Research Problem:

Study the interaction of laser with biomaterials such as blood and the effect of laser with it.

1.3 Literature review:

S.GUNASEKARANT, R.K.NATARAJAN, V.RENGANAYAKI and R.RATHIKHA (2008) Fourier Transform Infra-Red (FTIR) and UV-Visible spectroscopic techniques employed to study the spectral differences between a healthy serum and that affected with leukemia. The intensity ratio parameters (IRP) among the peaks were calculated in both the methods and it is found to be an indicator to differentiate a leukemic serum from the healthy one. Also to substantiate the findings, univariate statistical analysis has been made.

D.Yim, G.V.G. Baranoski, T.F.Chen, B.W.Kimmel and E.Miranda (2011) examined the biophysical processes responsible for the appearance attributes of whole blood, one the most fundamental of these materials. They describe a new appearance model that simulates the mechanisms

of light propagation and absorption within the cellular and fluid portions of this specialized tissue. The proposed model employs a comprehensive, and yet flexible first principles approach based on the morphological, optical and biochemical properties of blood cells. This approach allows for environment driven changes in the cells' anatomy and orientation to be appropriately included into the light transport simulations. The correctness and predictive capabilities of the proposed model were quantitatively and qualitatively evaluated through comparisons of modeled results with actual measured data and experimental observations reported in the scientific literature. Its incorporation into rendering systems is illustrated through images of blood samples depicting appearance variations controlled by physiologically meaningful parameters. besides the contributions to the modeling of material appearance.

V.H. GHADAGE , G.R. KULKARNI , B.N. ZAWARE (2015) Laser tissue interactions could be understood by using spectroscopic and advanced microscopic techniques such as scanning electron microscope (SEM).
Blood samples were collected from normal human subjects under standard laboratory conditions. Blood samples were irradiated by He-Ne laser (Wavelength λ= 632.8 nm, Power P = 3mW). The FTIR spectra for non-irradiated normal blood samples were compared with the FTIR spectra of irradiated blood samples. Significant changes were observed between the various bonds from the FTIR transmission spectra between C=O (Amide I), C-O (Anhydrides), N=O (Nitro), C-N (Amines) and C-H (Alkenes). The significant results were obtained when He-Ne laser irradiation was incident on whole blood for 30 and 40 minutes and the transmittance decreases due to denaturation of proteins. That would provide more inside into laser radiation - blood interactions and form a basic for therapeutic uses.

1.4 The Objective of this dissertation:

The main objective of the present work is to study the changes in FTIR and Uv-vis spectrophotometer spectra of whole blood in vitro due to Omega xp laser radiation ((λ= 675 nm, power = 30 mW) and report the changes in various functional groups .

1.5 Thesis Layout

This dissertation is consist of four chapters, the first one introduction and literature Review , chapter two deal basic concepts of laser, blood,

and light interaction with matter**,** chapter three about experimental part (The materials and device and method), chapter four consist Results and discussion and conclusion, recommendations finalyalist of reference.

Chapter Two

Basic Concepts

2.1 Laser:

The word (laser) is an acronym derived from Light Amplification by Stimulated Emission of Radiation. The light emitted by laser is different from that produced by more conventional light sources. laser is a device that generates or amplifies coherent radiation at frequencies in the infrared, visible or ultraviolet and other regions of the electromagnetic spectrum .

Lasers are distinguished from other light sources by their coherence. Spatial coherence is typically expressed through the output being a arrow beam which is diffraction-limited, often a so-called "pencil beam." Laser beams can be focused to very tiny spots, achieving a very high irradiance, or they can be launched into beams of very low divergence in order to concentrate their power at a large distance. (Mohamed Ahmed AbdElkareem, 2014).

Temporal (or longitudinal) coherence implies a polarized wave at a single frequency whose phase is correlated over a relatively large distance (the coherence length) along the beam. a beam produced by a thermal or other incoherent light source has an instantaneous amplitude and phase which vary randomly with respect to time and position, and thus a very short coherence length .

Most so-called "single wavelength" lasers actually produce radiation in several modes having slightly different frequencies (wavelengths), often not in asingle polarization and although temporal coherence implies monochromaticity , there are even lasers that emit a broad spectrum of light, or emit different wavelengths of light simultaneously. there are some lasers which are not single spatial mode and consequently their light beams diverge more than required by the diffraction limit.

However all such devices are classified as "lasers" based on their method of producing that light: stimulated emission, lasers are employed in applications where light of the required spatial or temporal coherence could not be produced using simpler technologies (Mohamed Ahmed AbdElkareem, 2014).

2.1.1 Properties of laser

Laser radiation shows as extremely high degree of monochromaticity ,coherence , directionality and brightness as compared to other noncoherent light sources.

A. Monochromaticity

TheMonochromaticity of laser radiation is a unique property of laser light, results from the circumstance that light oscillation sets in at one resonance frequency of the optical cavity, and owing to the balance between gain and loss in CW operation the line width ΔvL of the oscillating mode is ultimately limited by quantum noise. (Wsnaa Ali Eltayb Ibrahim, 2014).

B. Coherence:

The coherence of the laser radiation refers to the time period Δt in wich 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 is due to the fact that the gain medium is placed inside an open optical resonator. (Wsnaa Ali Eltayb Ibrahim, 2014).

D. Brightness:

The brightness of laser radiation is closely related to the directionality and stems from the capability of a laser oscillator to emit ahigh optical power in a small solid angle of space (Wsnaa Ali Eltayb Ibrahim, 2014).

2.1.2Laser construction

A laser system is constructed from three main parts:-

A. Pumping source

Pumping source is providing energy to the laser system for example electrical discharge, flash lamp, light from another laser, chemical reactions and even explosive devices. The type of pumping source uses principally depends on the gain medium, and this also determines how the energy is transmitted to the medium.

B. Laser Gain medium

Calls lasing medium results from stimulated emission of electronic or molecular transition from higher to lower energy state populated by a pump source.

C. The optical resonator or optical cavity

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

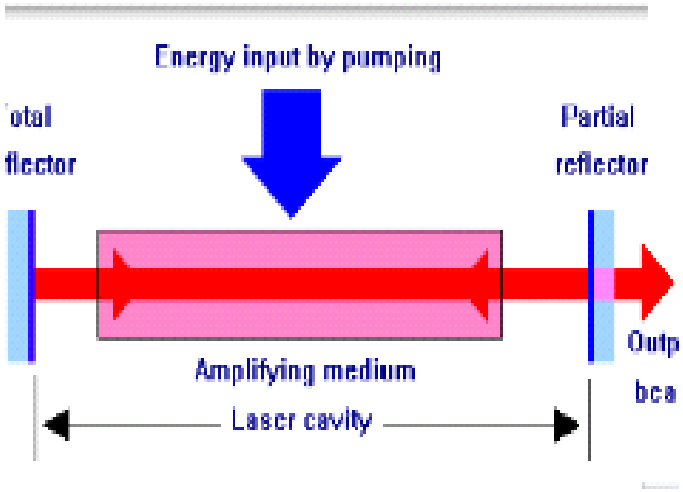


Figure 2.1 Laser system

2.1.3 Laser types

The various laser types developed so far display a wide range of physical and operating parameters. Indeed, if 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 lasers

A gas laser contains atoms or molecules. 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; CO2 laser; optically pumped gas lasers. (Karl F. Renk, 2011).

B. Solid state lasers

discuss solid state lasers that make use of 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 consists the ruby laser, the titanium–sapphire laser, neodymium-doped YAG laser, of other neodymium lasers, and of other YAG lasers. (Karl F. Renk, 2011).

C. Semiconductor lasers

Semiconductor lasers represent one of the most important class of lasers in use today, not only because of the large variety of direct applications in which they are involved, but also because they have found a widespread use as pumps for solid-state lasers.

These lasers will therefore be considered at some length. For the active medium, semiconductor lasers require a direct-gap material, so normal elemental semiconductors (e.g., Si or Ge) cannot be used.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) hence refered to as 1II-V compounds. (OrazioSvelto, 2002).

Omega XP laser

The omega XP laser system is a type of a semiconductor lasers (Gallium Aluminum Arsenide lasers) (GaAlAs) together with super luminous LEDs in a multi wavelength probes.

D. Liquid dye lasers:

Liquid dye lasers use a solution of complex dye material as the active medium. The dyes are large organic molecules, with molecular weights of several hundred. The dye material is dissolved in an organic solvent, like methly alcohol. Thus the active medium is a liquid. Dye lasers are the only types of liquid lasers which have reached a well-developed status.

One of the most important features that dye lasers offer is tunability, that is, the color of the output beam can be varied by adjusting the inter cavity tuning element and also by changing the type of the that is used. The monochromatic output of available dye lasers can be tuned over a broad range, from the ultraviolet, to the near infrared. Liquid dye lasers

that can be tuned to any visible wavelength, and to portions of the infrared and altraviolet, are commercially available in both pulsed and continuous models. Dye lasers are chosen for applications, like spectroscopy, in which tunability is important. (Ahmed Abdelrahman Ahmed Fadl,2007).

2.1.4 Laser applications:

Lasers are employed over a wide range of applications from scientific research, biomedicine, and environmental sciences to industrial material process, microelectronics and entertainment.

Some applications are: Industrial application like cutting , welding ,drilling by using CO_2 laser, ruby laser, argon ion laser , pulse N d:YAG laser.

Medical application like phototherapy of eye, tissue surgery, using (CO_2 laser, N d: YAG laser ,argon ion laser, and dye laser).

Military applications include range finders and beam weapons , by (CO_2 laser, N d:YAG laser, chemical laser, semiconductor laser). Other applications include Communication, information processing, super market scanners, printers, reading device for compact disc player, holography and spectroscopy.

Clinical and medical applications

One of the earliest applications of lasers in medicine was photocoagulation, using an argon-ion laser to seal off ruptured blood vessels on the retina of the eye. The laser beam 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. Today, lasers are used extensively in analytical instru mentation, ophthalmology, cellular sorting, and of course, to correct vision.

Many types of lasers are used in clinical applications including CO2, solid state, and diode lasers, as well as array of gas lasers covering the spectrum from the ultraviolet to the infrared.

(Basic Laser Principles)

2.2 Blood

Blood is composed 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, Blood constitute 8% of body mass . (https://ar.wikipedia.org/wiki/%)

Figure 2.2 shows the componants of blood.

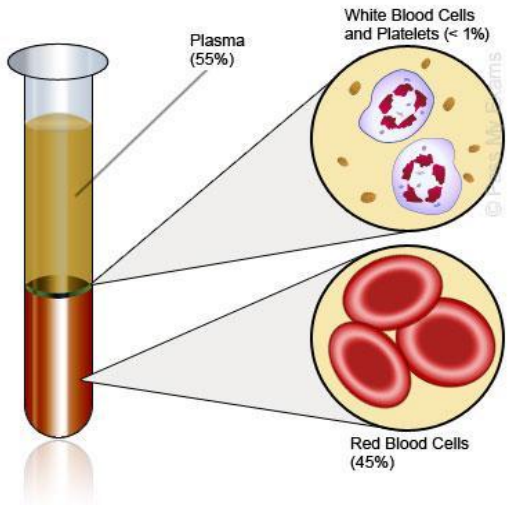


Figure 2.2. : Components of the blood

A. Plasma:

Plasma is material liquid transparent tend to yellowing and have an important role in water and solutes and also nutrients such as sugars, vitamins and hormones. There are 54% of the blood, such as article interstitial in the blood and is composed of 90% water (water a big role in terms maintains the body 's 37 heat ° C) and 10% other materials are dissolved ,such as 2% ion salts 1% of antibodies –hormones- dissolved gases. (https://ar.wikipedia.org/wiki/ %)

B.Red blood cells:

Cells are disc shaped concave – sided , its function transport of gases and concave surface in order to increase the gas exchange area, and features 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 the contents, the color red to the presence of material hemoglobin consists hemoglobin protein and iron,

the number nearly 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 is controlled by the kidneys by ahormone 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 hormonealaritropuecan 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. (https://ar.wikipedia.org/wiki/%)

Figure 2.3 shows the red blood cells

Figure 2.3: Red Blood Cells

C. 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 betweenseven 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. (https://ar.wikipedia.org/wiki/%)

There are five types of white blood cells , are: acidic and basic and neutral and lymphatic

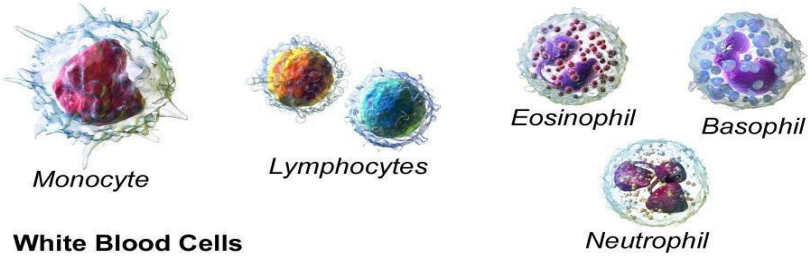


Figure2.4 White Blood Cells

And only it was broken by the appearance of the cytoplasm and 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 include lymphatic.

D. 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. (https://ar.wikipedia.org/wiki/%)

Figure 2.5 Platelets

2.2.1 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**.**
(https://ar.wikipedia.org/wiki/%)

2.2.2 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 a and in the same way for the group of blood B but in the absence of either of these Morttin be blood group O.
(https://ar.wikipedia.org/wiki/%)

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. (https://ar.wikipedia.org/wiki/%)

2.2.3 A 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**: Many of the tests do not show the presence of viruses in the blood directly. It is then screening for anti - virus in the blood, which is formed by the immune system to resist the virus.

- **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 begins hormonehCG 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** : frequent drinking alcohol spoil the blood. The number of white blood cells is reduced because of it . As part of the liquor

destroy red blood cells and grow in size. And measured those changes by setting the value of MCV.

- **Rheumatology** : Know autoimmune when the immune system to resist the healthy cells of thebody itself. In people who have that property left in their blood against the so- called ANA.

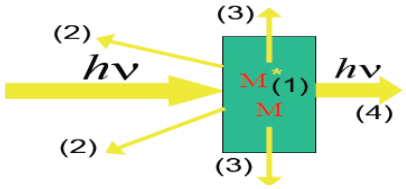
- **Thyroid** : people who labor quickly goes down they have the ability to concentrate may be suffering from poverty in the function of the thyroid gland. In that case , it appears in the blood high proportion of hormone TSH.

- **Cancer** : tumor caused by dead cells, moving in the circulatory system . There are ways to betested in the blood. They can see through genetic Tgieradtha know cancer causing type.

- **Anguish** : in the case of stress increases the secretion of the body of the hormonecorticosterone , which can be set in the blood. However , the high value of cortisone may arise from a reduction in blood sugar or a carrying case. (https://ar.wikipedia.org/wiki/%)

2.3 Light interaction with matter:

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



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

2.3.1Absorption:

If a light wave of a given frequency strikes 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 in

such a manner as to convert its vibration energy into thermal energy. Subsequently, the light wave with that given frequency is absorbed by the object .It is the transformation of radiant power to another type of energy, usually heat, by interaction with matter. In physics, absorption of

electromagnetic radiation is the way in which the energy of a photon is taken up by matter, typically the electrons of an atom. Thus, the electromagnetic energy is transformed into internal energy of the absorber, for example thermal energy. The reduction in intensity of a light wave propagating through a medium by absorption of a part of its photons is often called attenuation. Usually, the absorption of waves does not depend on their intensity (linear absorption), although in certain conditions (usually, in optics), the medium changes its transparency dependently on the intensity of waves going through, and satiable absorption (or nonlinear absorption) occurs.
The absorbance of an object quantifies how much of the incident light is absorbed by it. This may be related to other properties of the object through the Beer–Lambert law. The absorption coefficient determines how far into a material light of a particular wavelength can penetrate before it is absorbed. In a material with a low absorption coefficient, light is only poorly absorbed, and if the material is thin enough, it will appear transparent to that wavelength. The absorption coefficient depends on the material and also on the wavelength of light which is being absorbed.
Semiconductor materials have a sharp edge in their absorption coefficient, since light which has energy below the band gap does not have sufficient energy to excite an electron into the conduction band from the valence band.

2.3.2Reflection:

Reflection is the process by which electromagnetic radiation is returned either at the boundary between two media (surface reflection) or at the interior of a medium(volume reflection) .it is the change in direction of a wave front at an interface between two different media so that the wave front returns into the medium from which it originated. Common examples include the reflection of light, sound and water 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 seculars reflection. Reflection of light is either seculars (mirror-like) or diffuse depending on the nature of the interface. In specula reflection the phase of the reflected waves depends on the

choice of the origin of coordinates. (Wikipedia,2015). Diffuse reflection happen when light strikes the surface of a (non-metallic) material it bounces off in all directions due to multiple reflections by the microscopic irregularities inside the material and by its surface, if it is rough. Thus, an 'image' is not formed. This is called diffuse reflection. The exact form of the reflection depends on the structure of the material. Reflection and transmission of light waves occur because the frequencies of the light waves do not match the natural frequencies of vibration of the objects.

2.3.3Transmission:

It is the passage of electromagnetic radiation through a medium .The transmittance of a material is the proportion of the incident (approaching) light that moves all the way through to the other side.

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.

2.3.4Lightscattering:

Light scattering 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 (Kerker,M.1969)(Mandelstam ,L.I.1928). 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) (Bohren, C.F and Huffman, D.R 1983).

2.4 Spectroscopy:

Spectroscopy means study of the interaction between matter and radiated

energy and it used to refer to the measurement of radiation intensity as a function of wavelength .Spectroscopy is basically an experimental

subject and is concerned with the absorption, emission or scattering of electromagnetic radiation by atoms or molecules.

Electromagnetic radiation covers a wide wavelength range, from radio waves to grays, and the atoms or molecules may be in the gas, liquid or solid phase or, of great importance in surface chemistry, adsorbed on a solid surface.

Ultraviolet (UV) and visible radiation comprise only a small part of the electromagnetic spectrum, which includes such other forms of radiation as radio ,infrared (IR), cosmic, and X rays.

The energy associated with electromagnetic radiation is defined by the following equation:

$$E=hv$$

Where E is energy (in joules), h is Planck's constant (6.62 × 10-34 Js), and is frequency (in seconds).

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 are related by the following equation:

$$v=c/\lambda$$

Where v is frequency (in seconds), c is the speed of light (3 × 108 ms-1), and λ is wavelength (in meters).

In UV-visible spectroscopy, wavelength usually is expressed in nanometers (1 nm =10-9 m). It follows 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 radiation interacts with matter, a number of processes can occur, including reflection, scattering, absorbance, fluorescence/phosphorescence (absorption and reemission), and photochemical reaction (absorbance and bond breaking). In general, when measuring 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:

Total=Eelectronic + Evibrational + Erotational.

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

Eelectronic>Evibrational>Erotational.

In some molecules and atoms, photons of UV and visible light have enough energy to cause transitions between the different electronic energy levels. 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.

2.4.1 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. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state.

A hydrogen, deuterium or discharge lamp covers the ultraviolet range, and a tungsten filament (usually tungsten \halogen lamp) covers the visible range. The radiation is separated according to it‘s frequency\wavelength by a diffraction grating followed by a narrow slit. The slit ensures that the radiation is of a very narrow waveband it is monochromatic. The cells in the spectrometer must be made of pure silica. Detection of the radiation passing through the sample or reference cell can be achieved by either photomultiplier or photo diode, that converts photons of radiation into tiny electrical currents; or semiconducting cell (that emits electrons when radiation is incident on it) followed by an electron multiplier similar to those used in mass spectrometers. The spectrum is produced by comparing the currents generated by the sample and the reference beams.

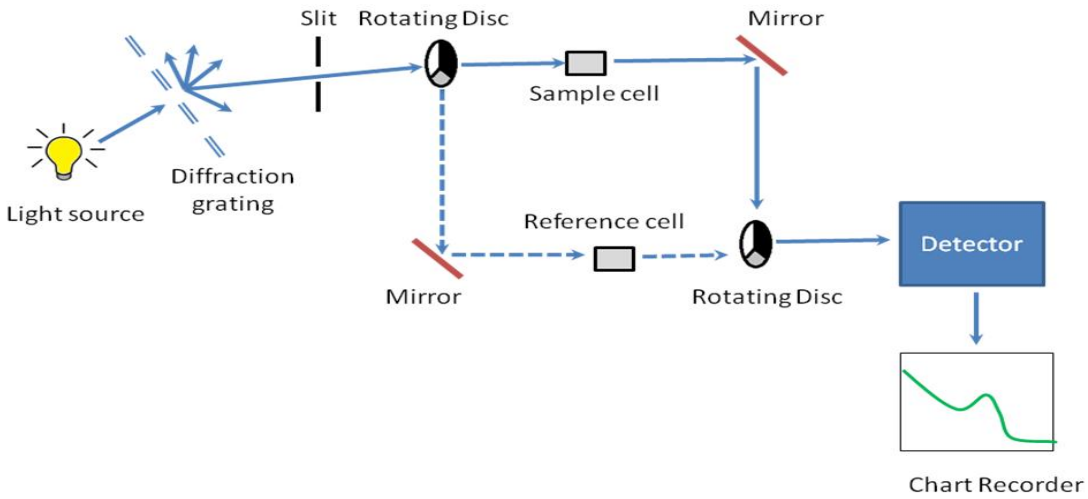


Figure 2.7 block diagram of Visible spectrometer

2.4.2 Fourier Transform Infrared (FTIR):

(FTIR) spectroscopy is a measurement technique that allows one to record infrared spectra. 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 data-processing technique called Fourier transform turns this raw data into the desired result (the sample's spectrum): Light output as a function of infrared wavelength (or equivalently, wavenumber). As described above, the sample's spectrum is always compared to a reference.
(https://en.wikipedia.org/wiki/Infrared_spectroscopy)

An alternate method for acquiring spectra is the "dispersive" or "scanning monochromator" method. In this approach, the sample is 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 virtually all modern infrared spectrometers are FTIR instruments.
(https://en.wikipedia.org/wiki/Infrared_spectroscopy)

ChapterThree

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:

Blood samples were collected from normal human subjects. the samples were irradiated by diode laser (Omega xp) with wavelength λ=675 nm, Power P = 30mW, the irradiation times were 1, 2 , 3, and 4 minutes. The samples of blood were obtained from two volunteers, and each sample was divided into five samples (1 ml for every one) for irradiation and control

Figure 3.1 Shows the samples while radiation

3.2.2 Devices:

3.2.2.1 Omega XP laser:

Omega xp 675nm, model XP serine number (2199) is founded in laser clinic. It used in many medical applications, the specification of laser Omega xp 675nm Wavelength 30 ~ 300 mW Output power, 3B Laser Classification
H:190 x D:300 x, W:260mm, Size Ga AlAs Medium

Figure (3.2) Omega xp laser

3.2.2.2 UV-VIS 1240 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 SHIMADZU contains a quartz cell of thickness 1 cm as a sample holder.

Figure (3.3) Photo of the The UV-VIS 1240 Spectrophotometer

3.2.2.3 FTIR spectrometer:

The Fourier transform infrared spectrometer used model IR300 Spectrometer made in USA.

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

3.3 Sample Preparation:

For UV-Visible spectral studies, each blood sample was diluted with normal saline (a volume of 1 mL of blood has been diluted with 10 mL of normal saline).

3.4 Procedure:

We collected the samples from two volunteers on EDTA containers, and pass power 30Mw of laser by Omega xp laser for different time (1,

2, 3 and 4 mints) for the samples of blood, and read the FTIR spectrometer of blood, then enter to UV –vis spectrophtometer and read the spectrum.

Chapter Four

Results and Discussion

4.1 Introduction:

This chapter summarize results obtained during the work. Results include photographs, figures and tables as shown below. Data fitting of experimental results was also shown, discussion and conclusion.

4.2 Results:

4.2.1 UV-Vis spectrophotometer Spectra

Figures (4.1) and (4.2) showed the spectra of UV-vis spectrophotometer for blood for normal sample and irradiated samples with diode laser (Omega xp) Wavelength λ=675nm, Power P=30mW and exposure times (1,2,3 and 4 min) .

Control Sample

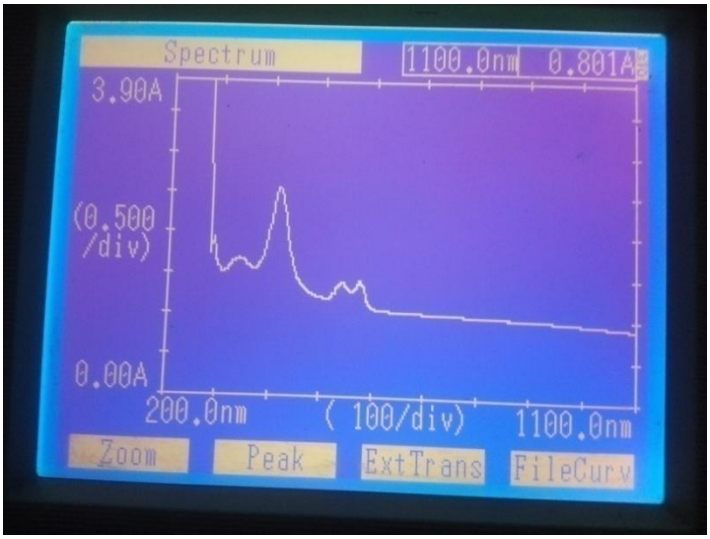
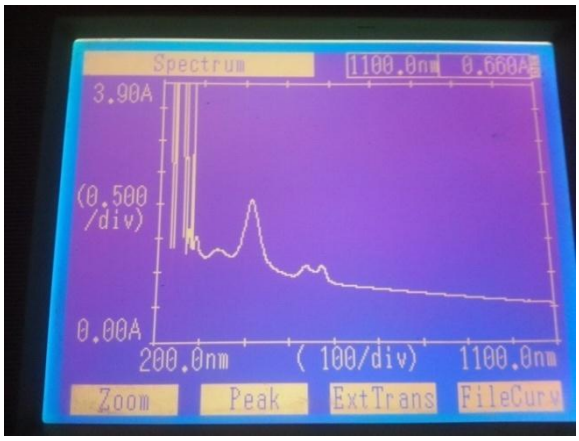


Figure (4.1) shows the spectrum of non- irradiated blood sample (control).

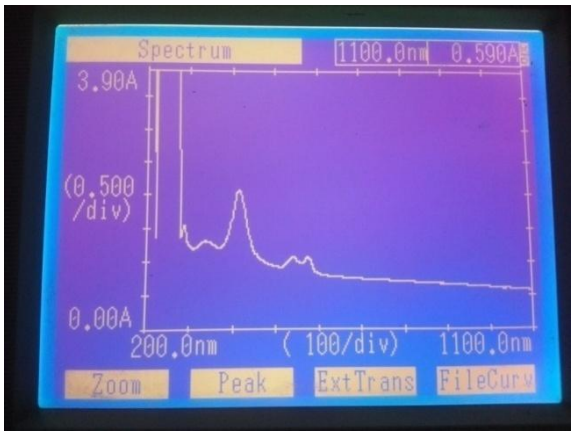
This spectrum referred to non- irradiated blood sample which specifid by peaks 576.0, 542.0, 416.0 and 340.0 with intensity 1.419, 1.399, 2.573and 1.682 respectively.

The results showed that as exposure time of laser radiation on whole blood increased, then photon dose also increases and the intensity of

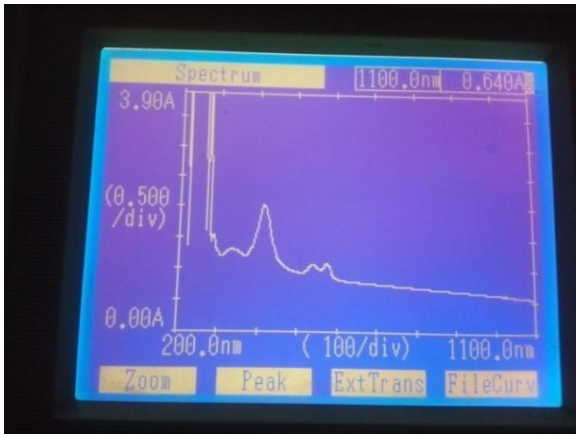
blood is decreases for all samples. As shown in figures 4-2 (a,b,c and d) below.



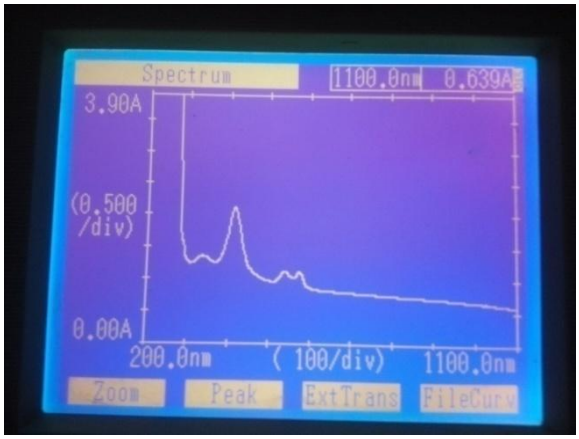
(a)



(b



(c)



(d)

Figure (4.2) Spectrum of irradiated samples (a,b,c and d) with different times (1,2,3 and 4)min respectively.

The table (4.1) below shows the values of UV-vis spectrophotometer peak spectra for blood, for the control sample that without radiation, and the other samples which exposure at different doses of radiation, and appears the intensity decrease with time of radiation.

Table (4.1) The intensity of normal and irradiated samples

wave numbers / cm^{-1} Peaks	Absorbance / (a u)				
	Control Sample	Sample 1 (1 mint)	Sample 2 (2 mint)	Sample 3 (3 mint)	Sample 4 (4 mint)
576.0	1.419	1.085	1.117	1.068	1.090
542.0	1.399	1.075	1.105	1.059	1.080
416.0	2.573	2.040	2.121	2.073	2.073
340	1.682	1.314	1.353	1.309	1.329
278.0	0.000	0.000	0.000	0.000	**4.000**

Different serum samples are analyzed quantitatively by calculating the intensities among the absorption peaks which is inversely proportional to the laser exposure time. This result indicate that there is photo degradation happened to the blood components.

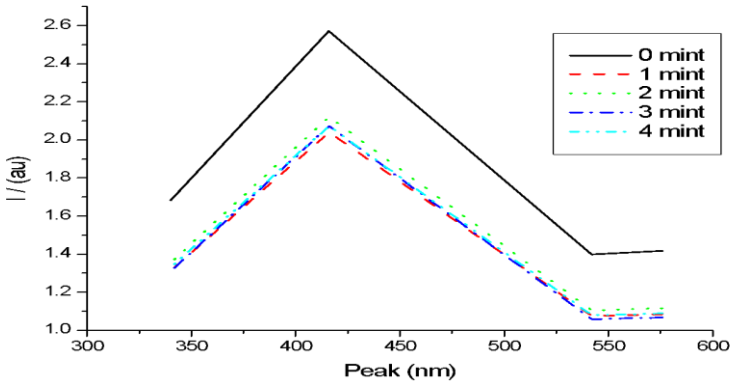


Fig (4.3) the Correlation between the Absorption Intensities and peaks of the different samples

Figure (4.3) shows that the absorbance of all irradiated samples decreased less than the non-irradiated sample and this due to decrease of blood component concentrations.

4.2. 2 Results of FTIR spectrometer:

Sample 1:

Control:

A satisfactory vibrational band assignment of absorption bands of the spectra is done with the help of the group frequency of the various constituents of the samples. Table (4-2) presents the vibrational band assignment of human serum.

Table4. 2. FTIR spectral analysis data

FTIR spectral data for normal blood (control)				FTIR spectrum of blood irradiated with Omega xp laser for duration 1, 2, 3 and 4 min.			
Sr.No	Wave number cm^{-1}	Group	% T	Irradiated Time (minute)	Wave number cm^{-1}	Group	% T
1	3325.93	O-H (free group)	54	1	3302.72	O-H (free group	55.33
2	1639.18	C=0 (Amide)	72.98		1637.58	C=O (Amide)	75.76
3	1221.29	C-0 (Anhydrides)	30.72		-	Absent C-O	-
4	772.18	C-H (Alkenes)	0.79		-	Absent C-H	-
5	671.317	C-H (Alkenes)	43.90		-	Absent C-H	-
6	3325.93	O-H (free group)	54	2	3302.92	O-H (free group)	73.80
7	1639.18	C=0 (Amide)	72.98		1647.98	C=0 (Amide)	86.43
8	1221.29	C-0 (Anhydrides)	30.72		-	Absent C-O	-
9	772.18	C-H (Alkenes)	0.79		-	Absent C-H	-
10	671.317	C-H (Alkenes)	43.90		-	Absent C-H	-
11	3325.93	O-H (free group)	54	3	3342.59	O-H (free group)	48.77

12	1639.18	C=0 (Amide)	72.98		3016.70	C-H (Alkenes)	75.17
13	1221.29	C-0 (Anhydrides)	30.72		1731.52	C=0 (Aldehyde)	27.38
14	772.18	C-H (Alkenes)	0.79		1629.03	C=0 (Amide)	36.49
15	671.317	C-H (Alkenes)	43.90		1363.18	N=O (Nitro)	24.20
16	-	Absent C-N	-		1218.25	C-N (Amin	21.48
17	3325.93	O-H (free group)	54	4	3316.97	O-H (free group)	15.40
18	1639.18	C=0 (Amide	72.98		1740.33	C=0 (Aldehyde)	14.18
19	1221.29	C-0 (Anhydrides)	30.72		1645.84	C=0 (Amide)	14.81
20	772.18	C-H (Alkenes)	79		1363.18	N=O (Nitro)	13.22
21	671.317	C-H (Alkenes)	43.90		1218.25	C-N (Amine)	12.06

The vibrational band at 3400 cm^{-1} is due to N-H stretching vibration of the secondary amides of protein. The asymmetric and symmetric stretching vibrations of the methyl group of the proteins and lipids are present in the region 3050-2800 cm^{-1}. It emerges from CH stretching vibrations of fatty acyl chains of all cellular lipids. The other two vibrational bands in the C-H stretching region are found to be present near 2922 and 2851 cm^{-1}, which are due to the asymmetric and symmetric stretching vibrations of the methylene group, respectively. The essential amide bands dominate in the region 1700-1500 cm^{-1}. The strong absorption band at 1655 cm^{-1} is assigned to C=O stretching of amide I of the proteins. The presence of band at 1548 cm^{-1} is due to the N-H bending vibrations of amide II that are strongly coupled to the C-N stretching vibrations of the protein amide group. The peaks at 1456 cm^{-1}, 1400 and 1315 cm^{-1} arise mainly from the asymmetric and symmetric deformations of methyl groups of proteins. The peak at 1400cm^{-1} may also be considered due to COO^- stretch of ionized amino acid chains, suggesting an increased contribution from carboxylate. The lipid phosphate band due to the asymmetric P-O stretching of PO2 occurs at 1240 cm^{-1}. The absorption bands at 1325, 1365, 1435 cm^{-1} arise dueto the CH bending of CH2 groups in a and β anomers. For glucose the optimal frequency range of 1250-925 cm^{-1} is used, since the mid IR spectrum of glucose includes several strong absorption bands in this region. The absorption peaks present at 1169, 1153, 1107, 1079 and 1035 cm^{-1} are

considered to be due to the different C-O stretching vibration of C-O-H and C-O-C bonds. The medium strength vibrational band present at 702cm^{-1} is assigned to N-H out of plane bending with the contribution of C-N torsional vibrations.

Figure 4.4 shows the FTIR spectrum for normal blood sample

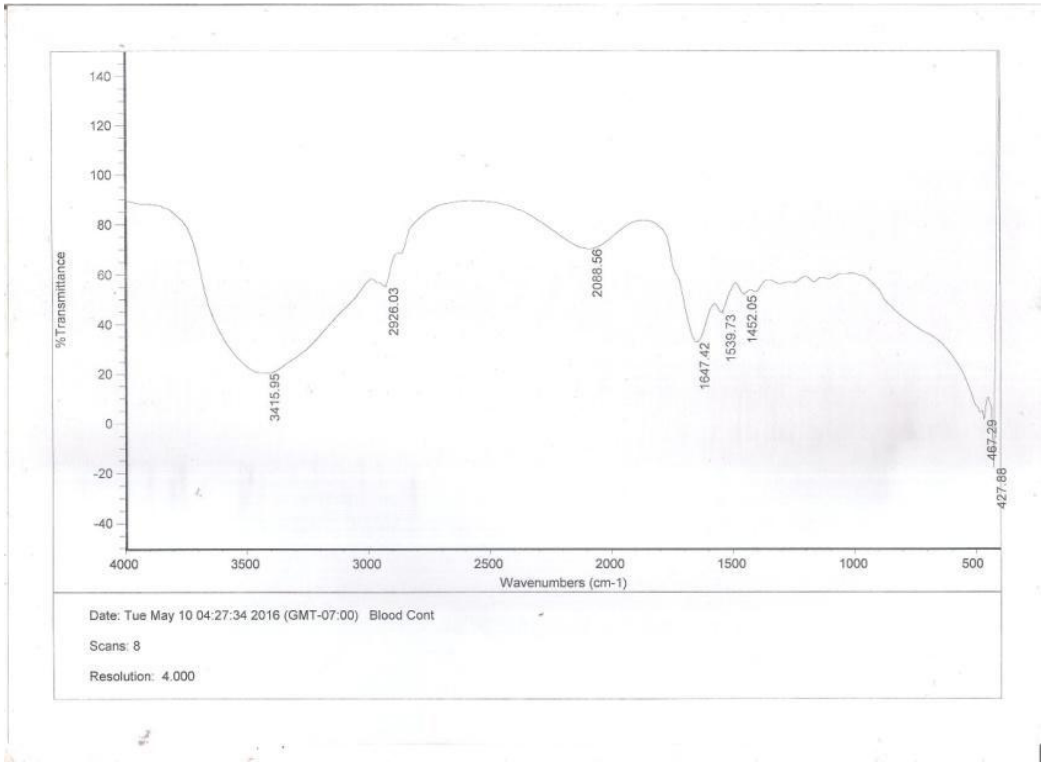


Figure (4-4) sample 1 FTIR spectrum for normal blood (control)

FTIR spectrum of normal whole blood laser radiation indicates the groups O - H, C = O, C - O and C - H.

As exposure time of laser radiation on whole blood is increased, then photon dose also increases. For more time of radiation on whole blood shows transmittance decreases to all bonds due to denaturation of proteins i.e. it breaks the polypeptide bonds due to conformational changes of proteins, as Figure (4-5) below

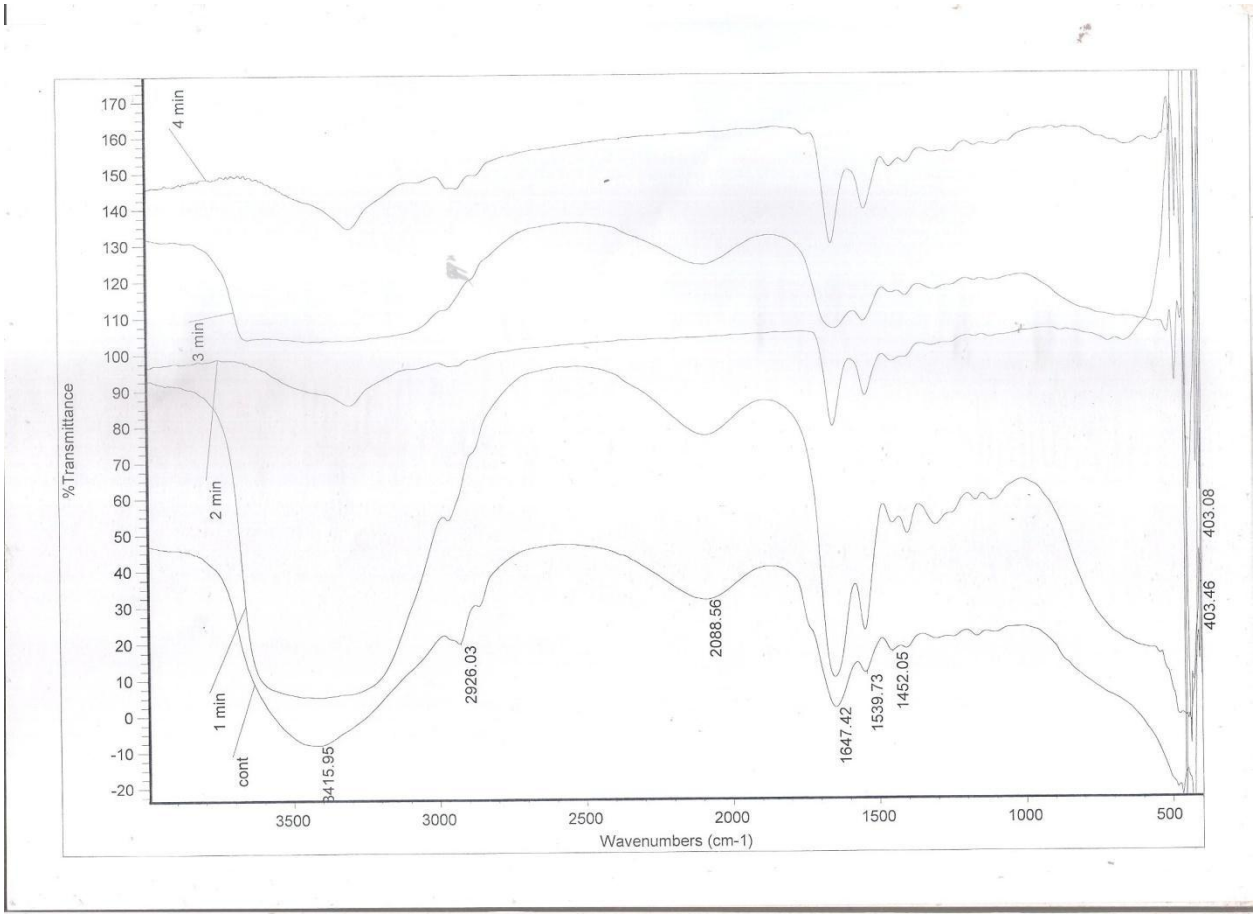


Figure (4-5) sample 1 FTIR spectra for whole blood

Sample 2:

Control

A satisfactory vibrational band assignment of absorption bands of the spectra is done with the help of the group frequency of the various constituents of the serum samples. Table-1 presents the vibrational band assignment of human serum.

The vibrational band at 3400 cm^{-1} is due to N-H stretching vibration of the secondary amides of protein. The asymmetric and symmetric stretching vibrations of the methyl group of the proteins and lipids are present in the region 3050-2800 cm^{-1}. It emerges from CH stretching vibrations of fatty acyl chains of all cellular lipids. The other two vibrational bands in the C-H stretching region are found to be present near 2922 and 2851 cm^{-1}, which are due to the asymmetric and symmetric stretching vibrations of the methylene group, respectively. The essential amide bands dominate in the region 1700-1500 cm^{-1}. The strong absorption band at 1655 cm^{-1} is assigned to C=O stretching of amide I of the proteins. The presence of band at 1548 cm^{-1} is due to the

N-H bending vibrations of amide II that are strongly coupled to the C-N stretching vibrations of the protein amide group. The peaks at 1456 cm^{-1}, 1400 and 1315 cm^{-1} arise mainly from the asymmetric and symmetric deformations of methyl groups of proteins. The peak at 1400cm^{-1} may also be considered due to COO^{-} stretch of ionized amino acid chains, suggesting an increased contribution from carboxylate. The lipid phosphate band due to the asymmetric P-O stretching of PO2 occurs at 1240 cm^{-1}. The absorption bands at 1325, 1365, 1435 cm^{-1} arise due to the CH bending of CH2 groups in a and β anomers. For glucose the optimal frequency range of 1250-925 cm^{-1} is used, since the mid IR spectrum of glucose includes several strong absorption bands in this region. The absorption peaks present at 1169, 1153, 1107, 1079 and 1035 cm^{-1} are considered to be due to the different C-O stretching vibration of C-O-H and C-O-C bonds. The medium strength vibrational band present at 702cm^{-1} is assigned to N-H out of plane bending with the contribution of C-N torsional vibrations.

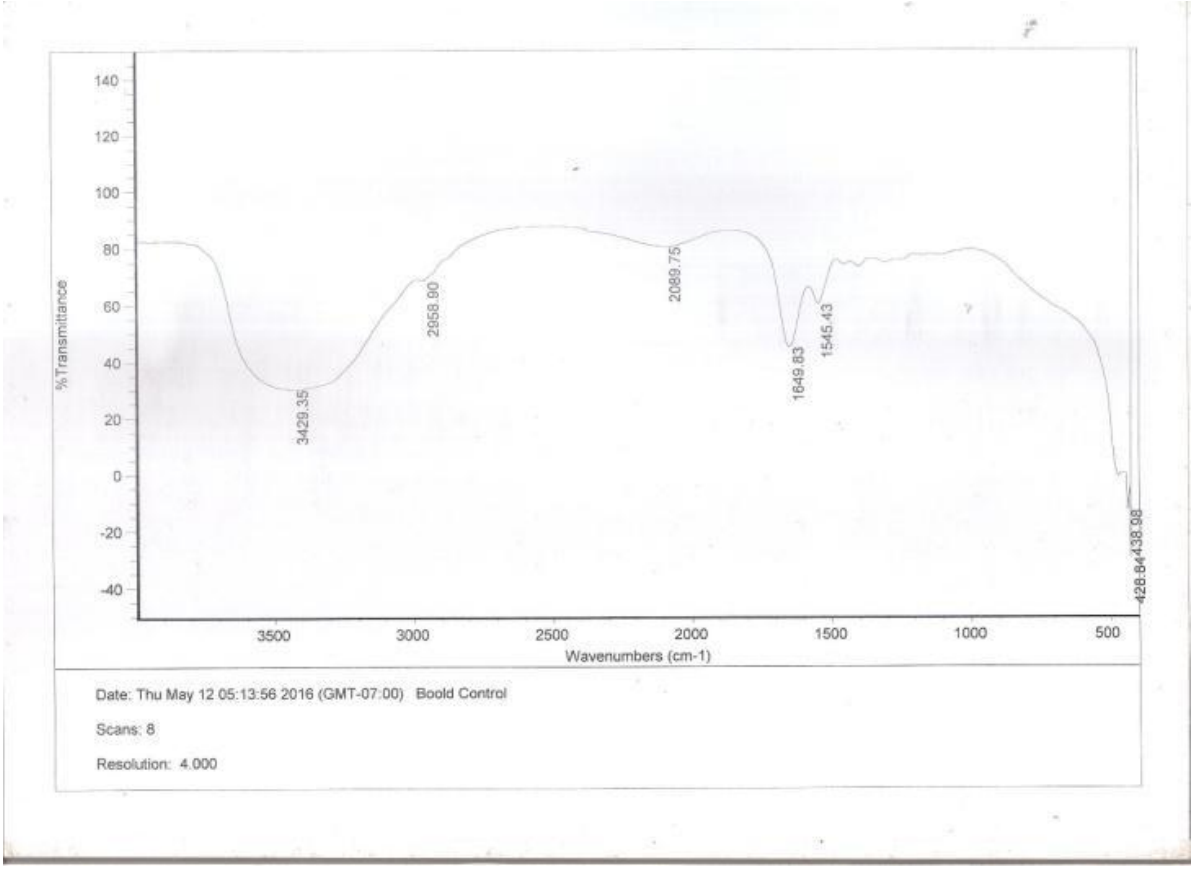


Figure (4.6) sample 2 FTIR spectrums for normal blood (control)

FTIR spectrum of normal whole blood laser radiation indicates the groups O - H, C = O, C - O and C - H.

As exposure time of laser radiation on whole blood is increased, then photon dose also increases. For more time of radiation on whole blood shows transmittance decreases to all bonds due to denaturation of proteins i.e. it breaks the polypeptide bonds due to conformational changes of proteins , as Figure (4 -7) below

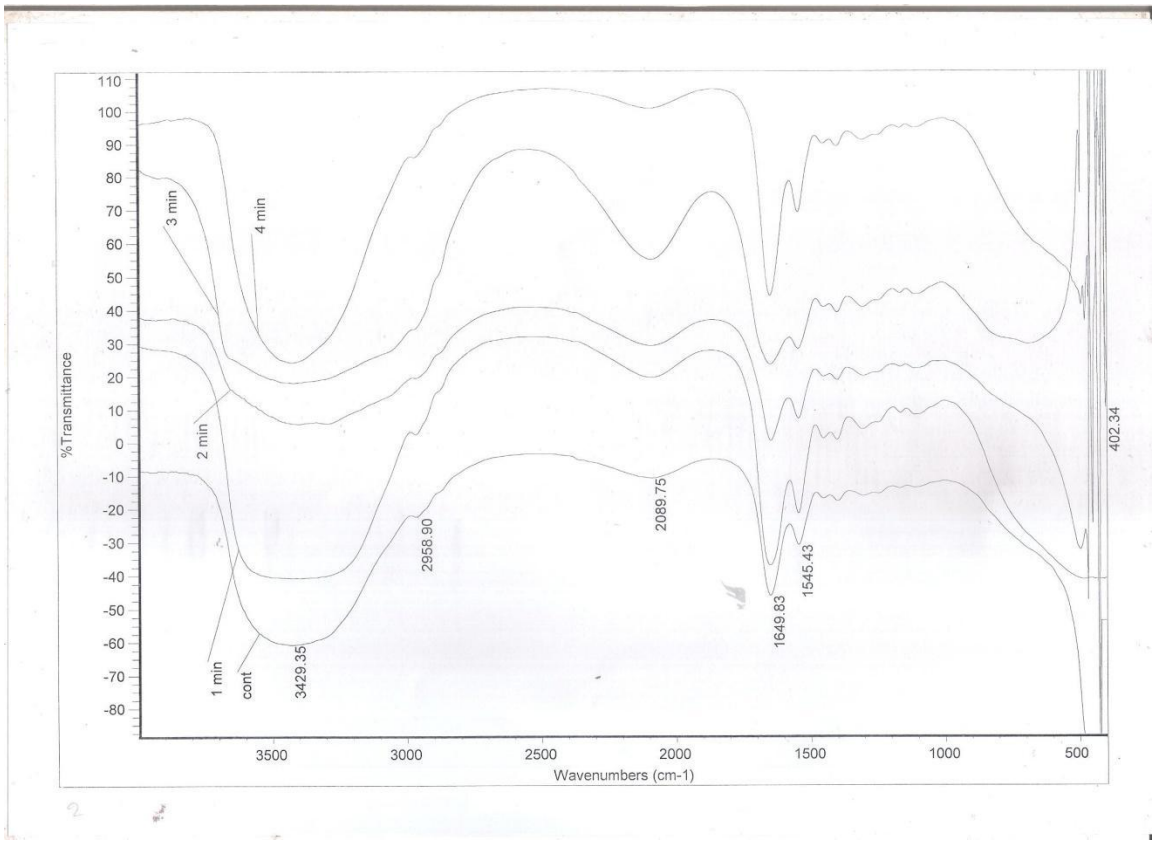


Figure (4.7) sample2 FTIR spectra for whole blood

In comparison with a previous study 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 laser diode (λ=675nm, power 30mW) was used with time exposure 1, 2, 3, and 4 minutes.

The results for the previous study

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 FTIR spectra show the groups O - H and C=O, and other groups C-O and C-H were absent at high doses.

UV-Visible spectra show that there was a decrease in the intensities of the irradiated four samples in comparison with the control sample, and there was no relation with variation in the dose with the intensities of the irradiated samples.

In addition this study shows clearly the decrease in the intensities of blood for samples with increasing time irradiation.

Conclusion:

From the obtained results the following conclusion is drawn:

The spectroscopic studies (IR and UV-Vis spectra) of blood samples for non-irradiation and irradiation showed some remarkable differences in terms of FTIR spectral profiles, absorption bands, wave numbers and the intensity and satisfactory analysis has been made. By increasing laser doses on whole blood shows transmittance decreases to all bonds due to denaturation of samples components.

Recommendations:

Future study could be done by:

1- Selecting different doses of laser by changing exsposure time, wavelength, power, modes.

2- Studying this effect in wide range of samples in different gender and edges.

3- Studying the possibility of using this technique in decreasing protein, amino, acids, in blood as treatment.

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Wikipedia 2015

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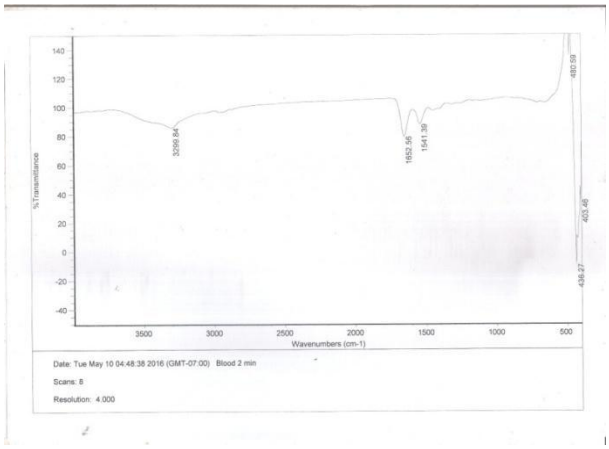
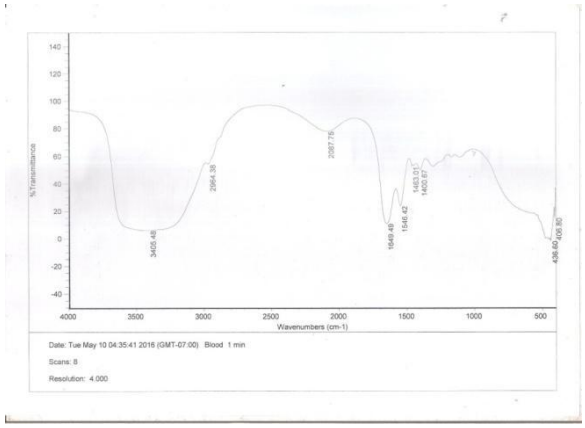
Appendix:

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

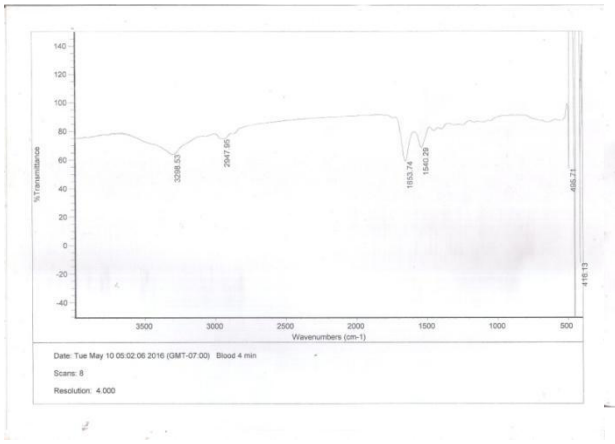
For experiment one:



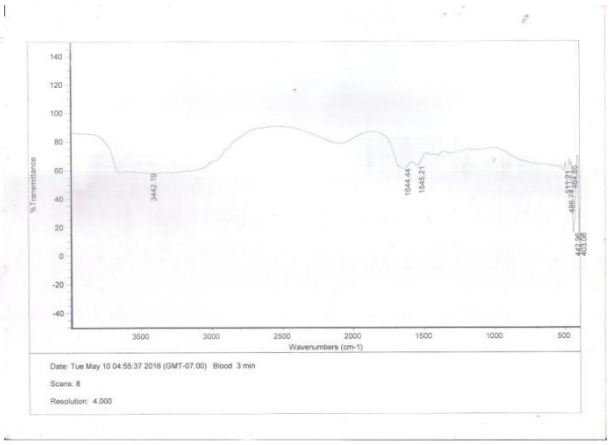
A



B



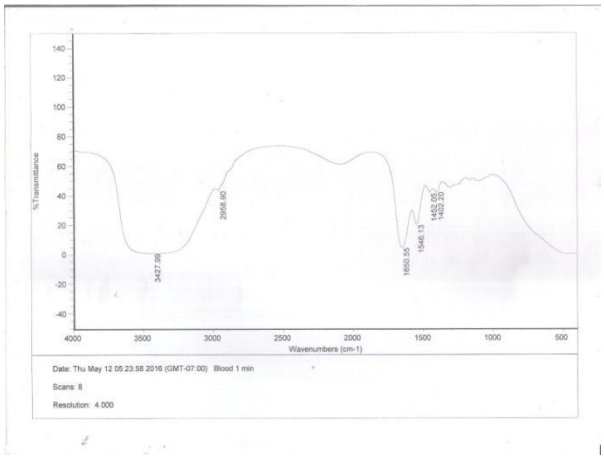
C

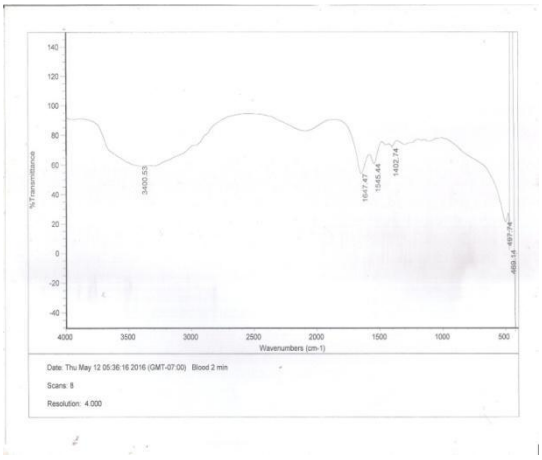


D

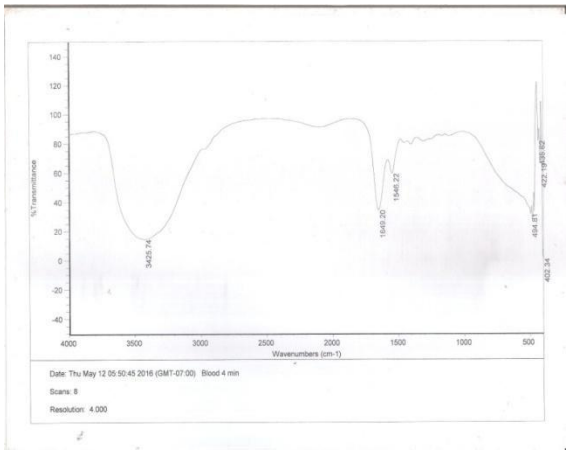
Figure (4.8) shows the spectra of irradiation blood samples (A, B,C and D) at time(1,2,3 and 4 min) respectively.

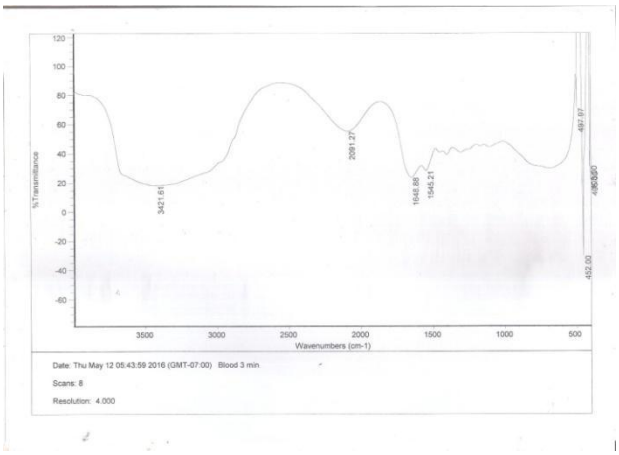
For expirment two:





A **B**





C **D**

Figure (4.9) shows the spectra of irradiation blood samples (A, B,C and D) at time(1,2,3 and 4 min) respectively.