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Department of Laser

Linear and Nonlinear Optical Properties of Blood Diagnoses for β-Thalassemia Patients

A Thesis

Submitted to the Council of the College of Science for Women / University of Babylon in Partial Fulfillment of the Requirements for the Master Degree in Laser Physics

By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿وَلَمَّا بَلَغَ أَشُدَّهُ آتَيْنَاهُ حُكْمًا وَعِلْمًا وَكَذَلِكَ نَجْزِي الْمُحْسِنِينَ﴾

صدق اللَّهُ العظيم

سورة يوسف(22)

Supervisors Certification

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Dedication

To the one who stood beside me and stayed up the nights and was patient to get to where I am (my mother) may God perpetuate her as an asset

To the one who supported me and was never stingy with anything (my father), may God protect him

To those who light the way for me and support me (my brothers), may God grant them success

To the comfort of my soul, good companionship and lasting love… my friend (Zainab Adnan)

Dedicate the results of this research to them

Ghufran

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Abstract

This study examines the possibility of using linear and nonlinear characteristics to diagnose some blood diseases and studying the ways through which this goal can be reached. This study was conducted in the laboratories of the College of Science for Girls and in cooperation with the Central Blood Bank in Babylon Governorate and the Thalassemia Center in the Children's Hospital for the period from December (2021) to July (2022).

The results obtained for the physical parameters (absorbance, transmittance, linear absorption coefficient, refractive index, extinction coefficient) for normal blood PCV (40%, 42%, 45%) and for concentrations 15%, 25%, 35%, NaCl, where the results for concentration 15% had the highest value of absorbance It is (0.688) in the case of the pcv45% sample, and the lowest value was for the pcv40% sample is 0.428, and this applies to the pcv42% sample. As for blood with high viscosity, the highest value of absorbance in the case of a concentration of 15% was 0.828 at pcv60% and the lowest value It is 0.758 when the sample pcv50%. Thalassemia is one of the common diseases in Iraq, and it results from the smallness and deformation of red blood cells. Samples were taken that have PCV (33%, 25%, 22%, 19%) and the highest value of absorbance in the case of concentration was 15% It is 0.405 when the pcv33% and the lowest value is 0.268 when the sample pcv19%. The blood components were separated into plasma and serum using only water to ensure complete cell lysis and dilution. It was noted that the highest absorbance was for blood viscosity samples at pcv50% (for hemoglobin, plasma and serum) was (0.948, 0.498, 0.393), respectively. This applies on the rest of the parameters, and the values of these components begin to decrease as the PCV decreases. On the contrary, it is noted that the emission spectrum in the case of diluted concentrations is higher in intensity and shifts towards the red region of plasma and serum for all samples and at a wavelength of 475 nm, noting that the wavelength of absorption was 414 nm. The study showed the nonlinear optical properties of samples (normal blood, blood viscosity, thalassemia) where the nonlinear refractive index and the nonlinear absorption coefficient were calculated based on the value of nonlinear transmittance and

then calculating the nonlinear susceptibility of the third order, where it was noted that each case had its own characteristics, so the natural blood samples were intermediate between viscosity and thalassemia, The nonlinear refractive index, nonlinear absorption coefficient and nonlinear optical susceptibility are of the third order for PCV45% (nonlinear refractive index 2.377 cm^2 /W, nonlinear absorption coefficient 28.019 m/W, nonlinear optical susceptibility 5.744 cm/W) for the same concentration of 15%, respectively. As for these parameters in the case of blood viscosity and for PCV60%, they were (nonlinear refractive index 2.864 cm^2 /W, nonlinear optical susceptibility 24.232 m/W, nonlinear absorption coefficient 4.624 cm/W) and for the same concentration, for thalassemia patients, it gave different results as it was for the same parameters mentioned for PCV33% (nonlinear refractive index 2.160 cm^2 /W, nonlinear absorption coefficient 23.017 m/W, nonlinear optical susceptibility 4.594 cm.

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Symbol	Meaning	Units
Hcts	Hematocrits	
RBCs	Red blood cells	
n	Refractive index	
He-Ne	*Helium–Neon* laser	
Nd: YVO4	Neodymium-doped yttrium *vanadate*	
NIR	Near infrared	

ATPases	Adenosine triphosphate synthase	
RI	Refractive index	
Hb	Hemoglobin	
DPSSLs	Diode pumped solid state lasers	
UV	Ultra Violet	
PCV	Packed cell volume	
LEDs	Light emitting diodes	
Epo	erythropoietin	
RBC	Red blood cell	
A	absorbance	
T	transmittance	
α	absorption coefficient	cm^{-1}
K	extinction coefficient	cm^{-1}/M
S_0	Ground energy level	
TEM00	Transition Electro Mode	
I_0	Incident Light Intensity	W/m^2
$\Delta\Phi_{\circ}$	Nonlinear phase shift	Rad
k	Wave Vector	cm^{-1}
λ	wavelength	Nm
U_x	Potential energy	J

χ	The linear susceptibility of the material	
χ^1	One-Order Nonlinear Optical Susceptibility	e.s.u
χ^2	Second-Order Nonlinear Optical Susceptibility	e.s.u
$\chi 3$	Third-Order Nonlinear Optical Susceptibility	e.s.u
P	Polarization	C/m^2
$\varepsilon_{\circ}$	Dielectric Constant	$C^2 N^{-1} m^{-2}$
n_o	The linear refractive index	
n_2	Nonlinear refractive index	cm^2 /mw
β	Nonlinear absorption coefficient	cm/mw
$\alpha_{\circ}$	Linear absorption coefficient	cm^{-1}
c	Speed of Light in Vacuum	m/s
v	Speed of light in media	m/s
ΔT_{p-v}	normalized transmittance difference between the top and valley	
S	The Linear Transmittance of the Aperture	

r_a	Aperture Radius	mm
ω_a	Beam Radius at the Aperture	mm
L_{eff}	Effective Length	mm
L	Sample Length	
EDTA	Ethylene Diamine Tetra Acetic Acid	
PMT	Photomultiplier Tube	
USB	*Universal Serial Bus*	

Chapter One

Introduction

(1-1): Introduction

Biophysics, or biological physics, is one of the well-known overlapping branches that link physics and biology [1]. Biophysics is concerned with the study of biological phenomena and processes in biological systems by applying the concepts, basics and theories of physics, laws and methods that are used in physics to biological systems [2,3]. The nature of functions can be understood. The various biological systems, by conducting physical analyzes and searching for appropriate explanations for the phenomena that result from the activities in the biological tissues, based on this, it can be said that the term biophysics includes applications of the concepts and methods of physics on the body of the living organism with its various living components such as tissues, cells and other organs in addition to the vital activities related to these components in both health and disease[4-7].

The light generated from laser devices interacts with the tissue in four different ways: transmission, reflection, scattering, and absorption. The most important for the biological effect of laser light is absorption. The tissue absorbs photon energy which, in turn, as radiant energy can be reemitted or transformed into heat, and increase the internal temperature of the tissue [5].

The emitted laser light has the form of a much focused, parallel monochromatic beam (with one specific wavelength) with very high intensity. The laser light differs from the light emitted by other sources by the beam, which is consistent, coherent, and its rays have the same wavelength [6].

Laser irradiation is described by several parameters. The wavelength is the most important. It determines the depth of the penetration by the light the higher the wavelength, the greater the laser penetration through the tissues [7].

This is also associated with the thermal effect caused by the light which increases with the increasing wavelength. The next parameters are the density of the laser energy, and the duration of radiation. Both parameters specify the general laser irradiation dose absorbed by the cells, which in turn differently affect the cell metabolism.

The last parameter of the laser impact on biological tissues is a type of impulse: continuous or pulsating [8].

Each tissue has specific absorption characteristics base on its composition and chromophore content. The principal chromophores tissue is: (Hemoglobin, Melanin, Water, Protein) [9].

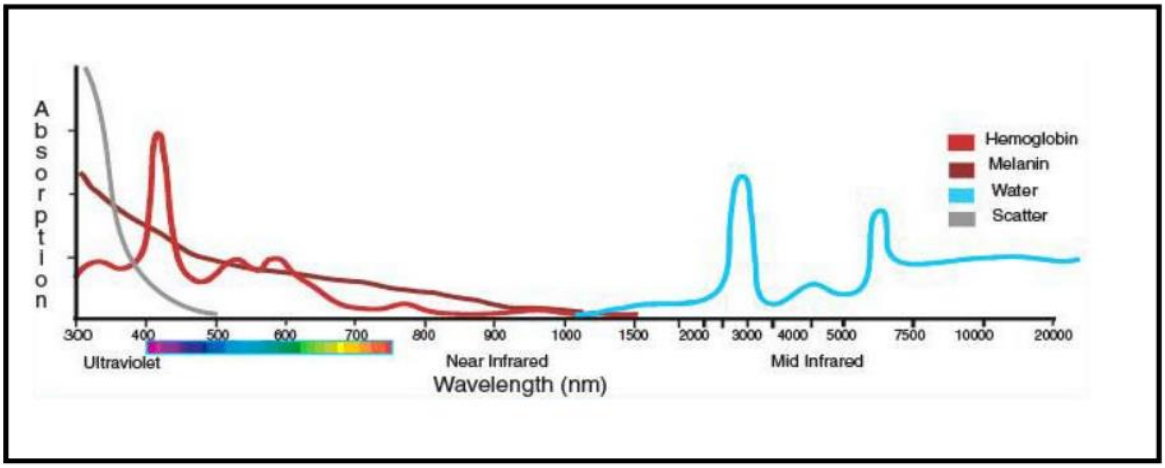

Figure (1- 1) Absorption of the main Chromophore [10]

Moreover, laser scattering in the biological tissue determines the intensity of light energy [11].

The scattering amount of energy of the laser is inversely proportional to the wavelength. The penetration of laser light in biological tissue increases with wavelength up to mid-infrared, where water that present in the tissues, absorbs the most energy of laser light [12].

The optical properties of materials are modified by high-intensity light such as a laser. The study of this phenomenon is called Nonlinear Optics.

This phenomenon is observed in a wide variety of materials like dyes, polymers, semiconductors, nanomaterials, Etc. After the invention of the first work laser by Theodore Maiman in 1960, Peter Franken et al demonstrated the first nonlinear optical phenomenon second-harmonic generation in 1961. This has been considered the beginning of the study of nonlinear optics [13]. One of the important applications of laser is its use in diagnosis and treatment The lasers enabled the creation and development of devices which by using the fluorescence phenomena combined with the monoclonal antibodies' methodology became a powerful tool to study cell metabolism and functions [14]. The biological and medical diagnosis of cases of disease in the tissues and components of the body and addressing its treatment requires knowledge of the properties of the biological tissue that interacts with the laser beams used, such as the optical and thermal properties that change as a result of the interaction process within the biological tissue [15,16].

(1-2): Literature review

The study of the interaction of laser radiation with tissues is an important subject, especially blood tissues, because of its clear effect in maintaining body balance, regulating its temperature, and many other functions. Some studies have also shown the study of linear and nonlinear optical properties of some diseases, so the following are the most important studies for this purpose.

Martina Meinke *et al.* (2007), explained absorption coefficient, scattering coefficient, and effective scattering phase function of human red blood cells (RBCs) in saline solution were determined for eight different hematocrits (Hcts) between 0.84% and 42.1% in the wavelength range of 250–1100 nm using field measurements. The reverse of the Monte Carlo technique [17].

Cholesterol and triglycerides in blood measurement by Z-scan technique is proposed by Dhinaa, A. N., and P. K. Palanisamy (2009), of. The nonlinear refractive index of cholesterol and triglycerides was found to vary linearly with concentration. Hence by calculating the nonlinear refractive index it is possible to measure their concentration in the sample. These measured values are found in equivalence with conventional colorimetric method [18].

Dhinaa, A. N., and P. K. Palanisamy (2010), study the Z-scanning technique used, which is a simple and effective tool, to determine the appropriate nonlinear optical properties of materials. This technique is used to measure urea and uric acid in the blood. The results of this method were found to be in good agreement with traditional color method [19].

Ansari, Mohammad Ali, and Ezeddin Mohajeran (2011), study the optical properties of biological tissues were known, such as absorption, scattering, penetration and fluorescence, and the effects of these properties on penetration according to that, lasers were classified in both the diagnostic and thera-peutic branches. This can help doctors to choose the optimal laser systems to perform the required treatment. [20].

In the same year Cruzado.et al concluded the optical parameters of the tissues are the basis for the application of light in diagnostic and therapeutic procedures in their study, so the optical properties were modified by cell death or cell aggregation, which cannot be avoided. Tested at 632nm using an optical setup consisting of double integrating sphere system [21].

Nonlinear Optical Properties of Sodium Fluorescence Dye in Water with Different Concentrations (1×10^{-5}, 2×10^{-5}, 5×10^{-5}, 7×10^{-5}, 1×10^{-4}) m with a constant thickness of 1 mm using the Z-Scan method explained by Nader R. (2015), to evaluate the nonlinear refractive index (n_2)

and the nonlinear absorption coefficient (β). It found that the nonlinear coefficients change with increasing dye concentrations [22].

In the same year Sun, Qiqi et al. The study of the fluorescence properties of heme and globin, the experimental results reveal that heme is the sole fluorophore of hemoglobin. Hemoglobin fluorescence can be effectively excited only via two-photon process, because heme has a centrosymmetric molecular structure and two-photon allowed transition is forbidden for single-photon process and vice versa due to the Laporte parity selection rule [23].

Alhussainy, Sadiq H. (2016), was found determination the effect of laser radiation on antioxidant system, the levels of catalase activity were progressively increase (P>0.05) in radiated blood samples. On the other hand, level of malondialdehyde (product of lipid peroxidation) pointed out a significant fall (p > 0.05) in both groups of radiated blood radiated blood samples. On the other hand, level of malondialdehyde (product of lipid peroxidation) pointed out a significant fall (p > 0.05) in both groups of radiated blood peroxidation compares to non-radiated blood samples.

The benefits of laser effects are included increase the efficiency of antioxidant system against oxidative stress in a whole-body tissue. The blood samples were exposed to two types of lasers, first laser was He-Ne laser with wavelength 632.8nm. With power 2mw. The second laser was Nd: YVO4 with wavelength 532nm and 4mw [24].

Lazareva, Ekaterina N., and Valery V. (2018), Tuchin focused on the measurements of the refractive index of hemoglobin solutions in the visible/near-infrared (NIR) spectral range at room temperature for characteristic laser wavelengths: 480, 486, 546, 589, 644, 656, 680, 930, 1100, 1300, and 1550 nm. Measurements were performed using the multi wavelength Abbe refractometer. Aqua hemoglobin solutions of different concentrations obtained from human whole blood were investigated. The

specific increment of refractive index on hemoglobin concentration and the Sellmeier coefficients were calculated [25].

Sadiq Hassan *et al*. (2018), explain how light energy of laser can be affected of ion pumps (ATPase) and concentrations of available ions across cellular membranes. A whole blood samples were collected and divided into three groups, The first subgroup was irradiated with laser 532nm, 4mw for 10 minutes and the second subgroup was irradiated with 650nm, 135mw for 10 minutes, there after these samples were used to determine the activities of ATPase and the obtained results showed a remarkable increase in the ATPase activities of irradiated blood samples when compared with non-irradiated blood samples [26].

Gienger, *et al*. (2019), noted that knowledge of the optical properties of biological cells is necessary to explain their interaction with light. A method is presented to determine the dependence between the refractive index (RI) of human erythrocytes and the intracellular hemoglobin (Hb) concentration. The procedure is based on the analysis of the mean extinction cross-section of the corresponding group $C^{-}ext(\lambda)$ [27].

In the same year Fatemeh Shahini et al. investigated nonlinear optical properties of blood serum using a continuous wave laser at a wavelength of 532 nm. Utilizing open and close aperture of a Z-scan technique enables a measurement of the nonlinear refractive index of blood serum. Our results show that increases because of increasing of the glucose content when the iron content is constant and measured the refractive index of this serum using Sheik-Bahae's and Z-scan models [28].

Ali Al - Saidi *et al*. (2020), founded that the absorption spectra of the control blood sample and the normal blood samples irradiated with the laser beam of the wavelength 671 nm for different irradiation times. It is noticed that the spectral peaks of the absorbance are dropped down compared to those of the normal blood samples irradiated with the laser beam of the

wavelength 532 nm. The absorbance values at the most intense peak at the wavelength 415 nm are, 1.129, 1.355, 1.548, and 1.903, which are smaller than the absorbance values of the normal blood samples irradiated with the laser beam at the wavelength 532 nm [29].

Nujhat Nuri Sultana *et al*. (2020), found the presence of the light induces a change in the refractive index which can be measured experimentally by means of a nonlinearity parameter n_2 organic materials yield a vast amount of information about the optical nonlinearity related to the type of bonding in the materials [30].

In the same year nonlinear optical techniques have been employed by Gautam et al. to provide a powerful tool to overcome such limitations and achieve enhanced transmission. For instance, two-photon fluorescence, sum-frequency generations, and coherent Raman scattering have been implemented to obtain deeper penetration and higher resolution [31].

Elblbesy, Mohamed A. (2021), show that the optical properties of biological tissue could offer much information that are used in medical diagnosis and therapy. The ability of tissues to absorb the different types of spectra is the basis for many therapeutic and diagnostic applications. Optical properties of tissue such as reflection, scattering, and absorption coefficients, scattering phase functions, and irradiance levels (light dosage) at tissue depth are under active investigation [32].

In the same year Mohammed, Raneem *et al*. study the effect continuous wave (CW) for diode pumped solid state lasers (DPSSL'S) were utilized in irradiate the samples of blood with wavelength (473 nm, 20 mW). The laser exposure time varied between (5 and10) min for each sample. Laser radiation has a great role in the absorption effects of the normal human blood after being compared with absorption spectrum of do-nors with thalassemia [33].

Also, in the same year Raji, Samaneh *et al*. showed the nonlinear optical responses of three biochemical analytes in blood serum, including glucose, triglycerides, and cholesterol are examined using the laser-based Z-Scan technique. Hence, different laboratory samples of blood serum with various concentrations of biochemical analytes are taken from patients for this purpose [34].

Ruixue Zhu *et al*. (2022), found that the Low-level laser radiation (LLLR) has been to have positive effects on the rheology of human blood. However, the detailed mechanisms of blood photo biomodulation remain unclear. LLLR with radiant fluence below 9.5 J/cm2 by 450 nm wavelength improved the RBC deformability and weakened the strength of cell-cell interaction. It also showed rejuvenating effects on RBC suspended in a harsh cell environment [35].

Taehyun Park *et al*. (2022), investigated the optical properties of blood components and the interactions between the photodetector and the blood components by illuminating two different wavelengths of ultraviolet radiation. Moreover, three different mixtures of blood components are successfully identified by SnO2 QD/FP-PD based on specific absorption phenomena, which provides a simple and effective technique for emergency medical care and health monitoring systems [36].

(1-3): Aim of the study

Using linear and nonlinear optical properties as a study to diagnose some blood diseases such as thalassemia and viscosity by using blood samples that contain different PCV and finding linear physical parameters such as (absorbance, transmittance, linear absorption coefficient, refractive index and extinction coefficient) as well as studying nonlinear parameters such as

(nonlinear absorption coefficient, nonlinear refractive index, phase shift and electrical sensitivity)as indicators for diagnosing the aforementioned blood samples and considering them as evidence of the presence of these diseases after comparing them with normal blood, and studying physical mechanisms such as fluorescence of blood components, plasma and serum, and comparing the emission spectrum with the absorption spectrum of these components.

Chapter Two
Theoretical Part

(2-1): Introduction

This chapter includes a general introduction to blood and its components, as well as the functions and physical characteristics that blood has and its great impact on the human body. The chapter also includes some of the diseases discussed in the study, such as thalassemia, blood viscosity, and how the size of red blood cells is affected by these diseases. It also shows the properties of the laser, the methods of its interaction with the tissues, the effects resulting from this interaction, the linear and nonlinear optical properties, and the techniques used to study these properties.

(2-2): Human blood and its components

Blood is a fluid connective tissue that flows inside the body of the organism within the blood vessels (veins, arteries and capillaries). Blood accounts for 7% of the human body weight, with an average density of approximately 1060 kg/m ^{3} [37,38]. Human blood consists of the main components: plasma, red blood cells and white blood cells in addition to blood platelets. Fig. (2-1): shown components of blood [39]. The following is a sincere explanation of these components.

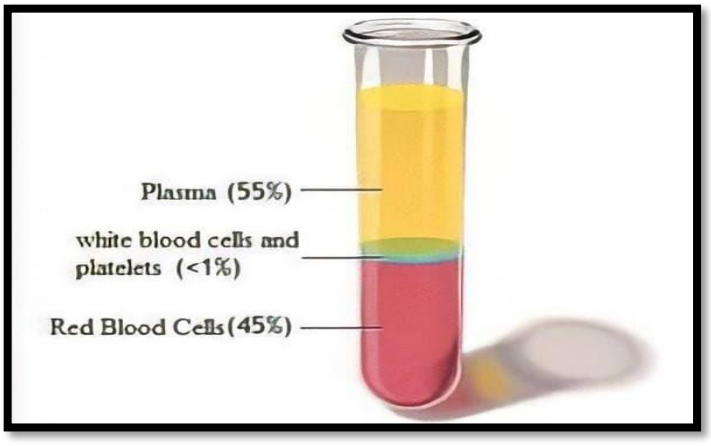

Figure (2- 1) Components of blood [40]

(2-2-1): Red Blood Cell (Erythrocytes)

They are cells in the form of concave discs with two surfaces, with a thin wall and no nucleus. Inside they contain hemoglobin, which is iron and protein, which gives blood its red color. One of the advantages of this compound is that it is easy to unite with oxygen, and that is why red blood cells are called oxygen-carrying. Red blood cells are renewed every 120 days, and they break down and die in the liver and go to the bile to participate in its contents [41]. Approximation the number of RBC in men's 4-5 million in the women's 4 - 4.5 million [42]. Fig. (2-2): shown Red Blood cell.

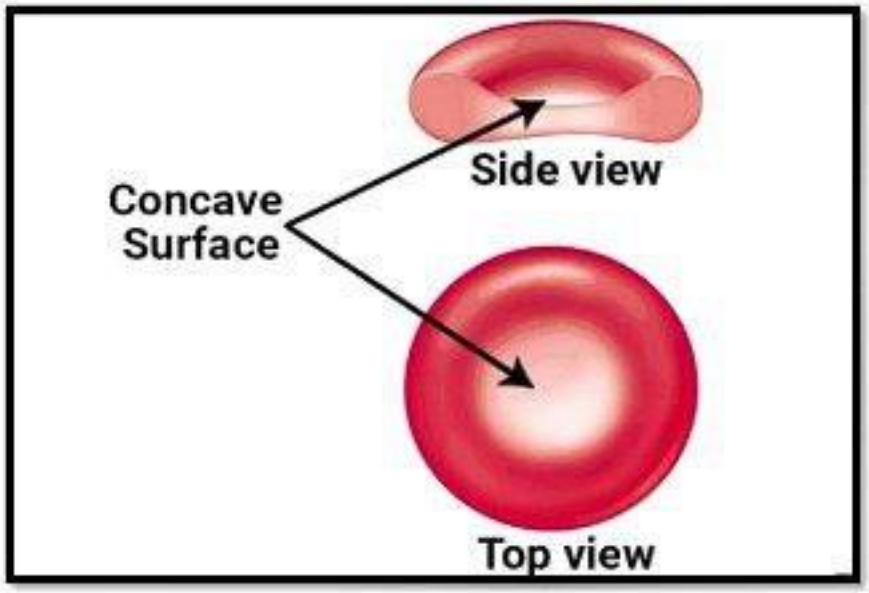

Figure (2- 2) Shape of Red Blood cell [40].

(2-2-2): White Blood Cells (Leucocytes)

White blood cells are a cellular component of the blood that lacks hemoglobin, has a nucleus, and is able to move. It is part of the immune system. It helps fight infection and defend the body against other foreign substances. White blood cells have different shapes. There are two main types of white blood cells. Granular blood cells and non-granular blood cells. All of these cells are made of bone cells, so any effect that occurs to the bone marrow affects the quantity or quality of blood cells. White blood cells are considered to be in the blood. Relatively inactive cells, as blood is only a means of transportation and

usually leave the body's vessels through amoebic movement through the walls of the vessels to reach the connective tissue surrounding these blood vessels and there, they can perform a number of their functions. White blood cells differ from red blood cells in the absence of hemoglobin, but they are distinguished from them by the presence of a nucleus. In fact, the original color of these cells is transparent, but appears white under the optical microscope as a result of light reflection on them. White blood cells can be distinguished into five types when examining the blood sample under the microscope, and this distinction depends on the shape of the nucleus and its divisions and on the type of dye color that the cell acquires when using some dyes for vital tissue. Fig. (2-6) represents schematic for the different types of white blood cells [42].

The differential count can be given for the different types of white blood cells, as follows: Neutrophils (about 5% to 6% of white blood cells and lymphocytes) about 25% to 40% of white blood cells form(Mononuclear cells)about 3% to 7% of white blood cells and form (Eosinophil) about 1% to 3% of blood cells white blood cells, and (basophiles) about 1% of white blood cells [43].

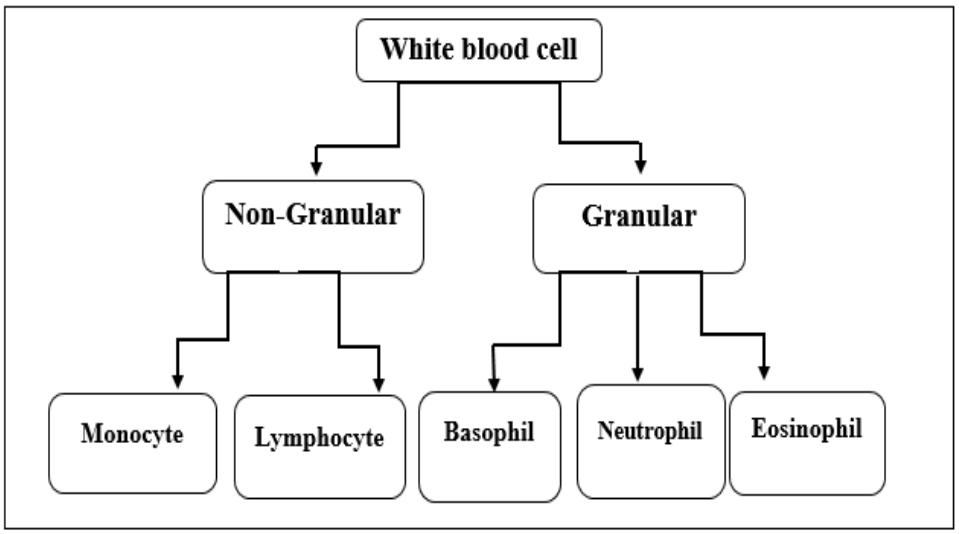

Figure (2- 3) Schematic for types of white blood cells [44].

(2-2-3): Blood Platelets (Thrombocytes)

They are cytoplasmic bodies found in the blood and they break down when they come into contact with the material, as the blood clots to stop the bleeding. The platelets do not have a specific shape, as they slip naturally in the blood and its main role is to transform the liquid protein in the blood (Fibrinogen to a solid substance called Fibrin). They are stiff threads that gather around the skin surface to prevent blood from escaping from the skin. Figure (2-6) shows the platelets [45].

Figure (2- 4) Blood Platelets (Thrombocytes) [40]

(2-2-4): Plasma

It is a liquid substance, in which blood components swim, and the plasma fluid has its own characteristics, for example, its tendency to yellow color, and this is due to the presence of bilirubin, as it is characterized by its high density, which

depends on blood proteins. plasma has a density of about 1.025gm/cm^3 and is made up of water and solid material, as water constitutes about 90% of the volume of the plasma, while it constitutes 10% of solid materials, 9% of which are organic materials and 1% inorganic materials,The most important of which are sodium, potassium and magnesium salts[46].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 lactic 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 [47].

(2-3): Blood Functions

1: Gas Exchange

The blood transports oxygen from the respiratory organs (lungs) to the tissues by means of hemoglobin for red blood cells and transports carbon dioxide from the tissues to the lungs to be excreted from the body [48]

2-: Nutritive

The blood transports and distributes nutrients from the digestive system to all tissues of the body [48].

3-: Regulation of body temperature

Blood helps regulate body temperature, as it distributes heat to different parts of the body [49].

4-: Regulating metabolism

The blood carries the various enzymes from the places of their manufacture to the organs different body in order to perpetuate the processes of construction and demolition in the cells of the body, and these processes are called (Metabolism) [39].

5-: Defense

The body is protected by white blood cells for their ability to devour the microbes entering the body and thus protecting the body from disease, as there are antibodies in blood cells that protects the body from bacterial infection [50].

6-: Transport and regulation of hormone

The blood regulates the secretion of hormones from its glands and maintains a balanced ratio in the blood, and the blood transports these hormones to their places of work [51].

7-: Water balance

The blood works to maintain the amount of water in the body, by removing excess water about the needs of the body through the kidneys and skin [52].

8-: Coagulation blood

The bleeding resulting from a blood vessel injury is stopped by blood clotting by the protein Fibrinogen found in the blood plasma. This protein is generated in the liver, which plays a key role in blood clotting [51].

(2-4): physical properties of blood

1-: Color of Blood

The color of blood is red due to the presence of hemoglobin, which gives this color, and the degree of redness in the arteries differs from that in the veins. It is bright red in the arteries, due to the presence of oxygen, and dark red in the veins due to the presence of carbon dioxide.

2-: Density of blood

The density of blood depends on the presence of dissolved substances in the plasma, such as red blood cells and protein. The average total blood density of a person is about 1060Kg/m^3in the normal case, this varies according to the state of the body.

3-: Temperature

The body temperature is constant, with some differences from one organ to another in order to carry out its normal function.

4-: Blood viscosity

Blood viscosity is the force of blood friction against the walls of the arteries and veins, and blood viscosity depends on the number of proteins present in the plasma, especially fibrinogen, and the role of blood viscosity is in maintaining blood pressure. there depending on the organ's need in order to carry out its normal function.

5-: Osmotic pressure

Osmotic pressure is generated in the blood due to the presence of crystals and salts in the blood plasma, and its importance is due to its important role in maintaining the state of equilibrium between salts and water inside and outside the cell (in the arteries and small blood vessels). Dehydration of cells, as for decreased salt, causes water to enter the cells and leads to what is known as water intoxication.

6-: Hydrogen concentration in the blood pH

It is the index of the concentration of hydrogen in the blood. It is a measure that determines whether the solution is acidic, basic, or moderate. This index tends to be basic in relation to the blood solution (that is, the blood is alkaline) and is equal to 7.4 pH in the arteries and 7.35 pH in the veins [52-54].

(2-5): Thalassemia

Thalassemia is a heterogeneous grouping of genetic disorders that result from a decreased synthesis of alpha or beta chains of hemoglobin (Hb) as shown in figure (2-5) [52].

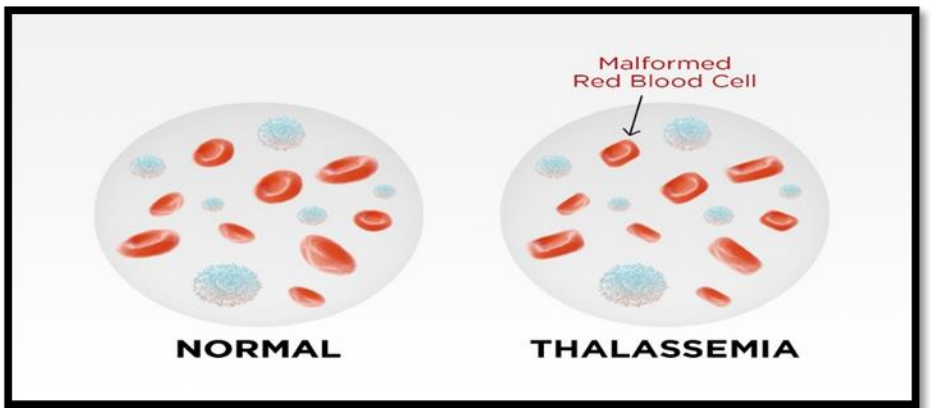

Figure (2- 5) Normal blood and thalassemia [52]

Hemoglobin serves as the oxygen-carrying component of the red blood cells. It consists of two proteins, an alpha, and a beta. If the body does not manufacture enough of one or the other of these two proteins, the red blood cells do not form correctly and cannot carry sufficient oxygen; this causes anemia that begins in early childhood and lasts throughout life as shown in figure (2-6). Thalassemia is an inherited disease, meaning that at least one of the parents must be a carrier for the disease. It is caused by either a genetic mutation or a deletion of certain key gene fragments.

Alpha thalassemia is caused by alpha-globin gene deletion which results in reduced or absent production of alpha-globin chains. Alpha globin gene has 4 alleles and disease severity ranges from mild to severe depending on the number of deletions of the alleles. Four allele deletion is the most severe form in which no alpha globin's are produced and the excess gamma chains (present during the fetal period) form tetramers. It is incompatible with life and results in hydrops fetalis. One allele deletion is the mildest form and is mostly clinically silent.

Beta thalassemia results from point mutations in the beta-globin gene. It is divided into three categories based on the zygosity of the beta-gene mutation. A heterozygous mutation (beta-plus thalassemia) results in beta-thalassemia minor in which beta chains are underproduced. It is mild and usually asymptomatic. Beta thalassemia major is caused by a homozygous mutation (beta-zero thalassemia) of the beta-globin gene, resulting in the total absence of beta chains. It manifests clinically as jaundice, growth retardation, hepatosplenomegaly, endocrine abnormalities, and severe anemia requiring life-long blood transfusions. The condition in between these two types is called beta-thalassemia intermedia with mild to moderate clinical symptoms [53,54].

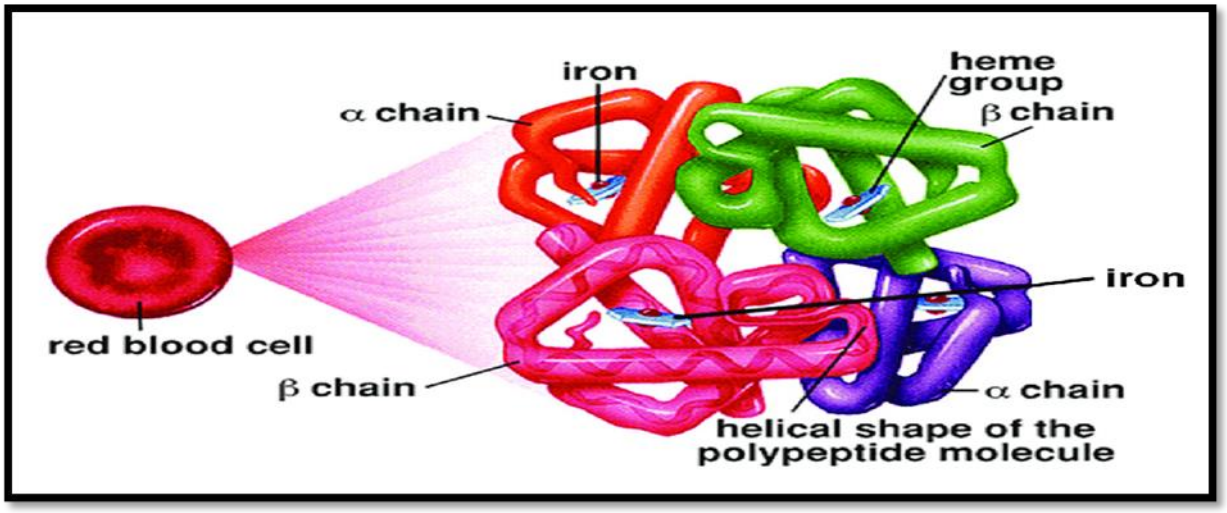

Figure (2- 6) Hemoglobin structure [55]

(2-6): Hemopoiesis

Is the process by which the formed element of the blood produced. the process is Regulated through a series of steps beginning with hemopoietic stem cells. Stem cells are capable of producing red cell of Immune system. For red cell production, Erythropoietin [Epo] is primary regulatory hormone produced and released by peritubular capillary lining cells within the kidney. In bone marrow the first morphological morphologically recognizable erythroid Precursor is pronormoblast this cell can undergo four to five cell divisions, which resulting the productional of 16-32 mature red cells [56].

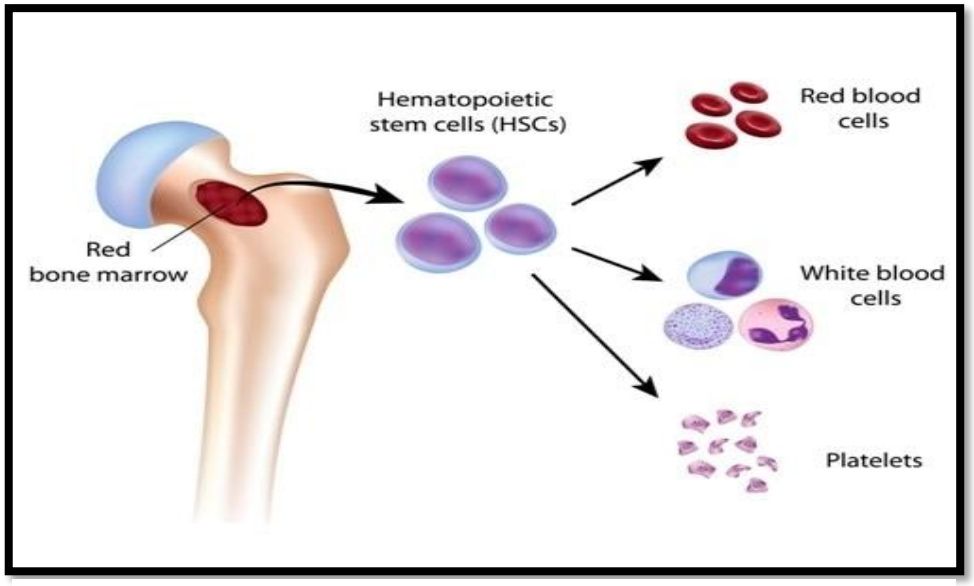

Figure (2-7) Blood cell formation in bone marrow [56]

(2-7): Polycythemia (blood viscosity)

Polycythemia, or erythrocytosis, refers to an increase in the absolute red blood cell (RBC) mass in the body. In practice, this is reflected by an increase in hemoglobin levels, or hematocrit, over what is considered physiologic for that age and gender.

The standard RBC mass does not usually exceed 36 ml/kg in males and 32 ml/kg in females. The reference ranges for normal hemoglobin levels and hematocrits vary depending on altitude, from ethnicity to ethnicity and country to country[65]. However, as a frame of reference, the hemoglobin, and hematocrit of a healthy adult male are 16 g/dL plus or minus 2 gm/dl and 47% plus or minus 6%, respectively. The hemoglobin and hematocrit of a menstruating adult female are usually 13 g/dL plus or minus 2 gm/dl and 40% plus or minus 6%, respectively. Polycythemia in the newborn is defined as a central venous hematocrit over 65% or a hemoglobin value above 22 g/dL [57].

(2-8): Laser -Tissue interaction mechanisms

There are many different mechanisms through which laser light can interact with tissues. For the most common interaction mechanisms for therapeutic and surgical applications will be divided into five broad classes: -

1. **Photochemical interactions**: Photons excite molecules or atoms, making the molecules more likely to undergo chemical reactions with other molecules. In photodynamic therapy, for instance, a photosensitizer (a molecule that becomes reactive when it absorbs light and can therefore induce chemical reactions within other molecules or tissue) causes reactive oxygen species to form which lead to (cell death). Photodynamic therapy is increasingly widely used in oncology to destroy cancerous tumors [58].

2. Photo thermal interactions: photons are absorbed by a chromphore (a light-absorbing molecule) and converted into heat energy, which can cause a range of thermal effects from tissue coagulation to vaporization. Applications include tissue cutting and welding in laser surgery [59].

3. Photo ablation: High-energy, ultraviolet (UV) photons are absorbed and, because they are more energetic than the chemical bonds holding the molecules together, cause the dissociation of the molecules. This is followed by rapid expansion of the irradiated volume and ejection of the tissue from the surface. This is used in eye (corneal) surgery, among other applications [60].

4. Plasma-induced photo ablation: A free electron is accelerated by the intense electric field in the vicinity of the laser beam. By colliding with a molecule and freeing another electron, it initiates a chain reaction of similar collisions, resulting in a plasma: a soup of ions and free electrons. One application of this is in lens capsulotomy to treat cataracts [61].

5. Photo disruption: It is the mechanical effects that can accompany plasma generation, such as bubble formation, cavitation, jetting and shockwaves. All these mechanisms illustrate as shown in figure (2-2) [62].

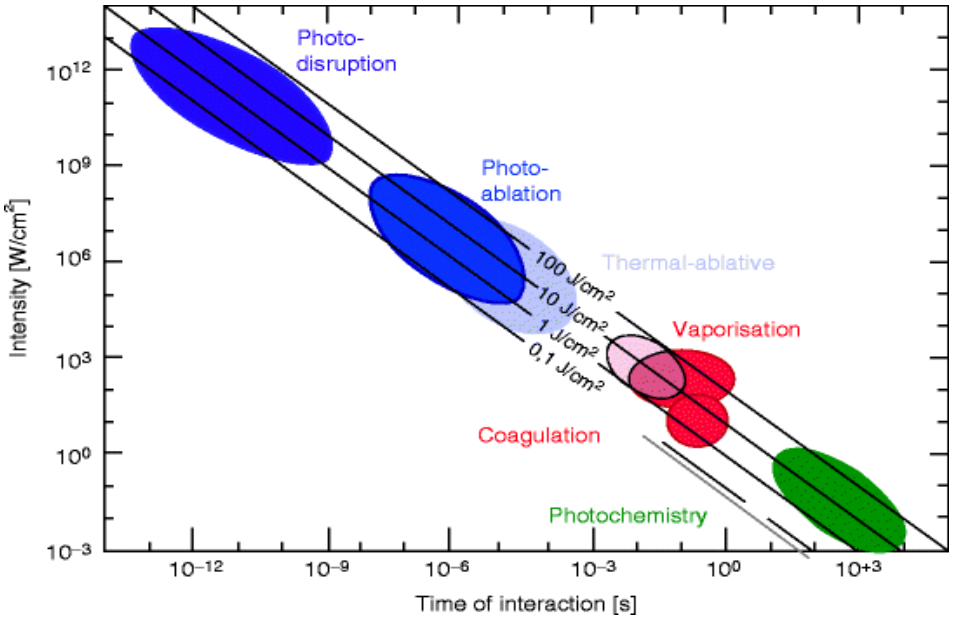

Figure (2- 8) Different types of laser-tissue interaction [63]

(2-9): Effect of Laser beam on Biological Tissue

The monochromaticity property of laser light, attached to its singularity of wavelength, is a determinant factor for the interaction with biological tissue, since it needs to be absorbed in order to interact with any tissue or matter. Biological tissues have light receptors (chromophores) that are highly selective to the wavelength it absorbs. In the case of biological tissues, some common chromophores include hemoglobin, oxyhemoglobin, melanin and water. The polarized characteristics of laser light also influences this interaction, as different polarizations of light can be absorbed to different degrees by different biological tissue or matter. Broad-band lamps and non-coherent light sources, such as light-emitting diodes (LEDs), have been successfully used in biophoton modulation therapy [64].

Laser radiation, when absorbed in matter, may lead to Vaporization and material removal. This process is extensively used in material processing, in laser cutting of materials, and in laser surgery. One of the problems that arises in the procedure is an excessive temperature increase in the surrounding areas. This is particularly important in medical applications, where the heat may cause undesirable tissue destruction. In laser surgery it is desirable to cut tissue at a

reasonable rate, yet to cause as little damage as possible to the neighboring tissue. It is therefore important to determine the temperature distribution in materials following laser irradiation, to maximize the tissue removal rate, and at the same time, to minimize the thermal damage.

The extent of tissue heating, damage, and removal depends on a variety of parameters: the laser wavelength (which determines the absorption depth), the mode of operation (cw or pulsed mode), the choice of fluence rate, pulse length, and repetition rate [65].

(2-10): Linear optical properties

The interaction between the nature and distribution of charges within the material (electronic, molecular, ionic) and the electromagnetic rays falling on the material lead to the appearance of the optical properties of the materials. A number of processes can occur when an electromagnetic ray falls on matter and their interactions together, where part of the light radiation is transformed into heat by being absorbed by the material, while the other part is called transmitted radiation as it passes inside the material without losing energy. As for the remaining part of the light radiation is reflected from the surface of the material (reflected) [66] In order to obtain information about the internal structure of the material and the nature or consistency of it, it is necessary to know the permeability, absorptivity and reflectivity of the electromagnetic beam falling on the material. The ultraviolet spectrum, but to know the field of practical applications in which materials are used, the visible spectrum must be studied [67].

1 -Absorbance

The absorbance (A) describes the number of absorbed photons by molecules, can be written as [68].

$$A = 1 - \log\frac{1}{T} \quad \ldots\ldots\ldots\ldots \quad (2-1)$$

Where *T*: is the transmittance of medium. The absorption of the incident rays by the material causes electronic activity that may lead to the dissolution of its molecules if the value of the absorbed energy is greater than the value of the dissolution of one of the bonds or its transition to a higher energy level, as the probability of absorption increases with the increase in the concentration of the material in the low energy level and with the increase in the number of photons of the incident rays [67].

2- Transmittance

The transmittance of a medium is defined as "the ratio of the intensity of the transmitted light (I) to the intensity of the incident light ($I_{\circ}$)", or it is "the energy of radiation transmitted from the medium to the energy of the radiation falling on it". [69]:

$$T = \frac{I}{I_{\circ}} \dots \dots \dots \dots (2-2)$$

The transmittance of the medium is related to the absorbance of the solution by the equation below [70]:

$A = -\log(1/T) = -\log(I / I_o) = \log(I_o / I) \dots \dots \dots \dots (2\text{-}3)$

It is clear from the above equation that the transmittance increases as the absorbance of the medium decreases [71].

3- Refractive Index

Light travels in all its wavelengths at its maximum speed through a vacuum, which is a constant quantity, and this value decreases in any other medium, and it changes in material media with different wavelengths.

The ratio of the speed of light in a vacuum to its speed in any given medium of wavelength is defined determined by the refractive index of the medium for that wave [72].

$$n=c / v \ldots \ldots \ldots \ldots (2\text{-}4)$$

whereas

c: the speed of light in a vacuum and v: the speed of light in a material medium.

The refractive index depends on the length of the electromagnetic wave and is not constant. The materials are used to change the direction of polarization of those waves [73]. Whereas, the greater the polarization, the greater the delay action, and the smaller the speed of light in the material, the greater the refractive index. Materials that do not have polarization do not have any delay in returning light, and therefore the refractive index has (n= 1). For most cases, the refractive index is greater than one and its value is proportional to the density of the medium, the higher the refractive index of the material, the greater the density of the medium, and the refractive index has no units of distinction. It can be obtained through the equation [74].

$$n = \left(\frac{4R}{(1-R)^2} - K^2\right)^{\frac{1}{2}} - \frac{(R+1)}{(R\ -\ 1)} \ldots\ldots\ldots\ldots (2-5)$$

4- Absorption Coefficient

The absorption coefficient can be defined as the ratio of the decrease in the energy flux of the incident radiation with respect to the unit distance in the direction of wave propagation within the medium, and the absorption coefficient depends on the photon energy (hυ) and on the properties of the material [75]. According to the Beer-Lambert law, the absorption coefficient is:

$$\alpha = \frac{1}{2.303A} \ldots\ldots\ldots\ldots (2-6)$$

5- Extinction Coefficient

extinction coefficient is an intrinsic property of material depending on their structure and is measure of how strongly a material absorbs light at a particular wavelength. It is given by the fraction of light lost due to scattering and absorption per unit distance in participating medium. It can be calculated from the Eq (2-7) .

$$K = \frac{\lambda\alpha}{4\pi} \dots\dots\dots\dots (2-7)$$

(2-11): Fluorescence

When a substance absorbs electromagnetic radiation, it will get excited and thus increase its energy this excited material can emit photons of different energies until it reaches a stable state in other words, when these particles return to the ground energy level (S_0), they will emit photons of the same a certain energy and a certain wavelength. Sometimes the radiating system absorbs high energy, causing Excitation of some electrons to an energy level higher than the stability level of the molecule in this case can The system can return directly to the level of stability by emitting photons with the same energy as the photons The absorbed electrons can also return to the ground state by emitting photons with an energy Low, it will have less energy and a longer wavelength than the wavelength of the absorbed photons The phenomenon is known as fluorescence [77,78]. Fluorescence is defined as a spontaneous emission process.it occurs between two levels that have the same plurality [78].

After the absorption process takes place after the molecules spend a very short period of time ranging from (10^{-9}-10^{-6}) seconds in the excited level known as the lifetime of the organic dye molecules. Fluorescence can occur when the particles return to the ground level emitting photons [79]. At room temperature the molecules are at the lowest ground level vibrational level. Therefore, the absorption takes place from the zero vibrational level of the ground level to one of the levels of the excited level. In this case, fluorescence can occur in a process

in which the molecules undergo transitions from the vibrational levels of the first excited electronic state to one of the vibrational levels of the electronic ground state emitting photons called fluorescence photons. This depends on the nature of the molecules and it is called normal fluorescence and it occurs in solutions and dense media [80].

The changes in the vibrational levels of the excited state that precede the occurrence of normal fluorescence is called vibrational relaxation. occurs with a period of time ranging from (10^{-13}-10^{-11}). Vibrational relaxation increases the temperature of the sample and causes the emission spectrum (fluorescence) to creep toward long wavelengths and is called (Stok's shift) [81]. The Stok's shift is the transformation spectrophotometer to lower energy between incident light and scattered or emitted light after interaction with the sample where the wavelength of these lines is longer than the wavelength of the excited ray responsible for the process absorption [82,83].

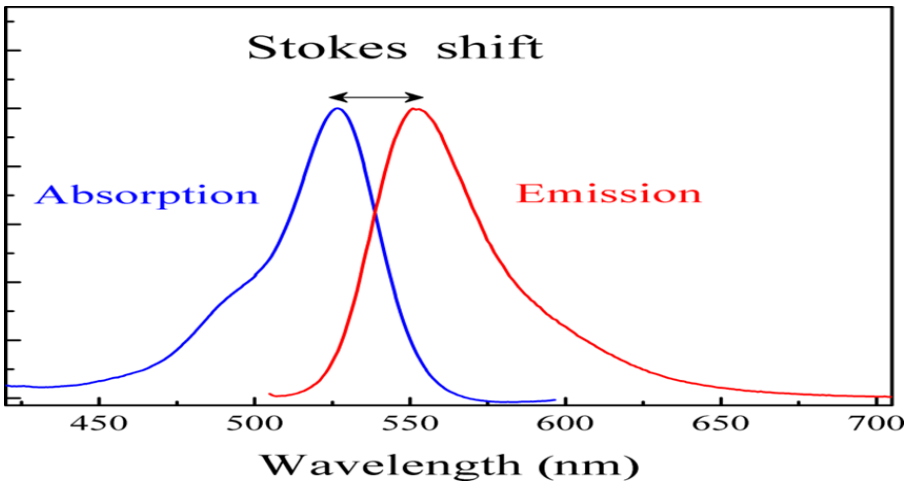

Figure (2-9) Stokes shift between the absorption spectrum and emission spectrum (fluorescence) [85].

A Stok's shift can be defined as the difference in wavelength or frequency units in the position of the absorption and emission (fluorescence) spectra of the same electronic transitions oscillatory at excited levels [84]. Figure (2-9) below shows the Stokes shift between absorption spectrum and emission spectrum (fluorescence) [85] .

Figure 2-10 shows a Jablonsky energy diagram, a diagram used to describe the most important processes optical physical. The absorbed light excites matter particles from the lowest ground state levels (S_{00}). to higher vibrational levels of the state (S_{1n}) and the thermal redistribution of the groups is carried out between series sub-levels within a very short time (~10^{-11}sec) [86].

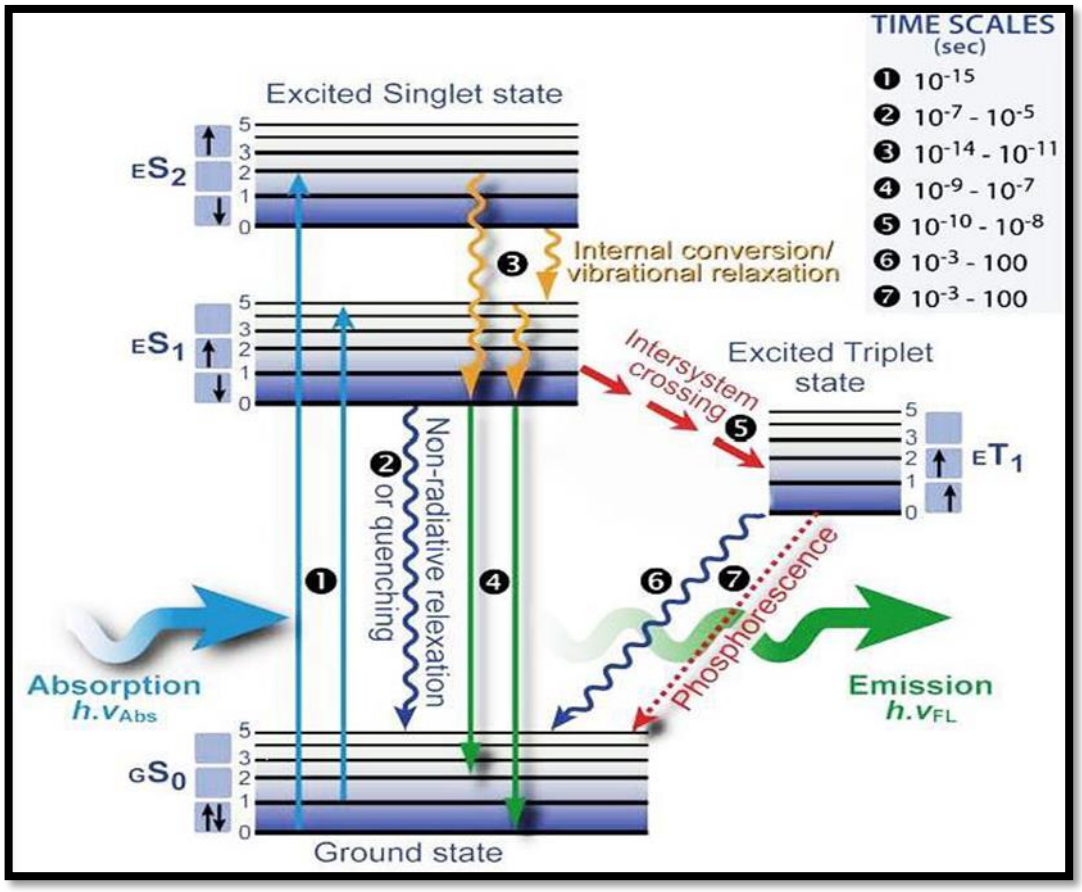

Figure (2- 10) Jablonsky scheme for optical spectral processes [87].

Distribution is achieved Boltzmann in chain with most of the excited particles that decay non-radiatively to the level(S_{10}). The excitation of the state (S_1) can also be affected by direct absorption from the ground state to state. The second excited singular (S_{2n}). For most organic dye solutions, the decomposition is from the. State (S_2 to S_1)non-radioactive and very fast (~$10^{-10}$to 10^{-11} sec) [87]. Can a molecule in (S_{10}) return to (S_{00}) by emitting a photon of light whose energy is less than the emission of the absorbed light. Thus, this spontaneous radiation

process (fluorescence) is converted to wavelengths longer than gentle wavelength absorption [87].

(2-12): Optical Nonlinearity Principle

The optical properties of materials are modified by high-intensity light such as a laser. The study of this phenomenon is called Nonlinear Optics. This phenomenon is observed in a wide variety of materials like dyes, polymers, semiconductors, nanomaterials, etc. After the invention of the first work laser by Theodore Maiman in 1960, Peter Franken et al demonstrated the first nonlinear optical phenomenon second-harmonic generation in 1961. This has been considered the beginning of the study of nonlinear optics [88].

When an electromagnetic wave propagates through a medium, it leads to the creation of an electrical polarization, P within the medium, and this is caused by the movement of electrons and nuclei in response to the electric field of the directed electric wave. Which leads to the vibration of the polarization at a specific frequency that is determined based on the properties of the medium and the wave frequency of the incident light, which leads to an overlap between the electric fields resulting from the polarization and the emitting fields. At low optical intensities, the polarization is proportional to the electric field of the incident wave, and the response of the medium is linear.

Many linear optical reactions can occur depending on the properties of the polarization, such as refraction, absorption, elastic and inelastic scattering and other linear parameters that occur when the intensity of the incident radiation is low.

However, when the intensity of the incident electromagnetic radiation is large, it will be sufficient to change the behavior of light in the material from linear behavior to nonlinear behavior and the emergence of nonlinear optical coefficients, and some of these nonlinear light interactions increase with the increase in the movement of electrons and ions as a result of applying a strong

electromagnetic field of light. In most materials, the electrons and ions are linked to a potential energy. For small displacements from the equilibrium position, the movement of electrons and ions is harmonic, but for large displacements, the movement becomes non-harmonic.[89]

Thus, when the optical intensity is low, the electron or ion moves within the harmonic field of the potential energy, and within this region, the polarization can vibrate according to the frequency of the incident wave only, and thus the response is linear. But in the case of a sufficiently large light intensity to change the motion, the motion becomes inharmonic in the potential energy, as in Figure (2-11). This is the first type of nonlinear response of matter.

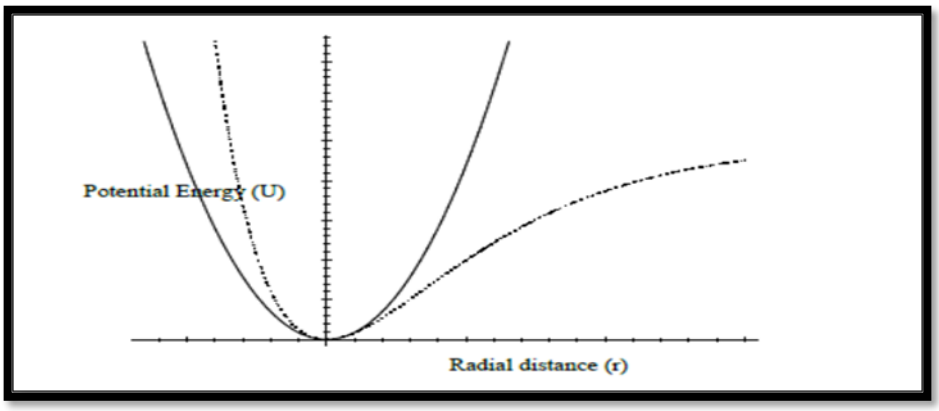

Figure (2- 11) The solid line expresses the harmonic potential energy, the dashed line expresses the non-harmonic potential energy

The total potential energy is given by the following relationship [90].

(2-8)

Where the first term expresses the harmonic potential energy, which is dominant in the case of small displacements X, and the rest of the terms express the non-harmonic potential energy in the case of large displacements. The additional limits in the potential energy led to the introduction of additional limits in the resulting polarization, and these limits depend on the second, third, and higher orders of the power of the applied field, which leads to an increase in the

nonlinear response [91]. As for the second type of nonlinear response, it results from a change in some properties The medium is due to the shedding of the light beam, when the light field is of great intensity, it will lead to a change in the basic properties of the medium, which in turn leads to a change in the way the medium affects the light beam, which leads to a nonlinear optical response. An example of this is the change in the refractive index of the medium as a result of shedding a light wave, and many of the propagation properties of a light wave are determined by the refractive index of the medium [92].

(2-13): Nonlinear Polarization

In the case of strong fields, the relationship between the electric polarization P and the field strength E is a linear part and a nonlinear part [93,94].

When an insulating medium is placed within an electric field, the medium becomes polarized, so if the medium does not have any transformation at the frequency of the applied field, then each particle of this medium represents a dipole that has a moment pi, and therefore the total dipole moment in unit size P is given by the relationship: -

… … … … (2-9)

where the sum is over the entire dipole's unit volume. The effect of the direction of the external field on the molecular dipoles depends both on the properties of the medium and on the strength of the field. Therefore, we can express the electric polarization, which is the sum of the dipoles per unit volume, with the following relationship as in the above equation

Where is called the polarity or electrical susceptibility of the medium, which expresses the properties of the medium, and ε_0 is the permittivity. Equation (2-10) is true for the field strength of conventional sources, the magnitude is the only

constant in the case of independence from E, and the value of the polarization is a function of the field frequency used.

In the case of a laser radiation of sufficient intensity, the polarization relationship becomes more general and is given by the following relationship: -

Since is the same amount as in the equation (2-10) and the coefficients , ,..... define the degree of nonlinearity of the material and are called non-linear electrical potentials.

If the field strength used is low, the equation (11) consists only of the first term expressing the resulting linear behavior, and as the field strength increases, the co-terms of higher orders in the equation (11) increase and this forms the basis of nonlinear light, and therefore the medium that has electrically polarization It is expressed by the equation from (11) called the nonlinear mean [95].

(2-14): Nonlinear (absorption, refraction)

The essential optical properties consisting of the interaction of light with matter are absorption coefficient V and refractive index W. When the material is illuminated, the energy of the absorbed photons makes it possible for the transition from the ground state to the excited state. This leads to the linear absorption, further excitation may be possible because of the plenty of incoming photons, this leads to the nonlinear absorption. There is also a change in the refractive index when a material is put in a strong electric field, actually, the refractive index becomes dependent on the intensity of the electric field, the refractive index is given by the relation [96].

$$n = n_o + n_2 I \ldots \ldots \ldots \ldots (2\text{-}12)$$

Where, n_o: is the linear refractive index, n_2: is the nonlinear refractive index. The absorption coefficient of the material is also too given by relation

$$\alpha = \alpha_0 + \beta I \ldots \ldots \ldots \ldots (2\text{-}13)$$

Where, α_0: is the linear absorption coefficient, β:is the nonlinear absorption coefficient [97]. The non-linear visual effect of the third order), which is the subject of the study, can lead to the nonlinear refractive index (n_2) and, (β) the nonlinear absorption coefficient, in general, the nonlinear effects can be expressed by the following equation [98,99]:

Whereas, it represents the real part of the nonlinear effect of the third order, which is expressed in terms of the nonlinear refractive index by the following relationship [100]:

where(c) is the speed of light in a vacuum and (n_0) is the linear refractive index of the material and (n_2) is nonlinear refractive index a What is the imaginary part (of the third-order nonlinear effect that (β) can be expressed in terms of the nonlinear absorption coefficient [101]:

Where (λ) is the wavelength of the incident ray.

(2-15): Z-scan technique

The use of Z-scan technology to measure and characterize nonlinear optical materials for its accuracy and simplicity, and the use of this method has spread in the study of nonlinear optical properties (nonlinear absorption and nonlinear refraction) [102,103]. The working principle is that the model is scanned for a long time through the focal region of the focused Caussian beam (TEM00)[104]

, the passage of the laser beam through a non-linear medium will change its intensity during the movement of the model along the z axis, due to the fact that the model faces a different laser intensity that depends on the z position For focus z = 0, the method is done by calculating the permeability through the model as a function of the position (z) of the model.. There are two systems of optical scanning method, the first is the closed aperture system for nonlinear refraction (n_2) and the open aperture system for detecting nonlinear absorption (β) (94,98).

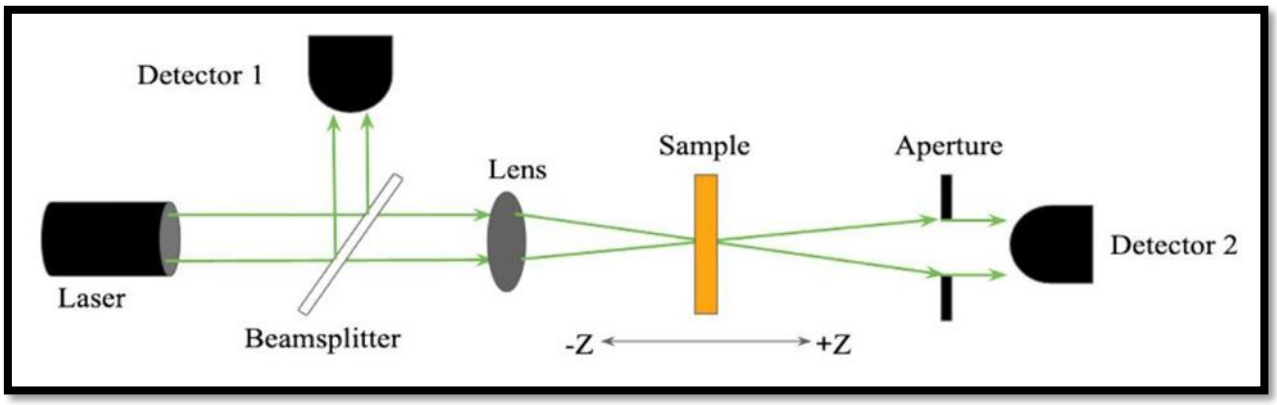

Figure (2- 12) Schematic of conventional Z-scan setup [99]

(2-15-1): Z-Scan Closed Aperture System

When the pattern is passed through the focal area of the beam as shown in Figure 1-2) then the detector measures the intensity passing through the aperture transmitted through the model and the intensity of the incident laser beam on the detector will vary due to Kerr lens generated in the material by the intensity of the laser beam [100]. To show how Scan-Z transmittance as a function of Z relates to the sample's nonlinear refraction if there is a medium with the linear refractive index is negative and the thickness is smaller than the diffraction length of the focused beam so it can be considered as a lens thin variable focal length.

At the far field(Z<<0) the beam intensity is low and the nonlinear refraction is negligible in this case the measured transmittance remains constant (i.e., independent of Z) when the sample approaches the focus, the intensity of the laser beam increases, which leads to the formation of an intrinsic lens in the sample. A negative subjective lens before the focus of the beam tends to collect the beam on

the aperture in the far field increasing the transmittance measured after the focus. Out of focus (Z>0) again the nonlinear refraction is low resulting in transmittance independent of Z. The maximum transmittance (peak) followed by the minimum transmittance (bottom) is evidence of a negative value of nonlinear transmittance (negative refractive index). While the scanning curve Z (i.e., the bottom followed by the top) characterizes the nonlinear transmittance (the material has a positive nonlinear refractive index). Figure (2-13) shown nonlinear transmittance represented by the positive and negative nonlinear refractive index value [101].

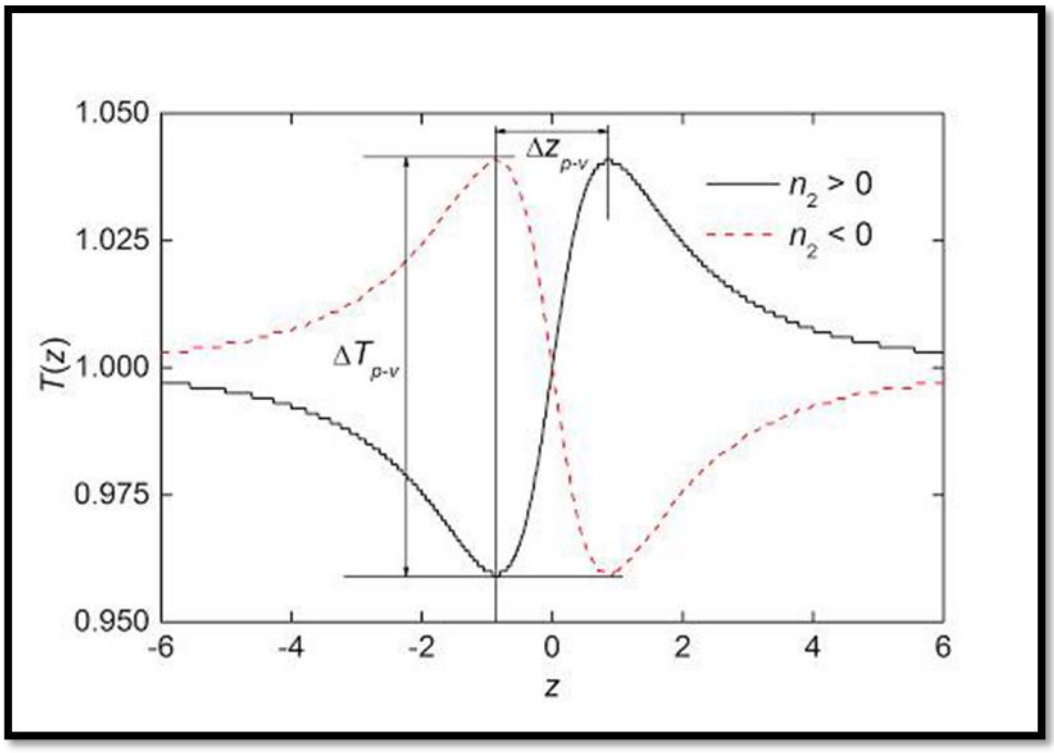

Figure (2- 13) Full z-scan technology for positive (black line) and negative (red line) non-linear refraction [102]

So, if the nonlinear refraction is positive (self-focusing occurs), that is, the bottom expands with a peak, and if the nonlinear refraction is negative, the top will follow a bottom (non-self-focusing occurs) and the amount of phase displacement it can be calculated by the change in the normal permeability between the top and bottom.

Calculate the nonlinear refractive index from the top to bottom difference for normal transmittance (experimental) with the following formula [103,104]:

$I_{\circ}$: - Incident light intensity

: nonlinear phase displacement of the vertex on the axis at focus

When:

$$\Delta T_{p-v} = 0.406(1 - S)^{0.25} |\Delta \Phi_{\circ}| \ldots \ldots \ldots \ldots \ldots (2\text{-}20)$$

Where is $|\Delta T_{p-v}|$ the change in the natural permeability between the top and the bottom and is equal to $|T_P\text{-}T_V|$ [103,105].

Where ( ) is the wavelength of the beam and (K) is the wave number where is

$$S = 1 - \exp\left(-2r_a^{\,2}/\omega_a^2\right) \ldots \ldots \ldots \ldots (2\text{-}22)$$

Where (S)is the linear permeability of the

(r_a): - The radius of the hole.

(ω_a): -The radius of the laser beam at the hole.

It is the effective length of the sample and it can be determined by the following relationship

whereas.

(L): - the length of the model

To calculate the intensity at the focal point, we use the following equation

Where (is the radius of the laser beam at the focus, and (P) is the laser power.

Chapter three: Materials and Methods

3-1 Introduction

This chapter includes an explanation of the materials used in studying the linear and nonlinear optical properties of some healthy blood samples, infected with blood viscosity and thalassemia in humans. The chapter also includes a method of preparation these samples, and an explanation of measurement methods, and devices used in measuring linear and nonlinear optical properties as shown in figure (3-1).

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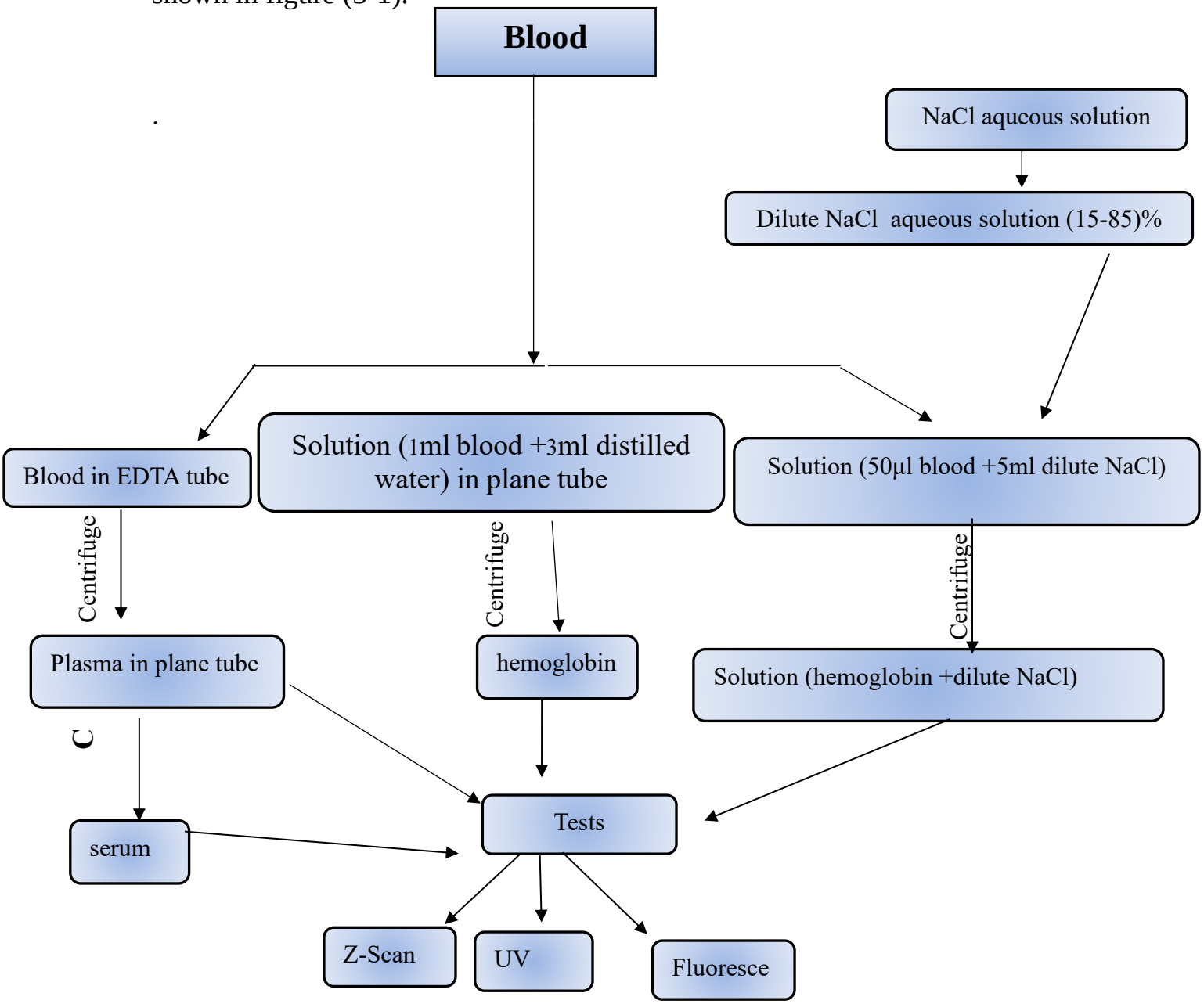


Figure (3- 1) work scheme

(3-2): The material used in the experiment:

(3-2-1): Microhematocrit centrifuges

Microhematocrit centrifuges (Sigma) are used for determination of volume fractions of erythrocytes (red blood cells). Micro-hematocrit centrifuges are very common when it comes to diagnose blood loss, polycythemia (an elevation of the erythrocyte count to above-normal levels), anemia, bone marrow failure, leukemia, and multiple myeloma. Because micro hematocrit centrifuges use micro capillary tubes as in the figure(3-1) to hold samples, very small samples of blood are required for testing .

Figure (3- 2) Shows a hematocrit centrifuge and a fine capillary tube

(3-2-2): Blood samples collection tubes (EDTA tube):

A number of cylindrical glass tubes were used containing an anticoagulant substance called Ethylene Diamine Tetra Acetic Acid (EDTA). To preserve the components of blood cells from damage after taking blood samples from people through the syringe, and after placing the blood in the anticoagulant tube, it is required to move the tube slowly until the anticoagulant substance (EDTA) is completely and homogeneously distributed over the blood components inside the tube with a purple stopper.

(3-2-3): Blood sampling tubes (normal tube):

They are cylindrical plastic tubes that do not contain anticoagulant elements, and are used to divide blood samples after adding them in EDTA tubes for the purpose of tests.

(3-2-4): Centrifuge

This device is used in medical laboratories to separate the components of blood from each other. The working principle is the centrifugal force that makes the particles that are denser in weight at the bottom of the tube and then the lower ones higher. It contains an electric motor that rotates at a high speed and its speeds can be controlled according to need.

The rotation helps to mix or isolate materials by centrifugation and this is done by the motor that rotates at a speed of 2000 to 3000 revolutions per minute and the rotation depends on the time specified by the operator by hand, figure (3-1).

Figure (3- 3) Centrifuge device

(3-2-5): Optical measurement (Double- Beam Spectrophotometer)

The absorption spectrum was measured using a spectrophotometer working in ultraviolet and visible regions(uv-visible) where aabsorbance within the range (900 - 190 nm). (In this study CECIL was used). ENGLAND (7200 CE). Figure (3-4) shows the spectrometer and its working principle:

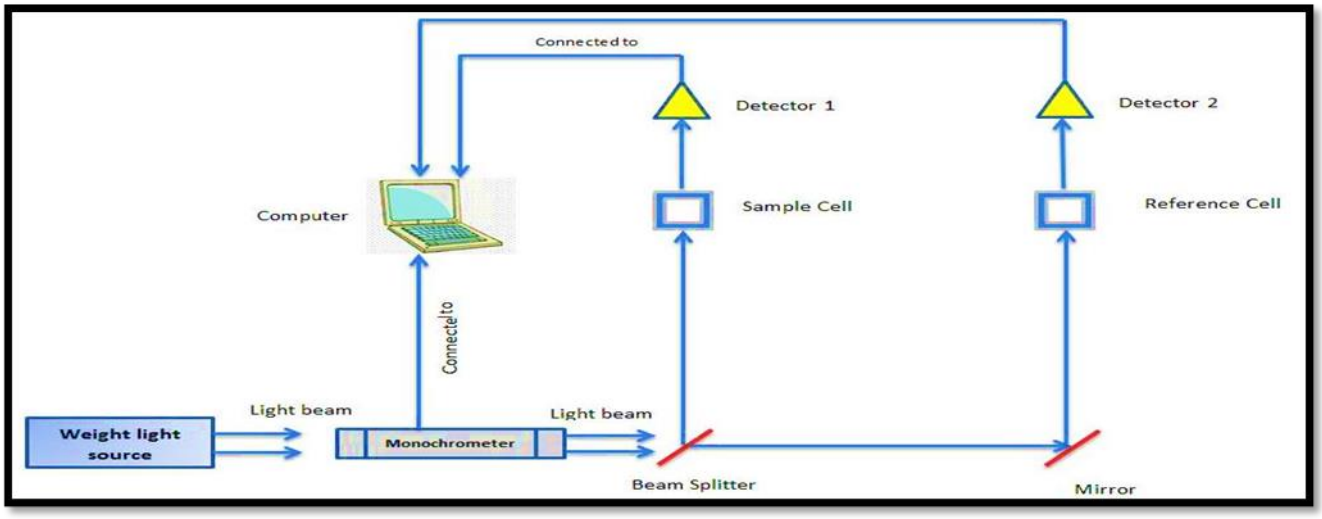


Figure (3- 4) Schematic diagram of the working principle of the machine

The device includes two sources of irritation, the first is a deuterium lamp. It includes the fiber unit located within the wavelength range (190-360 nm), the second is Tungsten lamp (includes a fiber lamp in the range of 360 wavelengths 1100 nm), this allows the use of the two lamps to change all UV and visible ranges of electromagnetic fibers, and the device consists of a monochromatic analyzer. To determine and study the wavelengths that excite the desired pattern, and the beam diffuser to split it, the light is split into two parts, one of which passes through the sample and the darkest passes through the sample only the solvent (the reference sample) and then for detectors that receive light transmitted from the sample and brighter than the sample containing the reference sample In turn, it is connected to a computer that has a computer program for calculating the absorption, where it works to subtract the value of the linear transmittance obtained from the reference sample from the product of the linear transmittance of the model. And in order to check the absorbance of blood samples, we put a quartz cell (1 cm thickness) that is filled with solvent (Distill

water) The user is in front of the beam coming from the source and we put a second cell, the same as the first cell, filled with the desired blood sample Check its linear absorbance in front of the beam coming from the source as well. After checking and recording the values. Absorbance in the device We divide all the results recorded from the absorbance test device by (3) because they are the highest values recorded by the device.

(3-2-6): Fluorescence spectrometry

Fluorescence spectrometer F5-2 was used to perform fluorescence spectrometry on prepared blood samples, as shown in Figure 12-3. It consists of a light source with a power of 150-watt Xenon arc lamp, scan rate (200, 400,600 nm/min), rate of emission and excitation spectrum (200 -700 nm), a calculator and a high sensitivity- photomultiplier tube (PMT) detector. (3-5) The mechanism of operation of the device.

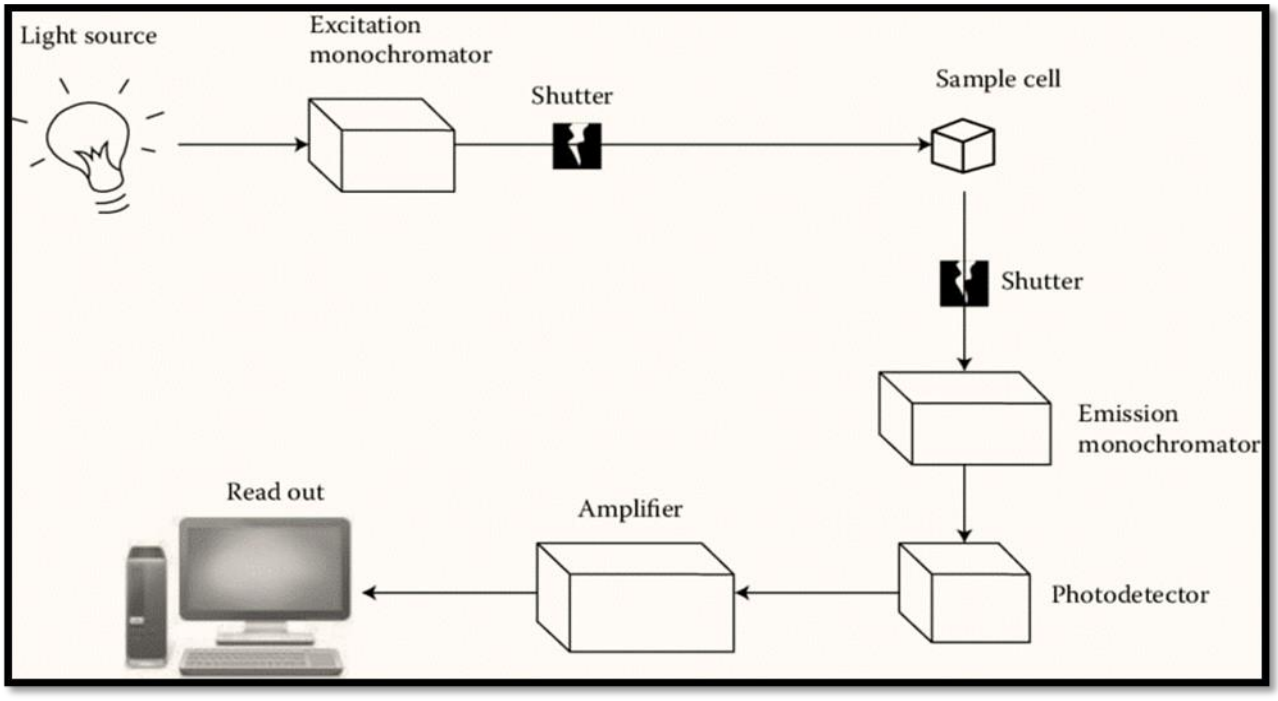


Figure (3- 5) Schematic diagram of the working principle of the fluorescence device

(3-2-7): Z-scan technique

To detect the nonlinear refractive index, the Scan-Z system was used, the parts of which are shown in the figure (ZSCAN-VER-8) (MAHFANAVAR) Company before this experience (3-6) It consists of:

1-Lasers of continuous pattern (CW) and of different wavelengths, (wavelength was used 532nm)

2- Different optical filters to control the intensity of the laser beam falling on the model.

3 - Optical lenses with different focal lengths (5.8, 5 cm)

4 - Splitter Beam to split the beam between the detectors.

5-The first detector is directly beyond the middle of the beam and is equipped with a 1 mm hole to detect nonlinear refraction and a detector beyond the middle of the beam to detect nonlinear absorption.

6- A lens before the nonlinear absorption detector to collect the equilibrium laser beam on it.

7- Connecting wires to transmit signals from the detectors to the Controller (and in turn connected to a wire (USB).

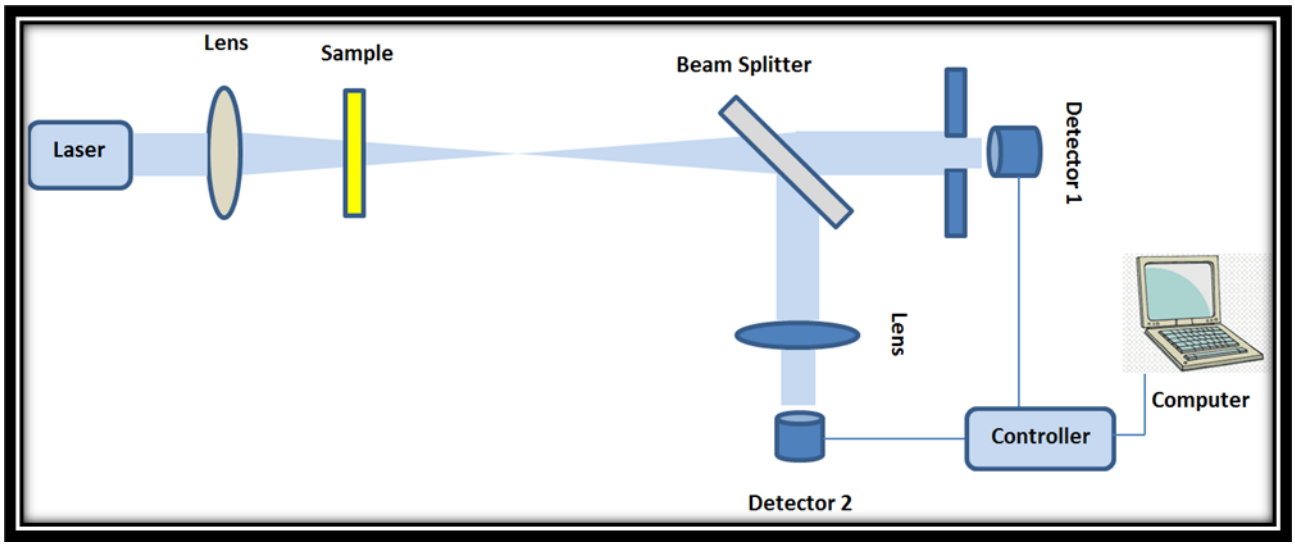


Figure (3-6) diagram and working principle of the technique

(3-2-8): Laser power meter

This device shown in Figure (3-7) below is used to measure the optical power of the laser beam transmitted from the model has a range of (0 to 40 mW).

This device is supplied by Sanwa Corporation (Japan). This works the device is within a wide spectral range (40-110nm), with a sensitivity that depends on the wavelength, as shown In Figure (3-7).

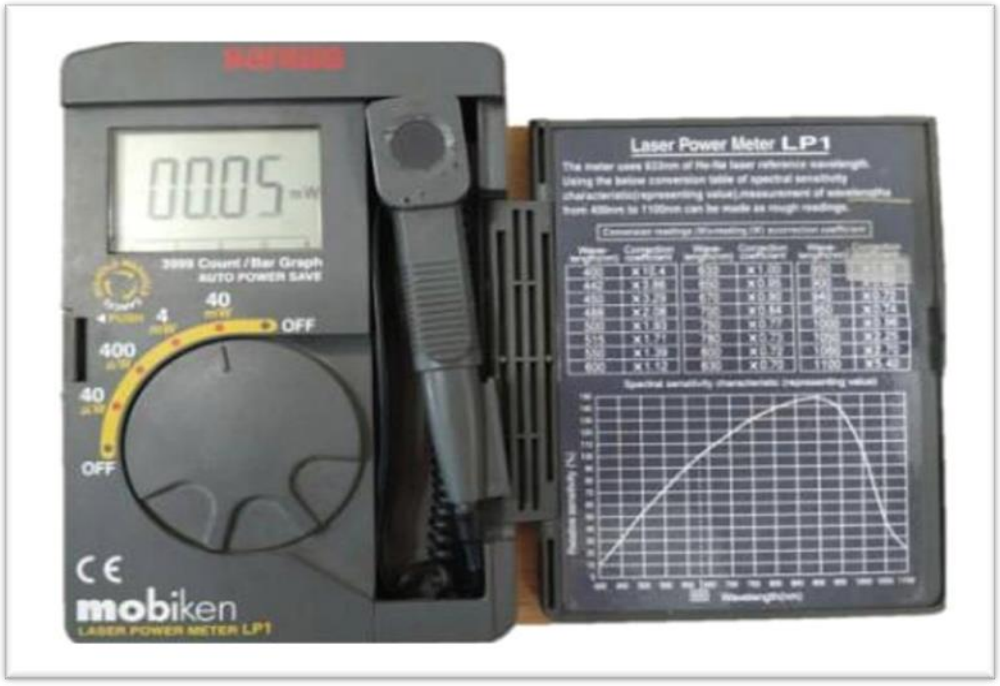


Figure (3- 2) Show the optical power device

(3-2-9): Refractometer

This instrument is used to directly determine the optical indices of refraction in liquids and solids.

Figure (3- 3) Refractometer device

The way it works depends on the principle of the critical angle between two media, and the device consists of two adjacent prisms faceted and made of flint glass, which has a high optical density and a large refractive index (NOVEX -HOLLAND). This device features the following: 1.7 :1.3 – Measuring rang, the measurement accuracy is up to 0.0002.

(3-3): Samples preparation

Blood samples were collected from 15 donors of different ages of children, women and men (12-50) years, where (5) of them were healthy and did not suffer from diseases, (4) children with thalassemia, (6) with blood viscosity.

(3-3-1): Prepare the solution

A solution of NaCl + distilled water is prepared by taking 10gm of salt with 1000 ml of water and in different concentrations (85-15%)) where they are kept in plastic containers for use in preparing blood samples as in the picture (3-7) which shows the concentrations Prepared for osmotic fragility calculation according to the table below (1)

Figure (3- 4) The picture shows the prepared solutions with different concentrations

Table (3- 1) The concentrations in the osmotic fragility experiment

Tube	NaCl 1% (ml)	Distilled water (ml)	NaCl %of final concentration
1	4.25	0.75	0.85
2	3.8	1.5	0.70
3	3.25	1.75	0.65
4	3.0	2.0	0.60
5	2.75	2.25	0.55
6	2.5	2.5	0.50
7	2.25	2.75	0.45
8	2.0	3.0	0.40
9	1.75	3.25	0.35
10	1.5	3.50	0.30
11	1.25	3.75	0.25
12	0.75	4.25	0.15

(3-3-2): Samples collection and preparation for osmotic fragility

Blood samples were collected in the morning and between eight and ten in the morning. The vein in front of the elbow joint (Anti-cubital vein) was selected for blood sampling. Prior to traction, the area was disinfected with methyl alcohol (70%) to prevent contamination and a tourniquet was tied 7 cm above the traction area for the purpose of vein support. During the withdrawal process, 22G syringes were used for the purpose of drawing blood. Two sets of tubes were prepared, the first set containing an anticoagulant (EDTA tubing) and the second set a normal blood-keeping tube for examination. 5µl of the prepared sample is taken with the concentrations mentioned in the above picture taken from each concentration of 5 ml and then placed in a centrifuge for 5 minutes at a speed of 3000 rpm as in the picture below (3-9). conducting tests for samples and for each concentration, but it was relied on the concentrations (35-25-15-NaCl) given that the decomposition had these concentrations.

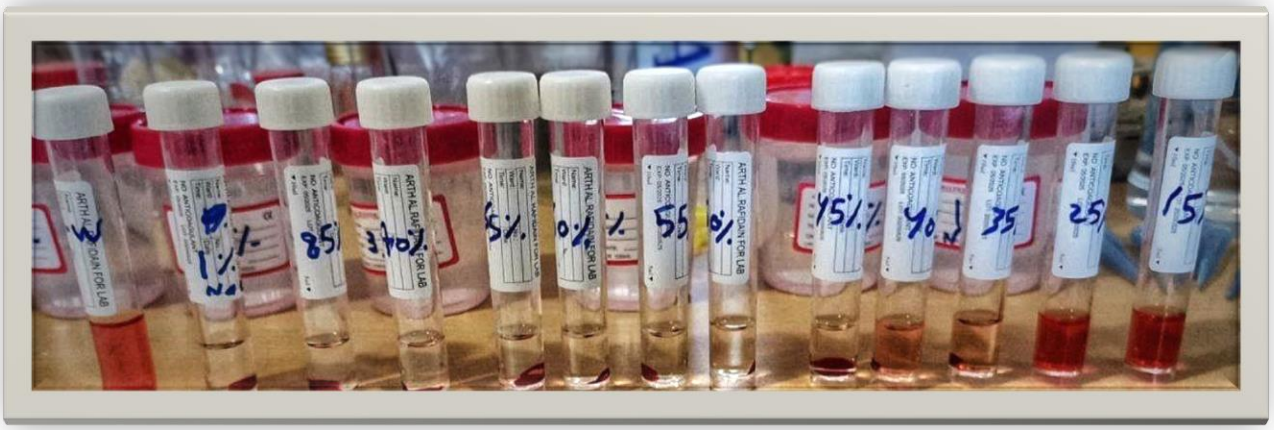


Figure (3- 5) The picture shows a sample of thalassemia distributed at different concentrations

(3-3-3): The second part is the separation of blood components

In this part, the blood sample was taken in the same way mentioned in paragraph 2, but here it will be divided into three samples, where in the first sample we take 5 ml of blood with 3 ml of Distilled water for dilution and take it to the centrifuge for 5 minutes and hemoglobin is obtained ,The second sample is placed the blood sample directly in (EDTA tube) and placed in the centrifuge in order to obtain the plasma, the third sample is taken part of the plasma in a plain tube and also to the centrifuge in order to obtain the serum. All prepared samples are diluted by adding 1ml it to 3 ml of water and then save it again in a plain tube and perform the required tests.

Chapter four:

Result and

Discussion

(4-1): Introduction

The optical properties of biological tissues (blood) can provide a lot of information used in medical diagnosis of diseases. In this chapter it had been discussed the empirical results, for linear and nonlinear optical properties of the samples (thalassemia, blood viscosity and healthy blood). These results include absorption and fluorescence spectrum. To study the linear optical properties (absorbance, transmittance, absorption coefficient and extinction coefficient) visible spectrometer were using for this purpose. As well as to study the nonlinear optical properties (refractive index n_2, absorbance coefficient β and susceptibility $\chi 3$), of the same samples it had been utilize z-scan technique, measurement of the emission spectrum of some samples.

(Plasma, serum) using a fluorescence device for comparison with the absorption spectrum. Blood consists of two main parts, plasma and cells, and red blood cells make up 99% of the blood cells. Therefore, the optical properties of blood depend on the physiological properties of red blood cells. The optical properties of whole blood are determined in the visible and near-red area by red blood cells, and this is due to the high concentration for cells (4-5.5 Million μL^{-1}) because their optical properties are more important than those found in blood contents [111].

Studying the optical properties of blood helps in determining the optimal wavelength that provides the maximum penetration depth for the radiation used in treatment or diagnosis [112]. The phenomenon of osmotic pressure is one of the most important physical phenomena found in the cells of the body, where the plasma membrane of the cells, especially the red cells, is one of the structures responsible for the osmotic or osmotic pressure [113]. When red cells are placed in solutions of different concentrations of salts, especially NaCl, then the solution consists of the solvent, which is water and the solute NaCl If the concentration of NaCl is from 0.9%-85%, then the number of solvent molecules entering through

the plasma membrane is equal to the number of water molecules leaving the cells through the plasma membrane to the outside, as the medium in this case is called isotonic, given that the molecules of the solute inside the cells of salts, minerals and organic compounds are equal to the concentration of NaCl Outside the cells, but when the solute molecules (NaCl) decrease and the concentration of the solvent molecules outside the cells rise, this leads to the entry of water molecules from the outside into the cells due to the decline in concentration, and the entry of water increases whenever the concentration of NaCl decreases. The plasma membrane has the property of elasticity, and the cell can bear the largest volume of water whenever the flexibility of the plasma membrane increases to stretch. When the dilution of the NaCl concentration reaches 35%, a section of cells begins to rupture their plasma membrane, especially the aging and abnormal cells, considering their membrane is less flexible and elastic. As for young and normal cells, the Its plasma membranes have a high ability to stretch. Therefore, the rupture of the plasma membranes and the exit of the hemoglobin dye into the medium is evidence of the percentage of broken red cells. The spectral properties, which are proportional to the concentration of the hemoglobin dye, are evidence of the amount of hemoglobin produced from the broken cells. It can be considered as a guide to measure and investigate the extent of the normal condition. For red cells, where it depends on the strength of elasticity of the plasma membrane, the penetration of molecules of the solvent (which is water) [114].

(4-2): Linear Optical Properties

Tests of the linear optical properties of osmotic fragility samples and blood separation by using UV-VIS spectrophotometer and Fluorescence spectrum for separation blood samples only.

(4-2-1): Normal blood samples

According to the osmotic fragility test of normal blood samples pcv(45%,42%,40%) with different concentrations for each sample (NaCl, 15%,

25%, 35%), and after conducting a UV-VIS spectrophotometer examination and using the linear optical properties program, the following results were obtained.

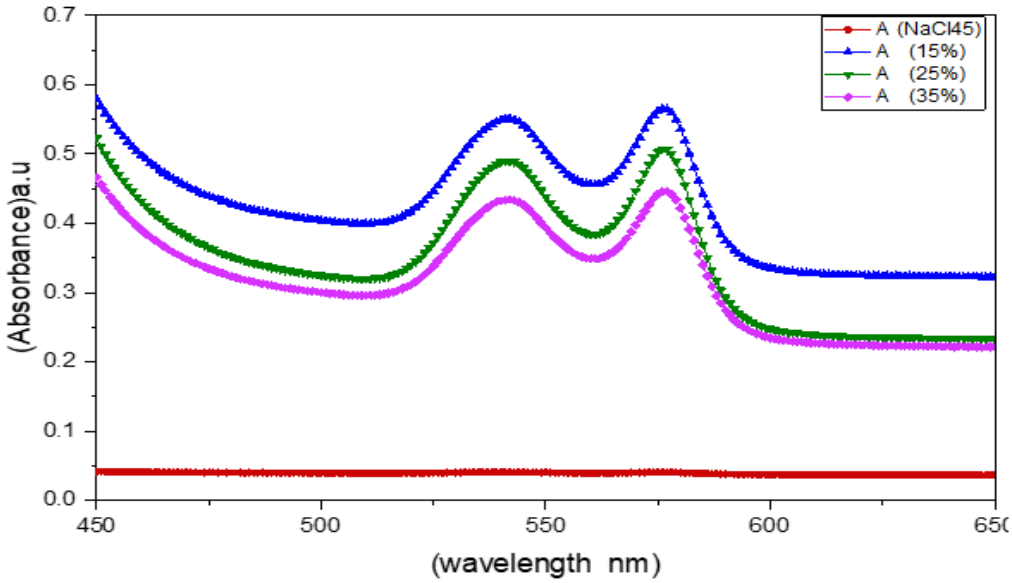


Figure (4- 1)linear optical properties of normal blood sample pcv45% absorbance

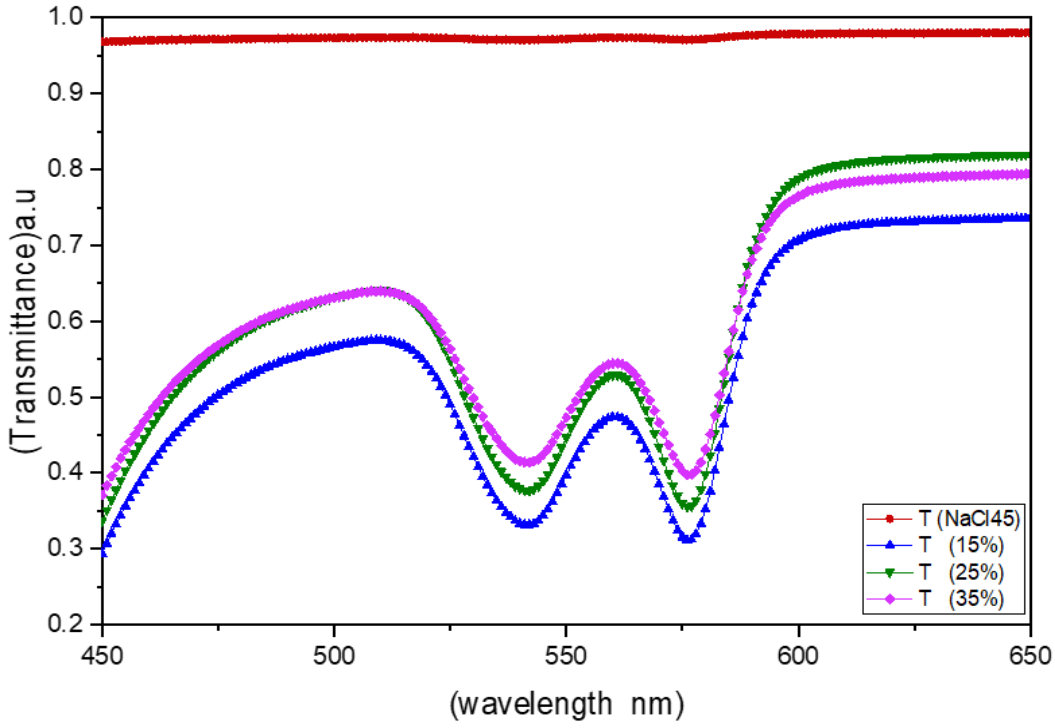


Figure (4- 1)linear optical properties of normal blood sample pcv45% transmittance

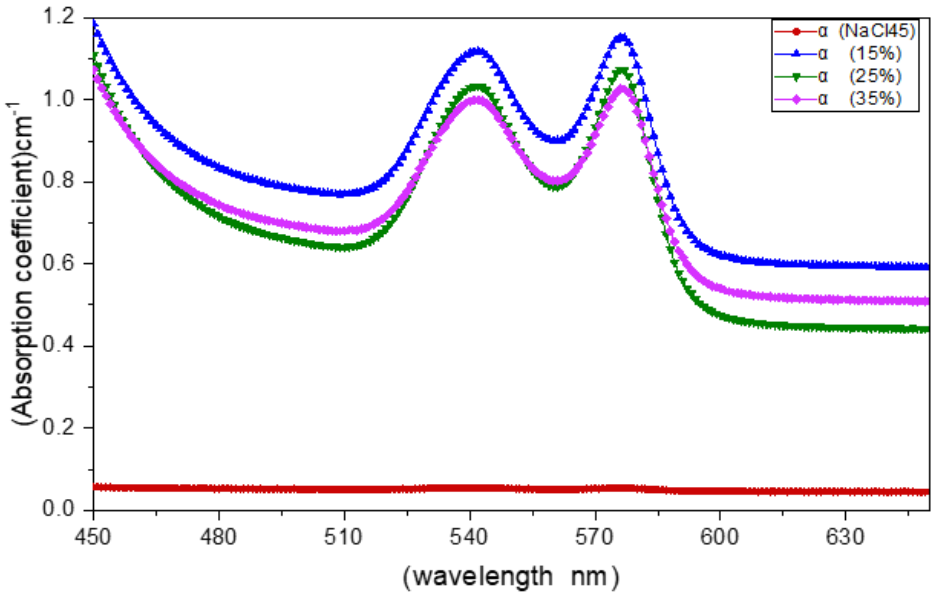


Figure (4- 2)linear optical properties of normal blood sample pcv45% absorption coefficient

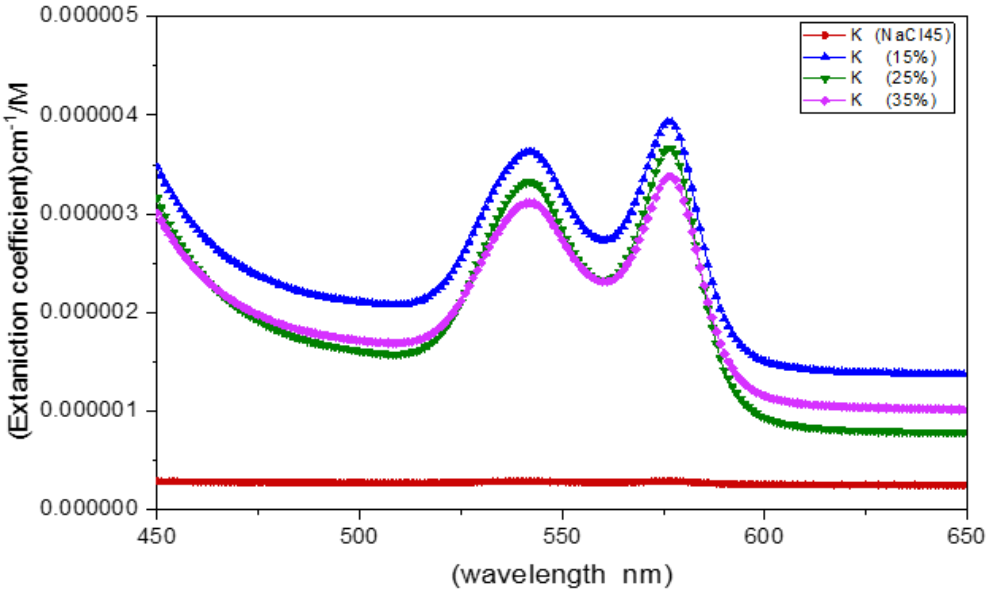


Figure (4- 3)linear optical properties of normal blood sample pcv45% extaniction coefficient

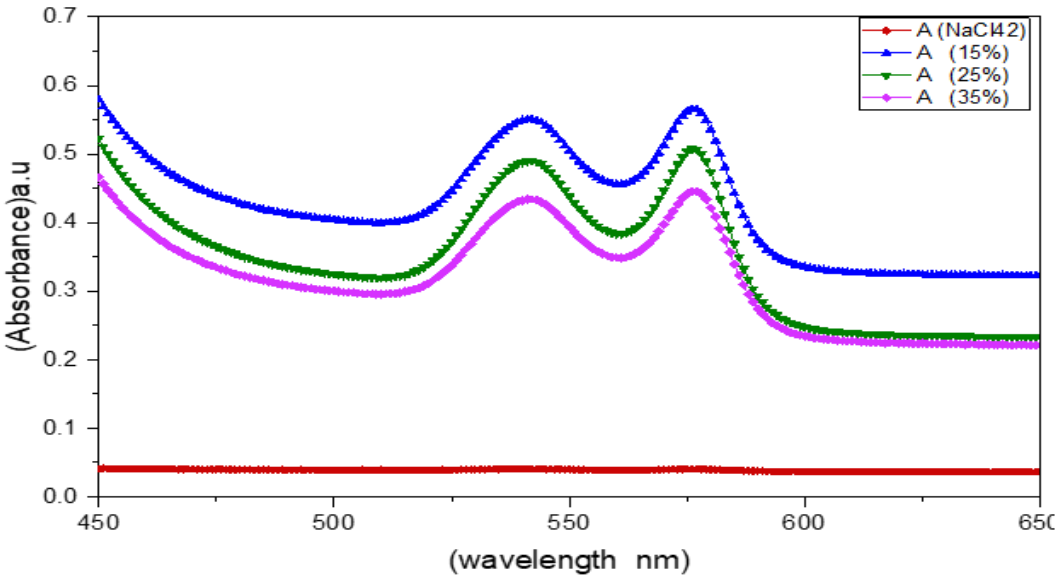


Figure (4- 4)linear optical properties of normal blood sample pcv42% absorbance

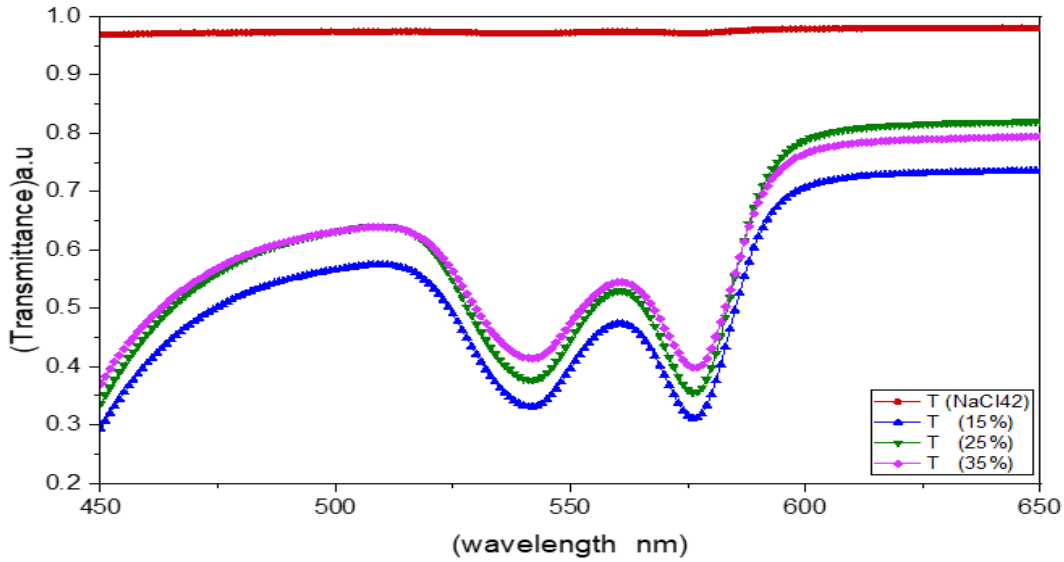


Figure (4- 5)linear optical properties of normal blood sample pcv42% transmittance

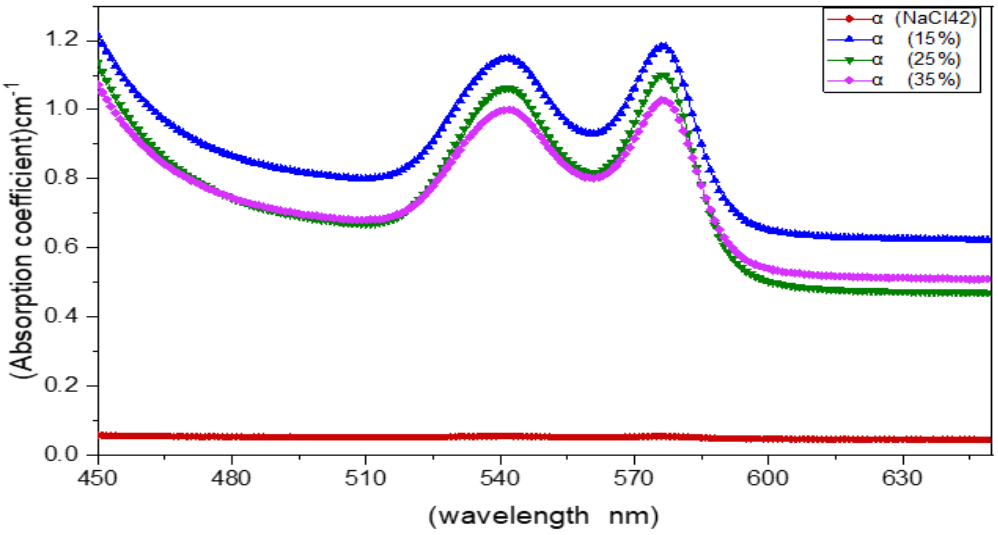


Figure (4- 6)linear optical properties of normal blood sample pcv42% absorption coefficient

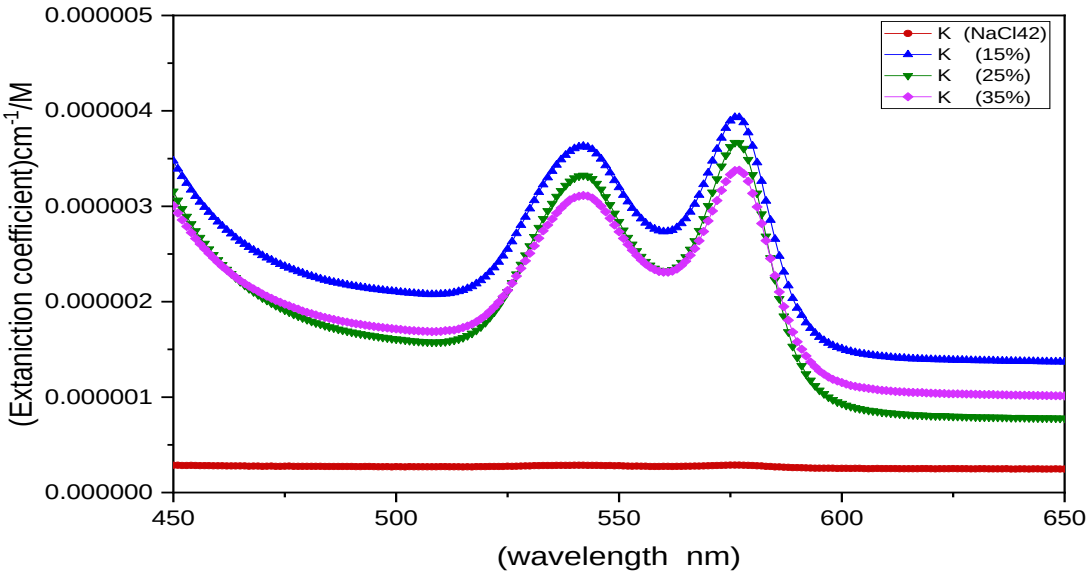


Figure (4- 7)linear optical properties of normal blood sample pcv42% extaniation coefficient

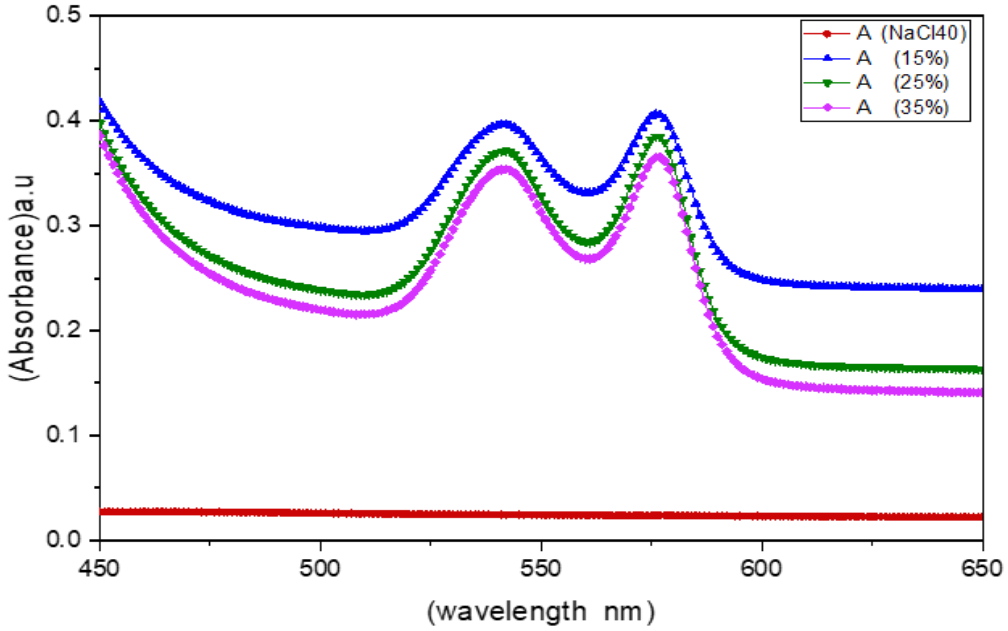


Figure (4- 8)linear optical properties of normal blood sample pcv40%, (a)absorbance

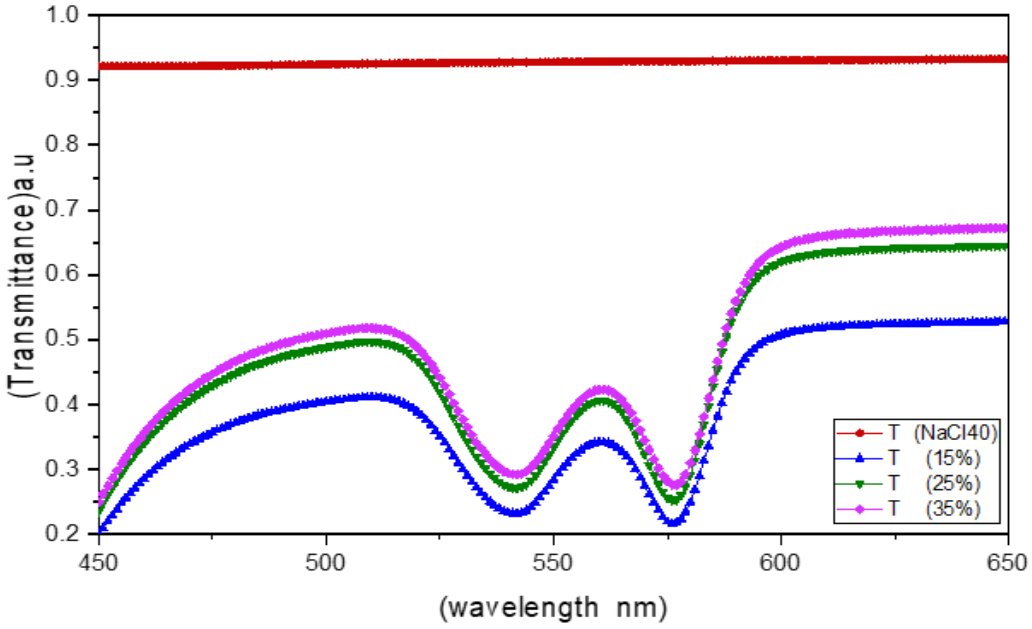


Figure (4- 9)linear optical properties of normal blood sample pcv40% transmittance

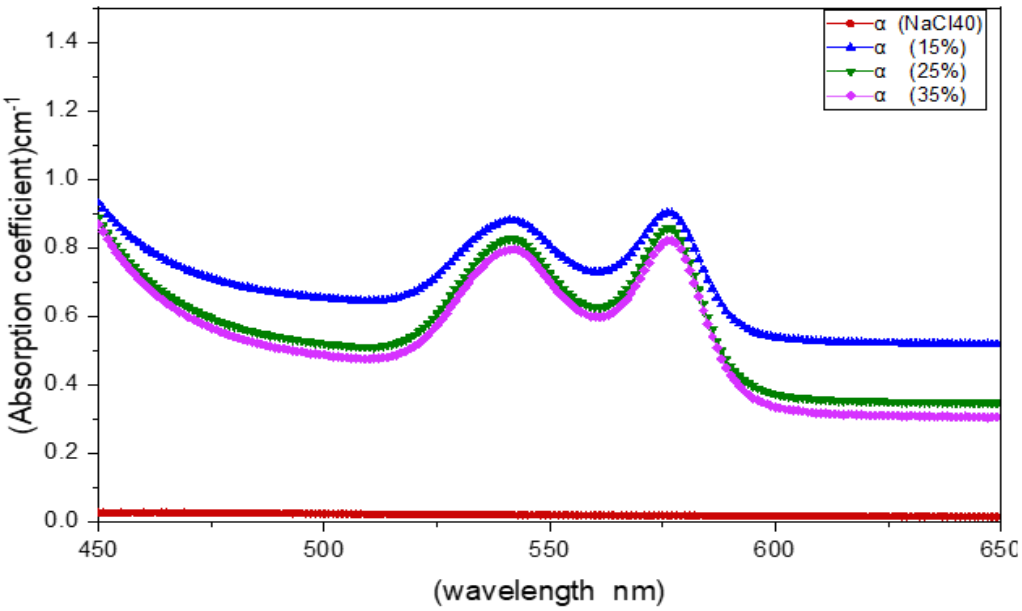


Figure (4- 10)linear optical properties of normal blood sample pcv40% absorption coefficient

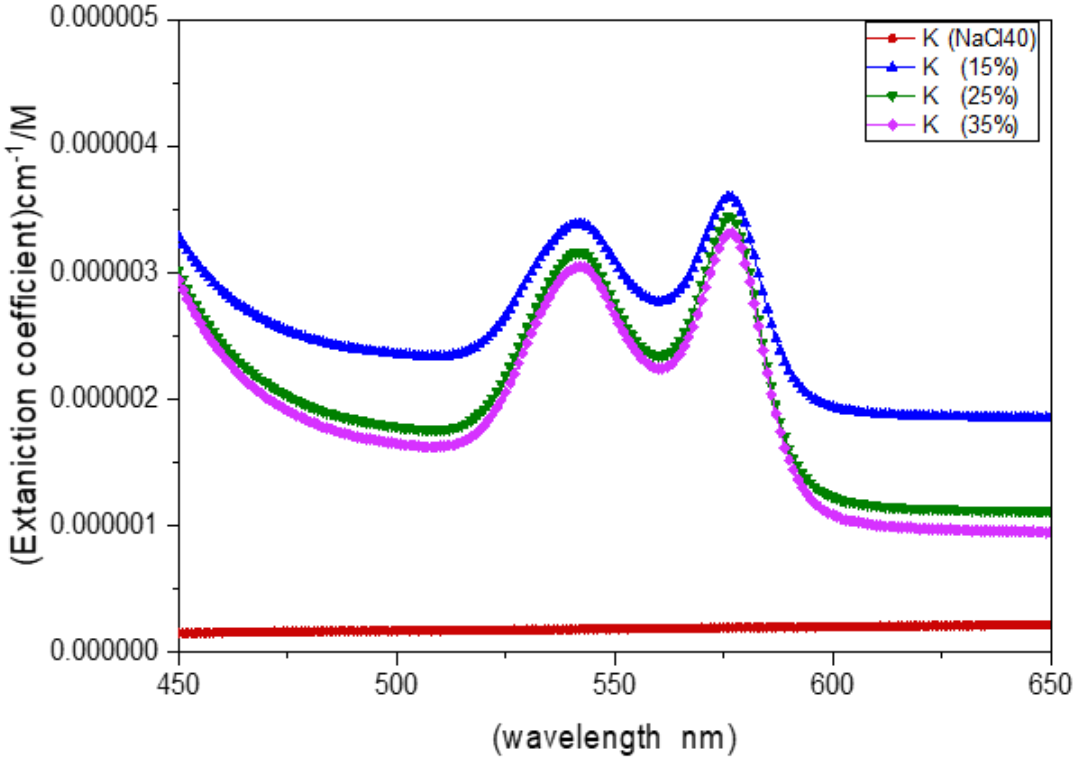


Figure (4- 11)linear optical properties of normal blood sample pcv40% extaniction coefficient

Table 4- 1 the linear optical properties of normal blood samples pcv 45%,42%,40%

normal blood samples	Concentrate Of NaCl	λ(nm)	A	T	α(cm^{-1})	n	K(cm^{-1}/M)
PCV45%	NaCl 1%	577	0.094	1.173	0.065	0.944	3.42×10^{-7}
	15%	577	0.688	0.311	1.275	2.118	4.95×10^{-6}
	25%	577	0.624	0.372	1.202	2.054	4.51×10^{-6}
	35%	577	0.531	0.411	1.054	1.974	3.32×10^{-6}
PCV42%	NaCl	577	0.042	0.969	0.051	0.956	2.90×10^{-7}
	15%	577	0.568	0.306	1.155	2.045	3.95×10^{-6}
	25%	577	0.510	0.341	1.075	1.986	3.67×10^{-6}
	35%	577	0.449	0.393	1.025	1.909	3.32×10^{-6}
PCV40%	NaCl	577	0.023	0.925	0.020	0.937	2.27×10^{-7}
	15%	577	0.428	0.236	0.905	1.927	3.60×10^{-6}
	25%	577	0.404	0.250	0.857	1.853	3.45×10^{-6}
	35%	577	0.3810	.265	0.826	1.764	3.29×10^{-6}

It was noted from table (4 -1) that the absorbance of the blood as a whole is in the visible region and at two peaks 540nm, 576nm, and we conclude that the linear parameters (absorbance, transmittance, absorption coefficient, refractive index, extinction coefficient) for blood samples increased with increasing PCV concentration (absorbed molecule) in the blood, where at pcv45% the parameters were as follows (0.688, 0.311, 1.275cm^{-1}, 2.118, 4.95 x$10^{-6}$$cm^{-1}$/M). Respectively, as shown in Table (4-1) and for a concentration of 15%, given that it is the concentration at which red blood cells are completely lysed, because the NaCl solution is less inside the cell, unlike water. As for the values of the linear parameters of the NaCl solution, they were Low due to the inability of red blood cells to completely dissolve due to the high concentration of salt solution inside the cell and for the same normal blood sample pcv45% as shown in the graphs (4-1) and were as follows (0.094,1.173, 0.065 cm^{-1}, 0.944, 3.42 x 10^{-7} cm^{-1}/m) respectively.

As for the pcv42% sample, the linear optical parameters were few because the concentration of PCV is lower than it was in the sample pcv 45%, and the results were as follows (0.568,0.306,1.155 cm^{-1},2.045,3.95 x $10^{-6}cm^{-1}$/ M) respectively as shown in the table and for the same concentration of 15%, as for the concentration of NaCl from the same sample, the results were (0.042,0.969,0.051 cm^{-1},0.956,2.90 x $10^{-7}cm^{-1}$/ M)respectively, as shown in the graphs in appendix A and Table (1-4).

It is also noted that the linear parameters of the pcv40%sample shown in the graphics (4-2) are as follows for the same concentration 15% (0.428,0.236,0.905cm^{-1},1.927,3.60x$10^{-6}cm^{-1}$/M), while in terms of the NaCl solution (0.023,0.925,0.020cm^{-1},1.020,2.27 x $10^{-7}cm^{-1}$/ M) respectively.

(4-2-2): Viscosity of blood samples

The linear optical properties of blood viscosity samples PCV (60% ,54 % , 52%,50%) have been calculated for people of both genders, as these people are distinguished by that the concentration of the number of red blood cells is large for them, so the pcv is high and the following are the most important results related to these samples.

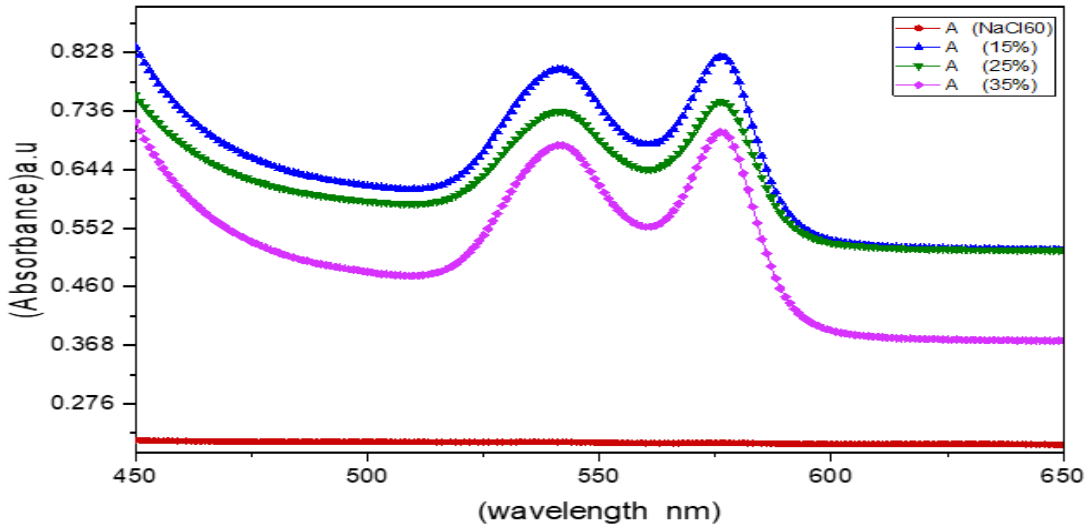


Figure (4- 12)linear optical properties of blood viscosity sample pcv 60% absorbance

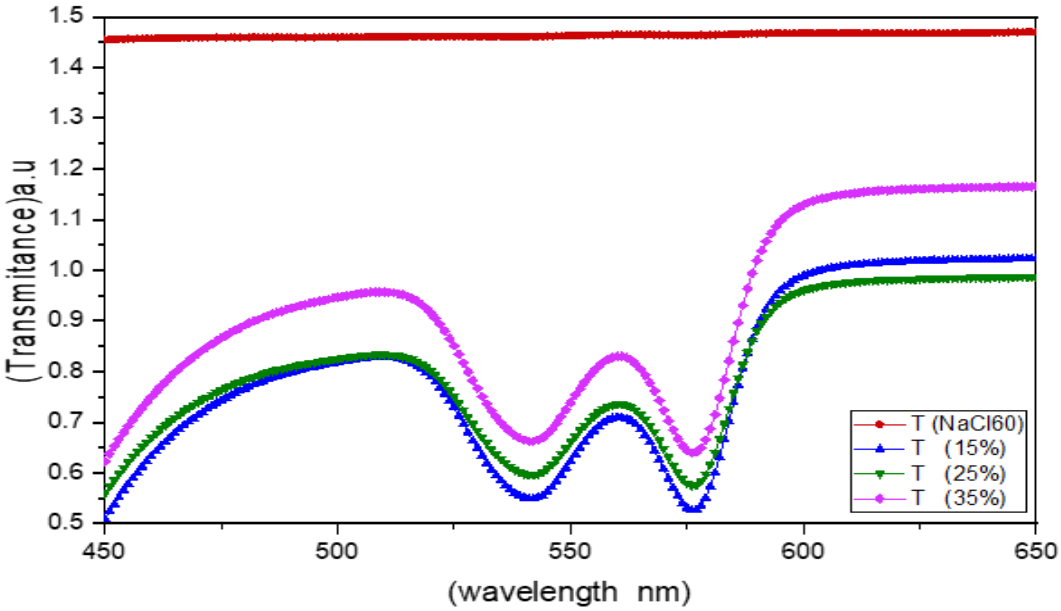


Figure (4- 13)linear optical properties of blood viscosity sample pcv 60% transmittance

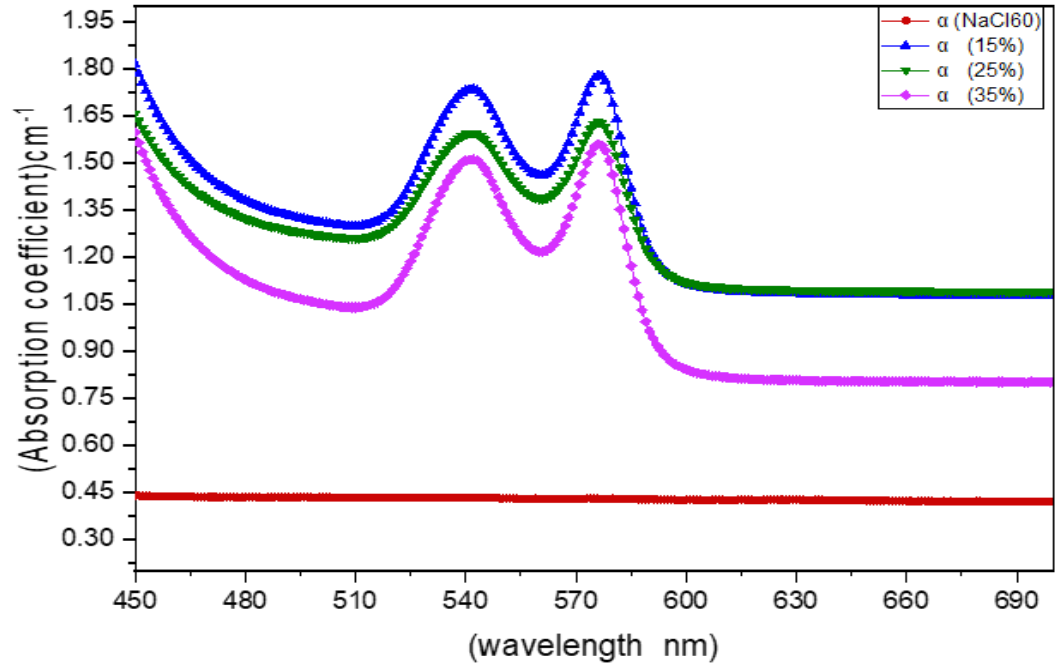


Figure (4- 14)linear optical properties of blood viscosity sample pcv 60% absorption coefficient

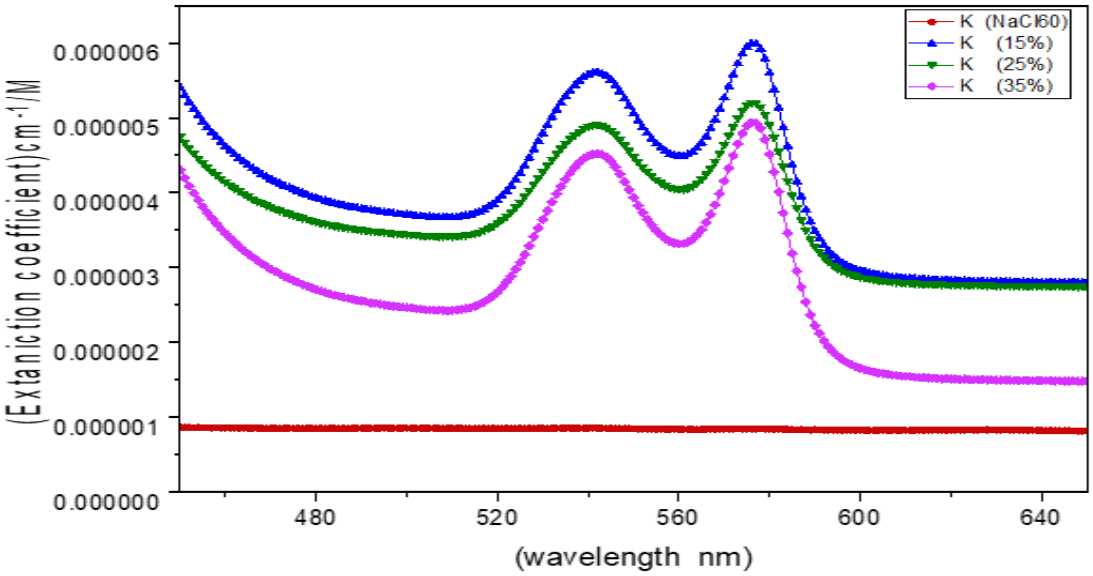


Figure (4- 15)linear optical properties of blood viscosity sample pcv 60% extaniction coefficient

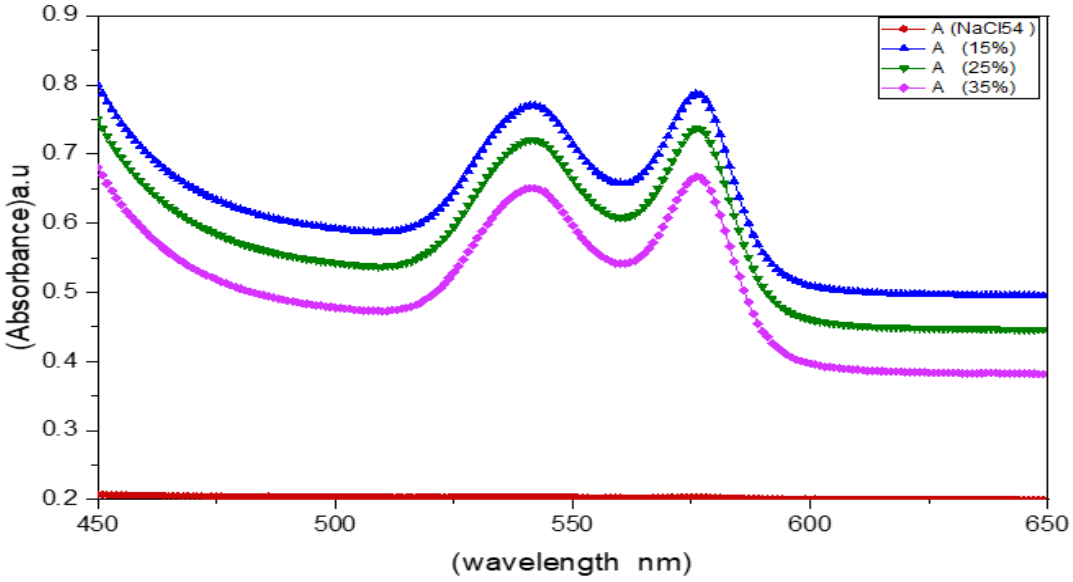


Figure (4- 16)linear optical properties of blood viscosity sample pcv 54% absorbance

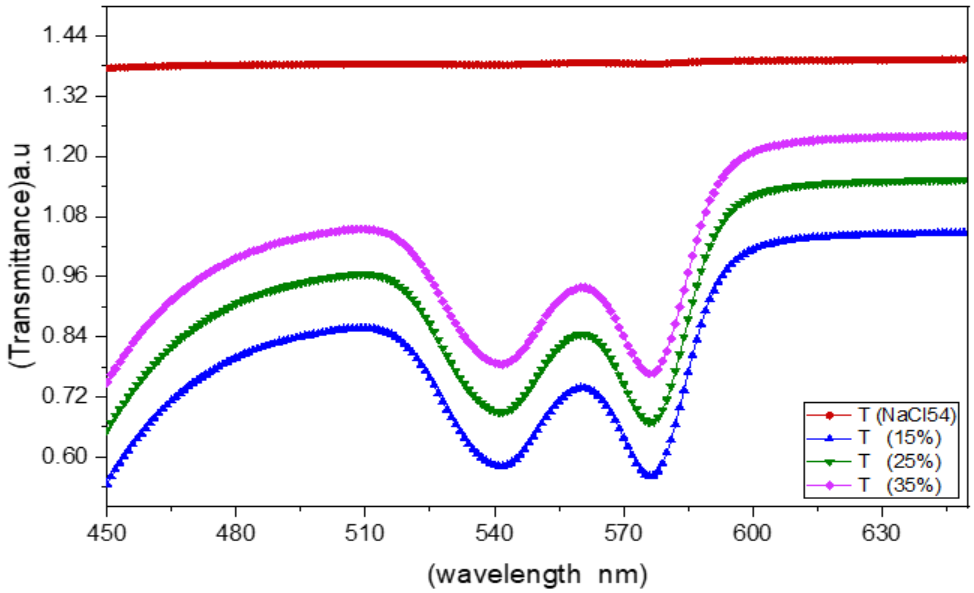


Figure (4- 17)) linear optical properties of blood viscosity sample pcv 54%transmittance

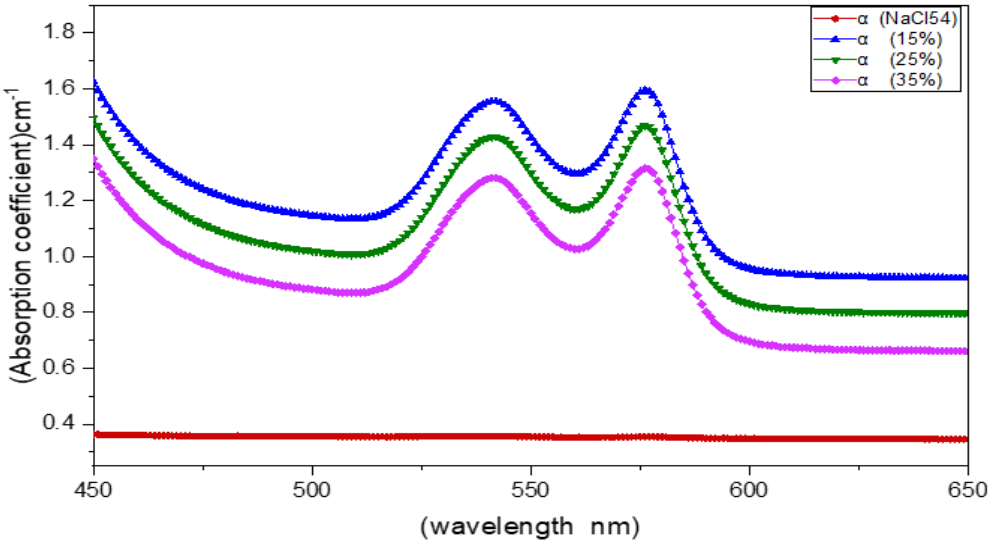


Figure (4- 18) linear optical properties of blood viscosity sample pcv 54% absorption coefficient

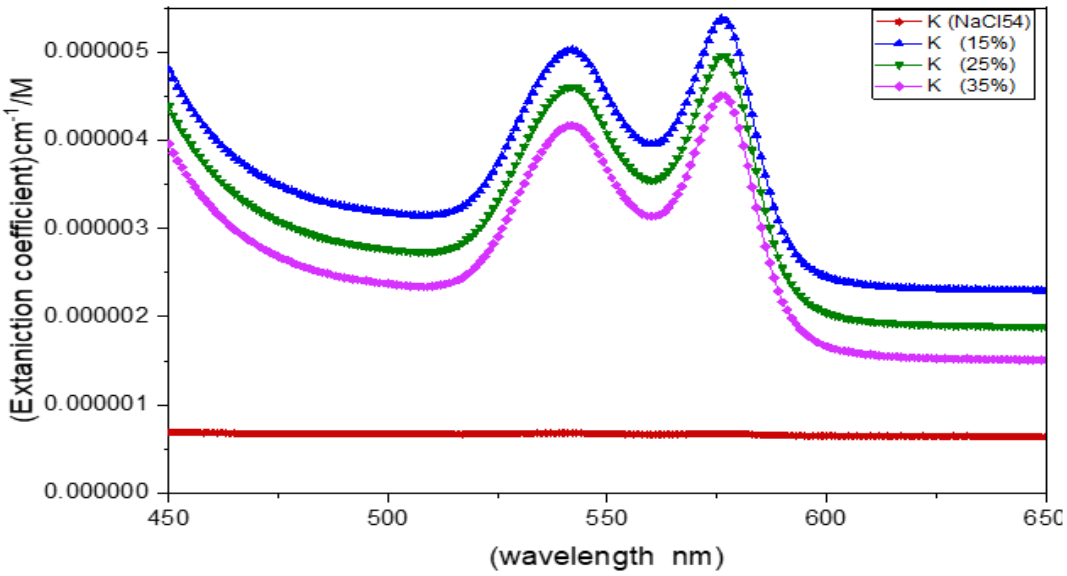


Figure (4- 19) linear optical properties of blood viscosity sample pcv 54%extaniction coefficient

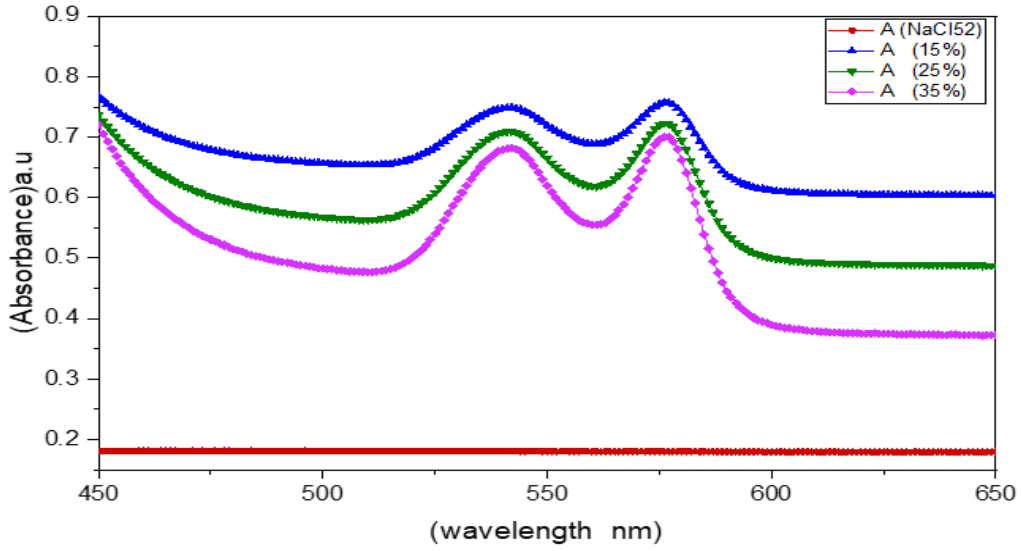


Figure (4- 20)linear optical properties of blood viscosity sample pcv 52% absorbance

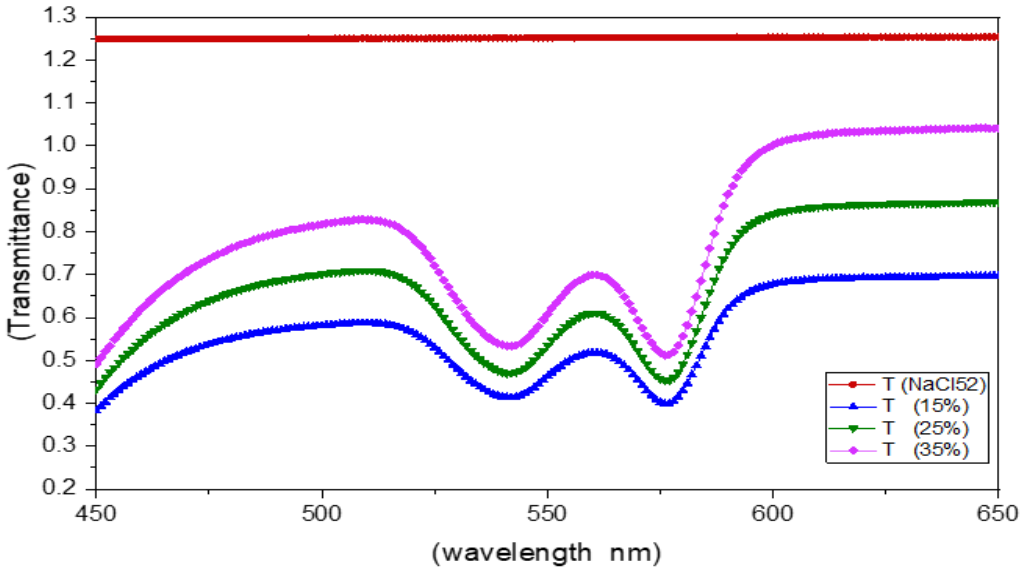


Figure (4- 21)linear optical properties of blood viscosity sample pcv 52% transmittance

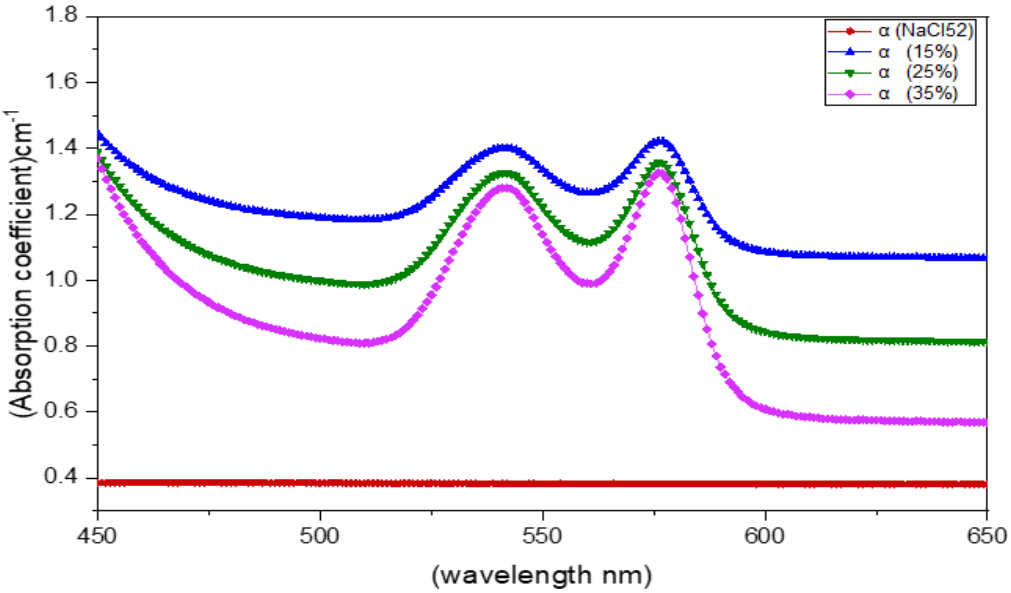


Figure (4- 22)linear optical properties of blood viscosity sample pcv 52% absorption coefficient

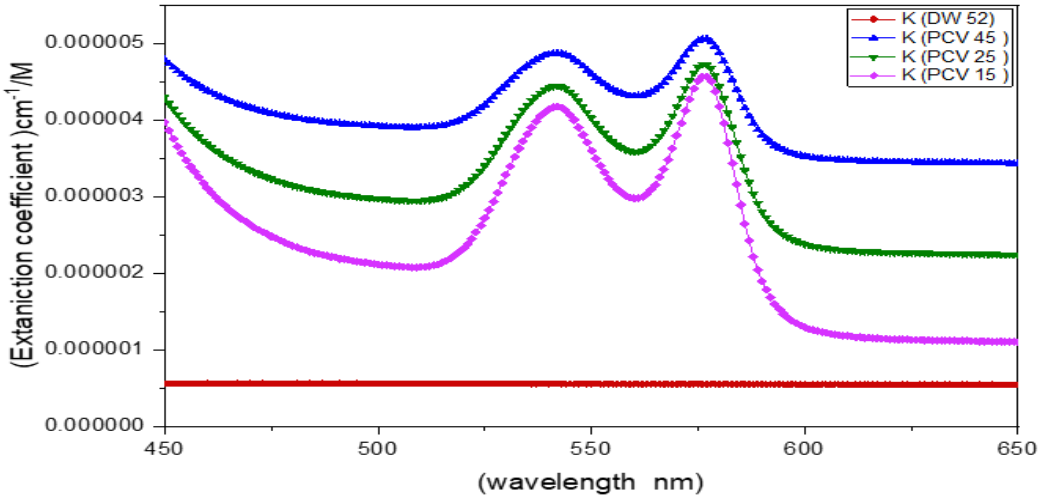
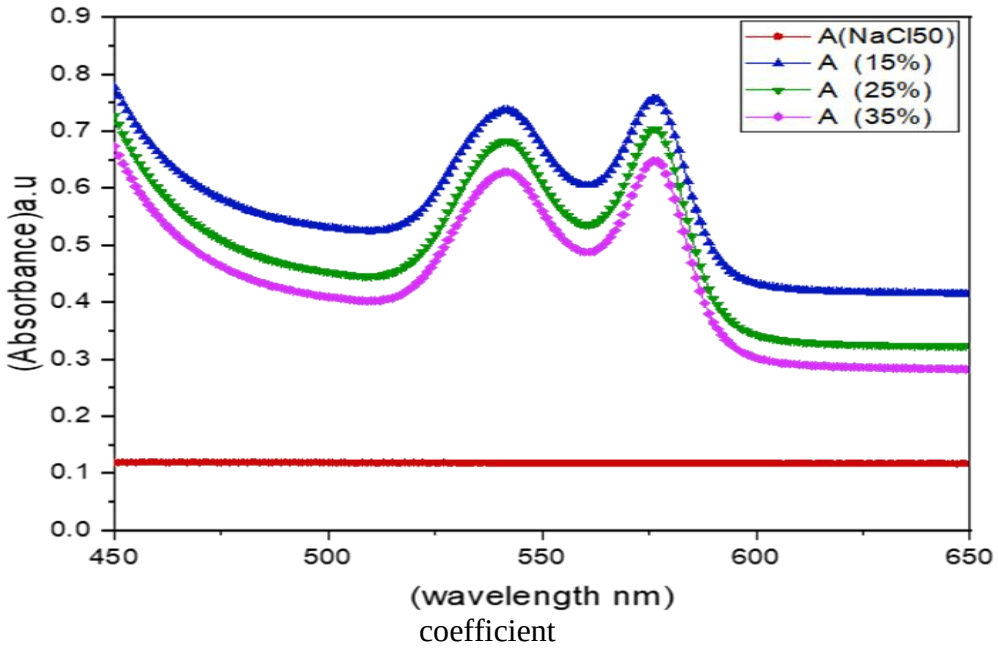


Figure (4- 23) linear optical properties of blood viscosity sample pcv 52% extaniction



coefficient

Figure (4- 24)linear optical properties of blood viscosity sample pcv50% absorbance

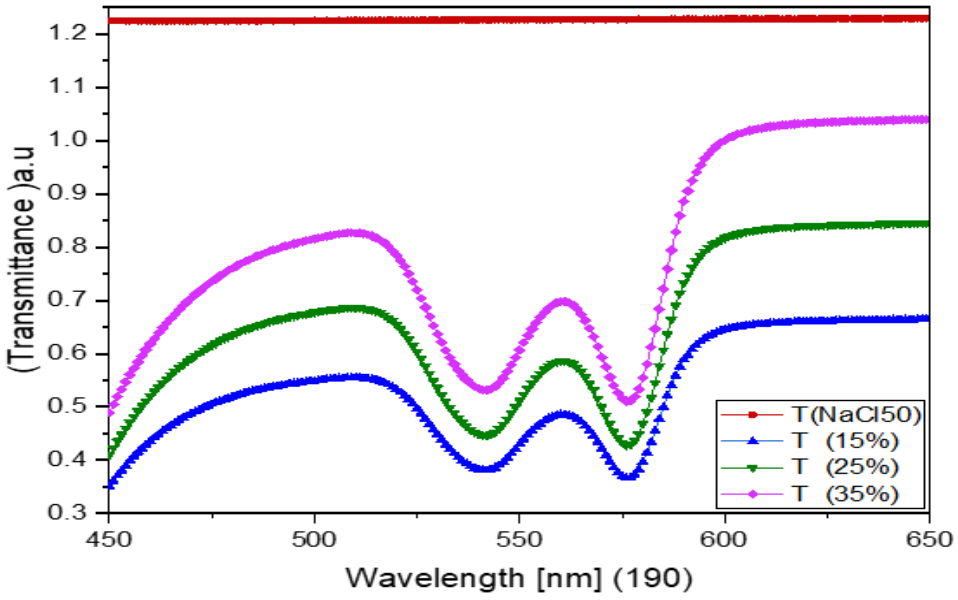


Figure (4- 25) linear optical properties of blood viscosity sample pcv50% transmittance

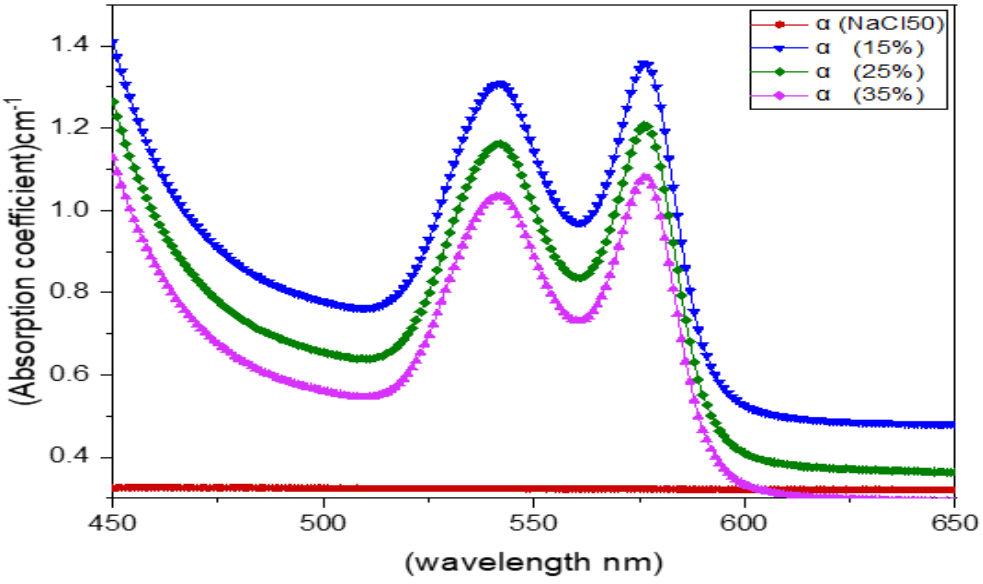


Figure (4- 26)linear optical properties of blood viscosity sample pcv50% absorption coefficient

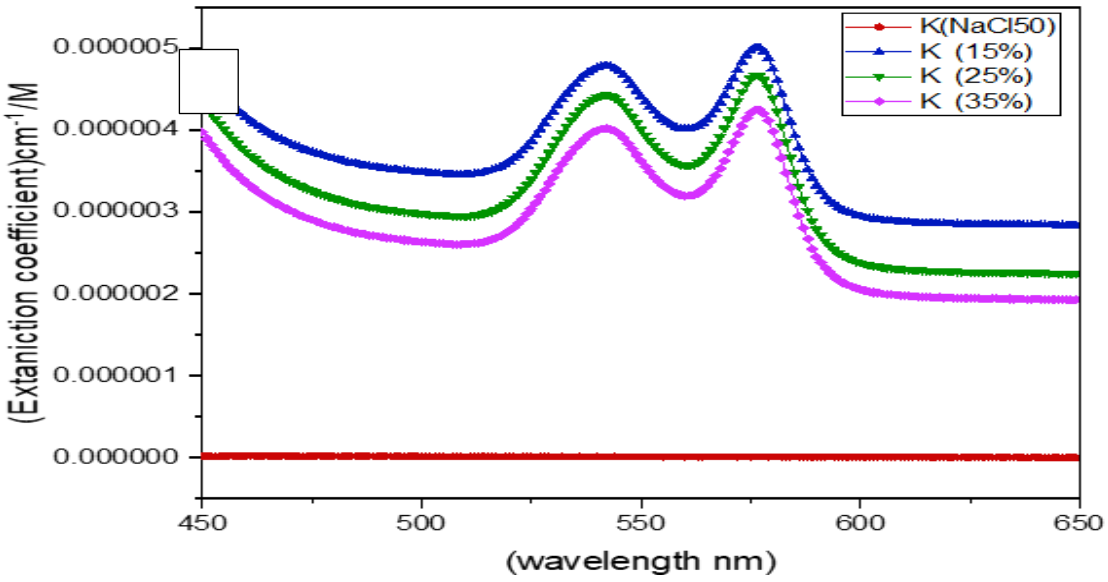


Figure (4- 27)linear optical properties of blood viscosity sample pcv50% extaniction coefficient

Table (4- 2) linear optical properties of blood viscosity samples pcv60%,54%,52%,50%

blood viscosity sample	Concentrate of NaCl	λ(nm)	A	T	$\alpha(cm^{-1})$	n	$K(cm^{-1}/M)$
PCV60%	NaCl 1%	577	0.216	1.469	0.435	1.192	8.77×10^{-7}
	15%	577	0.828	0.521	1.789	2.420	6.03×10^{-6}
	25%	577	0.754	0.576	1.638	2.320	5.24×10^{-6}
	35%	577	0.708	0.651	1.572	2.216	5.00×10^{-6}
PCV54%	NaCl	577	0.210	1.375	0.353	1.108	6.95×10^{-7}
	15%	577	0.790	0.463	1.592	2.278	5.35×10^{-6}
	25%	577	0.741	0.557	1.477	2.178	4.97×10^{-6}
	35%	577	0.671	0.632	1.327	2.116	4.50×10^{-6}
PCV52%	NaCl	577	0.178	1.251	0.383	1.004	5.41×10^{-7}
	15%	577	0.765	0.396	1.434	2.235	5.07×10^{-6}
	25%	577	0.679	0.451	1.358	2.127	4.74×10^{-6}
	35%	577	0.639	0.506	1.328	2.059	4.59×10^{-6}
PCV50%	NaCl	577	0.124	1.219	0.333	0.993	3.14×10^{-7}
	15%	577	0.758	0.364	1.365	2.178	5.01×10^{-6}
	25%	577	0.707	0.427	1.210	2.065	4.67×10^{-6}
	35%	577	0.654	0.503	1.086	1.940	4.27×10^{-6}

From the results shown in the pcv60% sample, the written optical characteristics of this sample and at a concentration of 15% are higher than in the case of normal blood due to the high concentration of the absorbed molecule (hemoglobin). The results are as follows as shown in table (4-2) (0.828,0.521,1.789cm^{-1},2.420,6.03x$10^{-6}cm^{-1}$/M), respectively. As for the NaCl solution, it was (0.216.1.469.0.435cm^{-1},192.8.77 x 10^{-6} cm^{-1}/M).

In the case of pcv54%, the linear results of this sample are lower for a concentration of 15% than in pcv60%, taking into account the low PCV concentration, and the results are as follows (0.790, 0.463, 1.592cm^{-1}, 2.278 ,5.35x$10^{-6}cm^{-1}$/M), as shown in Table (4-2), while in terms of the NaCl for this sample the optical linear parameters are (0.210,1.375,0.353cm^{-1},1.108,6.95 x$10^{-7}cm^{-1}$/M).

As for cases pcv52%,50%, the linear physical parameters at a concentration of 15% for these samples were lower than in the above cases for blood viscosity samples due to a decrease in the Packed cell volume (pcv) and the results were as follows for these samples (0.765,0.758,0.396,0.364,1.434cm^{-1}, 1.365,2.235,2.178cm^{-1},5.07x$10^{-6}cm^{-1}$/M,5.0107x$10^{-6}cm^{-1}$/M) respectively, as shown in the table

(4-2) and the graphs of the sample pcv54%, and sample pcv52% As for the concentration of NaCl for these samples, the results were as shown in the table (4-2), respectively for the two samples (0.178,0.124,1.251,1.124,0.383cm^{-1},0.333cm^{-1},1.004,0.993,5.41x$10^{-7}cm^{-1}$/M, 3.14 x$10^{-7}cm^{-1}$/M).

(4-2-3): Thalassemia blood samples (osmotic fragility)

Thalassemia is one of the common diseases in Iraq, and it results from small and deformed red blood cells, and the hemoglobin percentage in these people is low because of the small size of red blood cells, so this disease was taken in order to compare with normal blood and blood viscosity and study the linear optical

properties of it. And it was taken Four samples PCV (33%, 25%, 22% ,19%) with different concentrations (NaCl,15%,25%,35%).

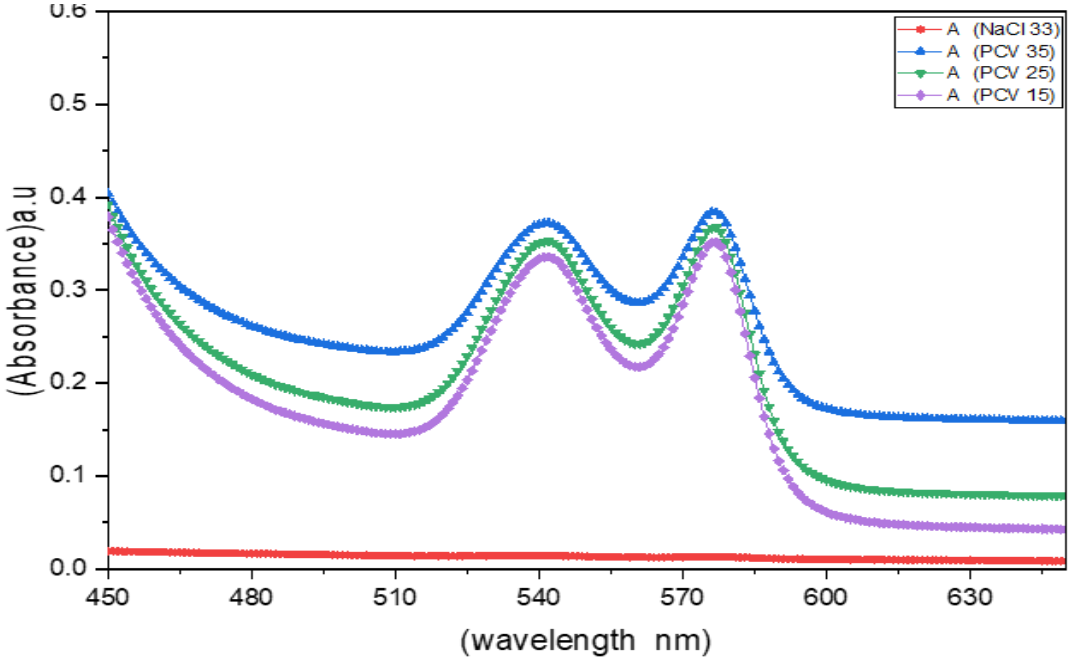


Figure (4- 28)linear optical properties of a thalassemia sample pcv33 % absorbance

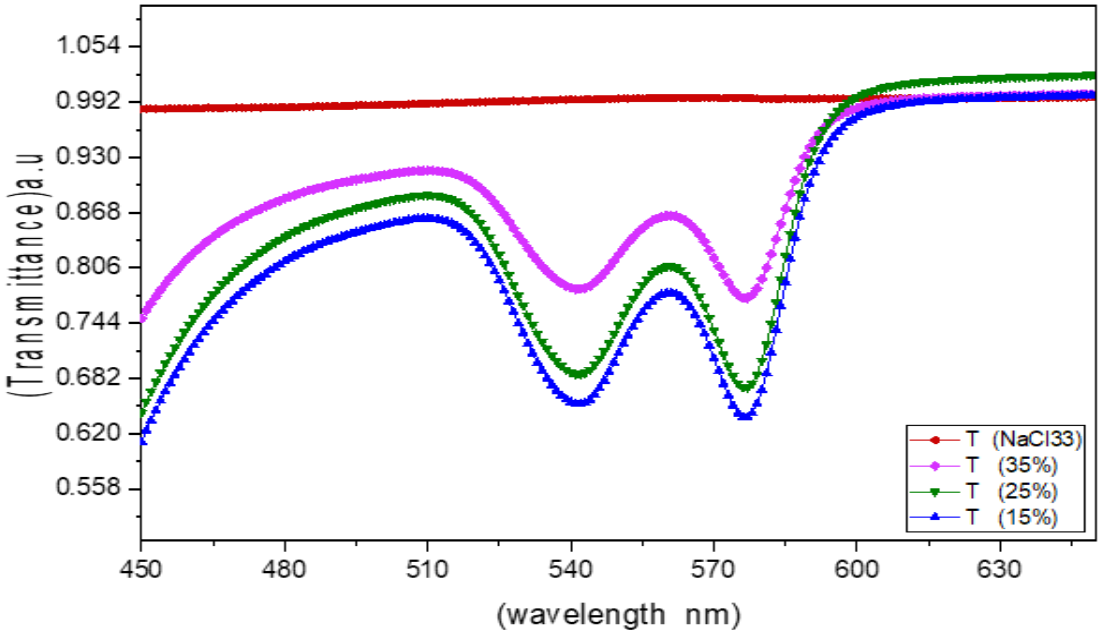


Figure (4- 29)linear optical properties of a thalassemia sample pcv33 % transmittance

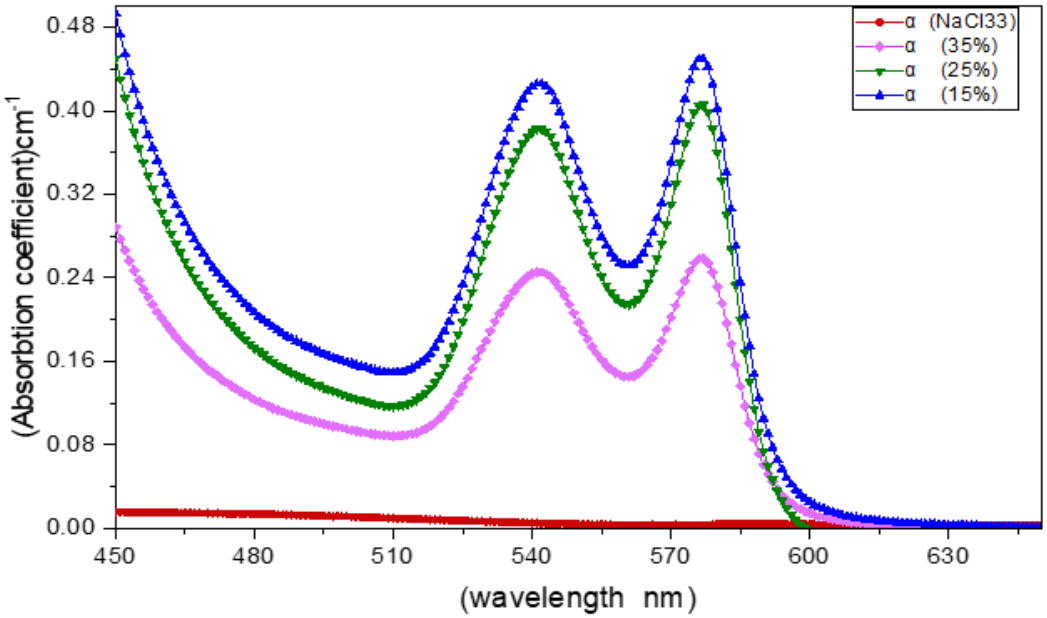


Figure (4- 30)linear optical properties of a thalassemia sample pcv33 % absorption coefficient

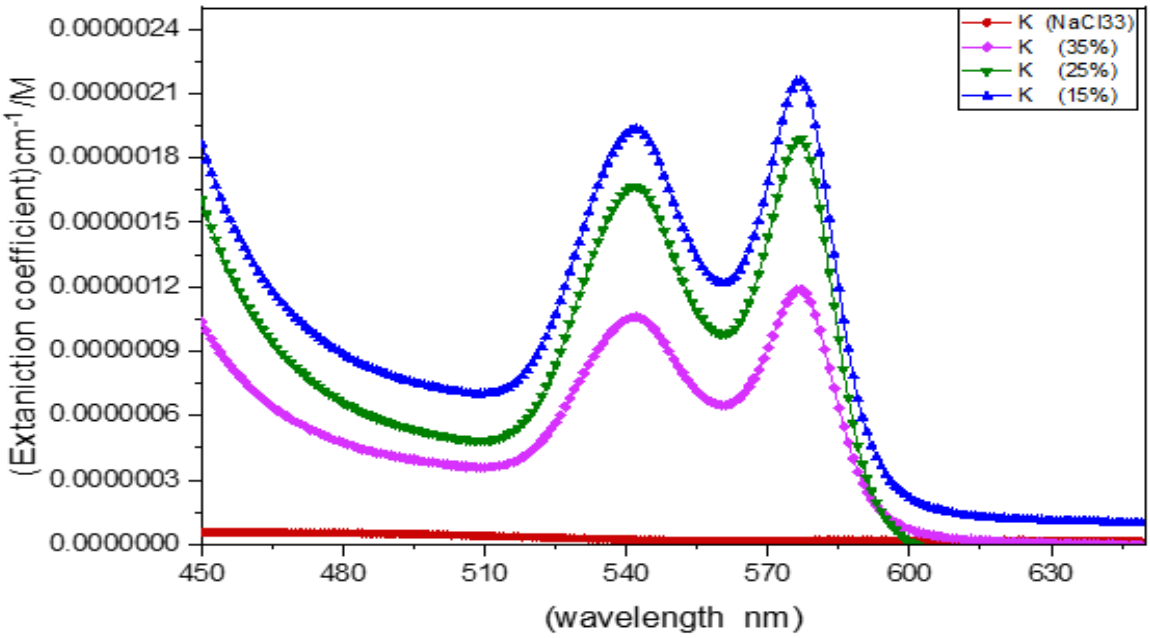


Figure (4- 31)linear optical properties of a thalassemia sample pcv33 % extaniction coefficient

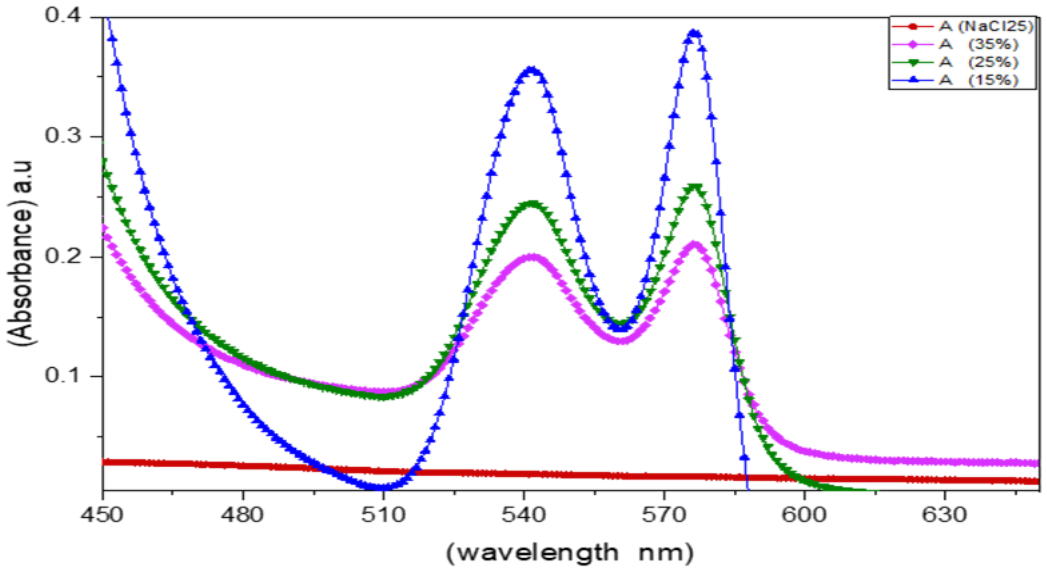


Figure (4- 32*)* linear optical properties of a thalassemia sample pcv25% absorbance

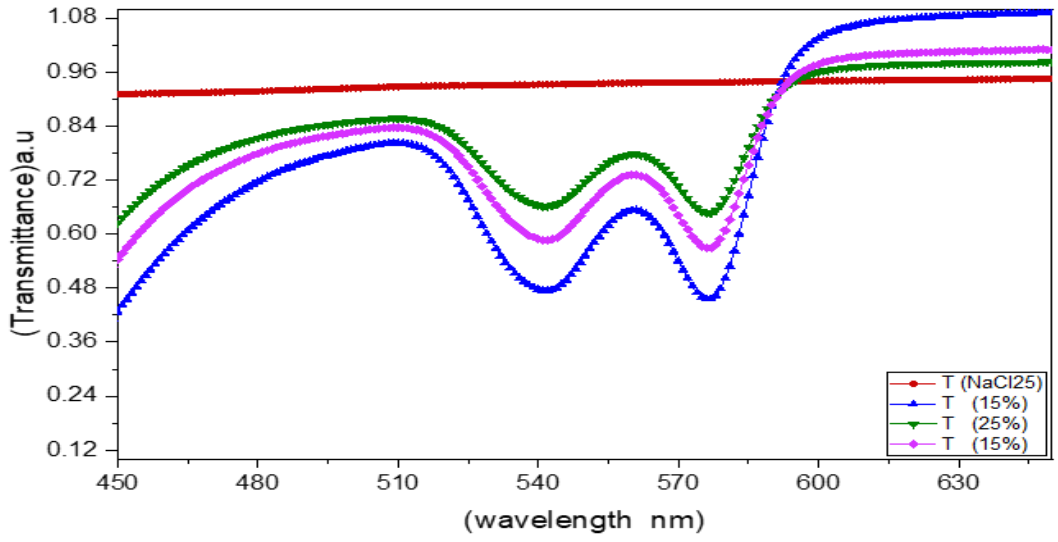


Figure (4- 33)linear optical properties of a thalassemia sample pcv25% transmittance

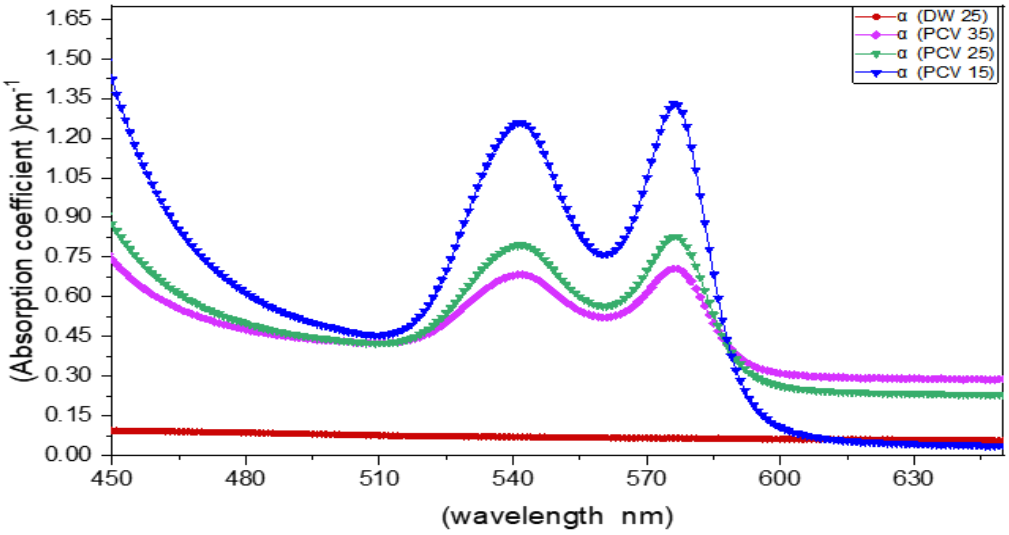


Figure (4- 34) linear optical properties of a thalassemia sample pcv25% absorption coefficient

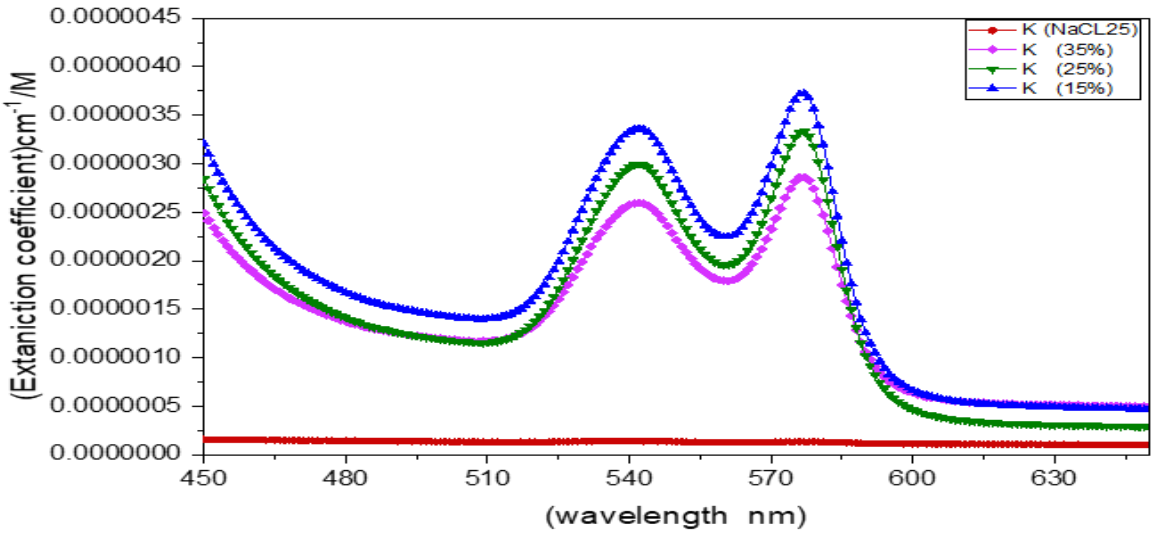


Figure (4- 35)linear optical properties of a thalassemia sample pcv25% extaniction coefficient

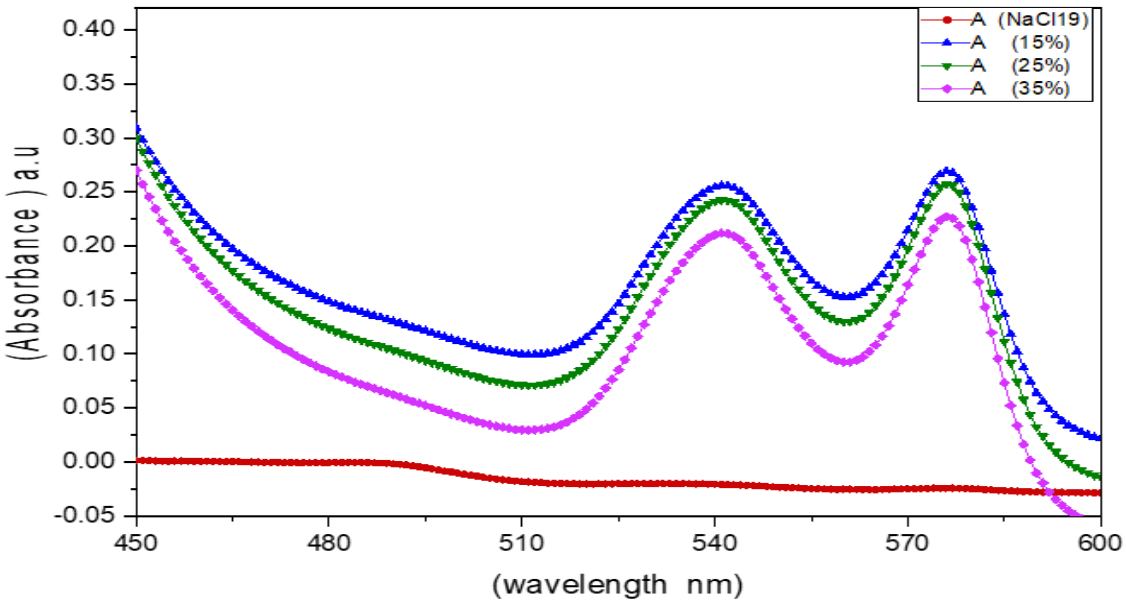


Figure (4- 36)linear optical properties of a thalassemia sample pcv 19% absorbance

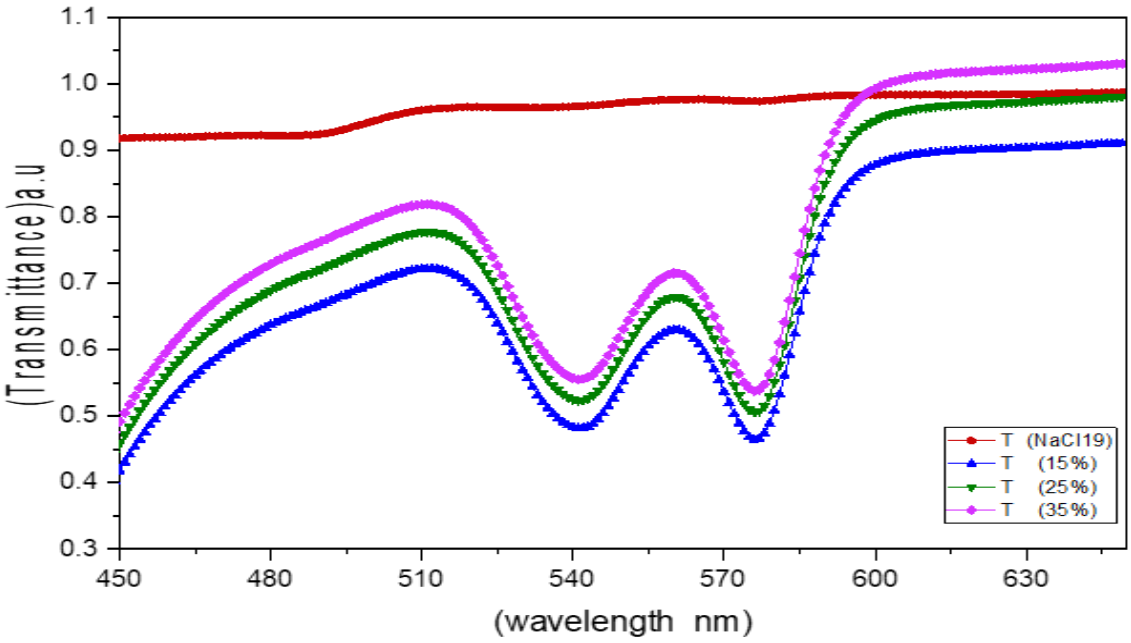


Figure (4- 37)linear optical properties of a thalassemia sample pcv19% transmittance

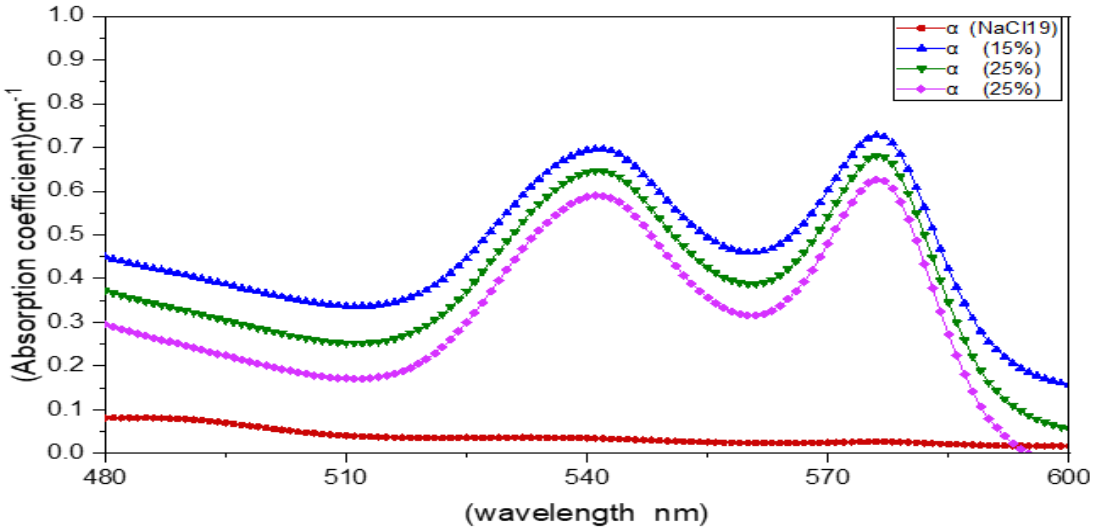


Figure (4- 38) linear optical properties of a thalassemia sample pcv19% absorption coefficient

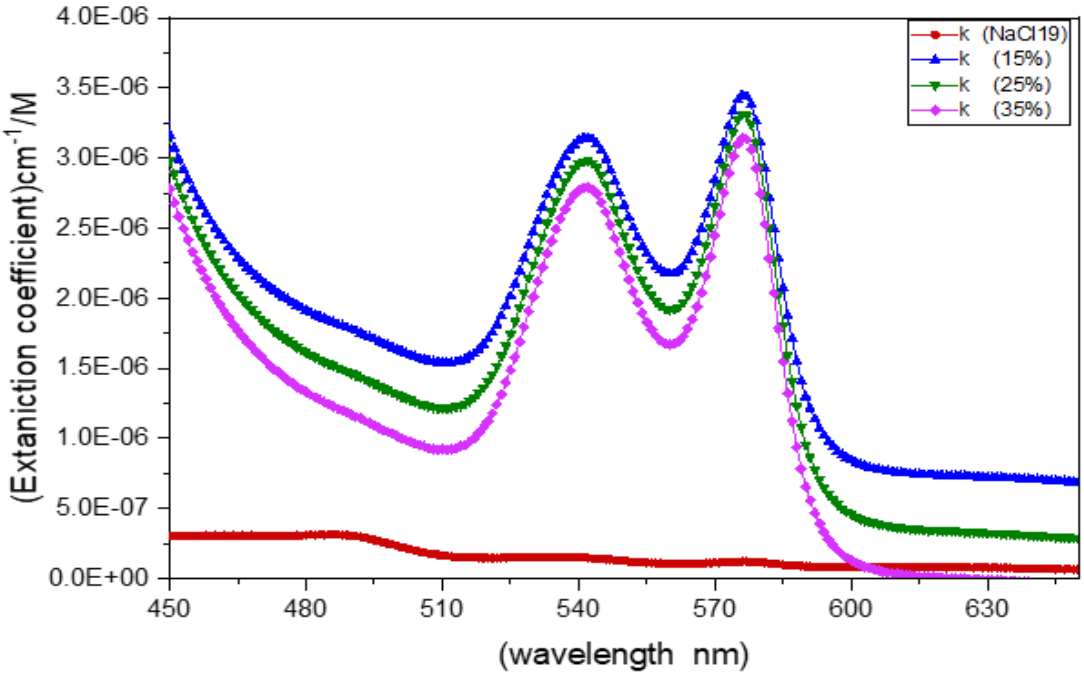


Figure (4- 39) linear optical properties of a thalassemia sample pcv19% extinction coefficient.

Table 4- 3 showing the linear optical properties of thalassemia samples of pcv33% ,22 %,25 %,19 %

Thalassemia blood samples	Concentrate of NaCl	λ(nm)	A	T	$\alpha(cm^{-1})$	n	$K(cm^{-1}/M)$

PCV33%	NaCl	577	0.006	0.990	0.007	1.020	3.42×10^{-7}
	15%	577	0.405	0.637	0.451	3.304	2.17×10^{-6}
	25%	577	0.389	0.668	0.407	2.354	1.87×10^{-6}
	35%	577	0.277	0.771	0.259	2.120	1.20×10^{-6}
PCV25%	NaCl	577	0.018	0.933	0.063	1.398	3.89×10^{-7}
	15%	577	0.364	0.463	1.336	4.227	6.13×10^{-6}
	25%	577	0.259	0.563	0.834	3.240	2.84×10^{-6}
	35%	577	0.217	0.641	0.716	3.020	2.05×10^{-6}
PCV22%	NaCl	577	0.013	0.965	0.037	1.097	1.55×10^{-7}
	15%	577	0.281	0.477	0.739	4.138	3.76×10^{-6}
	25%	577	0.255	0.502	0.685	3.063	3.36×10^{-6}
	35%	577	0.209	0.583	0.539	2.939	2.87×10^{-6}
PVC19%	NaCl	577	0.021	0.977	0.024	1.176	1.18×10^{-7}
	15%	577	0.268	0.466	0.732	4.0125	3.47×10^{-6}
	25%	577	0.258	0.505	0.685	2.975	3.30×10^{-6}
	35%	577	0.227	0.537	0.628	2.609	3.13×10^{-6}

It was noted from the table (4-3) of the pcv33% sample that the number of linear parameters for this sample is also increased by an increase in hemoglobin, and it is as follows for a concentration of 15%. (0.405,0.637,1.336cm^{-1},3.309,$6.13\times10^{-6}cm^{-1}$/M). It was noted from these values that the amount of permeability is higher than absorption, due to the fact that this disease is a few hemoglobin. Therefore, absorption is few, permeability, and stalking increases, and this has led to an increase in the linear refractive factor by law (2-5).

As for the sample, pcv25 %,22%, it is noted that the linear parameters of these samples and the concentration 15% are (0.364,0.281,0.463,0.477,0.739cm^{-1},0.732cm^{-1},$3.76\times10^{-6}cm^{-1}$/M ,$3.47\times10^{-6}cm^{-1}$/M) respectively.

With regard to the sample pcv19%, its behavior was in the visual properties, as in the previous samples, but few because the concentration of hemoglobin is little and the salt most. This led to the fact that the visual properties are few except

for transmittance, by relying on the absorption of the sample as follows (0.268,0.466,0.407cm^{-1}, 4.012 ,2.17×$10^{-6}cm^{-1}$/M) for 15% for concentration 15% of the same sample.

(4-2-4): Normal blood samples (separation components of blood)

In this section, blood components will be separated in order to know the absorbance of each component, as well as the emission spectrum of plasma and serum. Here are the most important characteristics obtained for normal blood samples.

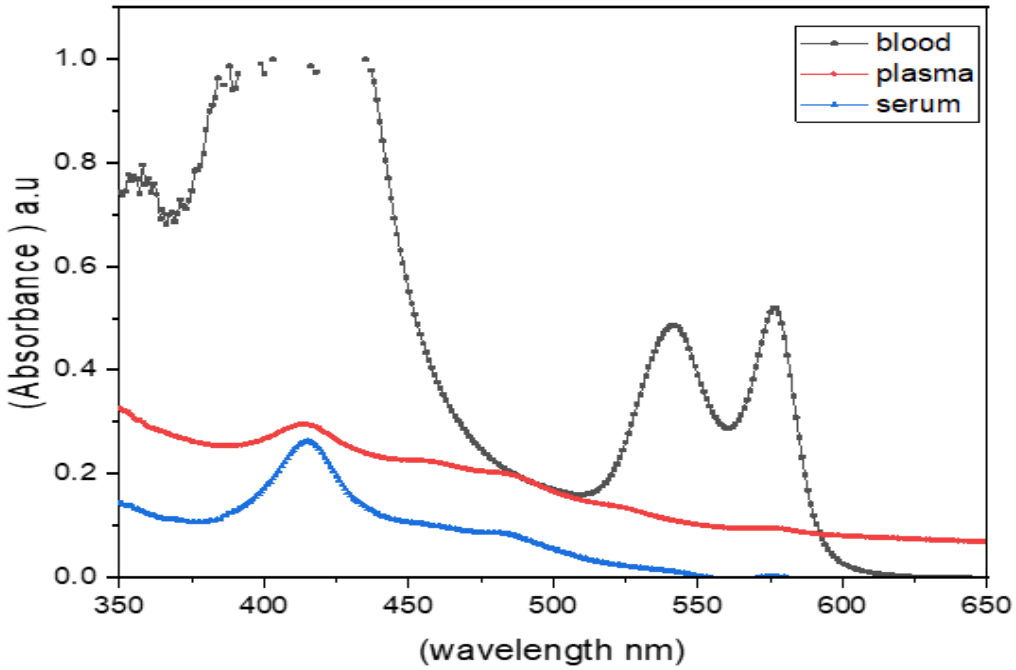


Figure (4- 40) linear optical properties of normal blood sample pcv42% absorbance

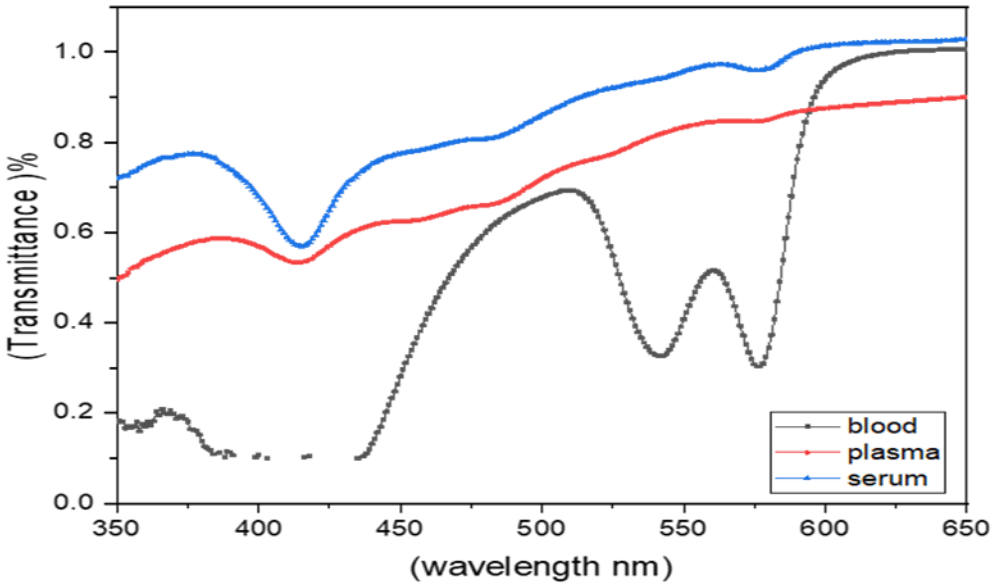


Figure (4- 41)linear optical properties of normal blood sample pcv42%, transmittance

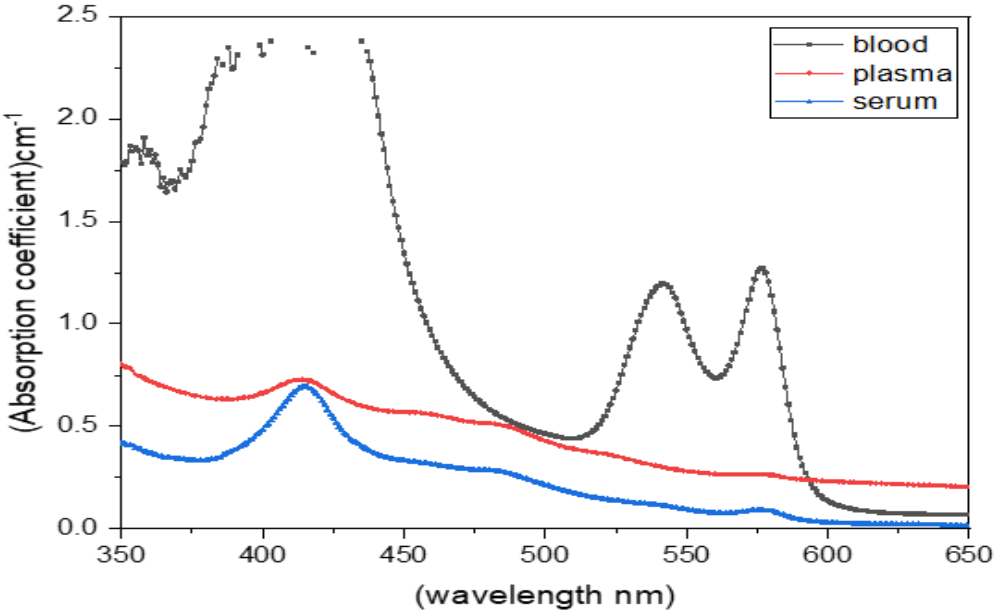


Figure (4- 42) linear optical properties of normal blood sample pcv42% absorption coefficient

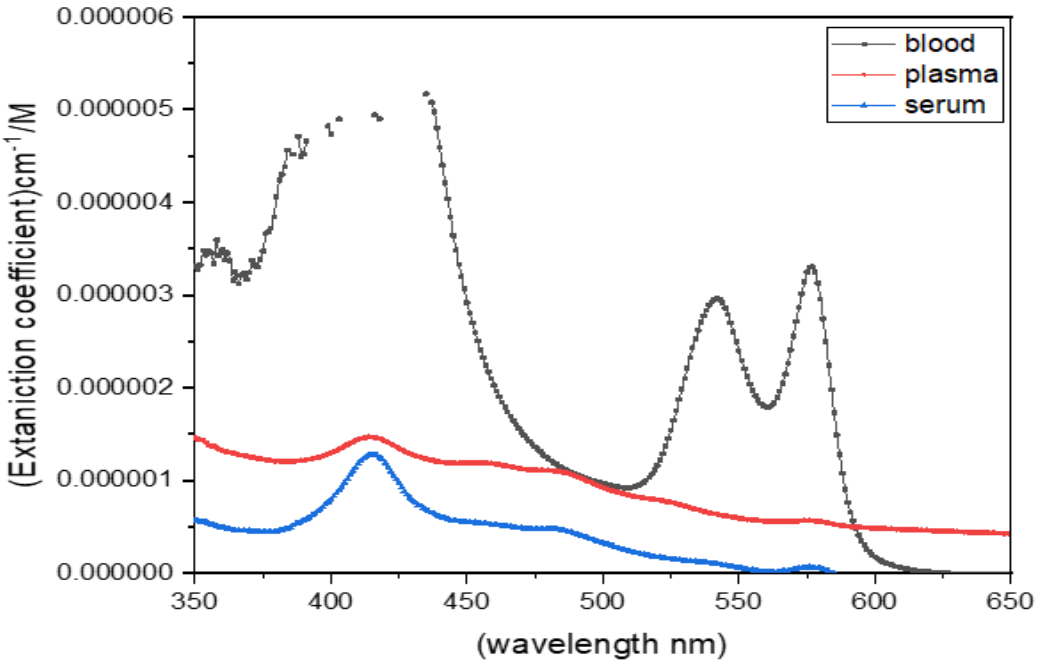


Figure (4- 43)linear optical properties of normal blood sample pcv42% extaniction coefficient

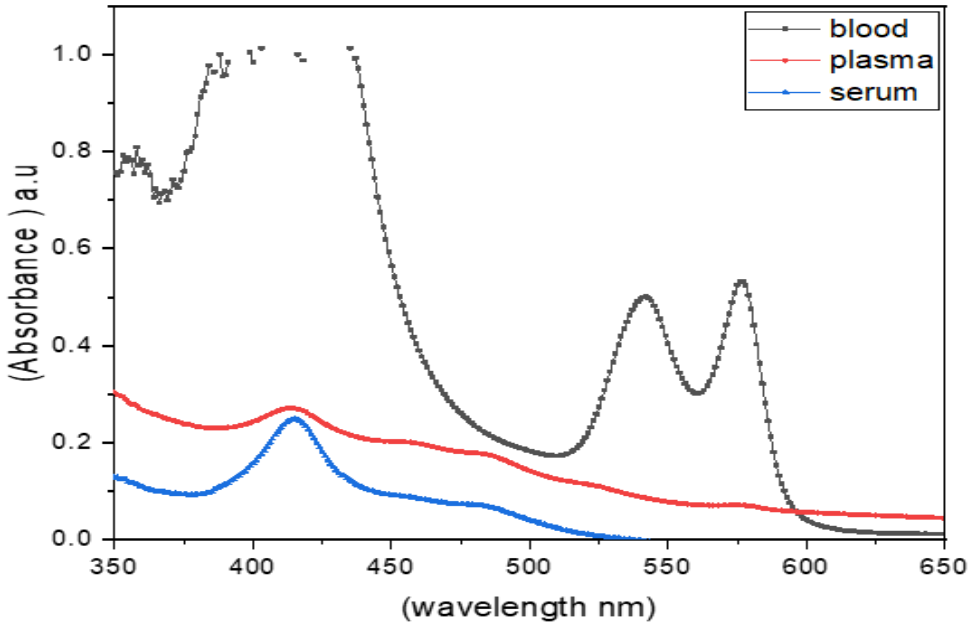


Figure (4- 44)linear optical properties of normal blood sample pcv39% absorbance

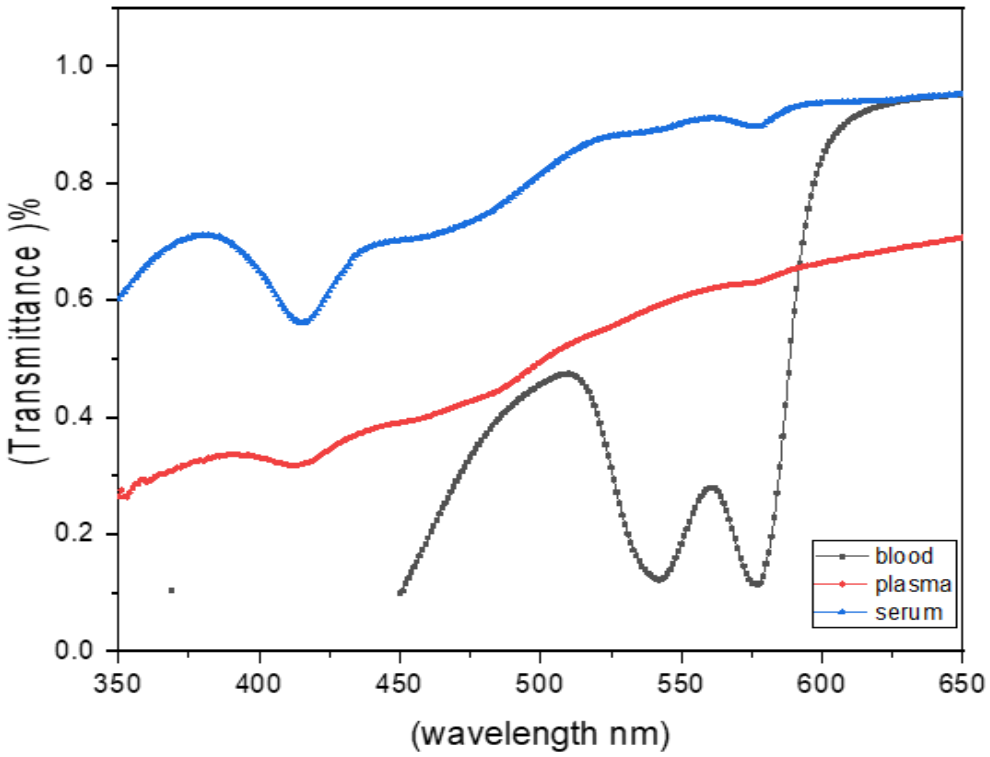


Figure (4- 45)linear optical properties of normal blood sample pcv39% transmittance

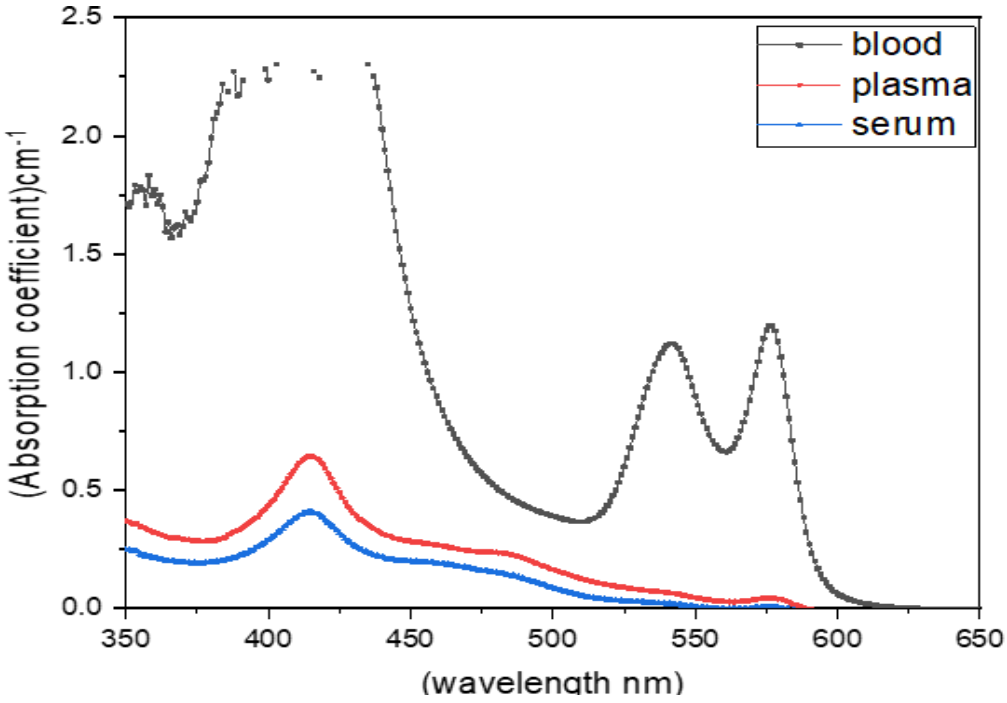


Figure (4- 46)linear optical properties of normal blood sample pcv39% absorption coefficient

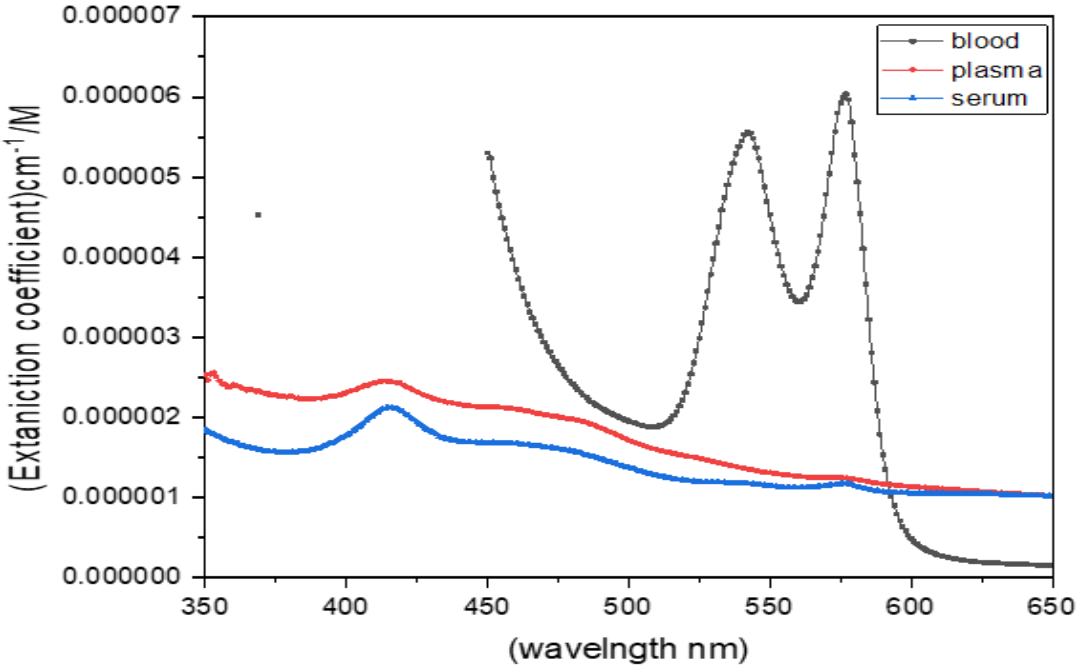
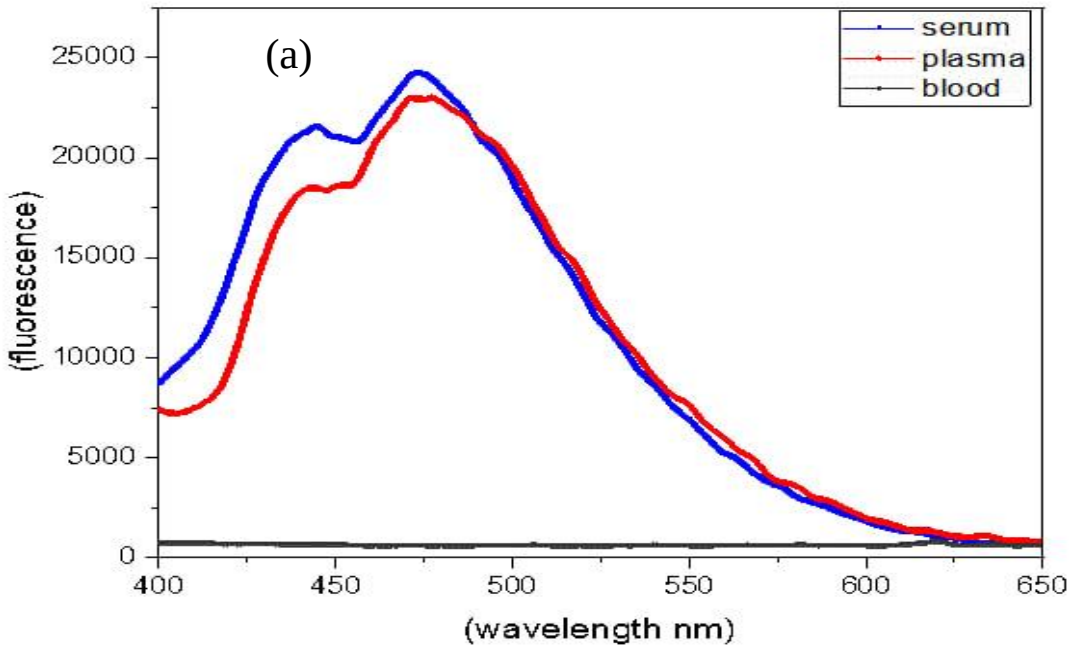


Figure (4- 47)linear optical properties of normal blood sample pcv39% extinction coefficient



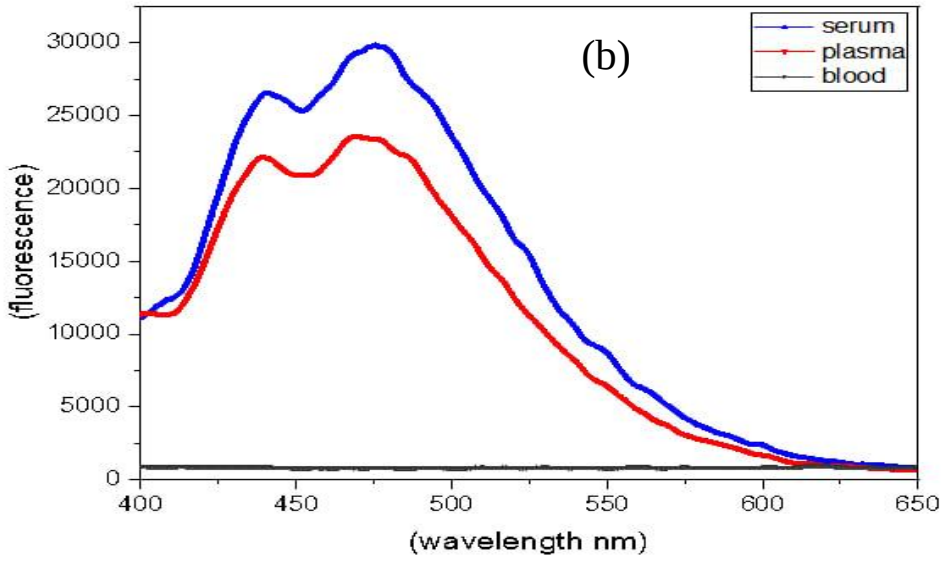


Figure (4- 48-) fluorescence spectrum of normal blood samples in the case of blood, plasma, and serum (a)intensity pcv42 and(b)intensity pcv39.

Table (4-4) the linear optical properties of normal blood samples pcv42%,39%

Normal blood sample	Sample	λ_a (nm)	λ_{emi}(nm)	A	T	α(cm^{-1})	n	K(cm^{-1}/M)
PCV42	blood	577	-	0.590	0.302	1.254	1.978	5.96×10^{-6}
	plasma	414	475	0.323	0.471	0.630	2.662	2.48×10^{-6}
	serum	414	475	0.301	0.607	0.410	1.870	2.15×10^{-6}
PCV39	blood	577	-	0.515	0.296	1.191	1.779	4.26×10^{-7}
	plasma	414	475	0.296	0.476	0.580	2.827	2.32×10^{-6}
	serum	414	475	0.266	0.604	0.542	2.212	1.08×10^{-6}

It is noted from the results obtained for normal blood samples after separating the blood components into plasma and serum, where we note that the absorbance of blood for all samples is in the visible region at the wavelength of 577nm as we previously reached. As for the case of plasma and serum, it was at 414nm

As for the rest of the linear parameters taken in the study (absorbance, transmittance, absorption coefficient, refractive index, extinction coefficient), they are as follows (0.590,0.323,0.301) respectively, absorbance of blood, plasma, and

serum of the pcv sample 42% and so on for the rest of the parameters, while for the sample pcv39% (0.515,0.296,0.266) as shown in table (4-4) for the two samples.

We also note that the value of the refractive index varies from one sample to another, depending on the values of absorbance, transmittance, and reflectivity, and by applying the law (2-5), which depends on the pcv for each sample, as shown in the table (4-4).

The fluorescence was measured for normal blood samples, where we notice that in the case of blood only there was a quenching in the fluorescence spectrum due to the concentration, where the quenching occurs due to the high concentration and this leads to a decrease in intensity, and this is similar to what was reached by Najbar, Jan, and Marek Mac [115]. As for the spectrum Absorbance of plasma and serum for these samples at 414nm, and after taking fluorescence, it shifted towards the red shift for both plasma and serum in 475nm due to a decrease in concentration. The intensity was higher for serum and lower than that for plasma. As shown in the fluorescence diagram (4-49).

(4-2- 5): blood viscosity samples (separation components of blood)

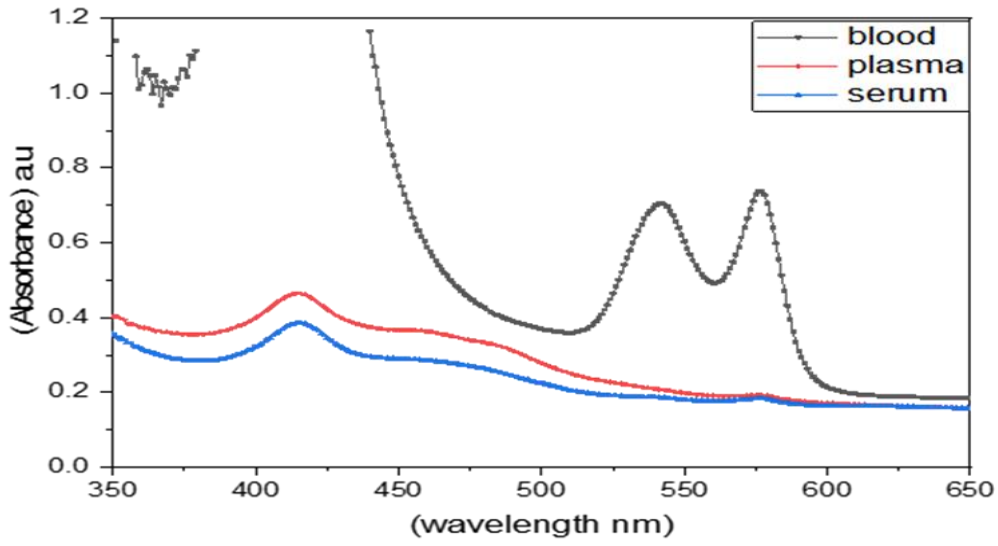


Figure (4- 49)linear optical properties of blood viscosity sample pcv50% absorbance

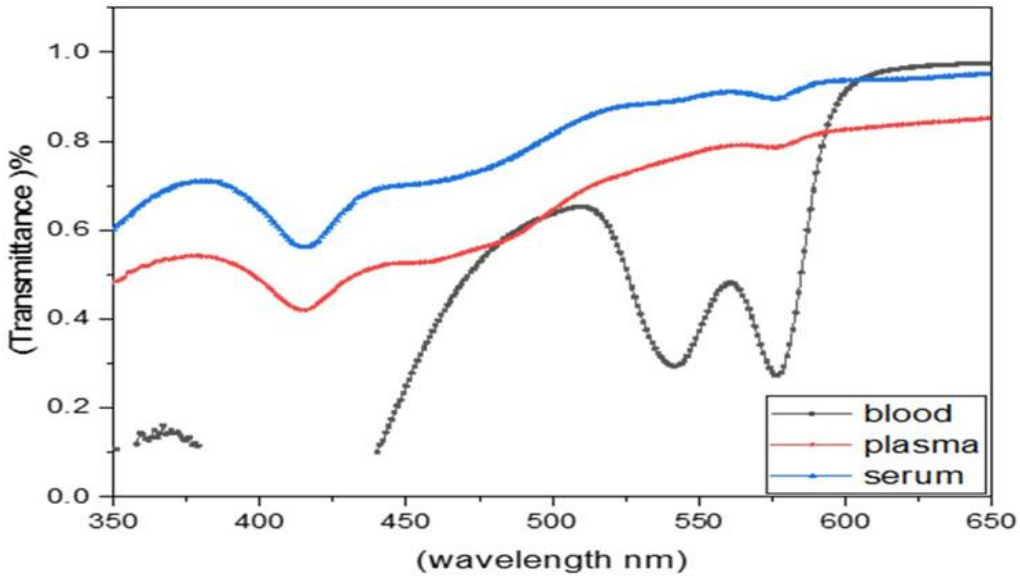


Figure (4- 50)linear optical properties of blood viscosity sample pcv50% transmittance

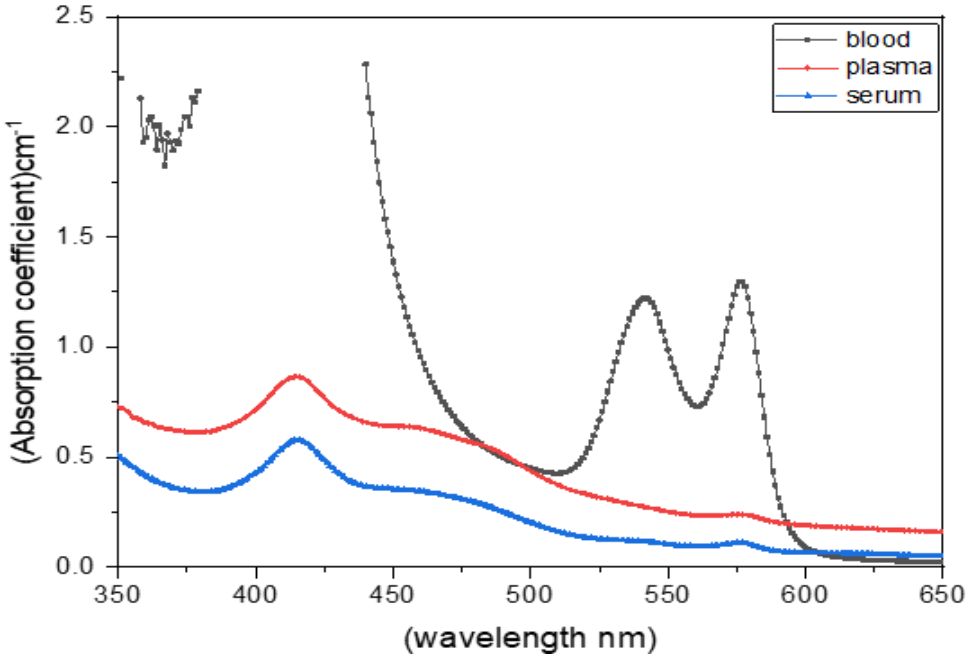


Figure (4- 51) linear optical properties of blood viscosity sample pcv50% absorption coefficient

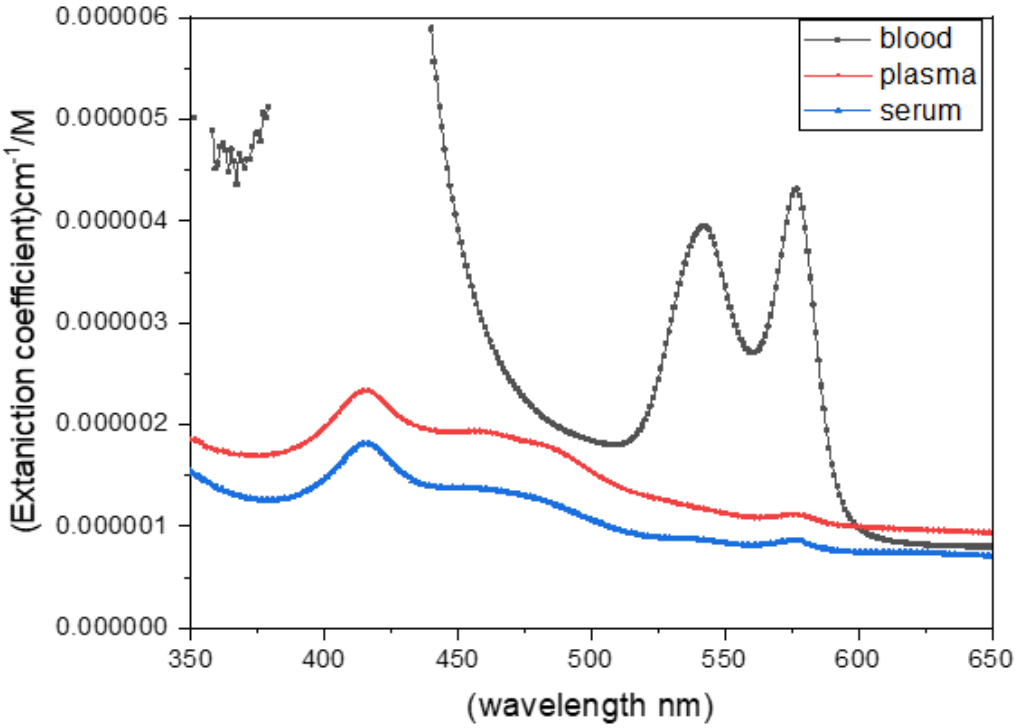


Figure (4- 52)linear optical properties of blood viscosity sample pcv50% extinction coefficient

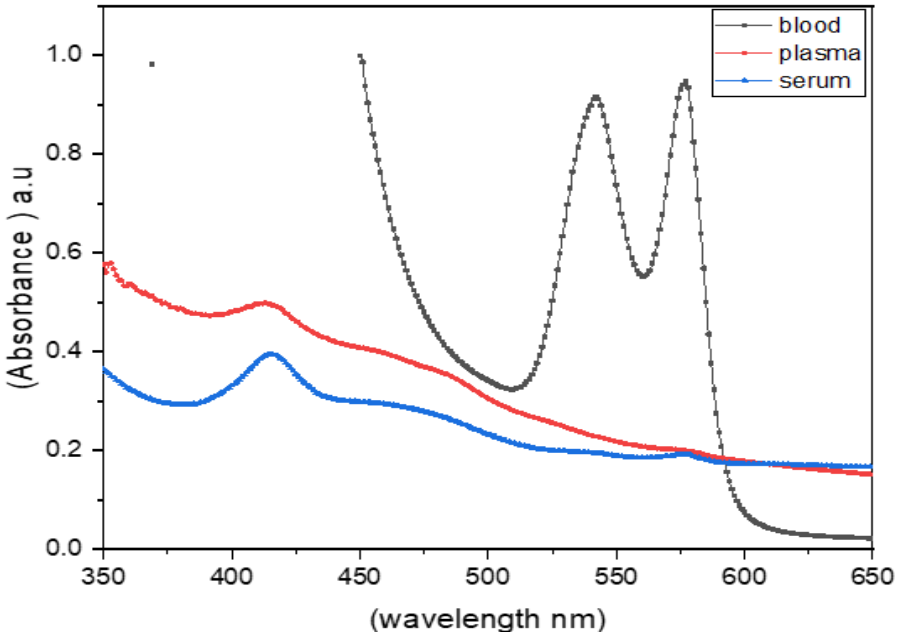


Figure (4- 53)linear optical properties of blood viscosity sample pcv48% absorbance

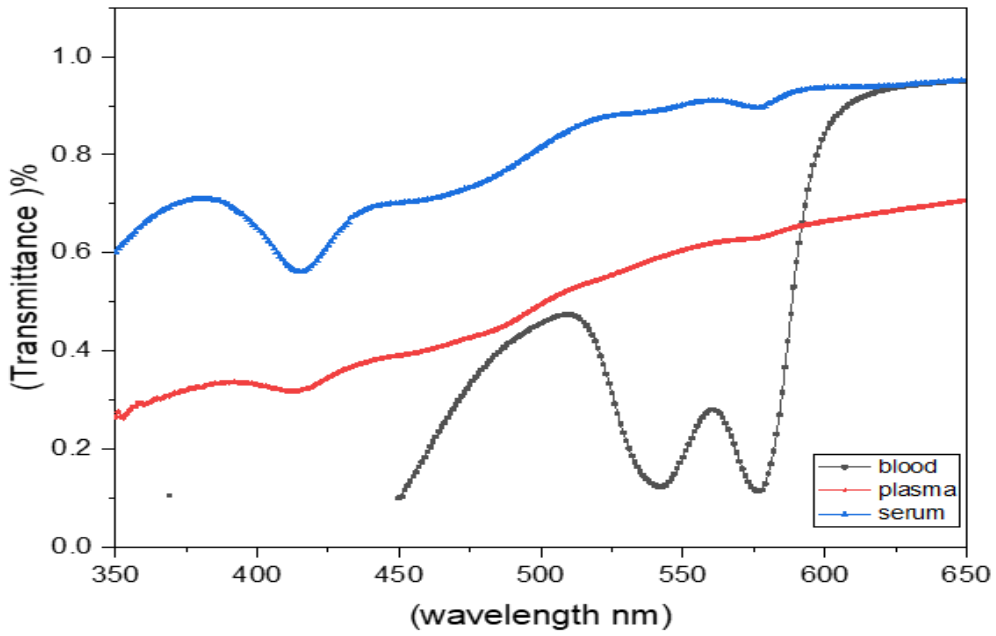


Figure (4- 54)linear optical properties of blood viscosity sample pcv48% transmittance

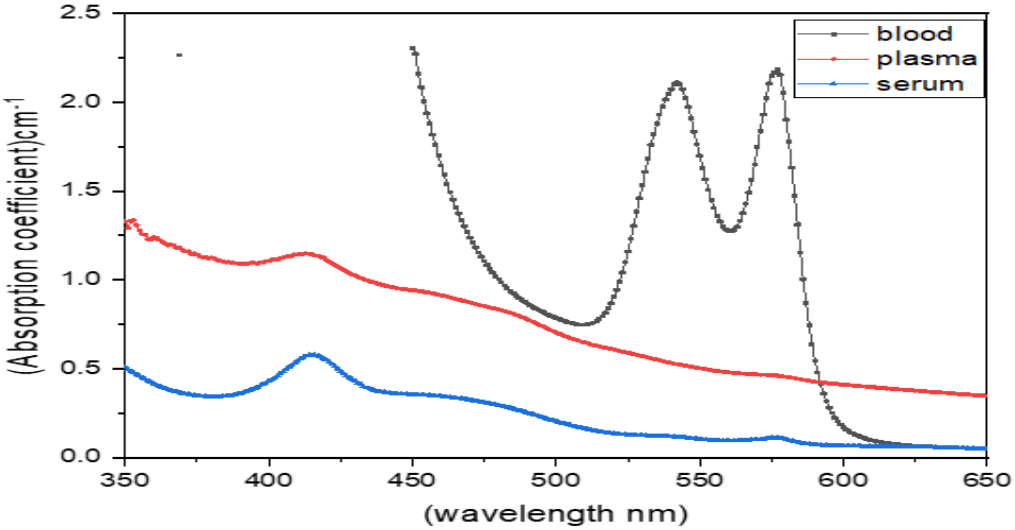


Figure (4- 55)linear optical properties of blood viscosity sample pcv48% absorption coeffcient

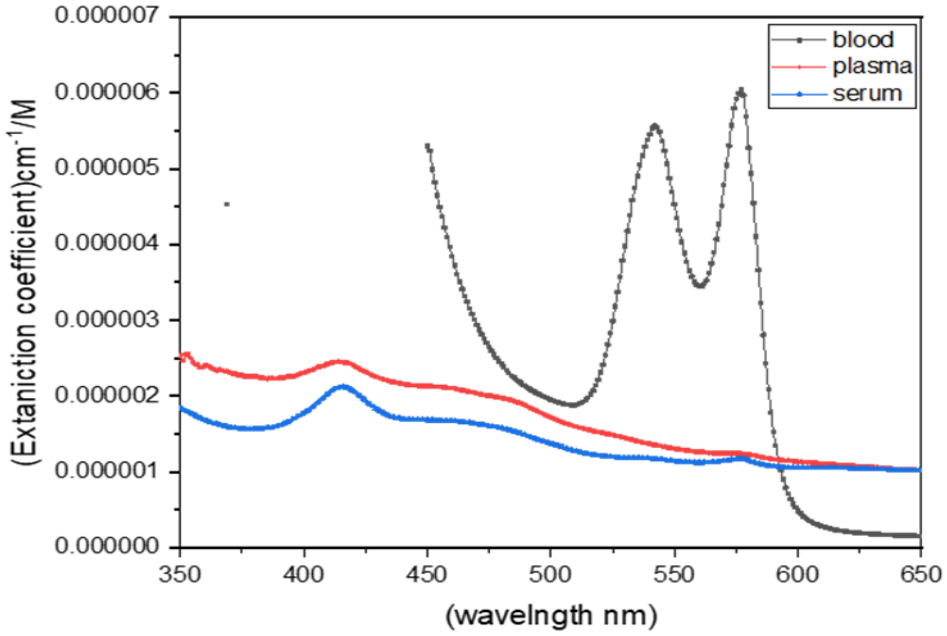
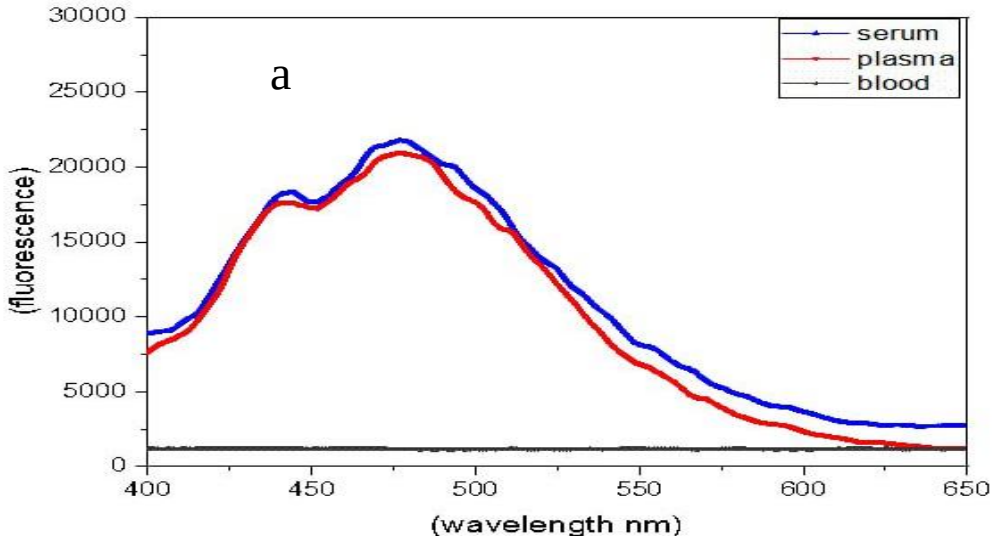


Figure (4- 56)linear optical properties of blood viscosity sample pcv48% extinction coefficient



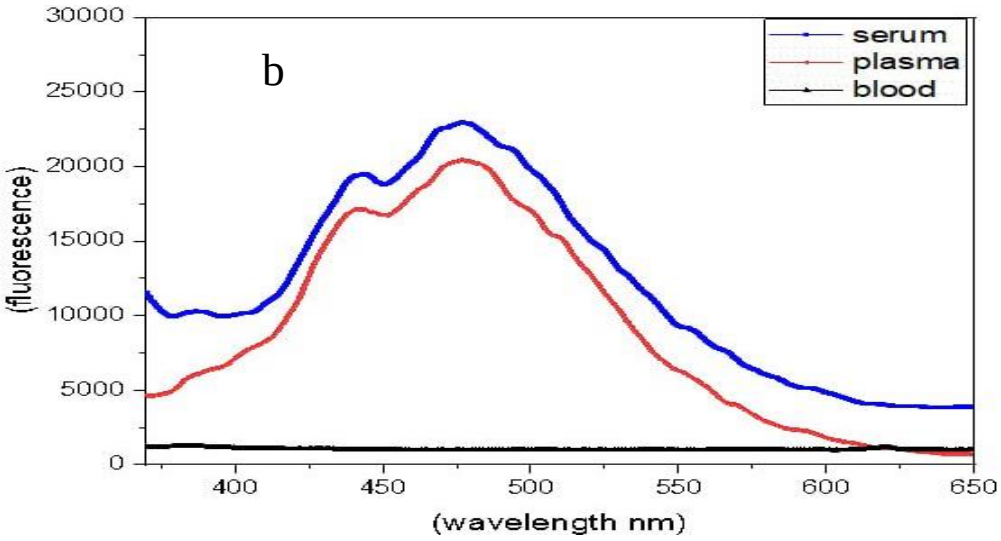


Figure (4- 57) fluorescence spectrum of blood viscosity samples in the case of blood, plasma, and serum, (a)intensity pcv50% and(b)intensity pcv48%.

Table (4-5) the linear optical properties of blood viscosity samples pcv50%.48%.

Blood viscosity sample	Sample	λ_a(nm)	λ_{emi}(nm)	A	T	α(cm^{-1})	n	K(cm^{-1}/M)
PCV50%	blood	577	_	0.948	0.271	2.185	2.530	5.96×10^{-6}
	plasma	414	475	0.498	0.421	1.146	1.795	2.48×10^{-6}
	serum	414	475	0.393	0.559	0.586	1.561	2.15×10^{-6}
PCV48%	blood	577	-	0.747	0.969	1.298	5.321	4.26×10^{-7}
	plasma	414	475	0.471	0.306	0.870	11.121	2.32×10^{-6}
	serum	414	475	0.384	0.341	0.580	3.205	1.84×10^{-6}

In the case of blood viscosity, the concentration of hemoglobin is high, so it affects the linear parameters mentioned in a study as in Table (4-5) where the absorbance of the sample is pcv50% for blood, plasma and serum as follows (0.948,0.498,0.393), where we noticed in the case of blood only that it is higher than that of plasma and serum.

As for the sample pcv 48%, it is less absorbent and the rest of the parameters are less than it is in the case of pcv50%, due to the low concentration of hemoglobin in the sample, and it is as follows (0.747,0.471,0.384).

In the case of fluorescence, too, a quenching in fluorescence spectrum occurred in the case of blood only, but in the case of plasma and serum, we obtained the results as shown in the graph (4-58) of the two samples, and also shifted towards to the red shift 475nm, after the absorption spectrum of these samples was 414nm as shown in the table (4-5).

(4-3) :Nonlinear optical properties (osmotic fragility)

In this section, nonlinear optical properties such as (difference transmittance ΔT_{p-v}, nonlinear phase shift $\Delta\Phi_{\circ}$, nonlinear refractive index n_2, nonlinear absorption coefficientβ, third-order nonlinear optical susceptibility χ^3 were calculated; calculated according to the normalized transmittance data that was Obtained from a z-scan setup with a close aperture.

From the lower numbers as pcv blood concentrations increase (45%, 42%, 60%, 50%, 33% and 19%), the transmittance ΔT_{p-v} increases peak and valley decreases, as shown in Table (4-6) and other tables in the case of the concentration 15% T_{p-v}. the other concentrations 25% and 35% for the same pcv mentioned in the study take the same direction.

(4-3-1): Normal blood sample

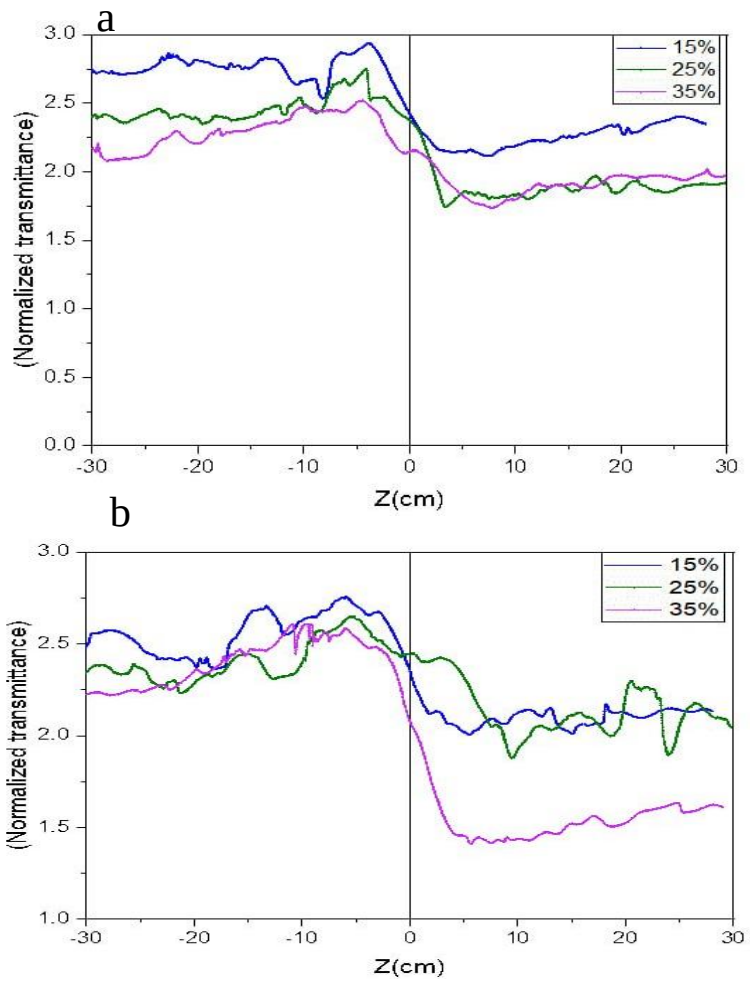


Figure (4- 58)nonlinear refraction of normal blood samples using a wavelength of 532nm(a) pcv45%and(b)pcv42%.

Table (4-6) shows the measured nonlinearities of normal blood samples pcv45%,42%

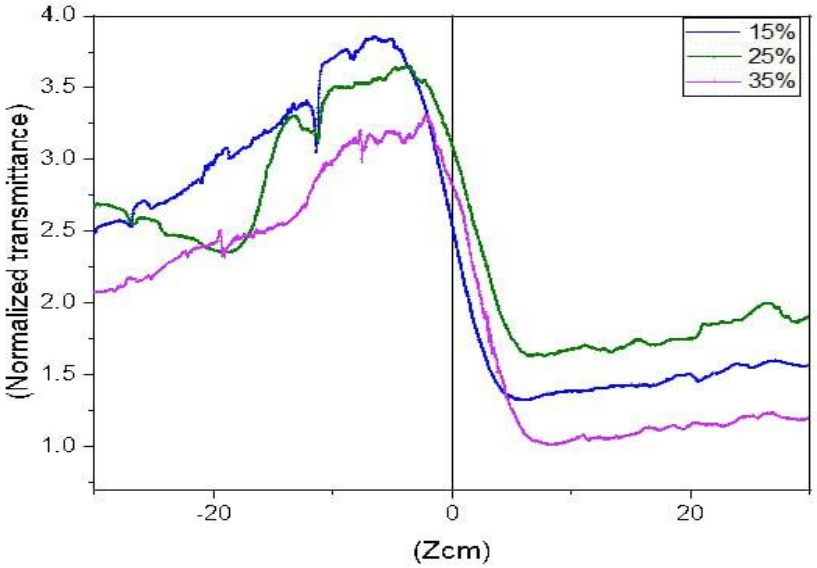
Normal blood sample	Concentration	ΔTp−v	$\|\Delta\Phi_o\|$	$n_2 \times 10^{-9}$ (cm^2/W)	β (m/W)	Re(χ3) ×10−7 (esu)(cm^2/W)	I_m(χ3) ×10−4 (esu)(cm/W)
Pcv45	15%	1.005	2.487	2.377	28.019	2.546	5.744
	25%	0.832	2.059	2.354	24.975	2.488	3.628
	35%	0.76	1.220	2.229	21.867	2.236	3.223
Pcv42%	15%	1.814	4.489	1.894	18.550	1.611	5.330
	25%	1.794	4.452	1.855	16.985	1.552	4.766
	35%	1.708	4.227	1.413	12.374	0.896	4.159

1. It was noted from table (4-6) the values of the nonlinear refractive index n_2, its amount increases with the increase of pcv. In the case of the pcv 45% and at a concentration 15%, the value of n_2 is equal to($2.377cm^2/W$), while pcv 42% the value of n_2 equal ($1.894cm^2/W$), with the same concentration, at a concentration 25% and 35%, the nonlinear refractive index n_2 values of pcv 45%, 42% were ($2.354,2.229,1.855,1.413cm^2/W$) respectively.

2. For nonlinear absorption coefficient β also are increased due to increase of the pcv and vice versa, as can be seen from the results obtained from normal blood (pcv 45% and 42%) with concentration 15% Where the value of β is (28.019m/W). for pcv 45% and for the case of pcv 42% it is equal to (18.550m/W). Noted that the intensity used in the measurement of normal blood samples was (11.6 mW/cm^2).

3. third-order nonlinear optical susceptibility χ^3 where it was measured for normal blood, and the results in the case of pcv45% were (5.744(esu)cm/W) and a concentration of 15%, while pcv45% was the amount (5.330(esu)cm/W). And when using the concentration of 25% and 35% for the same, the results were obtained (4.766,4.159,3.628,3.223, (esu)cm/W).

(4-3-2): blood viscosity samples



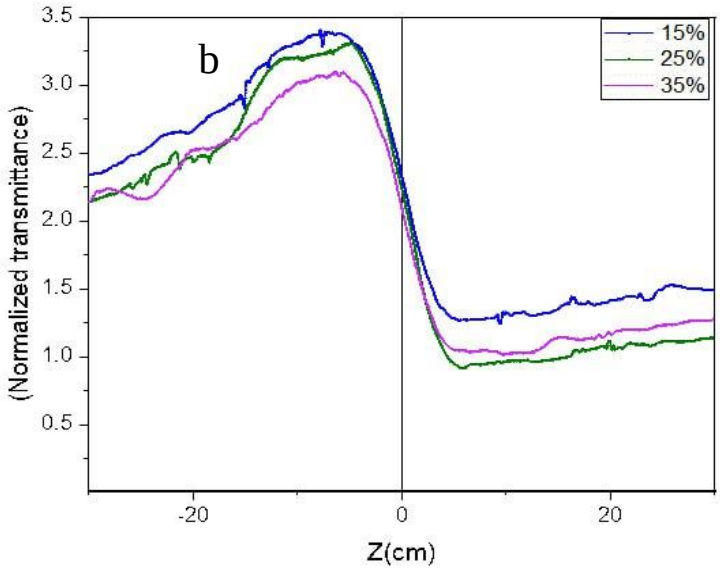


Figure (4- 59)nonlinear refraction of blood viscosity samples using a wavelength of 532nm, (a)pcv60% and(b) pcv50%.

Table (4-7) shows the measured nonlinearities of viscosity blood samples pcv60%,50%

Blood viscosity sample	Concentration of NaCl	ΔTp−v	$\|\Delta\Phi_o\|$	$n_2 \times 10^{-9}$ (cm^2/W)	β (m/W)	Re(χ3) ×10−7 (esu)(cm^2/W)	Im(χ3) ×10−4 (esu)(cm/W)
Pcv60	15%	10.717	6.293	2.864	24.232	1.632	4.624
	25%	2.278	5.637	1.906	16.952	1.552	3.053
	35%	2.012	2.059	1.576	14.978	0.896	2.856
Pcv50	15%	8.352	5.798	1.677	17.322	2.546	3.243
	25%	2.343	5.271	1.846	16.057	2.488	3.223
	35%	2.05	5.073	1.200	14.978	2.236	1.264

1-We noted from the graph (4-60) for blood viscosity samples pcv(60%,50%) that the amount of normalized transmittance difference between the top and valley transmittance values It increased when the pcv increased, that is, with an increase in the concentration of hemoglobin in the blood, and the results for it in a sample

were 60% pcv, and for the concentrations shown in the table (4-7)as follows (10.717,2.278,2.012). As for the sample pcv50% and for all concentrations, it was (8.352,2.343,2.050).

2- We note that there is a discrepancy in the values of the nonlinear refractive index, as it increases, but by a little less than it is in the case of normal blood samples due to the use of high optical intensity, which was (27.308mW/cm^2), which led to a decrease in the nonlinear refractive index due to the saturation of the linearity. The values of the nonlinear refractive index were for pcv(60%.50%) samples and for the concentrations mentioned in the table are as follows (2.864,1.906,1.576,1.677,1.846,1.200cm^2/W) respectively.

3- For the nonlinear absorption coefficient also increases due to the increase of pcv as evidenced by the results obtained from highly viscous blood samples (pcv 60% and 50%) at a concentration of (15%,25%,35) where the value of β is equal to (24.232,16.952,14.978m/W) for pcv 60% and for the case of pcv 50% equals (17.322,16.057,14.978m/W).

4- for third-order nonlinear optical susceptibility χ^3 measured for viscous blood samples using the two laws (2-17,18), and the results were in the case of 60% (4.624 (esu) cm/W) for concentration 15%, while pcv50% was the amount (3.243 (esu) cm/W). And when using a concentration of 25% and 35% for the same, the results (3.053,2.856,3.223,1.264 (esu) cm / W) were obtained. It is also noted through the table for blood viscosity samples that the amount of third-order nonlinear optical susceptibility was low. The reason is due to the use of high intensity because of the high hemoglobin concentration, which led to a state of saturation in the nonlinear optical properties, which led to a decrease in the nonlinear absorption coefficient. This is similar to what was reached before by Dorranian, Davoud, and Yasaman Golian [116].

(4-3-3): Thalassemia blood samples

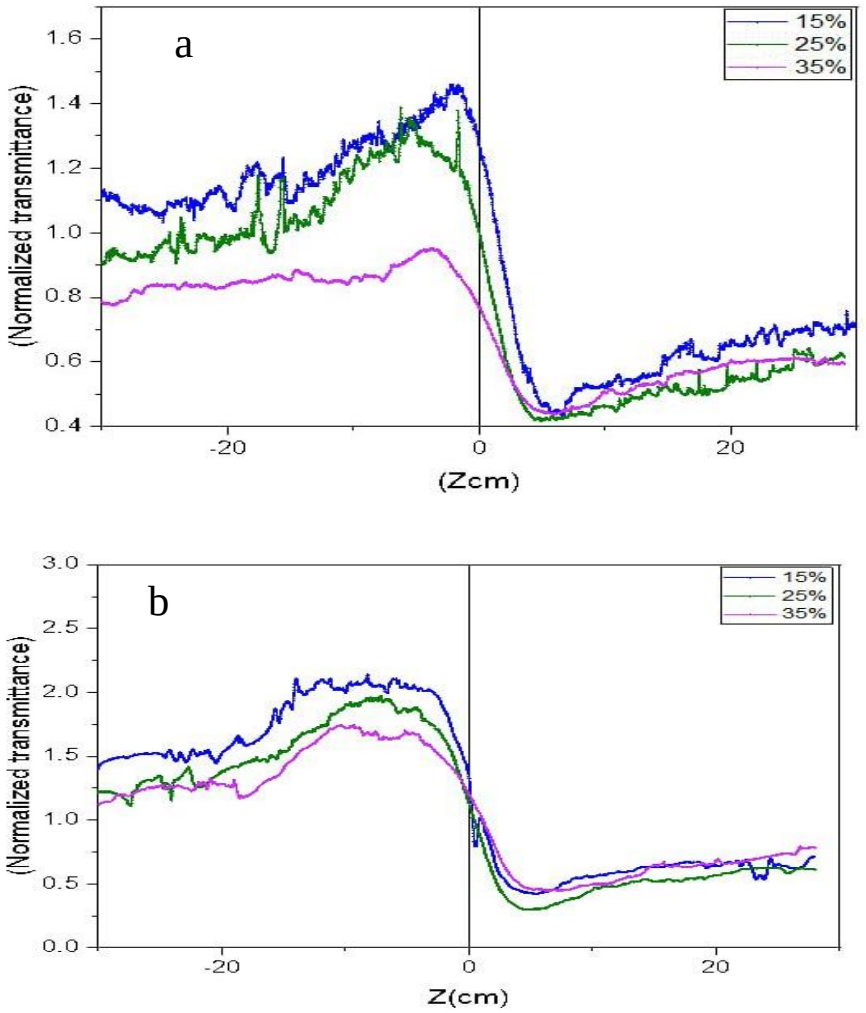


Figure (4- 60)nonlinear refraction of Thalassemia blood samples using a wavelength of 532nm, (a)pcv33% and(b) pcv19%.

Table (4-8) shows the measured nonlinearities of Thalassemia blood samples pcv33%,19%

Thalassemia blood samples	Concentration of NaCl	ΔTp−v	$\|\Delta\Phi_\circ\|$	$n_2 \times 10^{-9}$ (cm^2/W)	β (m/W)	Re(χ3) ×10−7 (esu)(cm^2/W)	Im(χ3) ×10−4 (esu)(cm/W)
Pcv33%	15%	1.195	2.943	2.160	23.017	2.378	4.594
	25%	0.766	2.326	1.395	22.742	2.003	4.280
	35%	0.758	1.269	1.392	22.109	1.961	3.370

Pcv19%	15%	1.009	2.905	0.237	21.901	2.236	5.126
	25%	0.94	1.868	0.235	21.614	2.194	4.388
	35%	0.513	1.820	0.229	12.592	2.166	4.220

1-The results in table (4-8) are the results of thalassemia samples, as people with this disease suffer from a decrease in blood concentration, which means that the volume of blood cells is small as we mentioned previously, and the nonlinear transmittance values of the sample are pcv33% for the concentrations taken in a study are as follows (1.195,0.766,0.758) for sample pcv19% was (1.009, 0.94 ,0.513).

2-For the non-linear refractive index calculated mathematically from the equation (2-19) the results were obtained for the thalassemia pcv33%,19% samples, where the intensity used in these samples was (16.450 mW/cm^2) and for all concentrations the results were obtained as shown in the table(4-8) (2.160, 1.395, 1.392 cm^2/W)for the sample pcv 33%, while for the sample pcv 19% the results were as follows(0.237,0.35,0.229 cm^2/W) and we noted The increase in the refractive index increases the concentration of hemoglobin depending on the decrease in the amount of salt in the sample.

3- The nonlinear absorption coefficient for thalassemia samples was higher than it is in the case of normal blood due to the increase in the optical intensity used, where the pcv was 33% (23.017,22.742,22.109m/W) and for all the concentrations mentioned in the table, respectively, but for the pcv19(21.901,21.614,20.592m/W).

4- for third-order nonlinear optical susceptibility χ^3 for thalassemia samples was pcv33% (5.126,4.388,4.220, (esu) cm/W) and for pcv19%(4.594,4.280,3.370(esu) cm/W). These values were reached by relying on the values of the refractive index and the nonlinear absorption coefficient, in addition to the wavelength used being 532nm.

Chapter Five
Conclusion and Future Studies

(5-1): Conclusions

From the results, we can conclude the following: -

1. The use of linear and nonlinear optical properties is an important tool for diagnosing some blood diseases, and this opens the door for diagnosis using spectroscopic methods away from the traditional methods used at the present time.

2. It is noted in the study of linear properties that the absorbance coefficient, the linear absorption coefficient and the molar inactivation coefficient increase with the increase in blood concentration.

3-The absorption spectra were measured using a UV spectrophotometer (Perkin Elmer-Lambda35), and peaks appeared at wavelength 542 and 577, and this means that there is good absorption of light by blood in these two regions and thus the importance of using UV in the study of blood. The reason for the presence of two peaks in absorption may be due to one of them due to the presence of hemoglobin and the other to the presence of other components in the blood such as water, serum and plasma.

4-The results of the emission spectrum show and here a fluorescence device was used to measure it, we noticed the emission spectrum shifts towards the red shift compared to the absorption spectrum of the same sample.

5-Nonlinear optics involves the study of the interaction and propagation of light of high intensity through matter. This leads to an effect on the refractive index and the absorption factor, as nonlinear properties appear on them.

6- The nonlinear permittivity of the third order, we note that the imaginary part increases with increasing concentration of the substance and the sample itself.

(5-2): Future studies

1. Study of linear and nonlinear optical properties to diagnose other blood diseases such as sickle cell anemia and red blood cell breakage.

2. Exposing blood samples to the laser beam directly and studying its absorption and emission spectrum and using it as a tool for diagnostic purposes.

3. Dependence on the number of nucleic acids after their isolation and the diagnosis of some diseases through linear and nonlinear spectroscopic studies.

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الخلاصة:-

تبحث هذه الدراسة إمكانية استخدام الخصائص الخطية واللاخطية لتشخيص بعض امراض الدم ودراسة السبل التي يمكن من خلالها الوصول الى هذا الهدف. وأجريت هذه الدراسة في مختبرات كلية العلوم للبنات وبالتعاون مع مصرف الدم المركزي في محافظة بابل ومركز الثلاسيميا في مستشفى الاطفال للفترة من كانون الأول (2021) الى تموز (2022) .

النتائج التي تم الحصول عليه للمعلمات الفيزيائية (الامتصاصية، النفاذية، معامل الامتصاص الخطي،معامل الانكسار الخطي,معامل الخمود المولاري) للدم الطبيعي (45%,42%,40%) Pcv وللتراكيز 15%, NaCl,25%, 35% حيث كانت النتائج للتركيز% 15 اعلى قيمة للامتصاصية هي(0.688) في حالة العينة pcv45% واقل قيمة كانت للعينة الـ pcv40% هي 0.428، وهذا ينسحب على العينة pcv42% .اما بالنسبة للدم ذات اللزوجة العالية فكانت اعلى قيمة للامتصاصية في حالة التركيز 15%هي 0.828 عند الـ pcv=60 واقل قيمة هي 0.758 عند العينة pcv= 50 .مرض الثلاسيميا من الامراض الشائعة في العراق وهو ناتج من صغر وتشوه كريات الدم الحمراء وتم اخذ عينات تكون لها الـ pcv= 33%, 25%, 22%,19% وكانت اعلى قيمة للامتصاصية في حالة التركيز 15%هي 0.405 عند الـ pcv=33% واقل قيمة هي 0.268عند العينة pcv=19%.

تم فصل مكونات الدم الى بلازما ومصل وذلك بأستخدام الماء فقط لضمان التحلل الكامل للخلية والتخفيف به.حيث لوحظ ان اعلى امتصاصية كانت لعينات لزوجة الدم عند pcv 50% (للهيموغلوبين,البلازما والمصل)كانت (0.948,0.498,0.393)على التوالي.وهذا ينطبق على باقي المعلمات ,وتبدأ قيم هذه المكونات بنقصان كلما قل الpcv على العكس من ذلك يلاحظ ان طيف الانبعاث في حالة التراكيز المخففة يكون اعلى شدة والازحة نحو المنطقة الحمراء للبلازما والمصل لجميع العينات وعند الطول الموجي 475nm,علما ان الطول الموجي للامتصاص كان 414nm.اوضحت الدراسة الخواص البصرية اللاخطية لعينات (الدم الطبيعي,لزوجة الدم,الثلاسيميا)حيث تم حساب معامل الانكسار اللاخطي ومعامل الامتصاص اللاخطي بألاعتماد على قيمة النفاذية اللاخطية ومن ثم حساب القابلية اللاخطية من المرتبة الثالثة حيث لوحظ ان لكل حالة خواص خاصة بها فعينات الدم الطبيعي كانت حالة وسطية بين اللزوجة والثلاسيميا ,فأن معامل الانكسار اللاخطي,معامل الامتصاص اللاخطي والقابلية البصرية اللاخطية من المرتبة الثالثة للpcv45%(1.894 cm^2/W, 18.550m/W, 5.330 cm/W)ولنفس التركيز% 15 على التوالي.اما هذه المعلمات في حالة لزوجة الدم وللpcv60% كانت (2.377×10^{-9} cm^2/W, 28.019 m/W , 5.744 cm/W)ولنفس التركيز ,بألنسبة لمرضى الثلاسيميا فقد اعطى نتائج مختلفة حيث كانت ولنفس المعلمات المذكورة للpcv33%(2.160 cm^2/W, 23.017 m/W, 4.594 cm/W).

جمـــــهوريـــــــــة العـــــــراق

وزارة التعليم العالي والبحث العلمي

جـــــــامعـــــة بـــــابـــــــــل

كليـــــــــة علوم البنات

قسم فيزياء الليزر

الخصائص الخطية واللاخطية البصرية لتشخيص الدم لمرضى بيتا- ثلاسيميا

رسالة

مقدمة الى قسم فيزياء الليزر في كلية علوم البنات /جامعة بابل وهي جزء من متطلبات نيل درجة الماجستير في علوم فيزياء الليزر

من قبل

غفران هادي كاظم

بأشراف

أ.م.د. صادق حسن لقتة

أ.م.د. داخل غني عمران

2023 م

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