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Effect of Different Low-Level Laser and Nanoparticles on Spectral Properties of Blood Proteins

A Thesis

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By

Noora Ali Obaid Albakri

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Supervised By

Prof. Dr. Wajehaa abd AL-Daim *Asst.Prof. Dr. Dakhel Ghani Omran*

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وَيَرَى الَّذِينَ أُوتُوا الْعِلْمَ الَّذِي أُنْزِلَ إِلَيْكَ مِنْ رَبِّكَ هُوَ الْحَقَّ وَيَهْدِي إِلَىٰ صِرَاطِ الْعَزِيزِ الْحَمِيدِ ﴿ 6 ﴾

صدق الله العلي العظيم

سورة سبأ الاية (6)

Certification

We certify that this thesis was prepared under our supervision at the Department of Laser Physics, College of Science for women, of Babylon University, as partial fulfillment of the requirements for the degree of Doctor of Philosophy of Science in Physics (laser and its applications).

Signature:	Signature:
Asst-Professor	Professor
Dr. Wajehaa abd AL-Daim	Dr. Dakhel Ghani Omran
Supervisor	Supervisor
Date: / /2022	Date: / /2022

In view of the available recommendation, we forward this thesis for debate by the examining committee.

Signature:

Assistant Professor

Dr. Jenan Ali Abd

Head of

Department of laser physics, College of Science for women, University Babylon

Date: / /2021

Noora

Dedication

To my dear father. …Who left for the homeland of the long sleepers…

To my affectionate mother..... I can't find words that can give her right, she is the epitome of love and delight for a lifetime, as well as a model of devotion and giving..

TO my wealth in this world and with their support, I reached here

(To my beloved husband)…….

To my wonderful brothers and sisters ……..

To my beautiful children....

To… All My Teachers …with Deepest Appreciation

Noora

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وزاة التعليم العالي و البحث العلمي

جامعة بابل

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تاثير الليزر منخفض المستوى المختلف والجسيمات النانويه على الخصائص الطيفية لبروتينات الدم.

اطروحة مقدمة الى

مجلس كلية العلوم للبنات / جامعة بابل و هي جزء من متطلبات درجة دكتوراه فلسفة في علوم فيزياء الليزر

من قبل

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بأشراف

أ.د. وجيهة عبد الدايم **أ.م.د داخل غازي عمران**

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الخلاصة:

تم استخدام شدة الليزر منخفضة المستوى (LLLT) للحث وتشخيص العديد من الأنشطة البيولوجية داخل الأنسجة والدم. تم تصميم دراسة المشروع لشرح آثار LLLT على خصائص الامتصاص والفلورة لمكونات الدم المختلفة بما في ذلك (البلازما ، المصل ، المصل بدون بروتينات ، وبروتينات المصل المترسبة. تم استخدام LLLT(473 نانومتر ، 532 نانومتر ، 650 نانومتر) لتشعيع الدم لمدة 10 دقائق بقوة (20 ميجا واط ، 28 ميجا واط ، 40 ميجا واط) أظهرت نتائج المكونات غير المشعة وجود ذروة عالية (امتصاص) للبروتين المترسب المتدفق بواسطة المصل والبلازما والمصل بدون بروتينات وأظهرت عينات البلازما المشعة زيادة في الامتصاص باستخدام الليزر الأزرق مقارنة بالمكونات الأخرى ، حيث أشارت عينات المصل المشعة إلى امتصاص عالي لليزر الأحمر مقارنة بالمكونات الأخرى ، حيث سجل بروتين المصل المُسجل زيادة في الكثافة الضوئية باستخدام الليزرات الخضراء مع أقل من السيطرة. أشارت عينات المصل بدون بروتينات إلى زيادة معنوية في الامتصاص في الليزر الأخضر والأزرق مقارنة بالسيطرة وأشعة الليزر الأخرى فيما يتعلق بخصائص الفلوريسنت للدم المكونات ، أظهرت عينات البلازما ذروة أعلى باستخدام الليزر الأحمر مقارنة بالعينات المشعة بالليزر الأخرى. من ناحية أخرى ، أكدت عينات المصل المشعة ذروة ملحوظة باستخدام الليزر الأخضر مقارنة بالعينات الأخرى. ومع ذلك ، كان بروتين المصل المشع المترسب ذروة مضان أقل بكثير عند مطابقته مع عينات التحكم. كما أظهرت عينات مصل الدم بدون بروتينات وتم تشعيعها بأطوال موجات ليزر مختلفة انخفاض ذروة التألق في المطابقة مع المجموعة الضابطة (عينات غير مشعة). يمكن أن تُعزى النتائج إلى كثافات مختلفة لمكونات الدم لأن عزل البروتينات (التخثر وبروتينات المصل) ، وكذلك التعديل الكيميائي الحيوي الضوئي أو التغييرات التي يسببها إشعاع الليزر ، يمكن أن يؤثر على قمم الامتصاص والفلورة لتلك المكونات ، نتائج عينة اليرقان المشع باستخدام الليزر المذكور أيضًا تؤدي إلى تقلبات في ذروة امتصاص اليرقان المشع الذي تم أخذ عينات منه منذ أن أشار الليزر الأخضر إلى ذروة عالية مقارنة بتلك ، ليزر آخر متبوعًا باللون الأحمر والأزرق ، هذه النتائج يمكن ينتج من تفاعلات مختلفة بين جزيئات البيليروبين والجزيئات العضوية الأخرى التي قد تتأثر بطاقة الليزر لتشكيل تفاعلات متعددة فيما بينها ، وقد تنتج هذه التفاعلات بسبب الأضرار التأكسدية الناتجة عن الجذور الحرة على تلك البروتينات. يتفاعل البيليروبين مع الضوء الساقط والضوء المنبعث ، لذلك يمكن لهذه الجزيئات أن تفضل الضوء الساقط 520-570 نانومتر على ضوء الليزر الآخر.

النتائج التي تم الحصول عليها من تشعيع عينات مرضى التهاب المفاصل (بلازما ، مصل ، بروتينات مترسبة ، بروتينات خالية من المصل) ، كما اختلفت أطياف الامتصاص بين العينات ، حيث أشارت عينات البلازما إلى ذروة انبعاث أعلى مع الاشعة الخضراء العينات المشعة ، بينما تعطي عينات المصل ذروة امتصاص في ليزر اللون الأحمر ، سجلت البروتينات المترسبة والبروتين الخالي من المصل انخفاضًا معنويًا أقل في أنشطة الامتصاص. صُمم البحث الحالي لشرح تأثيرات التركيزات المختلفة من CuNPs (10 ميكرومتر ، 50 ميكرومتر ، 100 ميكرومتر) على الامتصاص والأطياف الفلورية لمكونات الدم للصحة والتهاب المفاصل

واليرقان (في المختبر). تؤكد النتائج التي تم توثيقها من هذا العمل أن NPs تؤثر على أطياف امتصاص مكونات الدم بطرق مختلفة وتعتمد على نوع المكون لذلك سجلت البلازما والمصل زيادة كبيرة في أطياف امتصاص الدم السليم. في حين أن عينات مرضى التهاب المفاصل الذين عولجوا بـ NPs كان لديهم أطياف امتصاص أقل ، كما أن تركيز CuNPs العالي (100 ميكرومتر) يكشف عن مستويات ذروة عالية. عينة من مرضى اليرقان (البيليروبين) ، أوضحت النتائج انخفاض مستويات الامتصاص في معظم العينات المختبرة. فيما يتعلق بأطياف الانبعاث ، فقد لوحظ زيادة ذروة الانبعاثات في معظم العينات المعالجة لعينات الدم السليمة. وكذلك عينات مرضى التهاب المفاصل الذين عولجوا بـ NPs أشارت إلى انبعاث عالي المستوى في البلازما مقارنة بالتحكم ، من ناحية أخرى ، أظهرت نتائج العينات الأخرى أطياف انبعاث منخفضة في شدة ومهيجة في أطياف الضوء المرئية مقارنة بالسيطرة. أظهرت قمم عالية من الضوء المنبعث المعالج بـ CuNPs في معالجات عالية التركيز من CuNPs.

تتميز أطياف امتصاص الضوء للبروتينات الخالية من المصل والبلازما والمصل بمعدل امتصاص مرتفع مقارنةً بعينات مرضى اليرقان والتهاب المفاصل ، ويمكن أن تُعزى هذه النتائج إلى القدرة على التفاعل مع صبغة الأكريدين وزيادة ذروة امتصاصها على عكس اليرقان (فرط). البيليروبين) ، البيليروبين عبارة عن جزيئات كروموفور وله لون أصفر له نفس لون البيليروبين ، وقد يؤدي ارتفاع البروتينات الالتهابية والعلاج البيولوجي (الجسم المضاد) إلى عدة توزيع جزيئي في وسط العينات. إن الانبعاث الفلوري للأكريدين المختلط بالعينات المختبرة له أيضًا شدة مختلفة وفقًا لنوع العينات ، وهو ناتج عن تفضيل العلاقات وتفاعلات الأكريدين مع محتوى البروتين المختلف في العينات وأيضًا تأثير البيليروبين والحلقات العطرية للأحماض الأمينية المعنية مع وجود بروتينات من العينات هناك ، تختلف قمم الانبعاث بين العينات المختبرة.

Abstract:

Low-level laser intensity (LLLT) had been employed to induce and diagnose several biological activities within tissues and blood. The project study was designed to explain the effects of LLLT on absorbance and fluorescence features of different blood components including (plasma, serum, serum without proteins, and precipitated serum proteins). LLLT (650nm, 532nm, 473nm,) was used to irradiate blood drive component for 10 minutes with power (40mw, 28mw, 20mw). Results of non-radiated components showed a high peak (absorbance) of precipitated protein flowed by serum, plasma, and serum without proteins. The irradiated plasma samples showed increased absorbance with blue laser in comparison with other components whereas irradiated serum samples indicated high absorbance with red laser compared to other components precogitated serum protein recorded increase optical density with green lases with lower than control. Samples of serum without

proteins pointed out a significant increase of absorption in green and blue laser compared to control and other laser radiation. Regarding fluorescent properties of blood components, plasma samples showed a higher peak with red laser compared to other laser radiated samples. On the other hand, serum radiated samples confirmed a remarkable peak with green laser compared to other samples. However, precipitated radiated serum protein was a significantly lower fluorescence peak when matched with those control samples. Also, serum samples serum without proteins and irradiated with different laser wavelengths showed lowering fluorescence peaks in matching with control (non-irradiated samples). In conclusion, the results can be attributed to different densities of components of blood because isolation of proteins (clotting and serum proteins), as well as the photo-biochemical modulation or changes induced by laser radiation, can be an effected on absorption and fluorescence peaks of those component, the results of irradiated jaundice sample with mentioned laser also yield fluctuations in absorption peaks of irradiated jaundice sampled since green laser pointed out a high peak compared to those, other lasers followed by red and blue, These results can produce from different interactions among bilirubin molecules and other organic molecules that might be affected by laser energy go to form multiple interactions among them, these interactions may be results in because of oxidative damage resulting from free radicals on those proteins. Bilirubin interacts with antecedent light and emitted light, so these molecules can prefer the antecedent light 520 - 570 nm to other laser light. From a molecular point of view, the biochemical reaction of bilirubin molecules indicates two poorly overlapping bands of both absorption and fluorescent spectra.

The results which are obtained from irradiation of arthritis patients' samples (plasma, serum, precipitated proteins, and serum-free proteins), the absorption spectra were also different among the samples, since the plasma samples pointed out a higher emitted peak with green lager irradiated samples, whereas serum samples give a maximum absorption peak in red color laser the precipitated proteins and serum-free protein recorded a lower significant decrease of absorption activities. The present research was designed to explain the effects of different concentrations of CuNPs (10 µl , 50 µl , 100µl) on absorption and fluorescent spectra of blood components for healthy, arthritis, and jaundice (in vitro). The results which are documented from this work confirm that NPs affect the absorption spectra of blood components in different manners and their effects depend on the quality of blood

(type of component) so plasma and serum recorded a significant increase in absorption spectra of healthy blood. whereas samples of arthritis patients treated with NPs had lower absorbance spectra also the high concentration CuNPs(100 µl) reveal high peak levels. sample of jaundice patients (bilirubin), the results explained low levels of absorption in most tested samples. concerning emission spectra, it had been noted to increase emission peak in most treated samples of healthy blood samples. as well as samples of arthritis patients treated with NPs indicated a high-level emission in plasma compared with control, On the other hand, the results of other samples showed low emission spectra in an intensity and excited in visible spectra of light compared to control. showed high peaks of emitted light treated with CuNPs in high concentration treatments of CuNPs.

Whereas all the remaining treated samples recorded a significant lowering in emitted spectra compared to those nano-treated. from this finding, it can be concluded that the absorption spectra depend on the content proteins of samples and concentration of NPs also, emitted spectra depend on the quality of samples it has been that bilirubin decreases emitted spectra when treated with CuNPs.

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(List of Symbols and Abbreviation)

Symbols, Abbreviation	*Description*
DPSSL	Diode-Pumped Solid-State Laser
EDTA	Ethylene Diamine Tera Acetic Acid
WBC	White Blood Cell
RBC	Red Blood Cell
CW	Continuous Wave
Uv	Ultraviolet
LED	Light Emitting Diode
He-Ne	Helium-Neon laser
PRP	Platelet-rich plasma
TEM_{00}	Transverse single mode
A	Absorbance
T	Transmittance
I_o	Incident intensity
I_1	Transmitted intensity
λ	Wavelength
α	Absorption coefficient
k	Extinction coefficient
ℓ	Distance of light passed through material
ρ	Density
c	Specific heat
k	Thermal conductivity

t, b	Tissue and Blood domain
T	Temperature
$\mathbf{q_m}$	Heat generations due to metabolism
$\mathbf{W_b}$	Blood perfusion rate
$\mathbf{T_a}$	Arterial blood temperature
N	Complex refractive index
n	Refractive index
$\boldsymbol{\sigma}_{\mathbf{opt.}}$	Optical conductivity
$\boldsymbol{N}$	Electrons number
$V_{ext}(\vec{r})$	external potential
Ψ	wave function
$\hat{V}_{\text{elec}}$	electronic potential
$\hat{T}$	kinetic energy

Chapter One
General Introduction

1.1 Introduction:

Following the invention of the laser system, several studies were conducted to investigate potential interactions on tissue using various types of lasers and biological tissues [1]. While there are an infinite number of compositions for the experimental parameters, there are primarily five types of interactions. The interactions are chemical, heat, ablative photodecomposition, plasma-mediated ablation, and photo-disruption [2]. Prior to focusing on such encounters, it will show how important it is to research the optical characteristics of living tissues [3]. The influence of laser parameters and tissue optical properties on the adoption of light penetration in live tissues. As a result, once irradiated by a laser system, each biological tissue has its own class response functions [4]. Reflection, scattering, absorption, and fluorescence are the basic phenomena that occur when live tissue is bombarded by laser light as a result of light diffusion in tissue. The rule of Fresnel can suffocate reflection[5]. The optical features of live tissue, such as refractive index, scattering, and laser light absorption by living tissue, all influence laser light penetration inside living tissue [6]. The depth of penetration of a laser beam is determined by a variety of laser parameters (wavelength, power, duration, exposure time, spot size), as well as the color and consistency of the tissue.

Based on its composition and chromophore concentration, each tissue has unique absorption characteristics. (Hemoglobin, Melanin, Water, Protein) are the main chromophores in tissue [7].Water absorbs the bulk of infrared light, whereas hemoglobin and melanin absorb the majority of visible and ultraviolet light, respectively [8]. Scatter or absorption from covalent bands in proteins inhibits penetration depth in the range as wavelength drops toward the violet and ultraviolet, Figure (1-1)

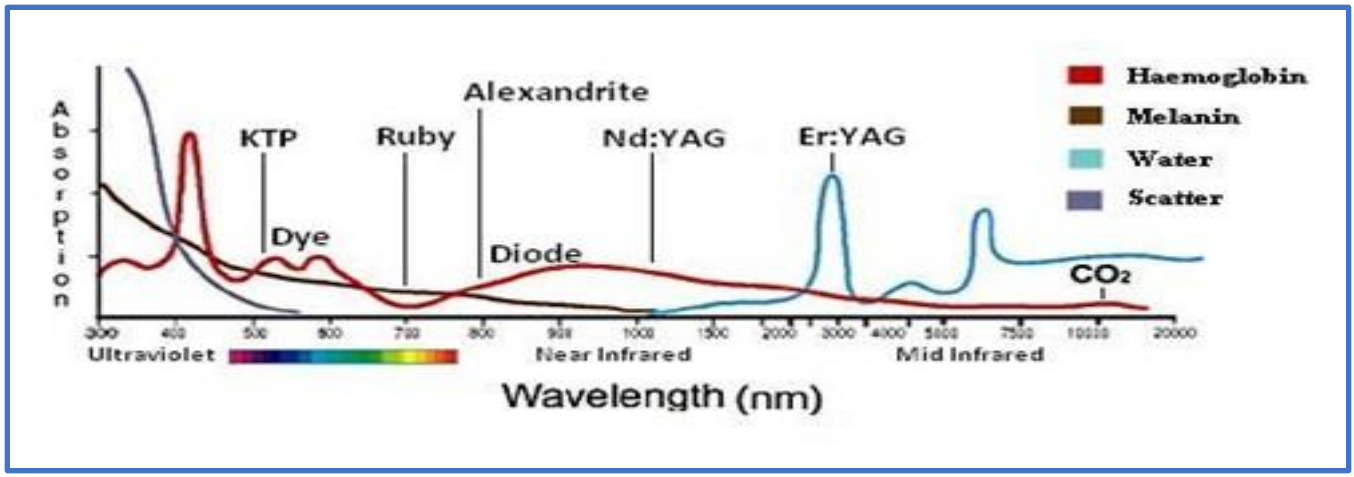


Figure 1-1: Main Chromophore Absorption [8]

When the laser beam is oriented towards the tissue, only about 3% of the incident light is reflected directly; the rest is absorbed and dispersed, and the rate of heat creation is determined by the rate of photon absorption inside the tissues. Outside the laser beam, the dispersed light absorbed may create heat [9], Figure(1-2).

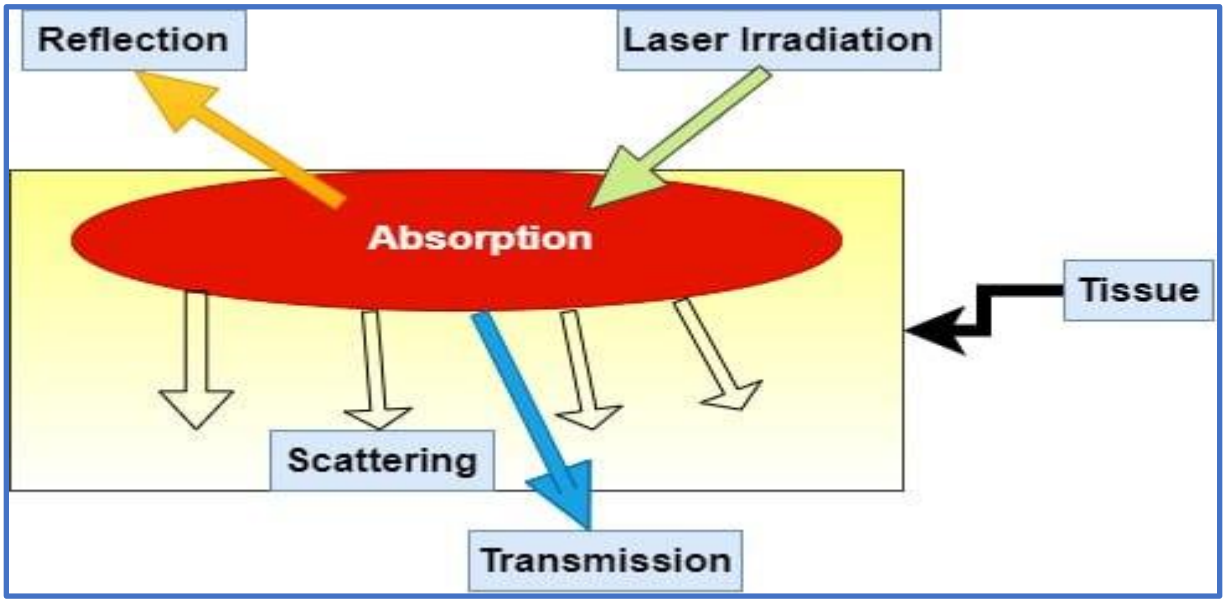


Figure 1-2 :The interaction of laser light with live tissue

Because the laser beam's intensity range produces cell necrosis and tissue coagulation, it can be employed in medical applications such as cutting or welding. This capability can be applied to a variety of surgical procedures and corneal refractive index correction.

In the medical field, there are two types of lasers: -

1. Low-energy lasers for medical use: This type is employed to activate the functioning of cells. Therapeutic lasers are commonly characterized as lasers that use energy densities less than the threshold limit energy where there can be unfavorable consequences in the activities of the cell. Biological impacts are not thermal, as they are when used in laser surgery.

2. High-power laser (heating): This type can be used in surgical procedures to cause tissue cutting, clotting, and necrosis, and these lasers are commonly referred to as surgical lasers because they are utilized instead of a scalpel in surgery [10].

There are two models for laser interactions with live tissues: (thermal and non-thermal). Vaporization and photocoagulation are two thermal mechanisms. Photo ablation and photo-dry-etching of tissues are non-thermal mechanisms [11]. The temperature at which the laser energy warms the tissue determines the degree of thermal damage. Table 1.1 shows the changes that occur when the laser beam is absorbed [8].

Table 1.1: Tissue Changes with Temperature Increases [8]

Temperature	Biological change
37-60° C	Warming, welding
60-65° C	Coagulation
65-90° C	Protein denaturization
90-100° C	Drying
100° C	Vaporization, carbonization

Where photons must be absorbed by electronic absorption bands, a low-power laser can have an effect on live tissue. The interaction of a laser at a given wavelength, power, and exposure duration is determined by the optical characteristics of the tissue.

Although lasers have several qualities, such as monochromatic, spatial and temporal coherence, directionality, and brightness, monochromatic lasers are of minimal value in most medical applications [11].

The brightness of the laser has the largest influence from the above features, and the laser beam, with or without focusing, causes localized specialized photochemical reactions and internal local heating. They can also work in CW or pulsed mode and produce ultra-short pulses with femtosecond pulses [12].

1.2 Interaction of Nanoparticles with Proteins: Relation to Bio-reactivity of the Nanoparticle

Nanoparticles (NPs) offer unique features that may be beneficial in a varied variety of applications, and hence they have gained great study. Particularly in the bio-medical arena, the usage of nano vaccinations and nano medicines are being aggressively investigated. Nevertheless, the understanding of the bio-compatibility

and hazards of exposure to nanoparticles is inadequate. Exposure to nanoparticles for people may be inadvertent, for example occupational exposure, or purposeful, for example via the usage of nano-enabled consumer items. There are a growing number of research that reveal harmful effects of nanomaterials on in-vitro cellular systems, there is an urgent need to understand the molecular pathways of nanoparticles-to-biological system interaction[13].

In a biological context, NPs may interact with bio-molecules such as proteins, nucleic acids, lipids and even biological metabolites owing to their nano-size and huge surface-to-mass ratio. Of particular relevance is the adsorption of proteins on the nanoparticle surface. The development of nanoparticle-protein complexes is usually referred to as the nanoparticle-protein corona (NP-PC) (NP-PC). A multitude of effects of protein adsorption on the NP surface may be predicted. Overall, the NP-PC may alter the biological reactivity of the NP [14].

1.3 Nanoparticle Protein Corona

Proteins are polypeptides with a specified shape and carry a net surface charge dependent on the pH of the surrounding medium[14]. Adsorption of proteins at the nano-bio interface is helped by numerous factors such as hydrogen bonds, solvation forces, Van der Waals interactions, etc[15]. The entire NP-PC creation is a multifactorial process that not only relies on the properties of the NP, but also on the interacting proteins and the media[16]. Specific attachment and dissociation rates for each protein define lifespan of their contact with the NP surface[17]. A "hard corona" is formed by irreversible (or at least long-term) protein binding to the NP, while a "soft corona" is defined by rapid reversible binding of proteins with quicker exchange rates [18].

Cellular proteins in serum and plasma are complex biological systems, and NPs may create Bio/Nano complexes in vivo when exposed to many, very distinct systems. Inhaled NP may pass past the mucosal layer, lung epithelial cells, and into the bloodstream. Similarly, after being phagocytized by a monocyte, the NP may be transported into endosomes, which eventually combine with lysosomes at the cellular level. Each of these proteomes reflects a distinct environment with distinct protein compositions, enzymatic activity, pH, ion compositions, and other characteristics. These conditions may lead the NP to go through a complicated series of alterations that are yet unknown. NP-protein interactions are continually altering even within the same environment. When exposed to blood plasma, for example, the nano-bio interface has been shown to vary over time owing to continual protein adsorption and desorption [1]. As a result, NPs that have entered the body must be seen as evolving systems that have been altered by successive exposure to various protein-rich environments. Several variables may alter the rate of protein adsorption on the NP surface. One aspect that might dramatically alter the NP-PC mix is the number of proteins available to interact with the NP surface. Monopoli and coworkers found that the proteins bound to NPs varied with plasma concentration, whereas the relative quantities of certain abundant proteins adsorbed on surfaces of silica or polystyrene NPs rose with increasing plasma concentrations [19]. In an vivo system, the NP surface may get pre-coated with certain proteins as it travels through diverse protein-rich environments. This may also help decide which new protein will bind to the NP-protein complex that has already formed. The subsequent adsorption of plasma proteins on the surface of multi walled carbon nanotubes (MWCNT) was demonstrated to be influenced by pre-coating of pulmonary surfactant proteins [20]. Also, even after multiple incubations with various biological fluids, silica or polystyrene NPs were shown to preserve a "fingerprint" of plasma proteins [20].

Proteins such as serum albumin, immunoglobulins, fibrinogen, apolipoproteins, and others make up a typical NP-PC in human plasma. Hell strand and coworkers found high density lipoproteins in the protein corona on polystyrene NPs in a recent research [21]. Blood proteins adsorb to foreign inorganic surfaces in a dynamic manner, with more abundant proteins like albumin and fibrinogen occupying the surface at first and then being replaced by proteins with a greater binding affinity for the surface. The Vroman theory underpins such a progressive binding pattern of plasma proteins[22].

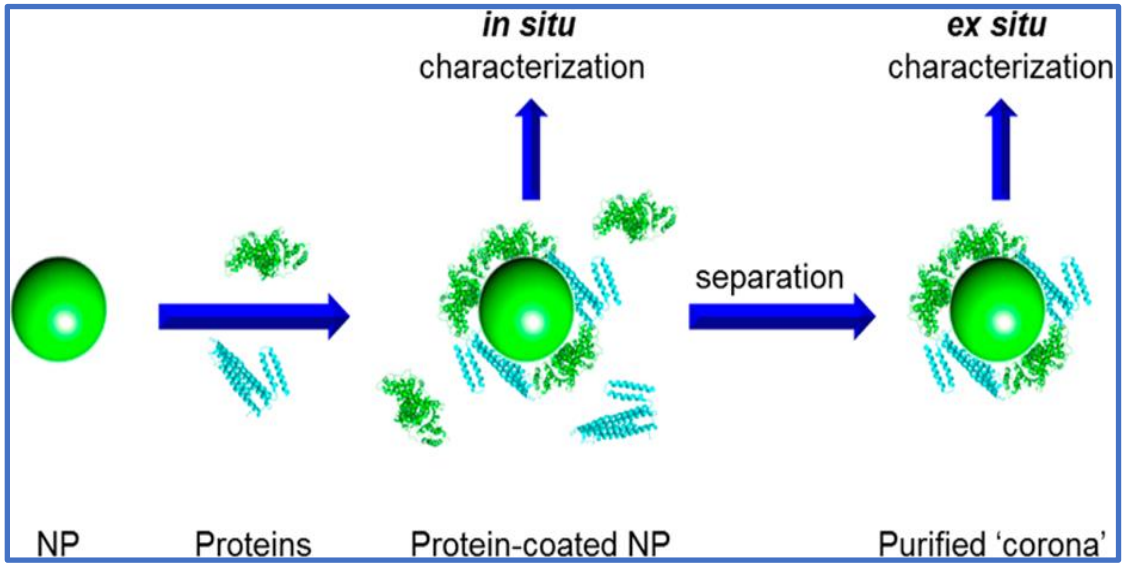


Figure 1-3 Schematic illustration of protein corona formation around NPs and subsequent in situ versus ex situ characterization. Two different proteins are depicted in cyan and green [22]

The affinity of a protein for the NP surface and its capacity to fully occupy the surface are additional factors in its adsorption on the surface. The arrangement of protein molecules on the NP surface may influence the latter's biological response at the cellular level [23].

1.4 Adsorbed Proteins' Structures are Altered by Nanoparticles.

The adsorbed protein's structure and function may be altered by the NP surface, altering the NP's overall bio-reactivity. The fate of proteins attached to the NP surface is further investigated in this section. When compared to flat NP surfaces, curved NP surfaces give more flexibility and surface area to the adsorbed protein molecules [24]. Curved NP surfaces may potentially influence protein secondary structures, causing permanent modifications in certain situations [25]. It's worth noting that the chemical characteristics of specific proteins, as well as their structural flexibility, play a role in controlling surface-driven secondary structure alterations [26]. Specific folding of the protein polypeptide chain produces conformational epitopes. Continuous epitopes, on the other hand, are areas of the protein main structure that include 10–12 amino acids and may provoke an immune response. The NP surface may cause anomalous unfolding of bound proteins, resulting in new conformational epitopes, or it may cause native protein structure unfolding, revealing buried epitopes. Such hidden epitopes may influence the functioning of bound proteins, eliciting an undesired immune response, for example. Deng et al. demonstrated that negatively charged poly (acrylic acid)-conjugated gold NPs bound fibrinogen from blood plasma and caused its unfolding, which activated the Mac-1 receptor on THP-1 cells, resulting in the release of inflammatory cytokines through the NF-B pathway [27]. Changes in protein structure might lead to a lack of tolerance towards oneself, which can trigger autoimmune reactions in the worst-case scenario [28].

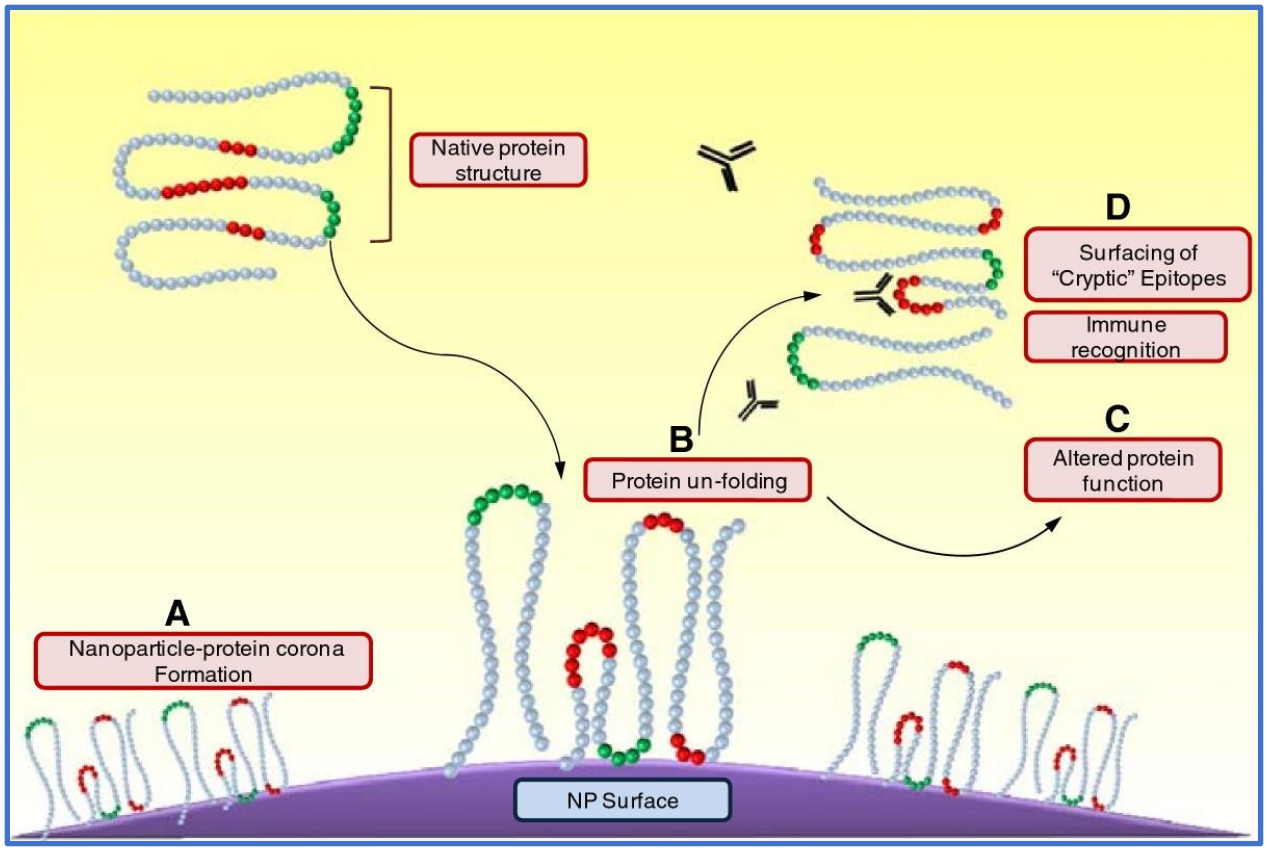


Figure1-4; **Schematic representation of NP surface induced unfolding of the interacting protein molecule and consequences.** (**A**) Protein molecules adsorb on to the NP surface, to form a complex termed as the (**B**) NP-PC.NP surface may induce conformational change to the native structure of the adsorbed protein molecule, causing it to unfold. Such protein conformational changes may either (**C**) alter the function of the native protein molecular even lead to (**D**) exposure of "cryptic" epitopes which may result in immunological recognition of the complex [24].

1.5 Literature Survey:

Series	Researcher & year	Subject
1	Shela Gorinstein, *et.al.,* (2000)	they revealed of human serum proteins (HSP) was studied by measuring the intrinsic fluorescence intensity at a wavelength of excitation corresponding to tryptophan's or tyrosine's fluorescence. the loosening of the HSP structure takes place primarily in various concentrations of urea before and after beer consumption[29].
2	Siposan and Adalbert, (2003)	They studied the effect of a low-power He-Ne laser on blood parameters. The authors noted that irradiation resulted in decreased viscosity, BSR, and changes in some erythrocytes [30].
3	Theodoro LH, *et.al.,* (2006)	Studied the effect of Er:YAG and Diode lasers on the adhesion of blood components and on the morphology of irradiated root surfaces. The Er:YAG laser did not caused the adhesion of blood components, whereas the Diode laser inhibited the adhesion [31].
4	Jianhua Zhou, *et.al.,* (2007)	This group explained the thermal damage in biological tissues caused by laser irradiation by

		using theoretical analysis .The tissue damage occurs sometime after laser irradiation[32].
5	John Zhang, *et.al.*, (2008)	Showed that the use of the laser on blood tissue cause a decrease in the value of blood pressure from (127)mHg to approximately (122)mHg [33].
6	Ahmed A. Ibrahim , (2009)	Explained the effect of (He-Ne) laser radiation and viscosity of red blood cells on erythrocytes sedimentation rate (ESR). Te laser radiation reduce the (ESR) of blood samples[34] .
7	MohammadAli Ansari and Ezeddin Mohajerani (2011)	They studied the mechanisms of laser-tissue interaction: optical properties of tissue and then explained the effects of these properties on laser penetration in tissue [9].
8	José Eduardo Cezar Sampaio , *et.al.* , (2012)	They showed the effect irradiation for Er,Cr:YSGG and Er:YAG laser on the adhesion of blood components on the root surface .They found (Er:YAG) laser was more effective in enhancing the adhesion of blood elements on root surfaces [35] .
9	Li Shang and G. Ulrich Nienhaus (2013)	They studied the Protein Adsorption onto Nanoparticles by Fluorescence Correlation Spectroscopy. when nanoparticles (NPs) are incorporated by a living organism, a protein adsorption layer, also known as the “protein corona”, forms on the NP surface. They showed This knowledge helpful for the safe and effective

		use of nanomaterials in science and technology[36].
10	Jaona H. R. *et.al.*, (2014)	Studied the effects of short pulsed laser radiation on transit heating of human tissues. They examined an increasing of local radiation absorption in the tissue with short pulsed radiation and gold nanoparticles (GNPs) embedded in human tissue [37].
11	Shikha rathore and Basharath Ali (2014)	They explained the effect of laser radiation (He-Ne) on electrical conductivity of blood and then found the conductivity of the blood was changed [38].
12	Flavia de paoli *et.al.*, (2015)	They studied the damage of DNA for blood cells caused by low –level laser . They showed the damage itoDNA of blood caused by exposure to low-level red and infrared lasers depending on fluence, power ,a,nd emission mode [39] .
13	Muhammed Zeeshan (2016)	Stated the mechanism of laser / light interaction at cellular and tissue and study of the influentfactorsctor for applications of low-level laser therapy. The interaction between tissue with different source of light (conventional light or LED) but the use of laser is to be more favorable in interaction with tissue and cells [40].

14	Deepali Verm,*et.al.*, (2018)	They made it clear protein nanoparticles tare he major grounds for the transformation of different properties of many conventional materials by virtue of their size and greater surface area which instigates them to be more reactive to some other molecules. A plethora of pronanoparticleicles applications via different routes of administration are explored and reported by eminent researchers.
15	Zahra Al-Timimi, (2019)	Showed the Impact of laser (Nd:YVO4 Crystals,532nm) radiation on white blood cells .When the laser dose time is increased there is no further change which is observed in WBCs , while at laser dose for shorter duration, there is a marked increase in the optical absorbance of the Hgb, which minimizes laser-induced heating[42].
16	Shanmugavel Chinnathambi *et.al.*, (2020)	They investigated the conformational changes of blood plasma proteins during the interaction with near-infrared light-emitting nanoparticles. the addition of nanoparticles causes the denaturation of the plasma proteins. However, it is noteworthy that the conformational recovery phenomena are observed for fibrinogen and transferrin, suggesting that the nanoparticle does not influence the ordered structure of proteins in the bloodstream[43].

17	N Suardi ,*et.al.*, (2021)	They studied Effects of Low-Level Laser Irradiation (continuous wave, 405nm wavelength) on Blood Protein The single exposure results demonstrated an overall increased protein level for all blood proteins. Global maxima (8 minutes' single exposure)[44].
18	Raneem Mohammed (2021)	studied the effect of laser irradiation with different wavelength on absorbance of normal human blood. she showed the Laser radiation has a great role in the absorption properties of the normal human blood after being compared with absorption spectrum of a non-irradiated sample[45].

1.6 Aims of the work:

1. Study the physical effects (especially density) of proteins in different blood components.

2. Effects of optical properties of those proteins when blood samples are divided into different parts that have different protein contents.

3. Determination of absorbance and fluorescent properties of blood components to that healthy samples and compared with those abnormal blood samples.

4. Studying the effect of electromagnetic radiation on (normal and abnormal) living tissues and compering between them.

5. Studying the optical properties of (normal) living tissues before irradiation with laser and comparing between them.
6. Studying the optical properties of (normal and abnormal) living tissues after irradiation with laser and comparing between them.

7. Explanation of the effects of bilirubin and inflammatory factors (arthritis samples) on optical and fluorescent properties of blood components.

8. Measurement interactions between Cu NPs and physical properties of blood components.

9. Measurement interactions between Acridine Orange and physical properties of blood components.

Chapter Two

Theoretical Part

2.1 Physics and Properties of Lasers:

A laser is a device that uses optical amplification to magnify the energy released by an electron when it is transported from an excited state to a lower energy state. The light generated by a laser differs from ordinary light in a number of ways. It's monochromatic since it's made up of photons with the same wavelength. This is in contrast to ordinary light, which is made up of seven colors and separates into its constituents as it passes through a prism. Laser light is coherent in nature, which means it has the same phase as other light. The interference property is employed in diagnostic medicine. Finally, laser light has a low divergence, implying a strong collimated beam [46].

The laser's emitted light is coherent, which means that all of the photons are in the same place at the same time. Because the laser beams light has a high intensity, it has a wave property called (Coherent), which means there is a constant relationship in the phase difference between the interfering waves, resulting in the constructive interference phenomenon [47].

The use of lasers in medicine has grown over time, and they now play a significant role in medical systems and surgery[48].They're employed in a variety of medical sectors, including cancer detection and therapy, dermatology, ophthalmology (Lasik and laser photocoagulation), and optical coherence tomography. Lasers are also used for cosmetic purposes, such as laser hair removal and tattoo removal [46].Continuous wave (CW) lasers and pulsed lasers are two types of laser systems. Whereas most gas lasers and to a lesser degree solid-state lasers belong to the first group, solid-state lasers, excimer lasers, and certain dye lasers belong to the second group[49] .

2.2 Biological Tissue Effects of Laser Properties:

Because laser light must be absorbed in order to interact with any tissue or substance, its monochromaticity quality, along with its singularity of wavelength, is a determining factor for its interaction with biological tissue. Light receptors (chromophores) in biological tissues are very selective for the wavelengths they absorb. Hemoglobin, oxyhemoglobin, melanin, and water are some of the most frequent chromophores found in biological tissues. This interaction is also influenced by the polarized features of laser light, since various polarizations of light can be absorbed to varying degrees by different biological tissue or substances. Biophotomodulation treatment has been effectively employed with broad-band lamps and non-coherent light sources, such as light-emitting diodes (LEDs) ,have shown to be effective in biophotomodulation treatment[50].

Photo induced biological processes are initiated in weak incoherent CW light employing a quantized radiation field and one-photon absorption from a pulsed coherent laser source or an incoherent thermal source of electromagnetic radiation.

Due to its collimation property, which permits the emission of non-divergent, parallel rays to create minimum beam spread as they travel over a distance, a laser may deposit a large amount of energy in a relatively tiny area (spot size). The quantity of energy produced by the laser is influenced by the diameter of the beam, as light energy is concentrated as the beam diameter is reduced. Ordinary light isn't collimated in any way. As it moves, its diameter expands, the diameter of the beam point expands, and the light loses energy It's on the way[51].It is difficult to determine the energy dose supplied to a target from a distance using non-collimated light beams unless the beam is in direct contact with the tissue [52].

2.3 Laser Types based on the Active Medium:

Lasers made of solid-state materials: Solid state lasers, such as the Ruby or Neodymium-YAG (yttrium aluminum garnet), have lasing material spread in a solid matrix. Infrared light emitted by Neodymium is typically at 1.064 micrometers (m)[53].

Gas Lasers: The most popular gas laser is the helium-neon laser, which has a wide range of output wavelengths, including red (632.8 nm) [53].

Excimer Lasers (also known as excimer lasers) are a kind Excimers combine reactive gases like fluorine and chlorine with inert gases like argon, xenon, or krypton to create an excimer. In reality, when a molecule or dimer is created, it may be electrically stimulated, resulting in the generation of invisible light When a molecule or dimer is created and lased, it can be achieved as a result of being electrically stimulated [54].

Dye Lasers: Can be tuned across a wide range of wavelengths and utilize complex organics as lasing media, such as rhodamine 6G in suspension or liquid solutions[53]

Semiconductor Lasers: These are not solid-state lasers and are sometimes referred to as diode lasers. The absorption of laser wavelength heats the target tissue in most medical laser applications. Light can be supplied in appropriately timed pulses proportionate to the size of the target structure to minimize undesired thermal harm to neighboring tissue, using the selective photothermolysis approach. Larger structures are treated with longer pulses, whereas smaller structures are treated with shorter pulses [55].

Diode Pumped Solid State Laser (DPSSL): A semiconductor laser is used to pump a solid-state crystal such as quartz in order to increase the performance of

a typical solid-state laser pumped using a flash lamp (Nd:YAG, Nd:YVO4) can achieve benefits such as high efficiency, extended lifetime, high reliability, and superb beam quality The pumping source (semiconductor laser), the solid crystal, and the resonator [56] are the essential components of this laser, which comprises of the basic ingredients for creating the laser beam.

2.4 Light propagation in tissue:

When a laser beam is projected into live tissue, four events occur that regulate the uneven propagation of light in the tissue:

2-4-1 Reflection

Reflection occurs at the interfaces of media with varied optical qualities, and the amount of light reflected from each medium is determined by the amount of input light as well as the refractive index of each material [12].

2-4-2 Transmission :

This means an increase in the depth of penetration of the laser light into the tissue, and that the depth of penetration is a function of the wavelength. An example of this is the absorption of water in the cell where it is little within the visible region of the electromagnetic spectrum (transmission increase), while an increase in water absorption is a function of the wavelength (decreased transmittance) [57].

2-4-3 Scattering:

The scattering process of the laser beam occurs by the tissue, as it is absorbed through a large area and thus its effect spreads and weakens. Most of the beam entering the tissue is affected by complete interference with water and cellular membranes. Long wavelength diffusion occurs more than short wavelengths of visible light and is absorbed and this leads to the generation of heat in the tissues,

and this effect is the cause of heat propagates from the treated area, and the damage may reach the adjacent tissue or tissue that is not targeted for treatment [57].

2-4-4 Absorption:

The absorption coefficient defines the process of transmitting energy from light to the target substance, which results in energy loss. Melanin, hemoglobin, and water are the major laser light absorbers in human tissue[58]. We can calculate the quantity of absorption from and all of the events depicted in figure (2-1) [12] using the Beer-Lambert formula[59].

$$A = \alpha \ell c \qquad (2\text{-}1)$$

$$\frac{I_1}{I_\circ} = 10^{-A} = 10^{-\alpha \ell c} \qquad (2\text{-}2)$$

Where :

$$A = \log_{10}\left(\frac{I_1}{I_\circ}\right) \qquad (2\text{-}3)$$

$$\alpha = \frac{4\pi\kappa}{\lambda} \qquad (2\text{-}4)$$

Where :

A: absorbance

I_o: incident intensity

I_1: transmitted intensity

λ: wavelength

α: absorption coefficient

κ : extinction coefficient

c: concentration of materiel

ℓ : distance of light passed through material

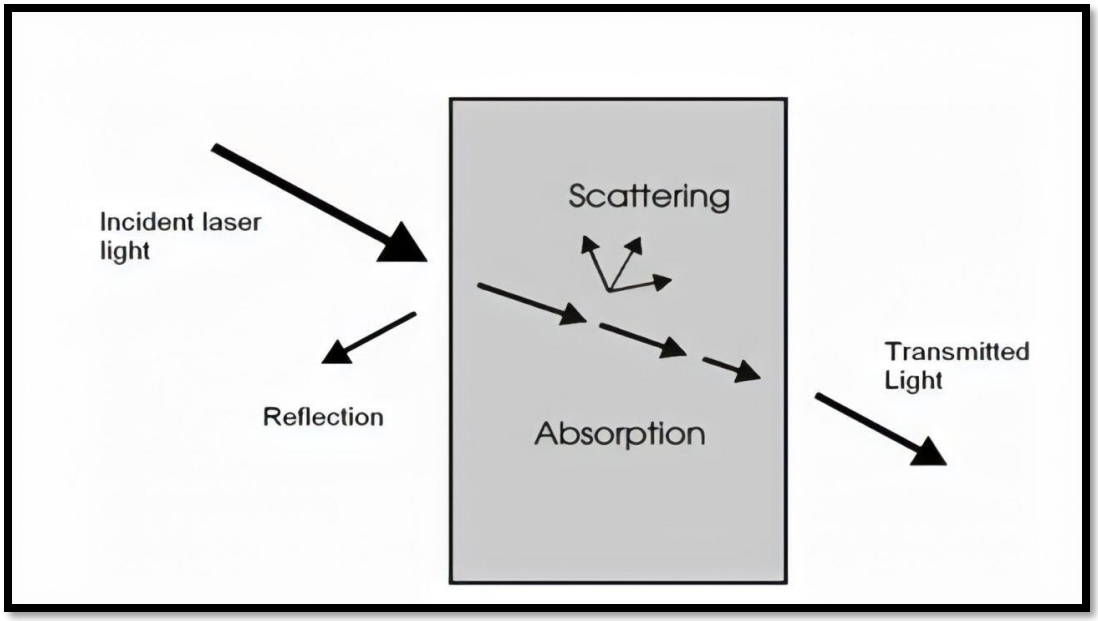


Figure 2-1: Light propagation in tissue [40]

2.5 Mechanisms of Laser-Tissue Interaction

Laser light interacts with tissues through a number of distinct processes. For therapeutic and surgical applications, the most prevalent interaction mechanisms will be categorized into five major categories:

1. Photochemical interactions: Molecules or atoms are excited by photons, making them more likely to perform chemical interactions with other molecules. A photosensitizer (a molecule that becomes reactive when it absorbs light and may thus stimulate chemical reactions within other molecules or tissue) causes reactive oxygen species to develop in photodynamic treatment, for example (cell death). In oncology, photodynamic treatment is becoming more commonly employed to eradicate malignant tumors [40].

2. Photothermal interactions: photons are absorbed and transformed into heat energy by a chromphore (a light-absorbing molecule). , which can result in a variety of heat

consequences, including tissue coagulation and vaporization. Tissue cutting and welding are two applications in laser surgery [60].

3. Photoablation: High-energy ultraviolet (UV) photons are absorbed and induce the dissociation of molecules because they are more energetic than the chemical bonds that keep the molecules together. After then, the irradiation volume rapidly expands and the tissue is ejected from the surface. This is employed in a variety of applications, including eye (corneal) surgery [61].

4. Plasma-induced photo ablation: The high electric field in the region of the laser beam accelerates a free electron. It starts a chain reaction of similar collisions by colliding with a molecule and liberating another electron, ending in plasma: a soup of ions and free electrons. This is used in the treatment of cataracts with lens capsulotomy [62].

5. Photodisruption: Mechanical phenomena such as bubble formation, cavitation, jetting, and shockwaves can occur as a result of plasma creation[49]. Figure (2-2) below depicts all of these mechanisms:

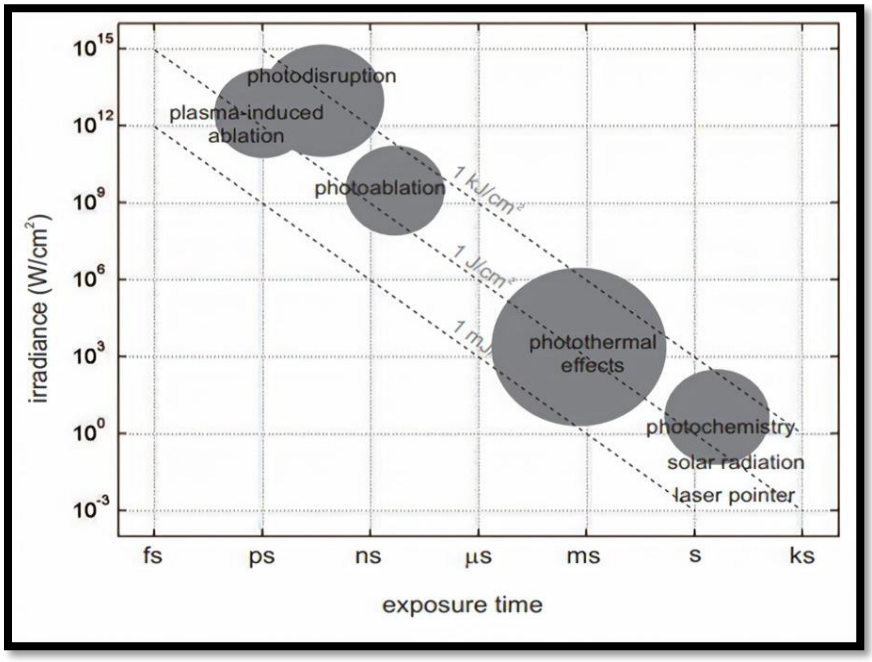


Figure (2-2) :Different forms of laser-tissue interactions[49]

2.6 Bioheat Transfer in Living Tissue:

The study of thermal energy transmission in living systems is known as bioheat transfer. Heat transport is important in biological systems because metabolic activities are temperature sensitive. Bioheat transfer techniques are also suitable for diagnostic and therapeutic applications requiring either mass or heat transfer since the mass transit of blood through tissue generates a thermal energy transfer.

The effects of blood flow on heat transmission in living tissue have been studied for over a century, beginning with Bernard's experiments in 1876. Since then, many physiologists, doctors, and engineers have been interested in mathematical modeling of the complicated thermal interplay between the vasculature and tissue [63]. The evaluation of the influence of blood circulation, which is the dominating route of heat evacuation and a significant cause of tissue temperature inhomogeneity, is a key concern for theoretical prediction of temperature distribution in tissue [64].

2-6-1 Photo Thermal Effects

In clinical practice, thermal effects are probably the most common kind of tissue-laser interaction. There is frequently a particular reaction route that leads to tissue damage in photochemical effects, such as photodynamic treatment. There is no unique mechanism here, and photons might be absorbed by any biomolecule while still causing a heat impact. The absorption of light and subsequent conversion to heat via vibrational relaxation deposit heat energy in the tissue. The tissue's temperature rises as a result of this. Heat will also permeate through the tissue, raising the temperature of the surrounding tissue. The tissue damage is determined by the temperature achieved and the length of time it is kept at that temperature. Thermal interaction is used in a wide range of medical applications, from the vaporization of

malignancies to the welding of gastrointestinal ulcers and the removal of skin markings such as port wine stain birthmarks or tattoos [65].

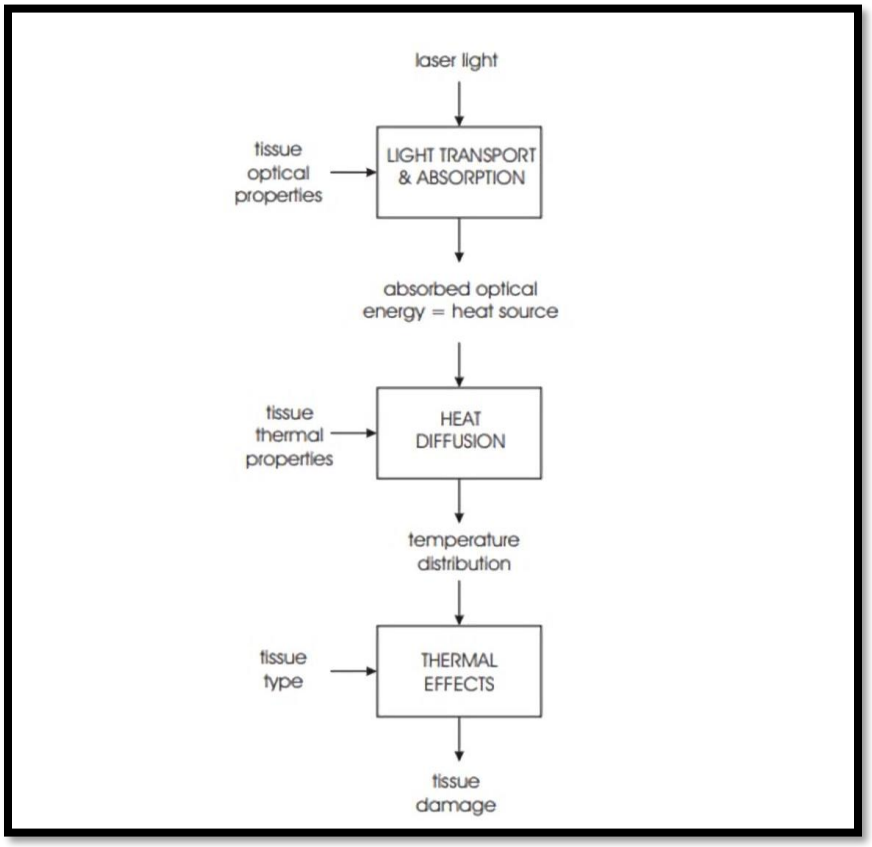
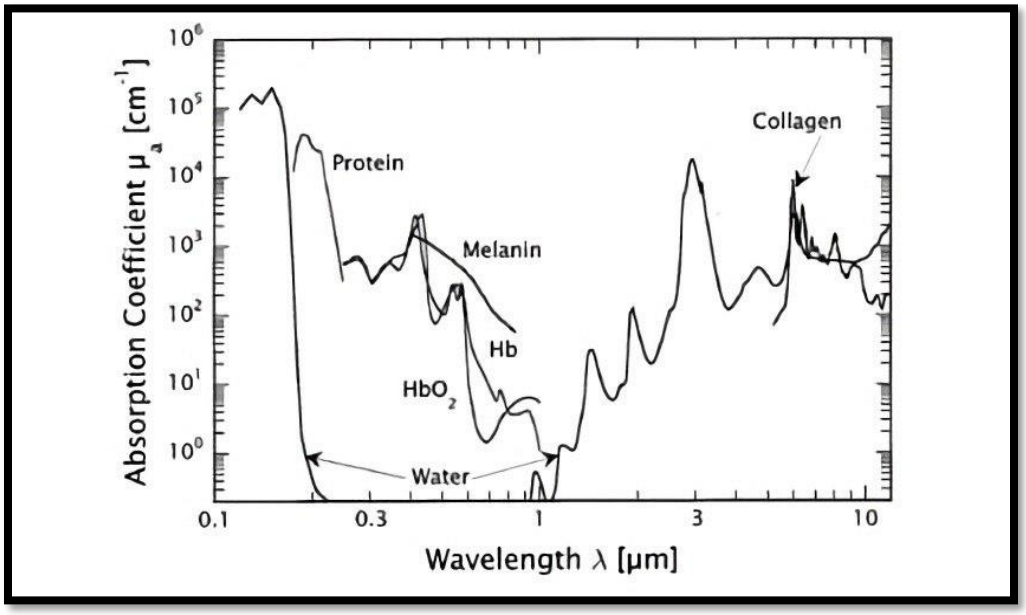


Figure (2-3): The many features of thermal light interactions with tissue [65]**.**

Figure (2-3) depicts how light interacts with tissue thermally, taking into consideration the fact that soft tissue transmits heat. Finally, the last portion of Figure (2-3) depicts what happens to the tissue after it reaches a specific temperature. Collagen, water, hemoglobin, and maybe a few additional chromophores, such as melanin, make up the tissue.

• Cells: Traditionally, scientists have focused on this component of the tissue. Although the proteins in particular cells can be critical to some applications, they are frequently viewed as water-filled for our purposes (such as the hemoglobin in red blood cells is to port wine stain treatment).

• Extracellular matrix (ECM): this is a fibrous framework in which cells nestle and which provides the majority of the stiffness and structure of the tissue. Collagen and elastin, as well as other glycoproteins and proteoglycans, are used to make it. The quantity of ECM to the number of cells varies greatly depending on the tissue type. The ECM content of the liver and muscle, for example, is minimal, but the ECM content of bone, tendon, and the retina is high. The collagen in ECM is of relevance when evaluating heat impacts since it breaks down at temperatures much below 100°C [49]. Figure 2-4 displays pictures of extracellular matrix.



(Figure 2-4): Absorption coefficient spectra for diverse tissue components. Note the peak in hemoglobin (Hb and HbO2) absorption at 577 nm, which is employed in the treatment of port wine stain [49]

2-6-2 The Penne's Model

is a model developed by Penne's. Penne's developed the first mathematical connection that represented the heat transport between blood and tissue in 1948. Penne's' model for explaining the energy balance of tissue metabolism and blood perfusion in live tissue was first developed to predict temperature fields in the human forearm. Penne's used three assumptions for the modeling analysis: the rate of heat production by tissue, the volume flow of blood per unit volume of tissue per second, and the tissue specific thermal conductivity were all assumed to be uniform across the forearm[66]. Although the Penne's bioheat model was developed using experimental data from the human brain, it may be used to calculate the rate of heat transfer in any perfused tissue. Many writers have used it to build mathematical models of heat transport in living tissues, and it has become known as the bioheat transfer equation. Penne's quantitatively assessed the brachial artery blood and tissue temperatures based on the human forearm temperature measurement findings, and then evaluated the application of heat flow theory to the forearm in terms of local rate of tissue heat generation and blood volume flow [67]. The rate of heat transfer between blood and tissue is proportional to the product of the volumetric perfusion rate and the differential between the arterial blood temperature and the local tissue temperature, according to Penne's' main theoretical contribution. Penne's assumed that the physical circumstances of the capillary circulation were nearly perfect equilibrium since he didn't know the amount of thermal equilibrium between capillary blood and surrounding tissue. The thermal energy balance for perfused tissue is given in the following form[66], according to his hypothesis that arterial blood temperature is consistent throughout the tissue.

$$\rho_t c_t \frac{\partial T}{\partial t} = \nabla . (k_t \nabla T) - W_b c_b (T - T_a) + q_m \qquad (2\text{-}5)$$

T is the temperature, (q_m) heat generations due to metabolism; (Wb) is the blood perfusion rate; and (Ta) is the arterial blood temperature, where(ρ) is the density, (c) is the specific heat, k is the thermal conductivity, with the subscripts t and b referring to tissue and blood domains, respectively, T is the temperature,($q_{m)}$ heat generations due to metabolism; (Wb) is the blood perfusion rate; and (Ta) is the arterial blood temperature. Wb varies from 0.0002 to 0.0005 g/(cm3.s) and qm is 0.000 lcal/(cm3.s) (cm3.s). The four variables in Equation (2-5) are meant to reflect thermal energy storage, diffusion, convective energy (i.e., energy perfusion of solids by liquids, typically blood) and metabolic heat generation [66].

2.7 The Blood:

Blood is a tissue made up of a suspension of cells suspended in a liquid called plasma. Blood makes up 7% of the human body weight and has a density of roughly 1060 kg/m3, which is extremely near to the density of pure water, which is 1000 kg/m3. Blood is made up of red blood cells, white blood cells, plasma, and platelets, as well as connective tissue, which is vital for many organisms, including humans and animals, and is crucial to jobs. Blood transports nutrients, vitamins, waste (carbon dioxide), and hormones to all of the body's tissues and cells, and its natural temperature is 37 degrees Celsius. The components of blood are shown in Figure (2.5) [68].

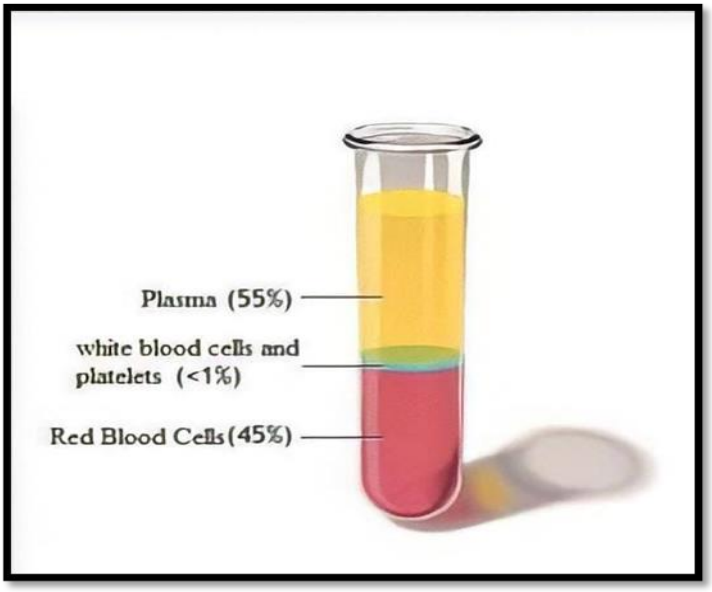


Figure (2-5): Blood Components [68]**.**

2-7-1 Plasma Proteins

Plasma proteins are the most abundant dissolved substances in the plasma. Plasma proteins are not used for energy and fall into three groups: albumins, globulins, and fibrinogen [69].

1. The albumins help maintain the osmotic pressure of the blood and account for 60% of the plasma proteins.

2. The globulins, comprising 36% of the plasma proteins, are as alpha, beta, and gamma globulins.

• Alpha and beta globulins function in transporting lipids and fat-soluble vitamins.

• Gamma globulins are a type of antibody.

3. Fibrinogen (4%) plays a primary role in blood coagulation.

2-7-2 Plasma Electrolytes

Plasma electrolytes are absorbed by the intestine or are by-products of Cellular metabolism. They include sodium, potassium, calcium, magnesium, chloride, bicarbonate, phosphate, and sulfate ions. Some of these ions are important in maintaining osmotic pressure and (pH) of the plasma. Ions are also critical for

nerve and muscle function, as potentials in these tissues are produced by the exchange of electrolytes between the extracellular fluid and the cell's fluid. Electrolytes enter and leave cells through proteins in the cell membrane called ion channels. Muscle contraction depends upon the movement of calcium, sodium and potassium through ion channels [70].

2.8 Physical properties of Human Blood:

2-8-1 Color:

The oxygen concentration in blood affects its hue. Bright red. due to high oxygen concentration. Dark red with little oxygen content [71].

2.8.2 Blood Viscosity:

Biochemical data, such as fibrinogen, cholesterol, or albumin levels, or globulin ratios, are associated with blood viscosity variables (such as blood viscosity, plasma viscosity, and red cell aggregation) from patterns of blood viscosity functions[72].

2-8-3 Osmotic Pressure:

Blood plasma includes adequate amounts of dissolved proteins, which is an essential chemical reaction. Salts and other biomolecules help the body maintain a healthy osmotic pressure. This implies that the plasma cannot both take water out of the cells and drive water into them. It cannot be hypertonic to water (having more salts and dissolved molecules) or hypotonic to water (containing fewer salts and dissolved molecules) [73].

2-8-4 Blood Density:

Density is defined as the mass per unit volume of a substance. Whole blood refers to the plasma and components of the blood. It is proportional to hematocrit, or the total protein content in blood; other plasma solutes only have a minimal impact on blood density. For determining distribution volumes and flow across organs, the density dilution approach can be utilized [74]. Its value for males ranges between (1.067-1.075) g/cm3 and is dependent on the presence of soluble components in the plasma such as red blood cells and protein. It varies from (1.0561-1.051) g/cm3 in women [75].

2-8-5 Temperature:

They are constant in the body, with slight variations from one organ to the next, depending on the organ's demand for heat to complete its primary function. The temperature of the liver, for example, is 40-41 degrees Celsius, whereas the temperature of the brain is less than (37 degrees Celsius), and the overall average body temperature is 36.8-37.8 degrees Celsius [76].

2.9 Blood Functions:

2-9-1 Respiratory:

Hemoglobin in red blood cells transfers oxygen from the respiratory organs (lungs) to the tissues, and carbon dioxide from the tissues to the lungs to be expelled from the body [77].

2-9-2 Nutritive:

The blood carries and distributes nutrients from the digestive system to all of the body's tissues[77].

2-9-3 Body Temperature Regulation:

Because blood transfers heat to various regions of the body, it aids in the regulation of body temperature [78].

2-9-4 Regulating Metabolism:

The blood transports many enzymes from their manufacturing sites to various organs throughout the body in order to maintain the construction and demolition processes in the body's cells, which are referred to as (Metabolism) [79].

2-9-5 Defense:

White blood cells guard the body by devouring germs that enter the body and thereby preventing it from sickness, since blood cells include antibodies that protect the body from bacterial infection [80].

2-9-6 Hormone Transport and Regulation:

The blood controls hormone release from glands and maintains a balanced ratio in the blood, and the blood distributes these hormones to their destinations [81].

2-9-7 Water Balance:

The blood keeps the body's water balance by eliminating surplus water beyond what the body requires via the kidneys and skin [79].

2-9-8 Blood Clotting:

which is caused by the protein Fibrinogen present in blood plasma, stops bleeding caused by a blood vessel damage. The liver produces this protein, which is important for blood clotting [81].

2.10 jaundice:

The yellow-orange coloring of the skin and sclera brought on by an abundance of bilirubin in the skin and mucous membranes is referred to as "jaundice." Jaundice is a symptom or indication of an illness rather than a sickness in and of itself. The major way that bilirubin is created is when the spleen converts the haem component of red blood cells into biliverdin and then unconjugated bilirubin. Since bilirubin is not water soluble, it travels from the spleen to the liver via the bloodstream while attached to the plasma protein albumin. Conjugated bilirubin is what it is called as in this form, and it is secreted into the gall. It is subsequently expelled in the feces after being further metabolized in the gut to other gall pigments. Only the unconjugated bilirubin levels are elevated in physiological jaundice due to an immature liver in the absence of any other sickness. There are underlying disorders that either cause pathological jaundice to produce more bilirubin or cause it to excrete less. The underlying problems must be treated in order to treat pathological jaundice[82].

2.11 Arthritis:

Rheumatoid arthritis (RA) is a painful, swollen autoimmune disease that can severely impair physical function and quality of life. Musculoskeletal pain, swelling, and stiffness are common presenting symptoms in clinical practice, so understanding how to diagnose and treat RA is critical. Infection, respiratory disease, osteoporosis, cardiovascular disease, cancer, and mortality are all higher in RA patients than in the general population. Early diagnosis, aggressive treatment, and expanded therapeutic options for disease-modifying antirheumatic drugs have significantly improved the management and long-term prognosis of RA in recent years[83].

2.12 Nanoparticles

The primary interest in nanoparticles stems from the fact that they are small enough to interact with cellular machinery and potentially to reach previously inaccessible targets, such as the brain. There are numerous areas where nanoparticulate systems are of significant scientific and technological interest, specifically for biomedicine leading to concern for the design of safe nanomaterials. Biomolecules (e.g., proteins, natural organic materials, detergents, and enzymes) when they come in contact with a biological medium. The absorption of biomolecules to such surfaces confers a new “biological identity” in the biological milieu, which determines the subsequent cellular/tissue responses. Due to their extremely high surface-to-volume ratio, NPs have very active surface chemistry in comparison to bulk biomaterials.

The interaction between nanomaterials and environmental biomolecules (protein) results in the formation of a biological corona on the NDP's surface that is quite dramatically different from that adsorbed on a flat surface of the same bulk material in the same experimental conditions the adsorbed protein layer that forms on flat surfaces of the same bulk material[84].

2.13 Acridine Orange Dye.

Acridine orange (AOH+) is a biologically important fluorogenic dye. It is widely applied as biological stain in cytochemistry for visualization and quantification of organelles like DNA, RNA and lysosomes. This dye is also used as an intracellular

pH indicator and in photodynamic therapy. Fluorescence properties of AOH+ is highly sensitive to its microenvironment , which makes it a very popular probe for investigating various important micro heterogeneous systems, such as

cyclodextrins, cucurbiturils, octaacid, pillarenes, microemulsions, micelles reverse micelles, ionic liquids, biomolecules,etc[85].

Chapter Three

Materials and Methods

Part One: Experimental Part

(3-1) Introduction:

This chapter explains the materials used in experiments to study the effect of laser beams at wavelengths of 473 nm, 532 nm, and 650 nm on (healthy, Jaundice and Arthritis component blood samples) of humans. It also explains the effect of copper nanoparticles on these samples and effect of Acridine Orange dye on the proteins for this sample. It also explains the method of preparing these samples and the measurement methods and devices used in optical measurements, as shown in figure (3-1).

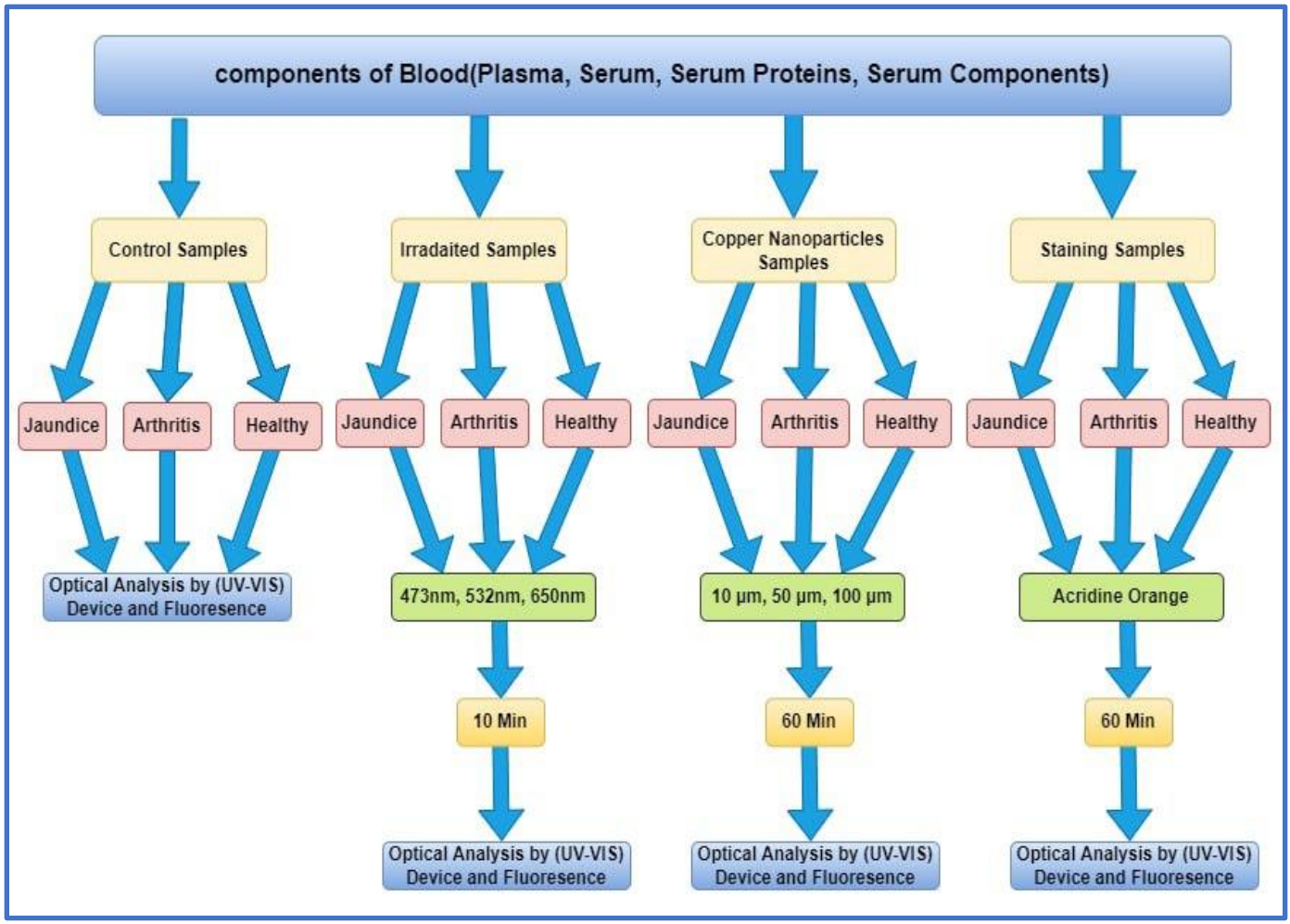


Figure (3-1) work scheme

(3-2) The Material used in the Experiment:

3-2-1 Blood Samples Collection Tubes (EDTA tube):

Ethylene Diamine Tetra Acetic Acid, an anticoagulant chemical, was employed in a variety of cylindrical glass tubes (EDTA). It is necessary 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, in order to protect the components of blood cells after taking blood samples from people through the syringe and placing the blood in the anticoagulant tube.

3-2-2 Blood Samples Collection Tubes (Plain tube):

It's a type of plastic cylinder tube that doesn't contain anticoagulants and is used to split blood samples after they've been added to EDTA tubes for the irradiation procedure.

3-2-3 Centrifuge:

In medical laboratories, this instrument is used to separate the different components of blood. The centrifugal force works by causing the heavier particles to fall to the bottom of the tube, while the lighter particles rise to the top. It has a high-speed electric motor that can be regulated.

The motor, which spins at a speed of 2000 to 3000 revolutions per minute, assists in mixing or isolating materials by centrifugation. The rotation is dependent on the duration chosen by the operator by hand, figure (3-2).

Figure (3-2) : Centrifuge device

3-2-4 Plastic Pipette:

The plastic pipette was employed, which is a manual pipette with gradations, one end of which is swollen and the other end of which is open .The plastic pipette is used to remove liquids and transfer them to the desired location, as the pipette tip is submerged in the liquid. With the fluid to be removed in hand, push on the swollen tip to draw blood and insert it in the appropriate location.

3-2-5 Glass cuvette:

In the current investigation, two glass cells with a thickness of 1 cm were employed in a double-beam spectrophotometer to measure and record the absorbance spectra of the blood sample.

3-2-6 Optical measurement (Double- Beam Spectrophotometer):

To measure the absorbance of the prepared blood samples and record their spectra, an English-made double beam spectrophotometer (CECIL CE-7500) functioning in the ultraviolet and visible areas with a range of wavelengths (190-1100nm) within

the electromagnetic spectrum was employed. A double-beam spectrophotometer is used to record the absorption spectra of the samples in terms of the wavelength of the light beam in figure (3-3). which shows a photograph of the device used in the measurement, a double-beam spectrophotometer is used to record the absorption spectra of the samples in terms of the wavelength of the light beam, this device is characterized by containing two beams of light where the source ray beam is divided by the beam splitter into two beams of equal intensity as it passes the first passes through the reference sample and is called the reference beam and then goes to photo detector No. (1) and the second passes through the sample whose absorbance is to be measured to photo detector No. 2. The two missing parts of the light rays through reflection or absorption by the material is equal for both paths, and the difference between the intensity of the two light beams represents the absorbance of the blood to be found only, where the examination sample was placed at the hole of the sample beam in the spectrometer, while the reference sample was placed at the aperture of the reference beam, and thus the absorbance of the sample was measured after subtracting Reference absorbance by the device self .

Figure (3-3): Double- Beam Spectrophotometer

3-2-7 LIF Spectrophotometer

To measure the emission of the prepared blood samples and record their spectra, The excitation wavelength of the samples should be known from the absorption spectrum. Fluorescence is based on the property of some molecules that when they are hit by a photon, they can absorb the energy of that photon to get into an excited state. Upon relaxation from that excited state, the same molecule releases a photon: fluorescence emission. The energy of the photon that is released is always lower than that of the photon that was absorbed. So, the photon that excites the dye always has a smaller wavelength than the photon that gets emitted. This is the so-called Stokes shift. Fluorescein-based dyes for example, get excited at a wavelength around 500 nm, blue light, but emit at around 530 nm, which is green light. Dyes don't get excited by just one exact wavelength, but by a range of wavelengths around a peak, the excitation spectrum. When people talk about the excitation wavelength of a dye, they usually mean the peak of the excitation spectrum. The same is true for emission, there is an emission spectrum, characterized by a peak in the spectrum. Companies

that sell fluorescent dyes usually provide the excitation and emission spectra. Excitation spectra are often graphed as relative efficiency in photon absorption (which leads to excitation). You need to know the emission and excitation spectra of a dye to be able to pick a proper filter set to measure that dye.

Figure (3-4): LIF Spectrophotometer

3-2-8 Lasers:

Two types of lasers (solid-state lasers and semiconductor lasers) and three wavelengths (532nm, 473, and 650 nm) with a continuous wave were employed in the current study's practical tests, with the output beam of the transverse single mode (TEM00) lasers with Gaussian beam distribution. For different wavelengths (532 nm, 473 nm, 650 nm), the beam waist lasers beam is (0.5 mm, 1 mm, 1.75 mm) correspondingly.

(3-3) Preparation of the Samples and the Laser Irradiation:

an adequate venous blood sample was collected from the anti-cubital vein of healthy and unhealthy adult subjects, The blood samples were divided into two parts, the first part was put in tubes containing anticoagulant (EDTA-Tubes) to ensure obtaining plasma after centrifugation at 3000 rpm for 5 minutes. The second part of the blood was transferred into gel tubes for separation of serum after centrifugation at 3000 rpm for 5 minutes. Some of the serum samples had been treated with trichloro acidic acid solution for precipitation of serum proteins and the remaining fluid of serum after protein precipitation was collected by other plain tubes. concerning precipitated proteins of serum had been dissolved by the addition of high NaCl concentrations.

All yield components (plasma, serum, precipitated proteins, serum without proteins) were diluted with distilled water :1 (3ml final volume).

furthermore, all components were irradiated with low ladder intensity (473mm, 532, and 632nm respectively) with power (20, and 28mw, 40mw, respectively) for 10 minutes. Then after, the absorbance and emission spectra were measured for all treated samples (control). As shown in Figure (3-5).

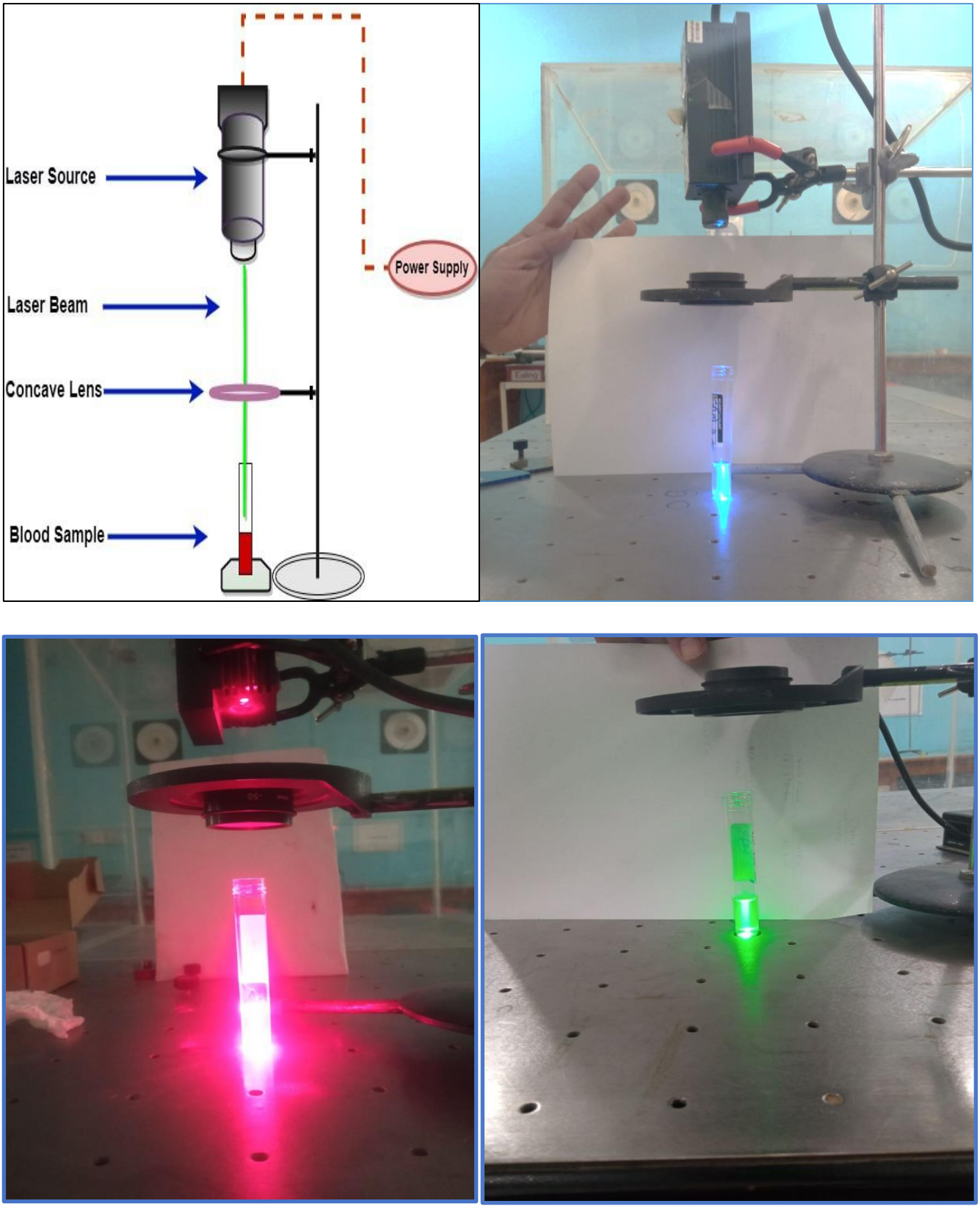


Figure (3-5): experiment setup

(3-4) Preparation of the Samples and Mix them with Copper Nanoparticles.

Available volumes of venous blood samples were taken from antecubital veins of patients with hyperbilirubinemia, arthritis patients as well as healthy control subjects the blood samples were divided into several components. The first component involved the separation of plasma from whole blood containing anticoagulant (EDTA) by centrifugation at 3500 rpm for 5 minutes. the second component is serum isolated from whole blood with our anticoagulant and centrifugation at 35000 rpm for 5 minutes. the third component is the precipitation of serum proteins by using the trichloroacetic acid solution and the precipitated proteins were dissolved with high NaCl concentration solutions.

The remaining fluid of serum after precipitation of serum proteins was kept to perform the required analysis. All component was mixed with 10 μm, 50 μm, and 100 μm of copper nanoparticles, and then the absorbance and emission spectra were evaluated. All blood components are diluted with distilled water (1 to 2) and the final volume becomes three milliliters.

(3-5) Preparation of the Samples and Mix them with Acridine Orange dye.

All prepared components of samples that were previously prepared were mixed acridine dye(0.5 to 1.5) and the final volume becomes two milliliters.

(3-6) Equipment Used in this Study :

Table (3-1) : Equipment used

Equipment	Manufacturer
UV-VIS Spectrophotometer	England
Fluorescence Spectrophotometer	China

Centrifuge	Japan
Laser device with wavelengths (473 nm, 532nm , 650 nm)	China
Copper nanoparticles	China
Acridine orange dye.	America
trichloro acidic acid	China
Plastic pipettes	China
Syringes	Germany
EDTA tube	Jordan
Stopwatch	America
plain tubes	Jordan
Capillary tubes	Germany
cotton wool	Turkey
Microscope slides	China
Tourniquet	China
Gloves	Malaysia
Power meter	China

Chapter Five
Conclusions and Future works

(5-1) Conclusions:

Through the results that were presented and discussed, a number of the following conclusions were reached:

1. Transportation of blood samples from plasma, serum, without proteins, and precipitated serum proteins give different absorbance peaks and fluorescent different densities of these samples because of different densities of these samples that result from the content proteins.
2. The presence of bilirubin in jaundice samples influences the optical proteins of samples that have different protein content and chromophores.
3. There is an interaction between CuNPs and blood components that resulted from indifferent absorbance and fluorescent properties.
4. There is a positive relationship between the content of protein samples and optical proteins.
5. The presence of biological therapy and inflammatory proteins in arthritis samples leads to increase absorbance and fluorescent activity because of increased fluorophores molecules.
6. These techniques can be employed to distinguish between blood component proteins and disease having effects on chromophores, fluorescent, and protein contents.

(5-2) Future works:

1. Isolation of proteins such as albumin globulin and clotting proteins to study what of these components have optical and fluorescent activities to diagnose those proteins alone.
2. stud of bilirubin optical properties and compared output results with hemoglobin because of bilirubin is derived from hemoglobin.
3. Isolation of other chromophores and fluorophore content of blood such as FAD, NADH, and cytochrome, and compared their optical properties with protein components of blood.

Chapter Four
Results and Dissection

Part One: Experimental Part

(4-1) Introduction:

This chapter dealt with the results of the optical properties before and after irradiation of components blood (plasma, serum, serum proteins, serum without proteins) (healthy, jaundice, and arthritis component blood samples), and the results of copper nanoparticles on the optical properties of components blood samples (healthy, jaundice and arthritis component blood samples) and results of acridine orange dye on the proteins for these samples using UV-visible spectrophotometers, fluorescence Spectrophotometer.

(4-2) Spectral Properties of Healthy Components Blood Samples before Irradiation:

Absorbance:

The absorption spectra of (healthy blood, Jaundice, and Arthritis) before and after exposure to irradiation were studied using a UV-Visible spectrophotometer, and the following are the results obtained from the study.

Figure (4-1) showed the absorption spectra of healthy blood components (plasma, serum, serum proteins, serum without proteins) before exposure to irradiation. Physically absorption is the transformation of part of the energy for the incident radiation on the material, and this energy acquired by the atoms or molecules of material is either thermal or vibration. Absorption depends on several factors, including the properties of the material, the concentration of the absorbent particles as well as the wavelength of the incident radiation, when the concentration of the material increases, this results in an increase in absorption.

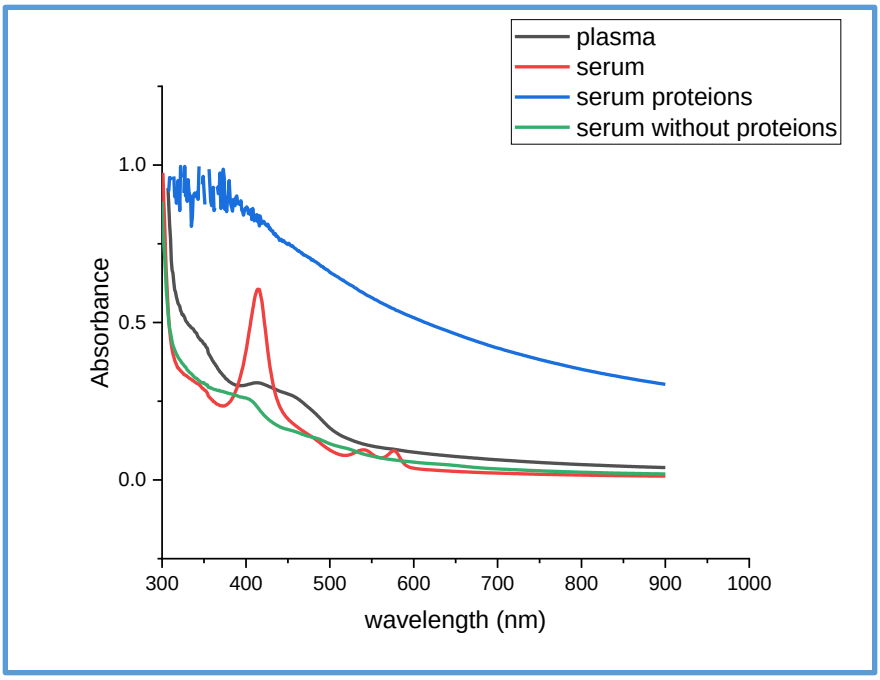


Figure (4-1): Absorbance Spectra of healthy component blood sample (plasma, serum, serum protein, and component of serum without protein).

Also, from these observations, it appears clearly that precipitated serum proteins were recorded with high levels of absorbance compared to other samples these results indicated that pure and high concentrations of proteins lead to a heightening of absorbance because of high levels of density of protein molecules. subsequently, serum recorded the second stage after precipitated proteins in the level of absorbance and these results indicated that serum has proteins molecules diluted with fluids of serum so that have low absorbance in comparison with those precipitated proteins because of fluid of serum diluted of proteins molecules and decrease its absorbance.

From the finding depicted in Figure(4-1), it was found clearly that density was a prominent factor that led to an increased absorbance of the sample. Plasma samples are denser than other samples because they have all proteins of blood especially clotting proteins and other proteins which elevate the optical density (absorbance of

plasma). On another hand, samples of serum-free protein showed lower levels of absorbance, and these findings confirm that proteins in blood have an effective factor in increasing the absorbance of light and also, they represent a major factor in the density of blood components.

(4-3) Spectral Properties of Healthy Blood after Irradiation.

A. Absorbance:

When laser light is beamed into the tissue, a tiny percentage of the light is reflected, as seen in Figure (4-2), but the majority of the laser light enters the tissue and is either absorbed or dispersed by the molecules. Water has two high absorption areas, one in the UV and one in the IR, Aromatic rings of proteins and nucleic acids have an absorption peak in the UV area between 260 and 280 nm. As a consequence, laser light in the UV range is extensively absorbed by water and proteins in the tissue, resulting in low light penetration. The same is true in the infrared area, which begins at 1.3 microns. Blood absorbs light in a wide wavelength range up to red light (630 nm), and absorption is available above 600 nm. Melanin absorbs light in the UV to the near-IR range. The absorption coefficient of tissue molecules is minimal between 600 nm and 1.3 microns, resulting in an intriguing optical window for laser light penetration into tissue[86].

Figure (4-2) shows the absorbance of samples after irradiation with (,473nm,532nm,650 nm). Three groups of the normal sample were irradiated with three wavelengths of the laser beam (650 473, and 532nm) for different output power (40, 20, and 28mw) at fixed exposure for 10 minutes for each group. One sample of blood component was left without irradiation and used as a control sample. Figure (4-2) depicts the absorbance of samples following irradiation with (473nm,532nm,650 nm) at output power (20nw, 28nw, 40nw) for a 10-minute

exposure duration. Because of the interaction between laser and molecules of material (blood component samples), the absorbance of healthy blood component samples increased after irradiation with different wavelengths. This means that the temperature of the medium increased due to the transformation of energy from incident photons to molecules of material, and then an increase in the vibrational energy of material molecules. The excitation state in medium molecules occurs when they absorb the energy of light photons, causing the medium temperature to rise. As a result, this process is significant in determining several physical properties, such as thermal conductivity.

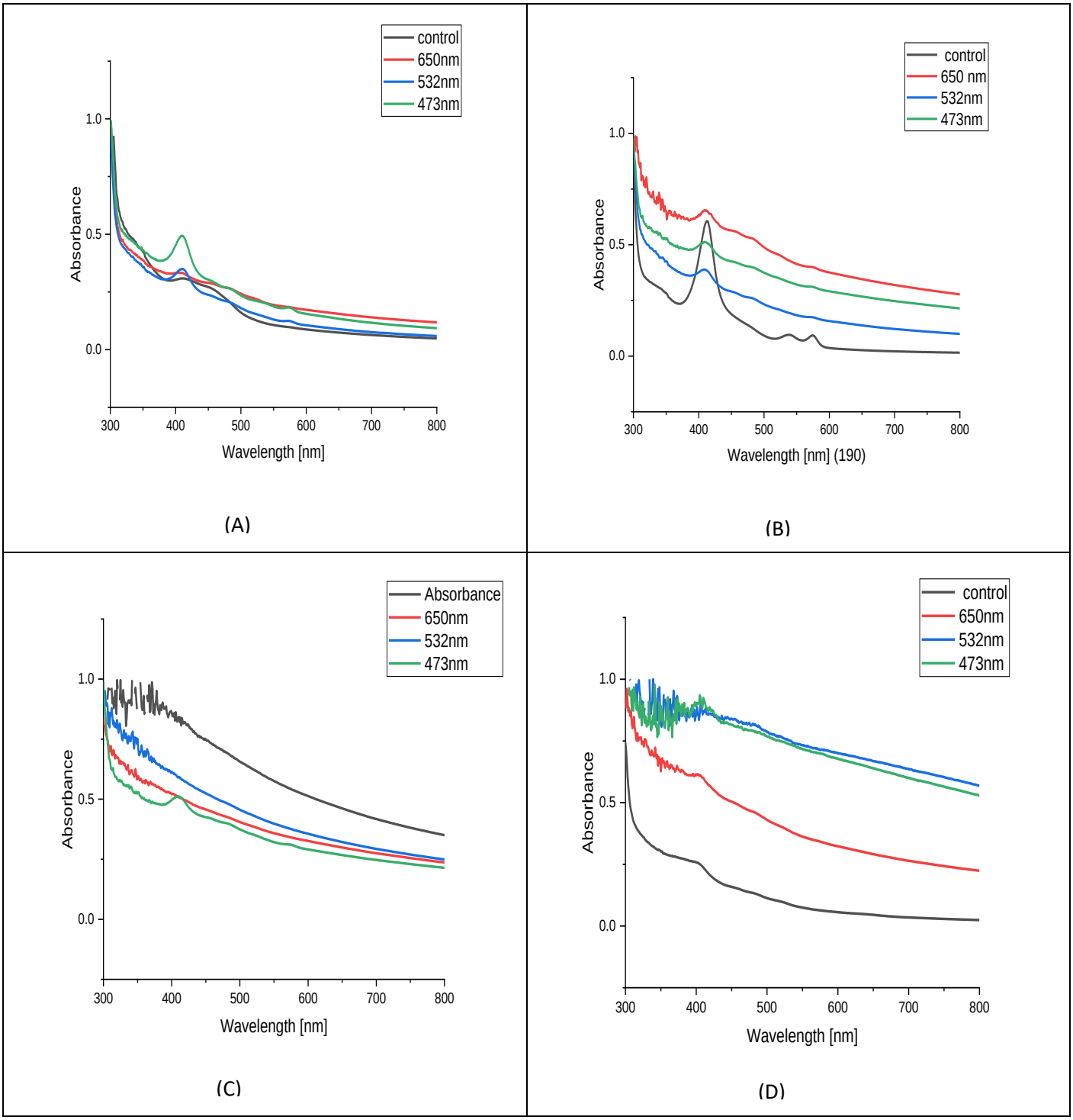


Figure (4-2): Absorbance of healthy component blood (A-plasma, B-serum, C-serum proteins D-components of serum without protein) before and after exposure to (473nm, 532nm, 650 nm)

Figure (4-2 A) showed an absorbance spectrum of plasma samples irradiated with different LLLT (473nm blue, 532nm green, and 632 nm red respectively). from data obtained from this figure, it appears to increase the absorbance (optical density) of all irradiated plasma samples in matching with no-irradiated samples (control), these findings can be attributed that laser radiation gave the ability to affect organic molecules of plasma other proteins. Also, there was found that the increase in absorbance is inversely proportionate with the wavelength of the laser beam. It appears that blue absorbance and then green 532nm and finally red 632nm, this is maybe attributed to of plasma absorbance because of conformational changes of molecules and increase their aggregation within the sample.

Regarding the effects of laser radiation on blood plasma, blood plasma was prepared and plasma samples were exposed to (650nm, 532nm and 473nm) laser beams for a period of ten minutes. It was noted that there was a difference in the peaks of the irradiated samples when compared with the non-irradiated ones. At the same time, it was noted that there was a difference in the peaks of (peaks) of samples irradiated with 532nm laser when compared with samples irradiated with 650nm laser and 473nm. The absorption spectra of a control plasma sample and a blood sample that was treated with a laser beam of wavelength (473 nm, 20 mw) because of the large increase in output power of the laser beam, the absorption value of the irradiated sample increases significantly during the exposure duration (10 minutes). The absorption spectra of control and irradiated blood sample with a laser beam of wavelength (532 nm, 28mW) for exposure time (10) min. The absorbance of irradiated sample for time (10) min increases where the peak of absorbance at (415nm) but these values increase gradually. Water absorption dominates plasma absorption in the near-infrared. Plasma absorbs a lot of light in the UV to the visible range because of the chromophores found in proteins and

other substances. Individual diversity in plasma absorption is caused by variable protein concentrations, nutritive substances, or medications, such as contraceptives. Even visual examination of plasma samples from various donors reveals a spectrum of hues spanning from yellow to green to orange to brown, as well as their transitions. Plasma 1 has a 450 nm absorption peak, which can be linked to bilirubin absorption. The absorption peak at 415 nm is frequently the most conspicuous, and it is caused by leftover RBCs or free hemoglobin from plasma formation. Furthermore, scattering particles like lipids can cause turbid plasma samples, which are normally ruled out during standard plasma quality checks.

Rayleigh scattering of protein molecules describes the scattering properties of pure plasma, resulting in a scattering cross-section that decreases with increasing wavelength. Plasma samples contain a variety of components, including molecules, and aggregations of molecules. Measuring the absorption and scattering of blood cells and plasma components determines the optical characteristics of blood. Data on blood's optical characteristics are crucial not only for various diagnostic and therapeutic applications in laser medicine but also for normal medical diagnosis. They are used in optical tomography, fluorescence diagnostics, diaphanoscopic, photodynamic treatment, and laser-induced thermotherapy to calculate the light distribution in blood-perfused tissues. The optical parameters can be used to explain light dispersion according to the transport theory. coefficient of absorption.

Plasma makes up roughly 55% of normal human whole blood. 90% of the time, it's water, and 10% of the time, it's proteins[87]. It believes that low-level laser irradiation affects the enzymes of lipid metabelian sum, improves the

cholesterol balance of plasma based on the divergence of absorption peak value change after low-intensity laser irradiation for plasma sample.

By comparing Figure (4-2 A), it can be seen that the absorbance of the irradiated sample is rising with the same range due to output power convergence, which results in an increase in the same rate of absorbance values. It was found that irradiation with low-level lasers such as 630.8 nm results in the formation of a single state of oxygen that enhances the production of reactive oxygen species (ROS) including H_2O_2, hydroxy radical, and super peroxide. ROS presented in blood serum proteins leads to damage and aggregation of serum proteins and increases their density[88].

In Figure (4-2 B) the absorption spectra of a control serum sample and serum sample that was treated with a laser beam of wavelength (632 nm, 28 mw) Because of the large increase in output power of the laser beam, the absorption value of the irradiated sample increases significantly during the exposure duration (10 minutes). It has a higher absorption rate compared to other lasers.

Regarding the effects of laser rays on blood proteins, the blood serum was prepared after the coagulation process took place. The serum samples were exposed to the laser rays (650nm, 532nm, 473) for a period of ten minutes. A difference in the peaks of the irradiated proteins was observed when compared with the non-irradiates in the same time. There is a difference in peaks of samples irradiated with 532nm laser when compared with samples irradiated with 650nm and 473nm lasers.

We noticed the appearance of the highest peak in the absorbance of the sample irradiated with the red laser, which is higher than the rest of the lasers because the red light penetrates deeper into the thick samples. The observation noted the Figure (4-2 C), revealed that all laser irradiation causes a decrease in absorbance spectra of precipitated serum proteins in a comparison with control samples. In addition, the

laser showed a variety of effects on the proteins, it showed that the green laser recorded a high peak of absorbance and then the red and blue respectively[89]. These data suggested that the effects of laser on pure proteins are different from those on plasma and serum proteins which are mixed with other molecules, and these effects may result because laser radiation exerts damage and break down of proteins also there are inter and intramolecular changes occurring in pure serum protein that led to decrease their absorbances compared to control.

A difference in the peaks of the irradiated proteins was observed when compared with the non-irradiated with the same Time There is a difference in peaks of samples irradiated with 532nm laser when compared with samples irradiated with 650nm and 473nm lasers. We noticed the appearance of an absorption peak at the blue laser (473) nm, and the reason is because it is the closest to the absorption spectrum of proteins.

Figure (4-2 D) showed the different results of absorbance of serum free from protein .It was revealed that together green and blue laser pointed out a high absorbance level at the same time the effectsfect were siilar ,on the other hand ,red laser showed low absorbance when compared with blue and green laser and a higher absorbance levelcomparisonared to control samples

In ,fact seSerumrotehave has a low density in matching with that plasma, serum, and precipitated serum proteins, but serum without protein has other organic biomolecules such as lipid, vitamins, carbohydrates as well as minerals, and ions. these components may be affected by laser radiation (green, blue, and red) because either high water contents and vitamines absorb light and also increase the heat of these molecules causing molecular interactions among described molecules and these changes were dependent inversely on a wavelength of light. It mark increasessrease absorbance with low wavelength.

(4-4) Effect of Laser Irradiation on Optical Properties of Healthy Blood sample:

From the above calculating the optical properties of healthy blood before and after irradiation, where in this paragraph the compared compered. In general, from the results the absorption is increased when exposing different wavelengths of the laser at exposure time (10) minutes, this is due to the absorption properties of light by molecules and this property depends on the molecular environment and mobility of chromophores. The absorption process provides information about the chemical composition and structure of the cell, when the light strikes the molecule suffers (absorption, transmission, and reflection), the magnitude of absorption in the sample depends on the concentration the of absorption component, when the component of absorption increase, absorption increased and the transmission decrease ed with small portion of reflection. The absorbed photon is exited to another state and release the energy to achieve stability, this phenomenon is important in effects in tissues.

There is a clear difference between absorbance spectra wavelengths at exposure time (10 min) because of an increasing in output powers of laser radiation were used and this illustrate in table (1):

Sample	Wavelength (nm)	Time of exposure (min)	Absorbance
plasma	412	10	0.47
serum	410	10	0.65
serum proteins	411	10	0.51
serum without protein	405	10	0.59

table (1): Peak of absorbance of healthy component blood (A-plasma, B-serum, C-serum proteins D-components of serum without protein) after exposure to (473nm, 532nm, 650 nm)

B. Fluorescence:

Fluorescence is a type of luminescence (emission of photons or light energy). It is known that when a molecule absorbs a photon (light), the acquired energy promotes the passage of the molecule itself from the ground state to an excited state. Conversely, when a molecule emits light, the energy of the molecule decreases by an amount equal to the energy of the released photon. Because some energy is lost in the process, the emitted fluorescent photon typically exhibits a lower frequency and a longer wavelength than the exciting photon it absorbed[90].

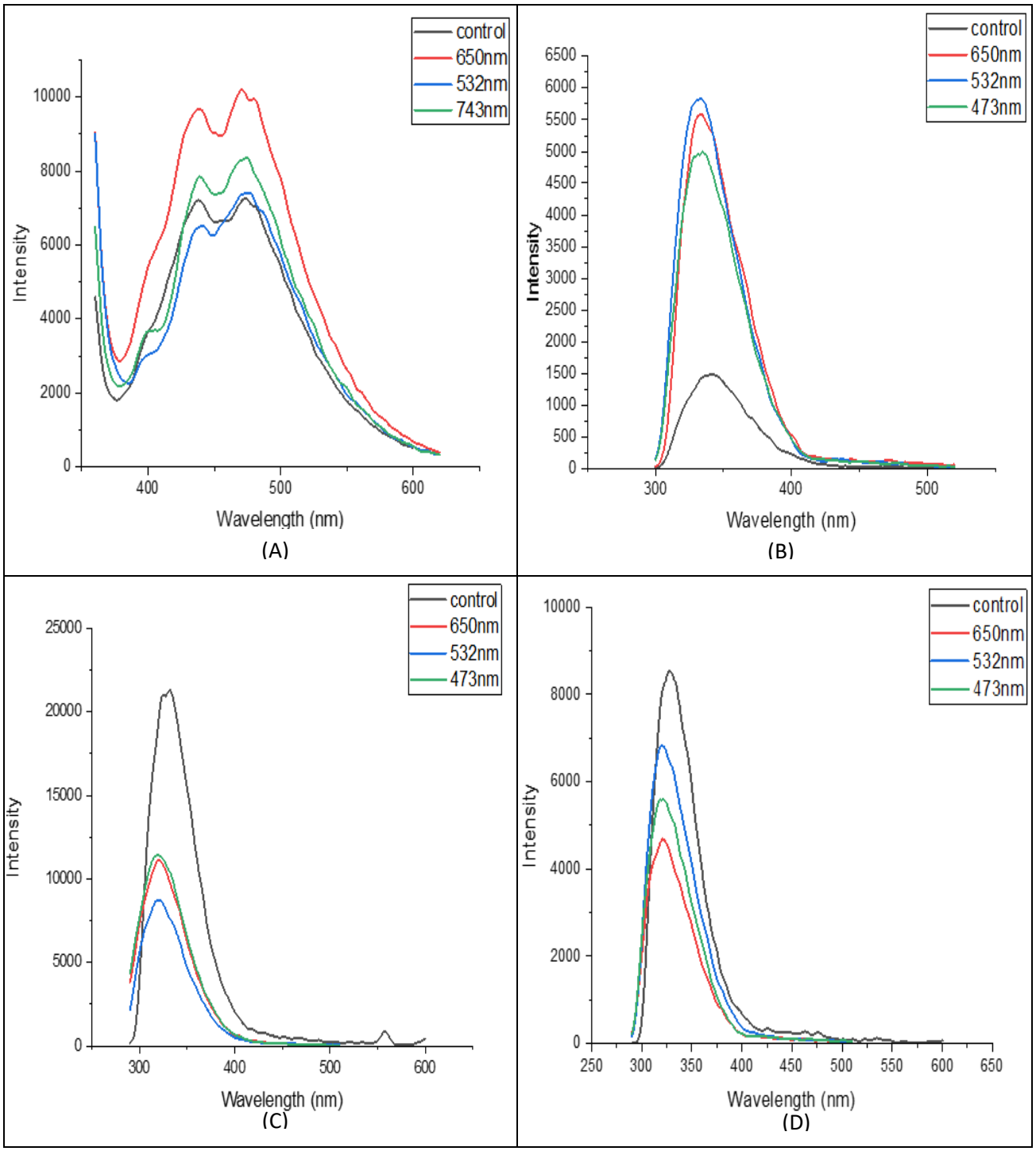


Figure (4-3): Emission spectra of healthy component blood samples (A-plasma, B-serum-serum proteins D-components of serum without protein) before after exposure to (473nm, 532nm, 650 nm)

In figure (4-3 A) It was well noted that results which were adopted in this figure, plasma samples which exposed to red laser showed high peak level of fluorescent intensity and there flowed by blue laser and finally green laser. these results were consistent with the facts indicated that red laser (high wave length) can be absorbed by components of plasma molecules in particular proteins that have fluorophores amino acids (tyrosine, tryptophan) and absorption of the red laser by water molecules increase heat content of plasma and laser maybe ender of biomolecules more excited.

Native fluorescence of bioliquids and tissues is extensively used for detection of pathological processes in the human organism. This approach is based on the evaluation of native molecular fluorophores emission, which depends on metabolic and pathological conditions of the organism. For instance, numerous works are devoted to cancer detection using tissue and (less frequently) blood serum fluorescence.

Figure (4-3B) Because of their similar biosynthetic origin, involvement in the same processes, and presence as significant extracellular components of the circulatory system, serum proteins form a dynamic system with varied biological activities. The intrinsic fluorescence intensity at a wavelength of excitation corresponding to tryptophan or tyrosine fluorescence, as well as surface hydrophobicity, were used to investigate the unfolding of human serum proteins (HSP). Human serum albumin (HSA) and human serum globulin (HSG) have maximum emission wavelengths (max) of 336.0 and 337.0 nm, respectively. A reduction in fluorescence intensity, a shift in emission maxima, and a rise in surface hydrophobicity, all of which indicated protein unfolding. Disruption of protein structure is blamed for differences in fluorescence behavior[91] .

Protein crystals are routinely imaged using their inherent protein fluorescence.

Tryptophan fluorescence accounts for the majority of this. Because protein fluorescence is so faint, it necessitates the use of very strong UV light sources and extremely sensitive cameras. However, because of the extended exposure durations necessary to get substantial data, intense UV light sources may damage the protein.

There are special characteristics of laser radiation having unpleasant and its usefulness in several applications one of them is based on excitation of chromones that absorb radiation visible or infrared wave length spectra, in biological fluids that exposed to infrared laser, molecules of water are excited and raise to maximum levels of vibrational state[92]. Also, there is evidence that proposed infrared laser can evoke photo-chemical interactions[93]. Figure (4-3C) The precipitated serum protein had low fluorescent peaks compared to that non-irradiated these data can be produced as a result of the effects of laser radiation on conformational structures of proteins and these changes occurs at a level of molecules of intra-molecules can attenuate the level of fluorescence compared with control.

Figure (4-3 D) Also, from these presentations appear that the levels of the fluorescent peak were low in irradiated samples of serum without proteins compared with control and these results were attributed to molecular changes of organic and inorganic molecules remaining in serum after protein precipitation and laser light can affect these molecules and changes the structures of the inflorescent molecules. Most, if not all tissues of body organs and fluids have many various fluorescent molecules other than protein in particular NADPH, Porphyrin, and Pyridoxal acid--. On the bases of results documented in Figure (10), it may be laser radiation affects fluorophores and maybe prevent the excitation of fluorophores molecules remaining in the serum without proteins.

Output power (mW)	Wavelength (nm)	Time of exposure (min)	The peak of fluorescence (Intensity)			
			plasma	serum	serum proteins	serum without protein
40	632	10	10056	5495	11056	4649
20	473	10	8341	4958	11396	5565
28	532	10	7383	5793	8418	6757

Table (2): Peak of Fluorescence of healthy component blood (A-plasma, B-serum, C-serum proteins D-components of serum without protein) after exposure to (473nm, 532nm, 650 nm)

(4-5) Spectral Properties of Blood Patient with Jaundice after Irradiation.

A. Absorbance:

The Figures (4-4) illustrated in (A) involved irradiation of plasm, serum, and proteins of Serum, without proteins (with different wavelengths (red 650 nm, green 532 nm, and blue 473 nm) The documents explained high peak absorption in laser 532 nm of plasma other than a laser, the effects of laser radiation on biological molecules (organic molecules) especially protein involved excitation of atoms of endogenous chromophores and liberation free radicals. in addition, the high intensity of plasma proteins can increase absorption wavelength this is occurring in samples of patients having high bilirubin content compared to control (without radiation)

the same samples of a patient with hyperbilirubinemia, the serum samples were irradiated with the different lasers the results in figure (B) the absorbance peaks of different lasers of samples showed there is blue laser no significant high peak absorption compared with other lasers, irradiated samples, these output results can be returned to that the absorption spectra of serum proteins similar among then and laser irradiation causing moderate effects of those proteins components and other biomolecules contain in serum samples of Jaundice patients

These are different peaks of absorption of precipitated proteins it the showed in figure(C) that blue laser recorded a significantly higher peak more than other lasers, and control, this is may resulted from proteins components can absorb the spectrum of blue laser other than laser of maybe the blue laser caused more excitation of molecules and atoms of bilirubin in this sample.

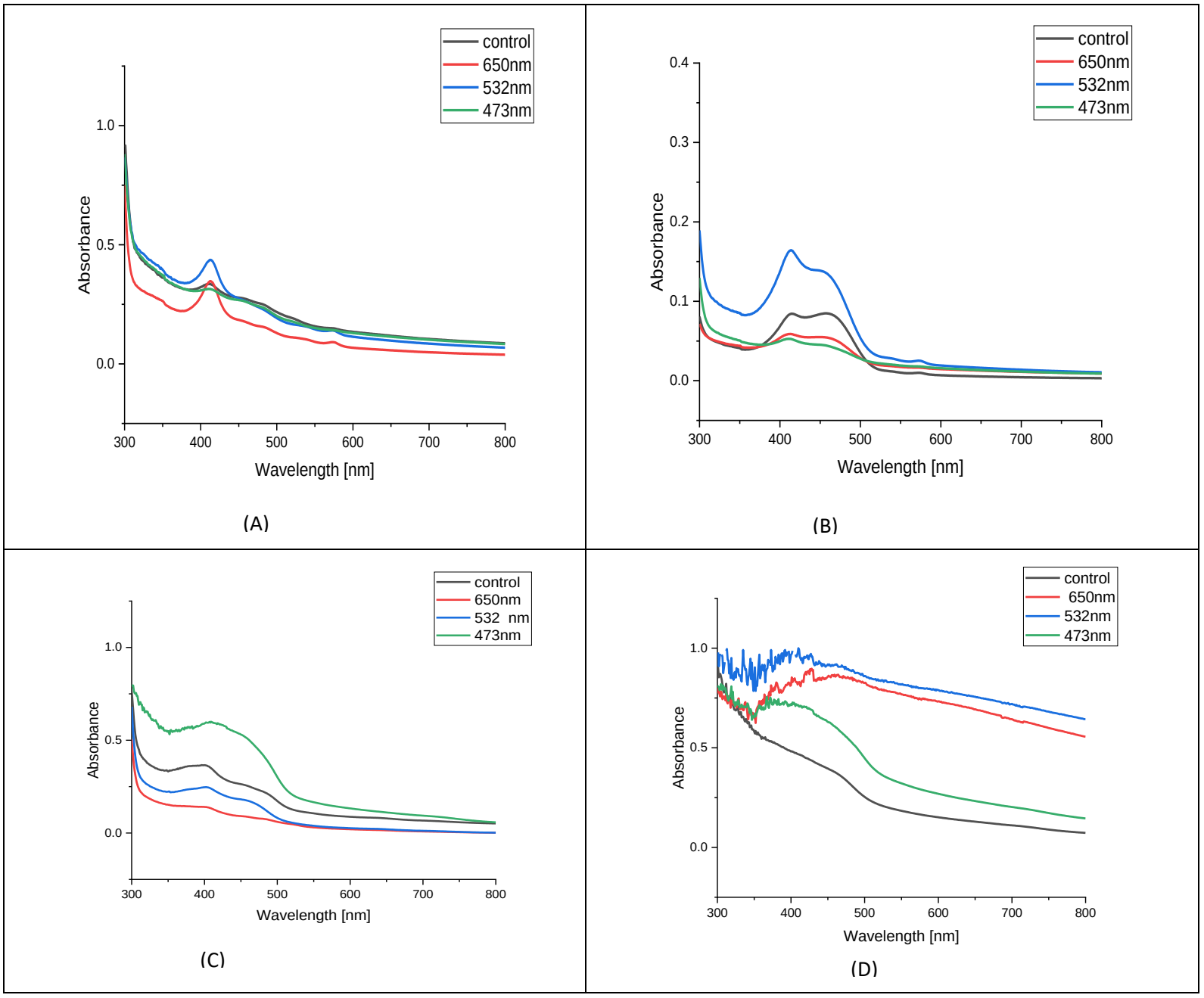


Figure (4-4): Absorbance of up healthy component blood (A-plasma, B-serum, C-serum proteins D-components of serum without protein) before and after exposure to (473nm, 532nm, 650 nm)

Samples of serum without proteins (D), the results of irradiated jaundice sample with mentioned laser also yield fluctuation in absorption peaks of irradiated jaundice sampled since green laser pointed out a high peak compared to those,

another laser followed by red and blue. these results can produce from different interactions among bilirubin molecules and other organic molecules that might be affected by laser energy which form multiple interactions among them, these interactions may be results in because of oxidative damage resulting from free radicals on those proteins.

Sample	Wavelength (nm)	Time of exposure (min)	Absorbance
plasma	412	10	0.46
serum	410	10	0.15
serum proteins	411	10	0.6

table (3): Peak of absorbance of unhealthy component blood (plasma, serum, serum proteins components of serum without protein) after exposure to (473nm, 532nm, 650 nm)

B. Fluorescence.

the fluorescent criteria of samples (plasma, serum, precipitated serum proteins, and serum-free proteins). the fluorescent peak fluctuated according to the types of the tested sample, it was well found that plasma and serum (A, B) recovered significant-high peaks of fluorescent activity in red laser compared to other samples irradiated with different laser wavelengths. On the other hand, the peaks picked from precipitated serum proteins and serum-free proteins indicated increased fluorescent waves emitted from irradiated samples with a blue laser and followed by other emitted light. These observations may return to different densities of examined samples, that is, the samples decrease their densities according to the isolated protein that intern affects quantum emitted and graded different absorption. In addition, bilirubin interacts with anticeident light and emitted light, so these molecules can prefer the antecedent light 520 - 570 nm to other laser light.

from a molecular point of view, the biochemical reaction of bilirubin molecules indicates two poorly overlapping bands of both absorption and fluorescent spectra.

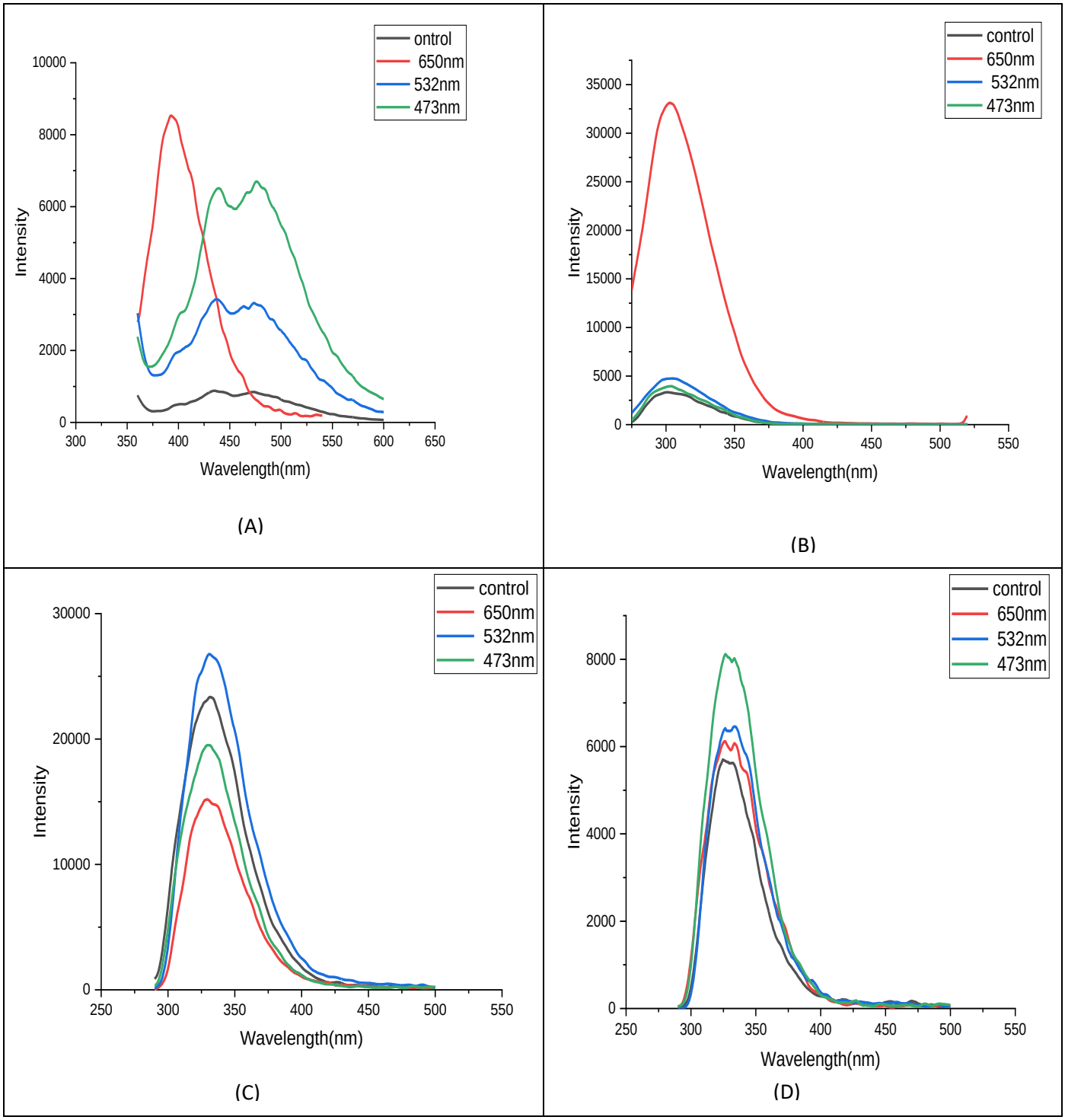


Figure (4-5): Emission spectra of jaundice component blood samples (A-plasma, B-serum-serum proteins D-components of serum without protein) before after exposure to (473nm, 532nm, 650 nm)

(4-6) Spectral Properties of Blood Patient with Arthritis after Irradiation

A. Absorbance:

The spectrum of absorbance for blood with arthritis before and after irradiation with (473nm,532nm,650nm) states in figure (4-6). The absorbance of blood with arthritis before irradiation (control sample) decreases compared with healthy blood before irradiation.

The results which are obtained from irradiation of arthritis patients' samples (plasma, serum, precipitated proteins, and serum-free proteins), the absorption spectra are also different among the samples, since the plasma samples pointed out a higher emitted peak with green lager irradiated samples, whereas serum samples give a maximum absorption peak in red color laser the precipitated proteins and serum-free protein recorded a lower significant decrease of absorption activities. It's not surprising that these irradiated samples have different densities, especially since they differ in protein contents that they have essential fluorescent molecules, however, the presence of inflammatory proteins and treatment with biological therapy (proteins in nature) can interfere with absorption and excitation properties of these molecules.

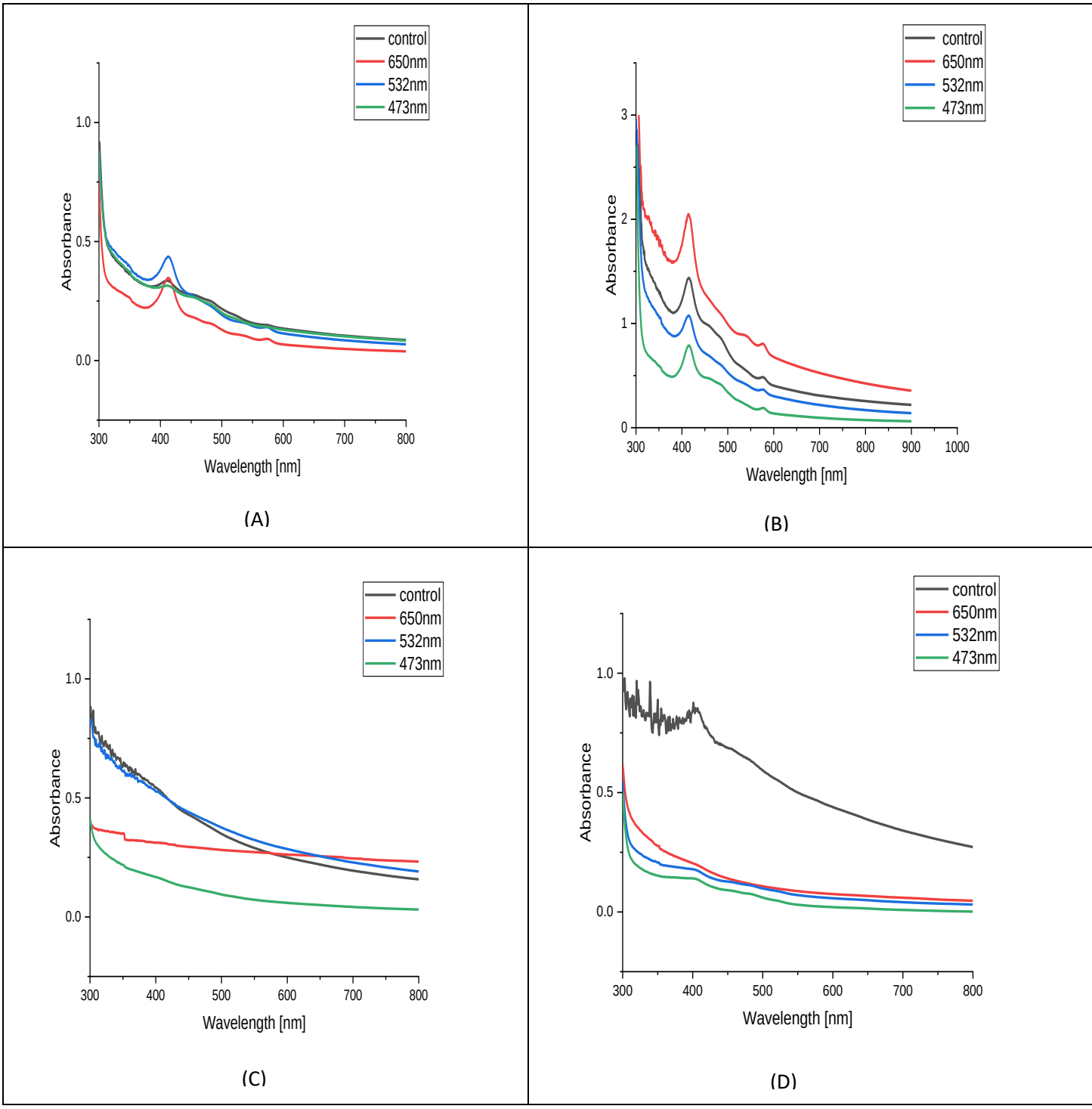


Figure (4-6): Absorbance of arthritis component blood samples (A-plasma, B-serum, C-serum proteins D-components of serum without protein) before and after exposure to (473nm, 532nm, 650 nm)

Sample	Wavelength (nm)	Time of exposure (min)	Absorbance
plasma	412	10	0.47
serum	410	10	0.15

table (4): Peak of absorbance of unhealthy component blood (plasma, serum) after exposure to (473nm, 532nm, 650 nm)

B. Fluorescence.

the quantum yield of emission spectra of an irradiated sample of patients with arthritis showed increased excitation intensity of irradiated plasma and serum samples with blue light laser and these results appear to conflict with emitted spectra of precipitated proteins and serum-free proteins since there is a blue laser and green laser-irradiated samples have the same intensity in precipitated proteins and no significant peak in free serum compared to control (without irradiated free serum proteins).

the recorded data produces from the various factors especially that affects the conformational stability of proteins molecules because of laser irradiation, since the laser can influence the stability of organic molecules in the blood (proteins) and the absorption spectra of the selected laser in addition to the intensity and type laser can affect the excitation of fluorophores molecules of proteins and tend to interact with low wavelength and high energy laser. The content and types of proteins in the different samples can produce different emission peaks depending on the type of proteins and the fluorescent molecules in their traction.

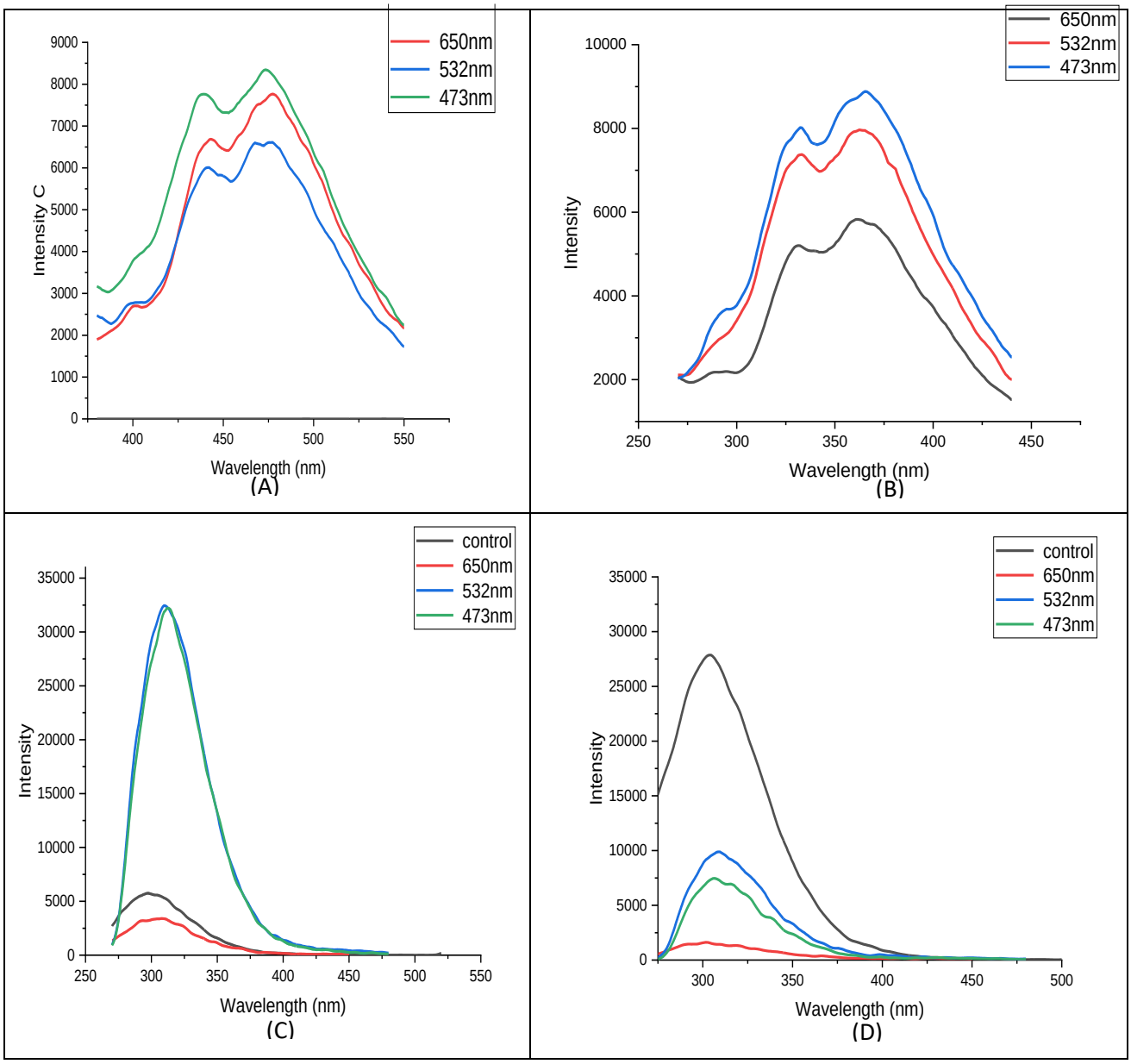


Figure (4-7): Emission spectra of arthritis component blood samples (A-plasma, B-serum, C-serum proteins D-components of serum without protein) before after exposure to (473nm, 532nm, 650 nm)

(4-7) Spectral Properties of Healthy Blood Samples Before and after Adding Copper Nanoparticles

A. Absorbance

protein binding to NPs results in changes in the NP or proteins' absorption spectra, which may be used to assess the binding[94]. The size and aggregation state of the NP protein complex, as well as the local dielectric surroundings, influence the shift and broadening of the absorption spectra [95]. Although quantitative and convincing re- findings are difficult to produce, UV/vis spectroscopy may be utilized to assess NP protein binding. When compared to bare nanoparticles, protein nanoparticle complexes have a distinct size distribution, with the possibility of forming NP dimer and NP trimer conjugates, which will contribute to the total absorption spectra in a different way. UV/vis is quicker, more versatile, and less difficult than other approaches, but it is not definitive and must be used in conjunction with other spectroscopic and structural studies[96] .

It's well known that nanoparticles when presented in biological fluids, cause interactions with organic molecules in particular proteins and this interaction forms protein corona adsorbed on the surfaces of proteins as a result of electrostatic, hydrophobic, and van der Waals forces[97]. Also, these interactions among proteins and nanoparticles lead to changes in stability, dispensability, and biodistribution of protein molecules[98]. The protein corona is established and surrounds the particles, these proteins make a complex ring adsorbed are albumin, immunoglobulin, glycoproteins, and apolipoproteins[99].

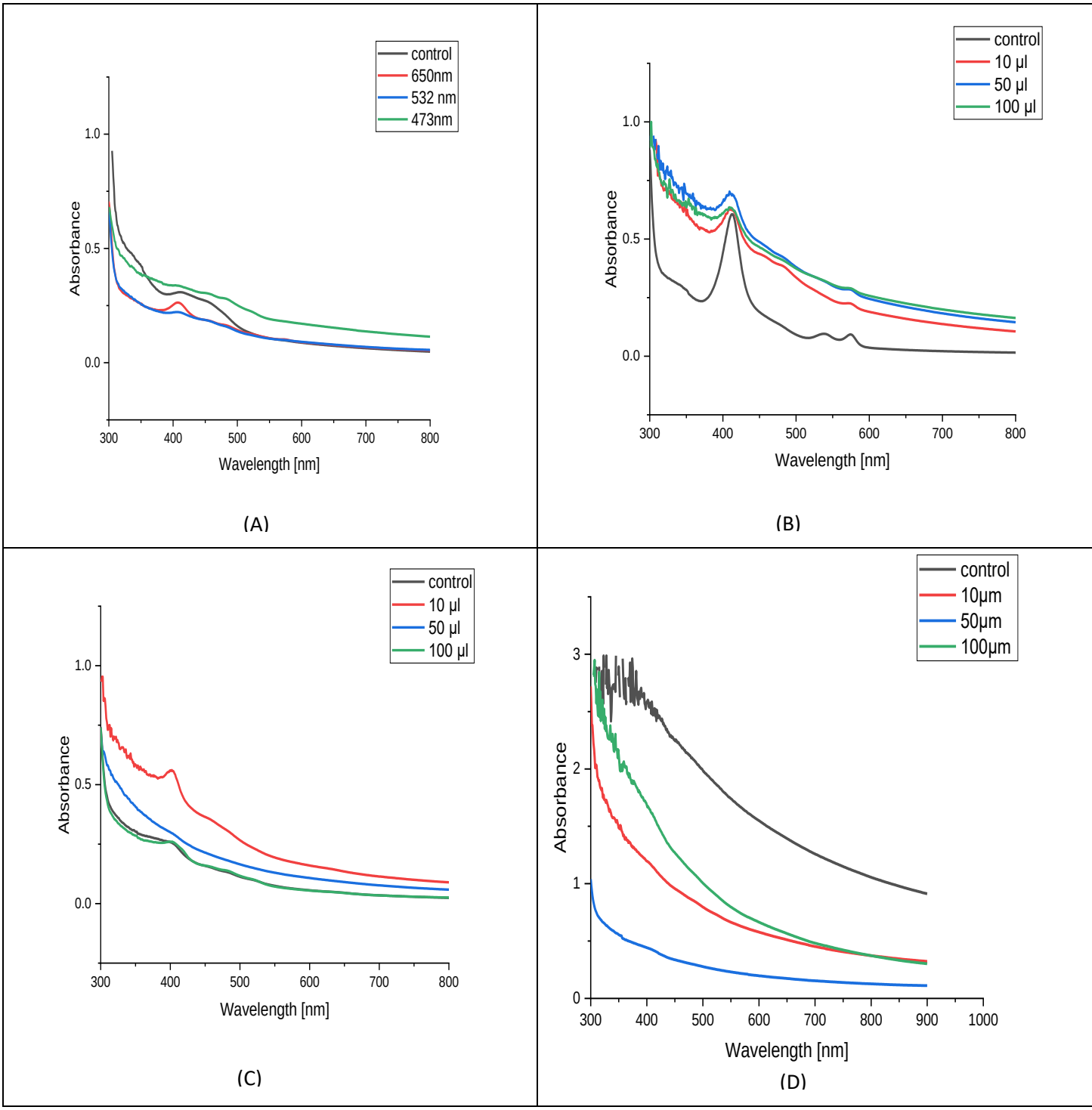


Fig (4-8) Absorbance spectra of normal component blood samples (plasma, serum, serum protein, component of serum without protein) mixed with three constructions from cu nanoparticles

From data documented in Figure (4-8), sample (A) they explained that plasma samples treated with CuNP indicated an increased peak of absorption of samples mixed with 100 µl of NP in matching with control and other treatments, on other hand, samples with 10 µl and 50 µl CuPN recorded lower absorption compared with control. These observations can result from a high concentration of CuNP having the ability to interact with protein ad increase its density and formation.

Serum samples (B) treated with CuNPs indicated high absorbance peaks of all treatments compared with the control that perhaps serum proteins without clotting proteins have high affinity for CuNPs and have high affinity to NP either due to the presence of electrical charges or there is a pathway enhance the formation of new molecular interaction among NPs and several serum proteins.

Precipitated serum proteins (C) display a significant increase in absorption of samples treated with NPs, especially with 10 µl and then 50 µl. These high-level absorption spectra indicated that these pure proteins have a high affinity to bind with NPs at different concentrations and these products and affinities result from proteins being pure and adsorb the NPs on their surface stronger than control.

Serum without protein (D): there are lower absorbance spectra of all treated samples compared with non-treated, these results may be the NPs non-interact with protein because no proteins presented. in addition, NPs might bind with non-protein organic compounds that have not absorbed light because of their distribution within the fluid of samples.

Sample	Wavelength (nm)	Time of exposure (min)	Absorbance
plasma	412	10	0.25
serum	410	10	0.7
serum proteins	411	10	0.6

table (5): Peak of absorbance of healthy component blood (plasma, serum, serum proteins.

B. Fluorescence.

Fluorescence spectroscopy may be used to analyze the structural and dynamic features of proteins that have been labeled with fluorescent probes. To minimize large conformational changes in the protein's natural structure, the fluorescent labeling must be well-designed. Furthermore, if the dye has a stronger affinity for the NP surface than the protein's functional groups, we may argue that adding a dye can change the protein's interaction with the NP. Because the excited fluorescent state lasts for nanoseconds, which is the time scale of many essential biological activities such rotational motion of protein side chains, molecule binding, and structural changes, fluorescence spectroscopy is sensitive to protein dynamics. Fluorescence emissions from NPs may be observed when they are innately luminous or tagged using fluorescence probes. Steady-state or time-resolved fluorescence spectroscopy may be used to detect NP protein binding [100].

Stepwise single-molecule photobleaching, also known as fluorescence resonance energy transfer (FRET). Trp groups' intrinsic protein fluorescence may also be utilized to track changes in the protein microenvironment as a result of NP binding. The interaction between proteins and NPs in a physiological buffer has recently been studied using fluorescence correlation spectroscopy (FCS). In a very tiny confocal volume, FCS measures the intensity of fluorescently tagged particles. When a tagged NP enters or exits the volume, the measured fluorescence changes,

and the time it takes the particle to cross the volume can be calculated, assuming Stokes-Einstein diffusion structural information about the item may be gleaned. The denaturation destabilizes proteins causing them to unfold, this leads to an increase in fluorescent intensity, tryptophan and tyrosine are the essential and natural fluorophores in proteins, and their fluorescent intensities are quenched when proteins are folded. As proteins become folded, this leads to removing quenching and increases the fluorescence intensity of targeted amino acids tyrosine and tryptophan.

Results of emissions of blood components treated with (10,50,100 µl) of CuPNs are depicted in figure (4-9) plasma fluorescent spectra (A) showed there is a significant elevation of emission spectra of treated samples (10,50,100 µl**)** NPs respectively compared to control these findings can be explained that plasma has high organic molecules especially proteins (clotting and non-clothing proteins) which have fluorophores amino-acids and maybe NPs can increase binding among protein molecules and enhance their fluorescent.

Serum components (B) treated with CuNPs showed a high peak of fluorescence in samples with 100 µl CuNPs. The peaks of 50 µl and 100 µ**l** samples are more closely similar and near control at a high peak in contrast with (10 µl**)** NPs. These results may be indicated that NPs with high concentration can increase fluorescent activity or increase electron excitations of targeted molecules, but low concentration gives the opposite effects.

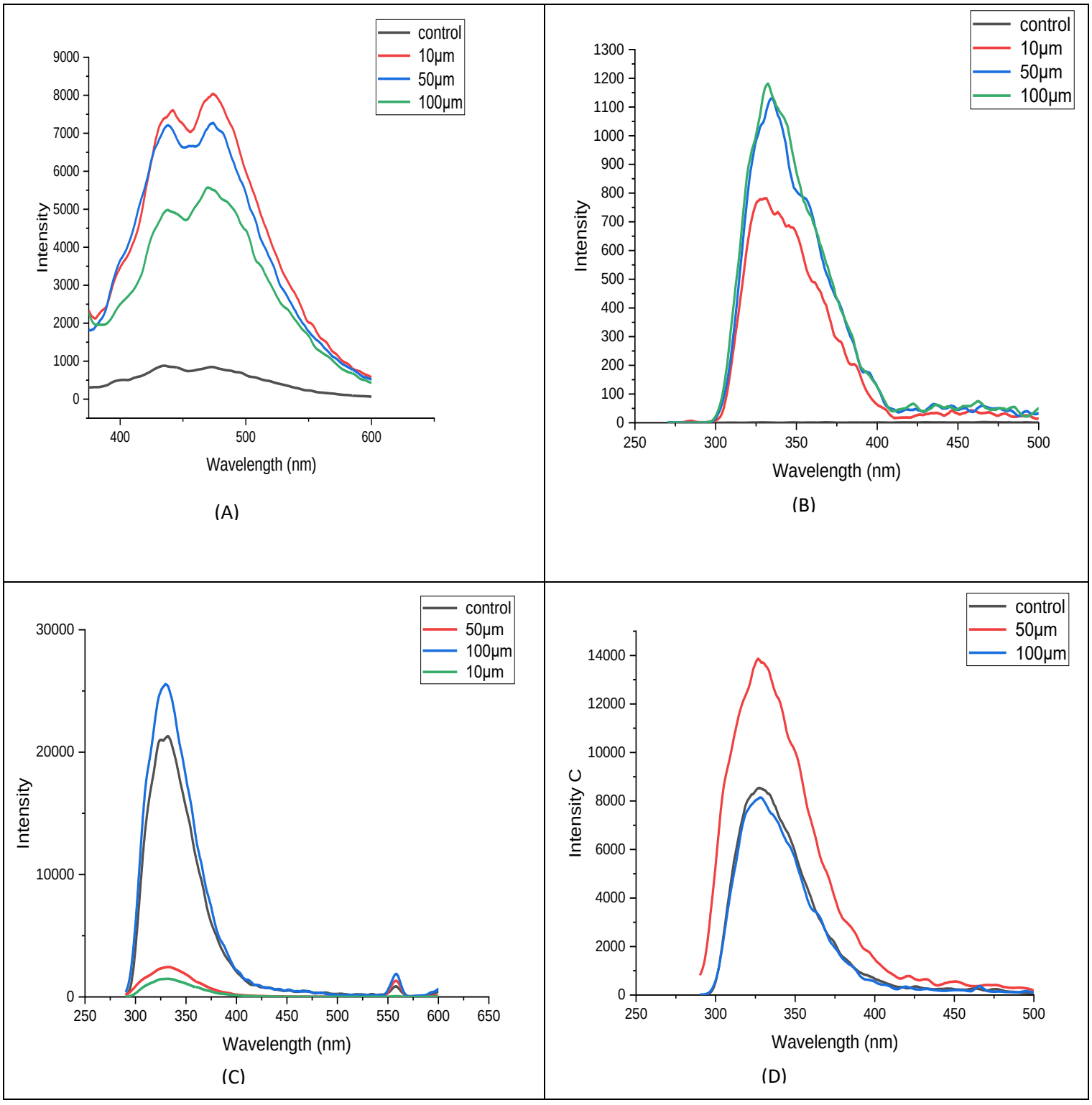


Figure (4-9) emission spectra of normal component blood samples (plasma, serum, serum protein, component of serum without protein) mixed with here construction from Cu nanoparticles.

Precipitated proteins (C) treated with CuNPs, their fluorescent spectra recorded a significantly higher peak at 50 µl CuNP with the lower peak at 10 and 100 µl CuNPs from these observations appears that proteins behave differently whatever absorption of accident light and their interactions with selective concentrations of NPs which nay intern fluorophores molecules of proteins.

Serum-free proteins (D) treated with CuNPs, indicated a significant increase in fluorescence spectra at (10 µl) CuNPs compared to other treatments, the serum-free proteins have been many fluorescent molecules including FAD, NADPH, pigment, and 50M fluorescent amino acids and these molecules may interact with different of NPs which might change their emission ability of affect electron excitations within fluorophore molecules.

(4-8) Spectral Properties of Arthritis Blood Samples before and after Adding Copper Nanoparticles

A. Absorbance

Results in Figure (4-10) of blood components isolated from patients addicted to arthritis and treated with biological therapies. The results pointed out different peak absorption spectra of different blood components (control), plasma, serum, precipitated proteins, and serum-free proteins of arthritic patients administered with biological fluids. and these blood components are treated with different CuNPs concentrations (10 µl, 50 µl, and 100 µl) respectively. it appears in figure A (plasma) that the concentration of 100 µl gives a high absorbance spectrum. this result showed that is multiple protein NPs interaction that might depend on the concentration of NPs or also clotting proteins have more affinity to NPs.

Result of serum (B) and serum-free proteins (C) indicated absorbance spectra

nearly equal and similar with differences in the peak of spectra with small moderate peaks in serum, these observations can be explained that proteins in serum are dissolved and mixed with other organic molecules which implicated among proteins to increase their density and arrangements.

Concerning results of serum-free proteins (D) there are different absorption spectra 50µl NPs recorded a high peak more than control in contract 10 µl then 100 µl recorded a lower peak. these fluctuations in absorbance spectra can be returned to the ability of NPs materials to bind with biomolecules whiter molecules have more affinity to react with NPs or those that can interact with a suitable concentration of NPs to increase the absorbance peaks.

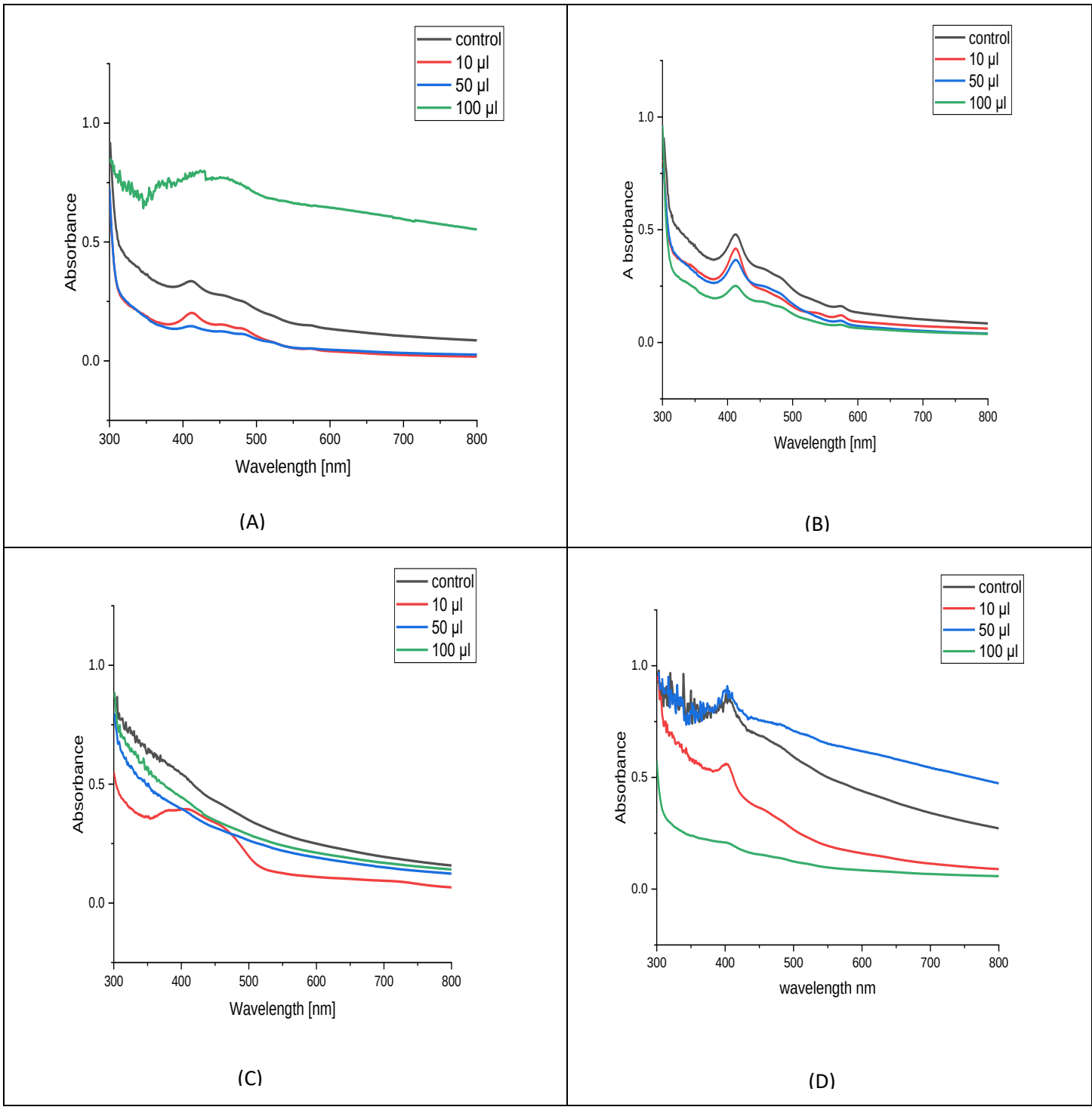


Figure (4-10) Absorbance spectra of up normal component blood samples (A -plasma, B-serum, C- serum protein, D component of serum without protein) mixed with three constructions from cu nanoparticles.

From a biological point of view, nanoparticles can interact with available biomolecules including proteins, nucleic acids, lipids, and even vitamins and other metabolites, these interactions are based on the specific properties of nanoparticles such as nanosized and large surface to mass ratio from this interaction is adsorption of proteins on the surface of nanoparticles[101].

Proteins contain specific amino acids that can absorb light at UV-spectrum, of these amino acids are tyrosine and tryptophan. is well documented that albumin has a maximum absorption band around 204 nm due to the n/p+ transition of C]O and a weak absorption band at 277 nm assigned to the P/P+ transition from the phenylalanine, these absorption peaks are still observable in UV-vis spectra[102].

Sample	Wavelength (nm)	Time of exposure (min)	Absorbance
plasma	412	10	0.36
serum	410	10	0.5
serum proteins	411	10	0.4
Serum without proteins	411	10	0.6

table (6): Peak of absorbance of unhealthy component blood (plasma, serum, serum proteins Serum without proteins).

B. Emission spectra:

Figure (4-11): Plasma samples (A) of patients with arthritis showed a high fluorescent peak with treated with 50 µl of CuNPs followed by 10 µl and then 100 µl of CuNPs compared to control. Plasma samples are rich with different types of protein in particular clotting protein, so it's not surprising to produce a significant peak of fluorescent spectra because of the high content of the differences among treated samples maybe fluorophores amino acids and other fluorophores molecules

but the differences among treated samples maybe back to different interaction pathway of CuNPs with fluorophores and their effects on excitation of electrons of these molecules.

Serum samples (B) of arthritis patients treated with different concentrations of CuNPs, recorded a significant elevation of emission spectra at the intensity with visible light but these peaks are closely related one to another so that these observations can be analyzed that serum proteins conduct the dame pathway to interact with NPs of excitation of their elections are different to lesser extent differ than od plasma proteins also inflammatory proteins and biological protein therapy might contribute in these output peaks. Precipitated proteins (C) of arthritis samples treated with CuNPs had been shown different internists of fluorescent spectra within UV and visible spectra of light and the results of 50 µl and 100 µl resemble intensity of control but are located in the visible range and they have a higher peak than 10 µl of CuNP. These data may reflect the interactions of NPs with pure proteins and their effects on electrons excitations which differ from one molecule to another and biological therapy and inflammatory proteins.

Serum-free proteins (D) of arthritis samples with NPs also resemble the precipitated proteins to a lesser extent and are different in their intensities and low than control and located near-visible spectrum. It can be suggested that non-protein molecules can excite at hearing visible spectra when treated with NPs and the absorption of light by their electron differ from control because NPs may affect the absorption of light and excitation of their electrons.

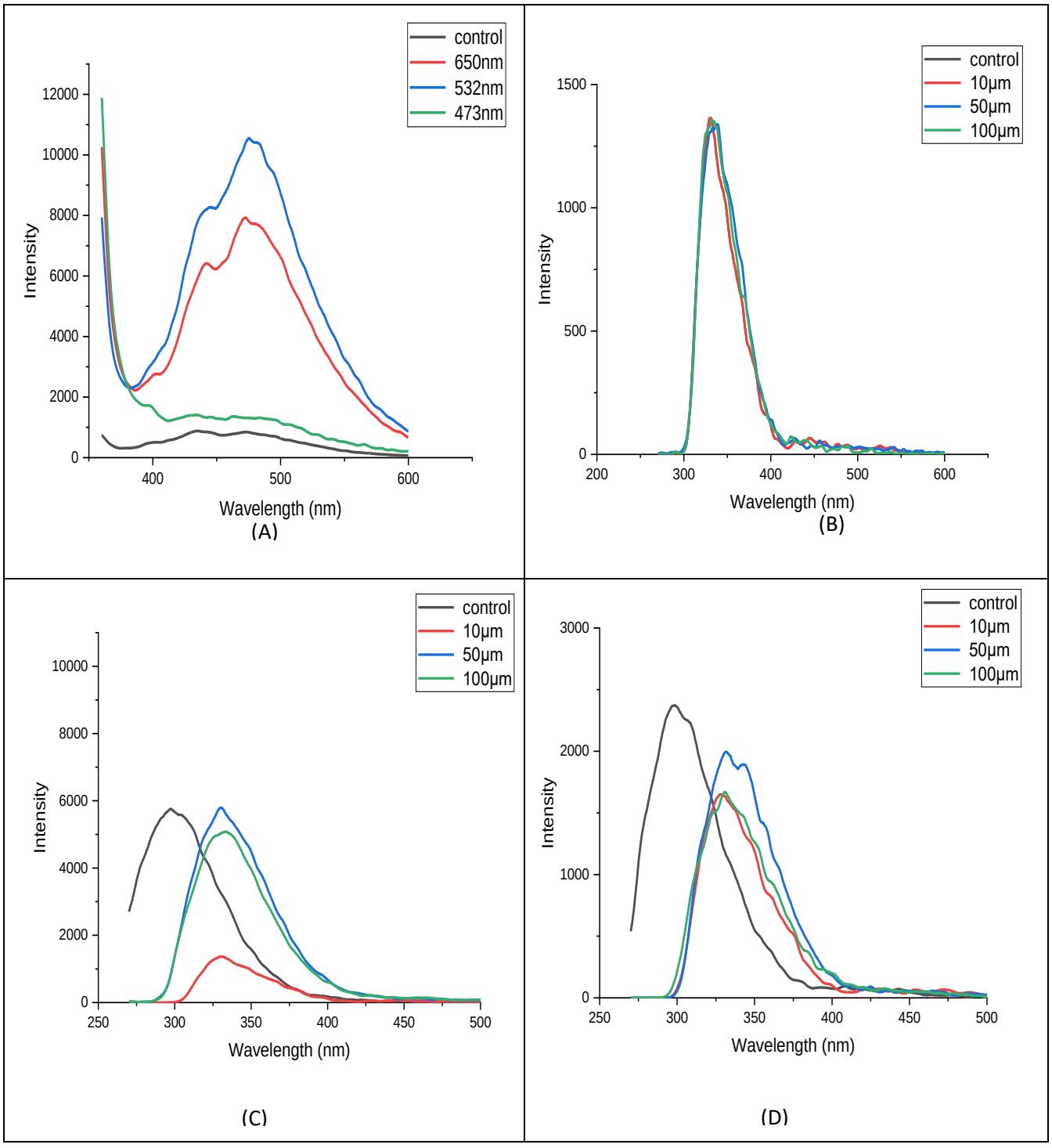


Figure (4-11) Emission spectra of up normal component blood samples (plasma, serum, serum protein, component of serum without protein) mixed with three constructions from cu nanoparticles

(4-9) Spectral Properties of Jaundice Blood Samples before and after Adding Copper Nanoparticles

A. Absorbance

Data shown in figure (4-12A) had been formed from the absorbance spectra of plasma samples of a patient with jaundice and treated with different concentrations of CuNPs (10 µl **,**50 µl**,**100 µl**)** showed high absorbance peaks in 10 NPs followed by 50 µl and 100 µl CuPNs compared to control. These observations may be a return to the high affinity of plasma proteins with NPs and their interaction may be more complex because of the presence of coagulation proteins to increase their densities.

Serum component (B), its absorbance peaks appear more similar, interacted, and approximately at the same scales. This is because serum proteins have the same affinity to bind and interact with NPs and go for the structure to absorb the light at the lower limit of the visible spectrum and there is no significant difference with control. Precipitated proteins (C) produced absorption spectra at different peaks when treated with CuNPs, of these results, there is a high peak at 100 µl NPs followed by 50 µl and 10 µl MNPs, these presented data perhaps return to the fact that precipitated proteins have abilities to bind with high concentrations of NPs and increase their density and its stereo-distributions, and the NPs can interact proportionate directly with its concentration.

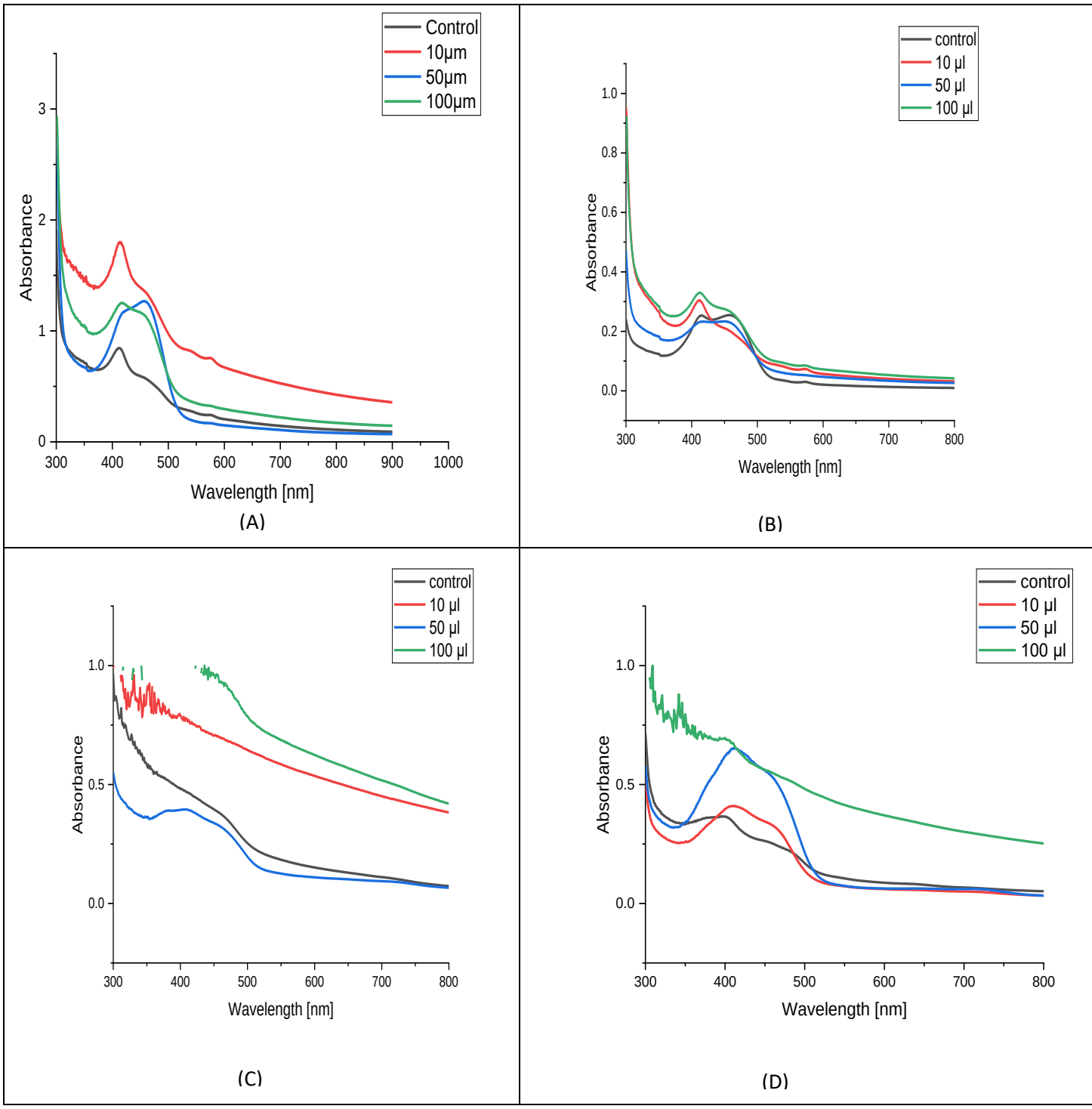


Fig (4-12) Absorbance spectra of up normal component blood samples (A-plasma, B serum, C-serum protein, D- component of serum without protein) mixed with three constructions from cu nanoparticles.

Serum without proteins (D) of parents with jaundice and teated with CuNPs, the results of absorption indicated a high peak with 100 µl and then with 10 µl on NPs, these findings can be explained on the basis that bilirubin alone or bounding with a high concentration of NPs increase density of the solution and also can absorb wavelength with the beginning of visible spectra or serum-free protein appear has selectively to interact with the specifications available concentration of NPs.

Sample	Wavelength (nm)	Time of exposure (min)	Absorbance
plasma	412	10	0.36
serum	410	10	0.65
serum proteins	411	10	0.4
Serum without proteins	411	10	0.6

table (7): Peak of absorbance of unhealthy component blood (plasma, serum, serum proteins Serum without proteins).

B. Emission Spectra:

Figure (4-13): Plasma samples (A) of jaundice treated with different concentrations of CuNPs (10, 50, 100 µl), It appears different emission spectra of treated samples since 50m and 100 µl NPs sample recorded high peak within visible spectrum in contrast to 10 µl NPs recorded lower intensity compared to control. These observations give simple explanations about the abundant protein content of plasma and their fluorophores contents also bilirubin that leads to differences in their interaction with NPs which perhaps change their absorption of antecedent lights and emitted lights.

Serum samples (B) of jaundice treated with CuNPs, these samples pointed out a significant lowering in the intensity of fluorescent spectra of all treatments

compared to those of non-treated (control), these findings can result from bilirubin which might be implicated in the decrease of excitation of fluorophores electrons as well as bilirubin (organic molecules) can absorb an antecedent light and decrease excitation of other fluorescent molecules or act as a barrier to prevent electron citation.

Precipitated proteins (C) of jaundice samples treated with CuNPs, the emission spectra of treated samples were similar to those o serum samples but at lower intensity compared to control. The marked explanation of these output spectra also results because of the role of bilirubin which can be exerted inhibitory of isolation barrier either absorption of drop lights and the binding of CuNPs either with proteins or with bilirubin molecules inhibit the fluorescent activity of fluorophores.

Serum-free proteins (D) it had also been noted that these lower emission spectra such as serum and precipitated proteins and the suggested explanation also can be a return to the presence of bilirubin which exerts inhibitory roles of fluorophores molecules other than proteins. In addition, the bindings of CuNPs if directly or indirectly with bilirubin and fluorophores can decrease or decrease the absorption and excitation of electrons to the source of light, it’s the major cause.

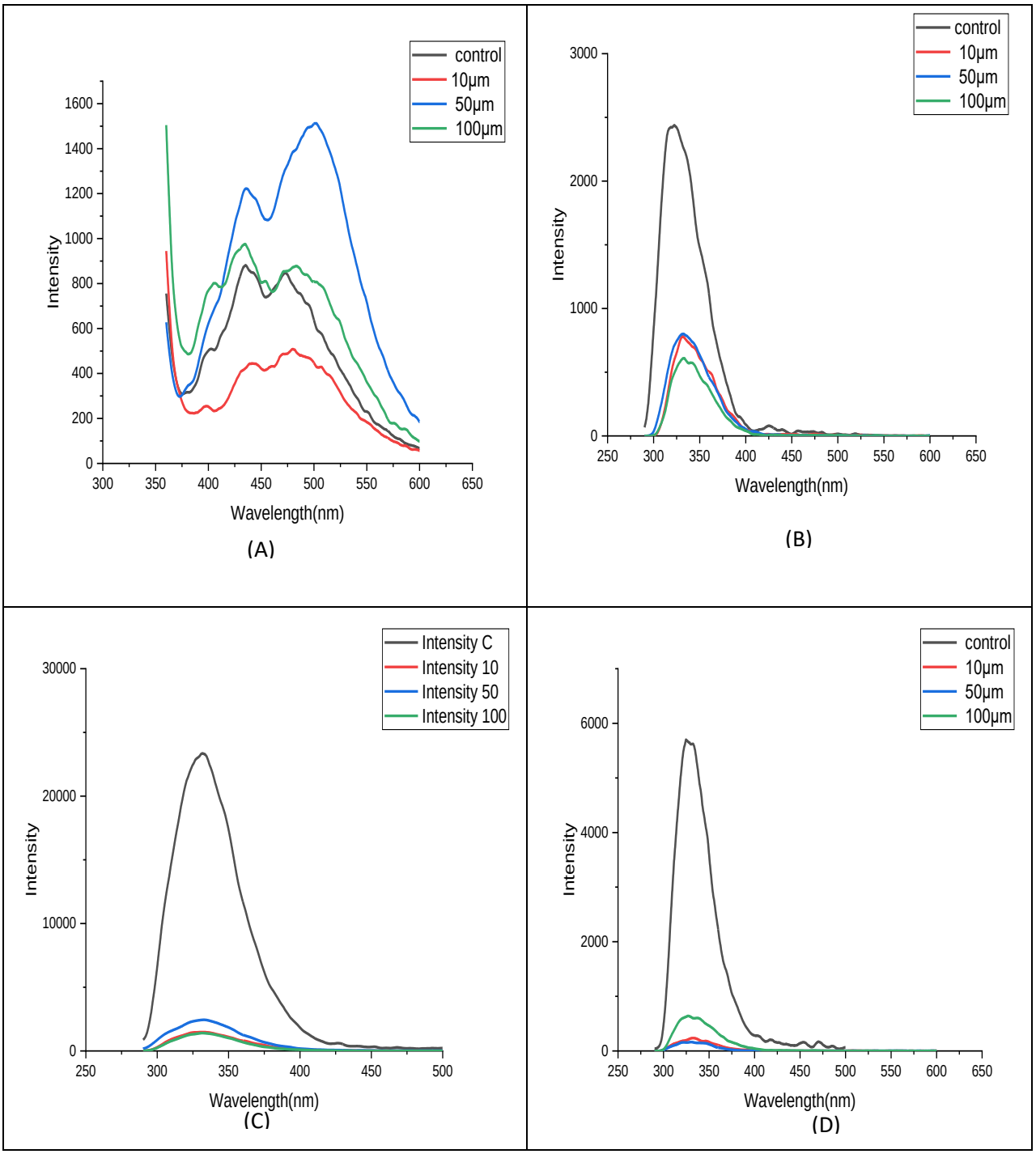


Fig (4-13) Emission spectra of up normal component blood samples (plasma, serum, serum protein, component of serum without protein) mixed with three constructions from cu nanoparticles

The chromophore of bilirubin explains the presence of at least two partially overlaying bands of both absorption and fluorescent emission spectra and accounts for interchromophore excitation transfer events responsible for emission sensitivity to the molecular environment and excitation wavelength. Bilirubin fluorescence emission under excitation at 366 nm at which bilirubin absorption is very low. Fluorescence excitation spectra of pure bilirubin in solution with solubilizing agents observed at 520 and 570 nm showed a wide region in the 430-510 nm range similar to the absorption profile[103].

(4-10) Spectral Properties of (Healthy, Jaundice and Arthritis) Components Blood samples after Adding Acridine Orange.

A. Absorbance

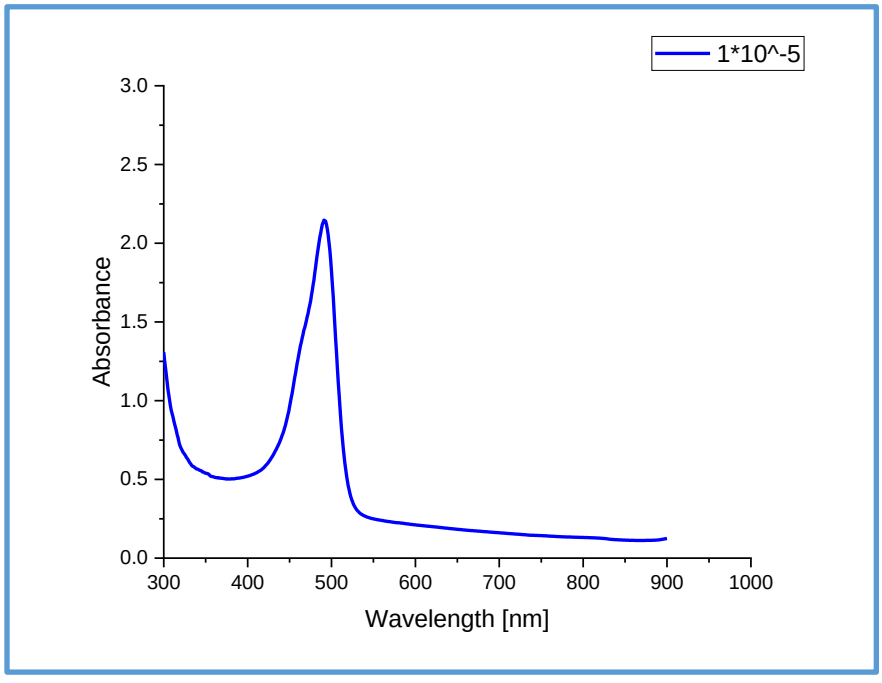


Fig (4-14) Absorbance spectra of acridine orange.

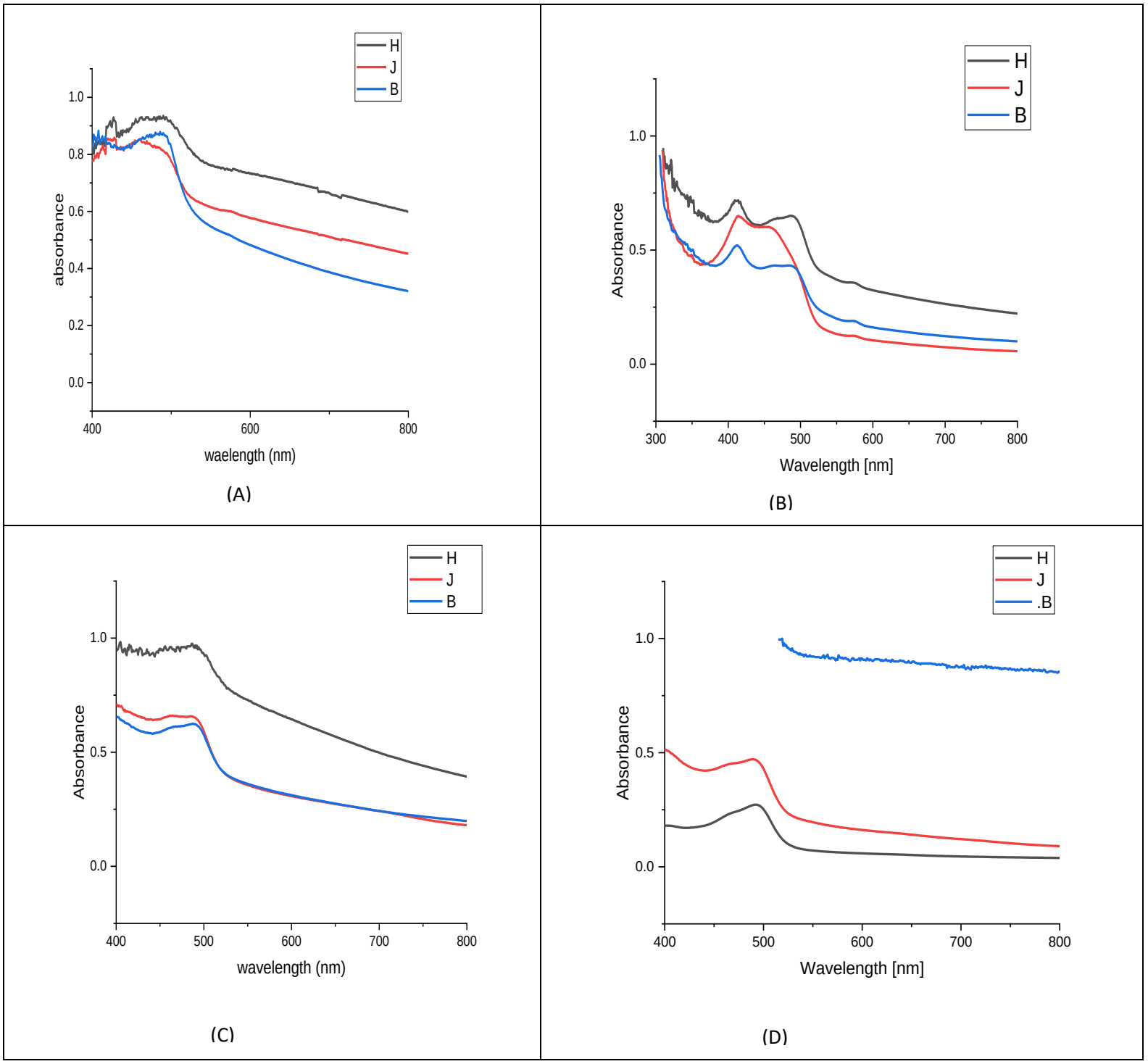


Fig (4-15) Absorbance spectra of normal and up normal component blood samples (A-plasma, B-serum, C-serum protein, D- component of serum without protein) mixed with acridine orange

The absorbance spectra of samples mixed with acridine dye (samples of (J) jaundice, (B) arthritis and (H) healthy) these samples also classified into plasma, serum, precipitated proteins and serum free proteins).

The absorption light spectra of plasma ,serum and serum free proteins have high absorption rate for healthy in compared with those samples of jaundice and arthritis patients ,these results can attributed to have ability to interact with acridine dye and increase its absorption peak in contrast with jaundice (hyper bilirubinuria),the bilirubin is a chromophore molecules and has yellow color which is the same color of bilirubin ,and elevation of inflammatory proteins and biological therapy (antibody) might several molecular distribution within medium of samples.

Sample	Wavelength (nm)	Absorbance
plasma	490	0.36
serum	412	0.7
serum proteins	490	0.68
Serum without proteins	488	0.48

table (8): Peak of absorbance of healthy and unhealthy component blood (plasma, serum, serum proteins Serum without proteins) .

B. Emission spectra

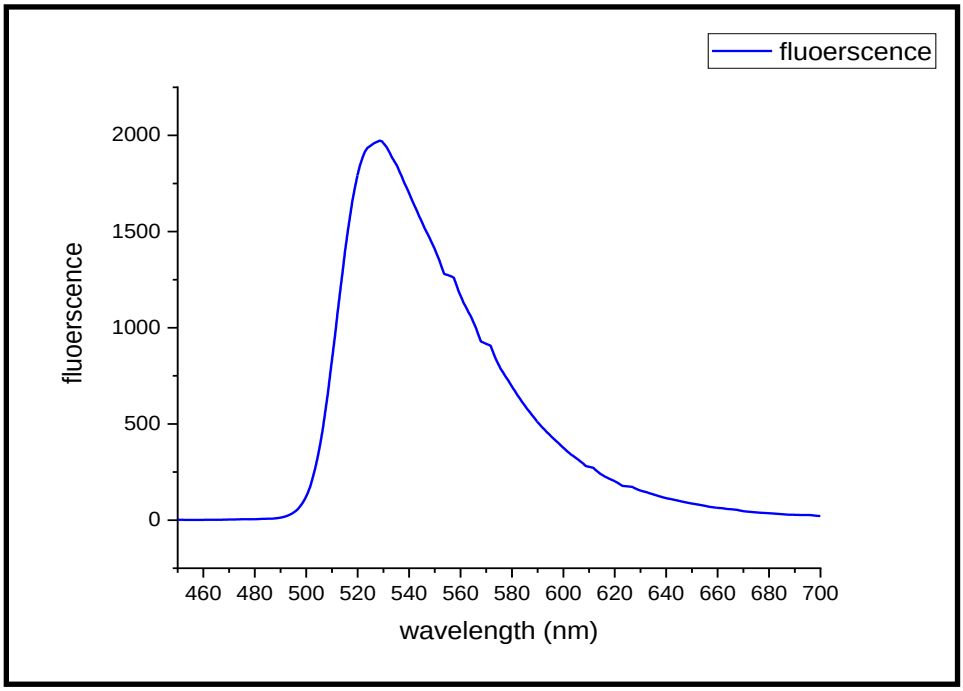


Fig (4-16) Emission spectra of acridine orange

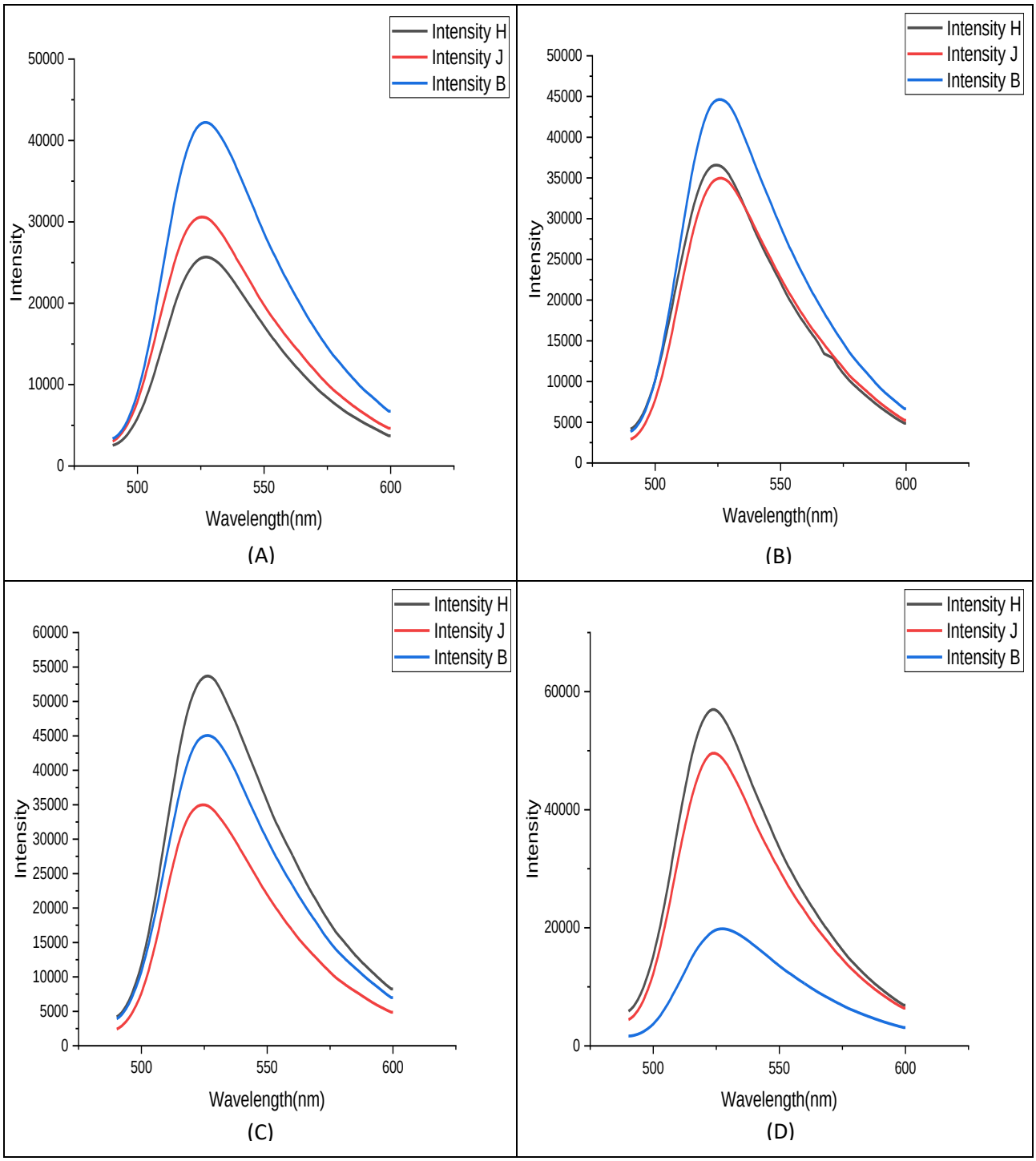


Fig (4-17) Emission spectra of normal and up normal component blood samples (A-plasma, B-serum, C-serum protein, D- component of serum without protein) mixed with acridine orange

The fluorescent emission of acridine that mixed with tested samples also have different intensities according to type of samples this is, it results from preference of relations and interactions of acridine with different protein content in samples and also effect of bilirubin and aromatic rings of amino acids involved with proteins of samples there for, the emission peaks are varies among tested samples.

As well as, the density of plasma, serum, precipitated proteins and serum free proteins are different causing additional factor in increase or decrease the content of fluorescent molecules that can synergize with acridine or interfere with it.

(List of Figures)

	component of serum without protein) mixed with acridine orange	

References:

References

[1] A. Katzir, *Lasers and optical fibers in medicine*. Elsevier, 2012.

[2] H. N. MARKOLF, *Laser-tissue interactions: fundamentals and applications*. SPRINGER NATURE, 2020.

[3] M. A. Ansari, R. Massudi, and M. Hejazi, "Experimental and numerical study on simultaneous effects of scattering and absorption on fluorescence spectroscopy of a breast phantom," *Opt. Laser Technol.*, vol. 41, no. 6, pp. 746–750, 2009.

[4] T. Vo-Dinh, *Biomedical photonics handbook: biomedical diagnostics*. CRC press, 2014.

[5] G. R. Fowles, *Introduction to modern optics*. Courier Corporation, 1989.

[6] R. W. Waynant, *Lasers in medicine*. CRC press, 2011.

[7] K. Freeman, "Lasers: The Perioperative Challenge," *AORN J.*, vol. 80, no. 5, pp. 959–960, 2004.

[8] M. L. Wolbarsht, *Laser applications in medicine and biology*, vol. 1. Springer, 1971.

[9] M. A. Ansari and E. Mohajerani, "Mechanisms of laser-tissue interaction: I. optical properties of tissue," 2011.

[10] B. Mvula, T. Mathope, T. Moore, and H. Abrahamse, “The effect of low level laser irradiation on adult human adipose derived stem cells,” *Lasers Med. Sci.*, vol. 23, no. 3, pp. 277–282, 2008.

[11] M. Csele, *Fundamentals of light sources and lasers*. John Wiley & Sons, 2011.

[12] P. Kaššák, L. Sikurova, P. Kvasnicka, and M. Bryszewska, “The response of Na/K-Atpase of human erythrocytes to green laser light treatment,” *Physiol Res*, vol. 55, pp. 189–194, 2006.

[13] E. Casals, T. Pfaller, A. Duschl, G. J. Oostingh, and V. Puntes, “Time evolution of the nanoparticle protein corona,” *ACS Nano*, vol. 4, no. 7, pp. 3623–3632, 2010.

[14] T. Cedervall *et al.*, “Detailed identification of plasma proteins adsorbed on copolymer nanoparticles,” *Angew. Chemie Int. Ed.*, vol. 46, no. 30, pp. 5754–5756, 2007.

[15] R. Landsiedel *et al.*, “Testing metal-oxide nanomaterials for human safety,” *Adv. Mater.*, vol. 22, no. 24, pp. 2601–2627, 2010.

[16] M. Lundqvist, I. Sethson, and B.-H. Jonsson, “Protein adsorption onto silica nanoparticles: conformational changes depend on the particles’ curvature and the protein stability,” *Langmuir*, vol. 20, no. 24, pp. 10639–10647, 2004.

[17] M. Lundqvist, I. Sethson, and B.-H. Jonsson, “Transient interaction with nanoparticles ‘freezes’ a protein in an ensemble of metastable near-native conformations,” *Biochemistry*, vol. 44, no. 30, pp. 10093–10099, 2005.

[18] S. S. Karajanagi, A. A. Vertegel, R. S. Kane, and J. S. Dordick, "Structure and function of enzymes adsorbed onto single-walled carbon nanotubes," *Langmuir*, vol. 20, no. 26, pp. 11594–11599, 2004.

[19] M. P. Monopoli *et al.*, "Physical− chemical aspects of protein corona: relevance to in vitro and in vivo biological impacts of nanoparticles," *J. Am. Chem. Soc.*, vol. 133, no. 8, pp. 2525–2534, 2011.

[20] M. Gasser *et al.*, "The adsorption of biomolecules to multi-walled carbon nanotubes is influenced by both pulmonary surfactant lipids and surface chemistry," *J. Nanobiotechnology*, vol. 8, no. 1, pp. 1–9, 2010.

[21] E. Hellstrand *et al.*, "Complete high-density lipoproteins in nanoparticle corona," *FEBS J.*, vol. 276, no. 12, pp. 3372–3381, 2009.

[22] L. Vroman, "Effect of adsorbed proteins on the wettability of hydrophilic and hydrophobic solids," *Nature*, vol. 196, no. 4853, pp. 476–477, 1962.

[23] C. Ge *et al.*, "Binding of blood proteins to carbon nanotubes reduces cytotoxicity," *Proc. Natl. Acad. Sci.*, vol. 108, no. 41, pp. 16968–16973, 2011.

[24] A. Verma and F. Stellacci, "Effect of surface properties on nanoparticle–cell interactions," *small*, vol. 6, no. 1, pp. 12–21, 2010.

[25] J. W. E. Worrall, A. Verma, H. Yan, and V. M. Rotello, "'Cleaning' of nanoparticle inhibitors via proteolysis of adsorbed proteins," *Chem. Commun.*, no. 22, pp. 2338–2340, 2006.

[26] S. H. D. P. Lacerda *et al.*, "Interaction of gold nanoparticles with common

human blood proteins," *ACS Nano*, vol. 4, no. 1, pp. 365–379, 2010.

[27] Z. J. Deng, M. Liang, M. Monteiro, I. Toth, and R. F. Minchin, "Nanoparticle-induced unfolding of fibrinogen promotes Mac-1 receptor activation and inflammation," *Nat. Nanotechnol.*, vol. 6, no. 1, pp. 39–44, 2011.

[28] A. E. Nel *et al.*, "Understanding biophysicochemical interactions at the nano–bio interface," *Nat. Mater.*, vol. 8, no. 7, pp. 543–557, 2009.

[29] S. Gorinstein *et al.*, "Intrinsic tryptophan fluorescence of human serum proteins and related conformational changes," *J. Protein Chem.*, vol. 19, no. 8, pp. 637–642, 2000, doi: 10.1023/A:1007192017291.

[30] D. Siposan and L. Adalbert, "Experimental study of low level laser radiation effects on human blood cells," *Vopr Kurortol Fizioter Lech Fiz Kult*, vol. 4, pp. 10–13, 2003.

[31] L. H. Theodoro, J. E. C. Sampaio, P. Haypek, L. Bachmann, D. M. Zezell, and V. G. Garcia, "Effect of Er: YAG and Diode lasers on the adhesion of blood components and on the morphology of irradiated root surfaces," *J. Periodontal Res.*, vol. 41, no. 5, pp. 381–390, 2006.

[32] J. Zhou, J. K. Chen, and Y. Zhang, "Theoretical analysis of thermal damage in biological tissues caused by laser irradiation," *Mol. Cell. Biomech.*, vol. 4, no. 1, p. 27, 2007.

[33] J. Zhang, N. Marquina, G. Oxinos, A. Sau, and D. Ng, "Effect of laser acupoint treatment on blood pressure and body weight—a pilot study. J Chiropr Med

[Internet]. 2008 Dec [citado 7 Mar 2012]; 7 (4):[aprox. 7p.].” .

[34] A. A. Ibrahim, “The Effect Of Laser Radiation And Viscosity Of Red Blood Cells On Erythrocytes Sedimentation Rate (ESR),” *Tikrit J. Pure Sci.*, vol. 15, no. 3, 2010.

[35] G. J. P. L. de Oliveira, L. H. Theodoro, E. Marcantonio Junior, J. E. C. Sampaio, and R. A. C. Marcantonio, “Effect of Er, Cr: YSGG and Er: YAG laser irradiation on the adhesion of blood components on the root surface and on root morphology,” *Braz. Oral Res.*, vol. 26, no. 3, pp. 256–262, 2012.

[36] L. Shang and G. U. Nienhaus, “In Situ Characterization of Protein Adsorption onto Nanoparticles by Fluorescence Correlation Spectroscopy,” *Acc. Chem. Res.*, vol. 50, no. 2, pp. 387–395, 2017, doi: 10.1021/acs.accounts.6b00579.

[37] J. H. Randrianalisoa, L. A. Dombrovsky, W. Lipiński, and V. Timchenko, “Effects of short-pulsed laser radiation on transient heating of superficial human tissues,” *Int. J. Heat Mass Transf.*, vol. 78, pp. 488–497, 2014.

[38] S. Rathore and B. Ali, “Effect of laser radiation on Electrical conductivity of Human blood,” *Int. J. Sci. Environ. Technol.*, vol. 3, no. 1, pp. 286–290, 2014.

[39] L. P. da S. Sergio *et al.*, “DNA damage in blood cells exposed to low-level lasers,” *Lasers Surg. Med.*, vol. 47, no. 4, pp. 361–368, 2015.

[40] M. Z. Khalid, “Mechanism of laser/light beam interaction at cellular and tissue level and study of the influential factors for the application of low level laser therapy,” *arXiv Prepr. arXiv1606.04800*, 2016.

[41] D. Verma, N. Gulati, S. Kaul, S. Mukherjee, and U. Nagaich, “Protein based nanostructures for drug delivery,” *J. Pharm.*, vol. 2018, 2018.

[42] J. M. A. Zahra, “Impact of laser (Nd: YVO4 Crystals, 532nm) radiation on white blood cells,” *Iraqi Laser Sci. J.*, vol. 1, no. 3, pp. 1–6, 2019.

[43] S. Chinnathambi, N. Hanagata, T. Yamazaki, and N. Shirahata, “Nano-Bio Interaction between Blood Plasma Proteins and Water-Soluble Silicon Quantum Dots with Enabled Cellular Uptake and Minimal Cytotoxicity,” *Nanomaterials*, vol. 10, no. 11, p. 2250, 2020.

[44] N. Suardi, H. C. Sing, S. J. Gemanam, E. B. B. Ong, and W. Z. Pei, “In Vitro Effects of Low Level Laser Irradiation on Blood Protein,” *J. Phys. Conf. Ser.*, vol. 1892, no. 1, 2021, doi: 10.1088/1742-6596/1892/1/012029.

[45] R. Mohammed and S. Hassan, “Studying the effect of laser irradiation with different wavelength on absorbance of normal human blood,” in *Journal of Physics: Conference Series*, 2021, vol. 1999, no. 1, p. 12144.

[46] G. Mohit, “Analyzing Thermal and Mechanical Effects of Pulsed Laser Irradiation on Tissues,” *MSc. Biomed. Eng. Florida Inst. Technol.*, 2014.

[47] C. F. Gmachl, “Suckers for light,” *Nature*, vol. 467, no. 7311, pp. 37–39, 2010.

[48] A. D. Klose and E. W. Larsen, “Light transport in biological tissue based on the simplified spherical harmonics equations,” *J. Comput. Phys.*, vol. 220, no. 1, pp. 441–470, 2006.

[49] M. H. Niemz, *Laser-tissue interactions*, vol. 322. Springer, 2007.

[50] V. Heiskanen and M. R. Hamblin, “Photobiomodulation: lasers vs. light emitting diodes?,” *Photochem. Photobiol. Sci.*, vol. 17, no. 8, pp. 1003–1017, 2018.

[51] P. Brumer and M. Shapiro, “Molecular response in one photon absorption: Coherent pulsed laser vs. thermal incoherent source,” *arXiv Prepr. arXiv1109.0026*, 2011.

[52] S. Bordin-Aykroyd, R. B. Dias, and E. Lynch, “Laser-tissue interaction,” *EC Dent Sci*, vol. 18, pp. 2303–2308, 2019.

[53] V. M. Arutyunyan, “Physical properties of the semiconductor-electrolyte interface,” *Sov. Phys. Uspekhi*, vol. 32, no. 6, p. 521, 1989.

[54] H. Abramczyk, *Introduction to laser spectroscopy*. Elsevier, 2005.

[55] Y. Zhang, J. Cai, C. Li, J. Wei, Z. Liu, and W. Xue, “Effects of thermosensitive poly (N-isopropylacrylamide) on blood coagulation,” *J. Mater. Chem. B*, vol. 4, no. 21, pp. 3733–3749, 2016.

[56] M. De Santis, E. Cesari, E. Nobili, G. Straface, A. F. Cavaliere, and A. Caruso, “Radiation effects on development,” *Birth Defects Res. Part C Embryo Today Rev.*, vol. 81, no. 3, pp. 177–182, 2007.

[57] R. Steiner, “Laser-tissue interactions,” in *Laser and IPL technology in dermatology and aesthetic medicine*, Springer, 2011, pp. 23–36.

[58] H. Golnabi and M. H. Mahdieh, “Trend of laser research developments in global level,” *Opt. Laser Technol.*, vol. 38, no. 2, pp. 122–131, 2006.

[59] R. P. Marshall, “Spectral dependence of laser light on light-tissue interactions and its influence on laser therapy: An experimental study,” *Arch. Med.*, vol. 5, no. 1, p. 5, 2020.

[60] B. Cox, “Introduction to laser-tissue interactions,” *PHAS*, vol. 4886, pp. 1–61, 2007.

[61] A. P. Castano, P. Mroz, and M. R. Hamblin, “Photodynamic therapy and anti-tumour immunity,” *Nat. Rev. Cancer*, vol. 6, no. 7, pp. 535–545, 2006.

[62] S. Parker, “Laser-tissue interaction,” *Br. Dent. J.*, vol. 202, no. 2, pp. 73–81, 2007.

[63] M. H. Neimz, “Laser-tissue interactions: Fundamentals and applications,” *Springer, Berlin*, 2003.

[64] L. Zhu, “Heat transfer applications in biological systems,” *Biomed. Eng. Des. Handb.*, vol. 1, pp. 2–33, 2009.

[65] P. N. Prasad, *Introduction to biophotonics*. John Wiley & Sons, 2003.

[66] T.-C. Shih, H.-S. Kou, and W.-L. Lin, “Effect of effective tissue conductivity on thermal dose distributions of living tissue with directional blood flow during thermal therapy,” *Int. Commun. Heat Mass Transf.*, vol. 29, no. 1, pp. 115–126, 2002.

[67] T.-C. Shih, H.-S. KOU, C.-T. LIAUH, and W.-L. LIN, “Thermal models of bioheat transfer equations in living tissue and thermal dose equivalence due to hyperthermia,” *Biomed. Eng. Appl. Basis Commun.*, vol. 14, no. 02, pp. 86–

96, 2002.

[68] G. Elert, “The Physics Factbook™—An encyclopedia of scientific essays,” *Vol. Blood a Hum.*, 2012.

[69] M. Marinkovic, M. Diez-Silva, I. Pantic, J. J. Fredberg, S. Suresh, and J. P. Butler, “Febrile temperature leads to significant stiffening of Plasmodium falciparum parasitized erythrocytes,” *Am. J. Physiol. Physiol.*, vol. 296, no. 1, pp. C59–C64, 2009.

[70] S.-W. D. Tsen, T. Chapa, W. Beatty, K.-T. Tsen, D. Yu, and S. Achilefu, “Inactivation of enveloped virus by laser-driven protein aggregation,” *J. Biomed. Opt.*, vol. 17, no. 12, p. 128002, 2012.

[71] D. G. Siposan and A. Lukacs, “Effect of low-level laser radiation on some rheological factors in human blood: an in vitro study,” *J. Clin. Laser Med. Surg.*, vol. 18, no. 4, pp. 185–195, 2000.

[72] L. DINTENFASS, “Blood viscosity, hyperviscosity, and hyperviscosaemia((Book)),” *Lancaster, England, MTP Press. Ltd., 1985, 494*, 1985.

[73] M. A. Srour, Y. Y. Bilto, M. Juma, and M. R. Irhimeh, “Exposure of human erythrocytes to oxygen radicals causes loss of deformability, increased osmotic fragility, lipid peroxidation and protein degradation,” *Clin. Hemorheol. Microcirc.*, vol. 23, no. 1, pp. 13–21, 2000.

[74] A. Mochizuki, T. Ogawa, K. Okamoto, T. Nakatani, and Y. Nitta, “Blood

compatibility of gas plasma-treated diamond-like carbon surface—Effect of physicochemical properties of DLC surface on blood compatibility," *Mater. Sci. Eng. C*, vol. 31, no. 3, pp. 567–573, 2011.

[75] M. Shmukler, "Density of Blood-The Physics Factbook," *Retrieved Oct.*, vol. 4, p. 2006, 2004.

[76] اكرام, ك . ج , " دراسة تأثير اشعة الليزر للطولين الموجيين 532 nmو 671 nm على عينات الدم السليم و الدم المصاب بفقر الدم للذكور " , رسالة ماجستير , جامعة البصرة , العراق , 2020 .

[77] N. Cherry, "Schumann Resonances, a plausible biophysical mechanism for the human health effects of Solar," *Nat. hazards*, vol. 26, no. 3, pp. 279–331, 2002.

[78] J. E. Lesch, "Science and medicine in France," in *Science and Medicine in France*, Harvard University Press, 2013.

[79] R. I. Handin, S. E. Lux, and T. P. Stossel, *Blood: principles and practice of hematology*, vol. 1. Lippincott Williams & Wilkins, 2003.

[80] L. Garby, *The respiratory functions of blood*. Springer Science & Business Media, 2012.

[81] N. Beck, *Diagnostic hematology*. Springer Science & Business Media, 2008.

[82] H. Brits *et al.*, "The prevalence of neonatal jaundice and risk factors in healthy term neonates at National District Hospital in Bloemfontein," *African J. Prim. Heal. Care Fam. Med.*, vol. 10, no. 1, pp. 1–6, 2018.

[83] M. Cully, “CD40L-targeting protein cools rheumatoid arthritis.,” *Nat. Rev. Drug Discov.*, vol. 18, no. 6, pp. 417–418, 2019.

[84] M. Sayed, D. M. Tom, and H. Pal, “Multimode binding and stimuli responsive displacement of acridine orange dye complexed with p-sulfonatocalix [4/6] arene macrocycles,” *Phys. Chem. Chem. Phys.*, vol. 22, no. 23, pp. 13306–13319, 2020.

[85] C. F. Klingshirn, *Semiconductor optics*. Springer Science & Business Media, 2012.

[86] M. A. Ansari and E. Mohajerani, “Mechanisms of laser-tissue interaction: I. optical properties of tissue,” 2011.

[87] M. C. Meinke, G. J. Müller, J. Helfmann, and M. Friebel, “Optical properties of platelets and blood plasma and their influence on the optical behavior of whole blood in the visible to near infrared wavelength range,” *J. Biomed. Opt.*, vol. 12, no. 1, p. 14024, 2007.

[88] V. E. Ivanov, A. M. Usacheva, A. V Chernikov, V. I. Bruskov, and S. V Gudkov, “Formation of long-lived reactive species of blood serum proteins induced by low-intensity irradiation of helium-neon laser and their involvement in the generation of reactive oxygen species,” *J. Photochem. Photobiol. B Biol.*, vol. 176, pp. 36–43, 2017.

[89] A. de Souza da Fonseca, G. A. Presta, M. Geller, F. de Paoli, and S. S. Valença, “Low-intensity infrared laser increases plasma proteins and induces oxidative stress in vitro,” *Lasers Med. Sci.*, vol. 27, no. 1, pp. 211–217, 2012.

[90] M. Monici, “Cell and tissue autofluorescence research and diagnostic applications,” *Biotechnol. Annu. Rev.*, vol. 11, pp. 227–256, 2005.

[91] S. Gorinstein *et al.*, “Intrinsic tryptophan fluorescence of human serum proteins and related conformational changes,” *J. Protein Chem.*, vol. 19, no. 8, pp. 637–642, 2000.

[92] K. T. V Grattan, “Book Review: Laser-tissue interactions: fundamentals and applications, M. Niemz; Springer, Berlin, 2002, price£ 56, ISBN 3-540-42763-5,” *Opt. Lasers Eng.*, vol. 42, no. 1, pp. 119–120, 2004.

[93] X. Gao and D. Xing, “Molecular mechanisms of cell proliferation induced by low power laser irradiation,” *J. Biomed. Sci.*, vol. 16, no. 1, pp. 1–16, 2009.

[94] A. M. Petrosyan, “Comment on ‘Growth and characterization of glycine picrate single crystal’ by T. Uma Devi et al.[Spectrochim. Acta A71 (2008) 340–343],” *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.*, vol. 75, no. 3, p. 1176, 2010.

[95] I. Delfino and S. Cannistraro, “Optical investigation of the electron transfer protein azurin–gold nanoparticle system,” *Biophys. Chem.*, vol. 139, no. 1, pp. 1–7, 2009.

[96] H. Cao, X. Qian, C. Wang, X. Ma, J. Yin, and Z. Zhu, “High symmetric 18-facet polyhedron nanocrystals of Cu7S4 with a hollow nanocage,” *J. Am. Chem. Soc.*, vol. 127, no. 46, pp. 16024–16025, 2005.

[97] C. Auría-Soro *et al.*, “Interactions of nanoparticles and biosystems: microenvironment of nanoparticles and biomolecules in nanomedicine,” *Nanomaterials*, vol. 9, no. 10, p. 1365, 2019.

[98] R. Barbir *et al.*, “Protein corona modulates distribution and toxicological effects of silver nanoparticles in vivo,” *Part. Part. Syst. Charact.*, vol. 36, no. 8, p. 1900174, 2019.

[99] S. J. Park, “Protein–nanoparticle interaction: corona formation and conformational changes in proteins on nanoparticles,” *Int. J. Nanomedicine*, vol. 15, p. 5783, 2020.

[100] S. R. Saptarshi, A. Duschl, and A. L. Lopata, “Interaction of nanoparticles with proteins: relation to bio-reactivity of the nanoparticle,” *J. Nanobiotechnology*, vol. 11, no. 1, pp. 1–12, 2013.

[101] S. Jokar, A. Pourjavadi, and M. Adeli, “Albumin–graphene oxide conjugates; carriers for anticancer drugs,” *RSC Adv.*, vol. 4, no. 62, pp. 33001–33006, 2014.

[102] M. Mahmoudi, I. Lynch, M. R. Ejtehadi, M. P. Monopoli, F. B. Bombelli, and S. Laurent, “Protein-nanoparticle interactions: Opportunities and challenges,” *Chem. Rev.*, vol. 111, no. 9, pp. 5610–5637, 2011, doi: 10.1021/cr100440g.

[103] A. C. Croce, A. Ferrigno, G. Bottiroli, L. G. Di Pasqua, C. Berardo, and M. Vairetti, “Fluorescence excitation properties of bilirubin in solution and in serum,” *J. Photochem. Photobiol. B Biol.*, vol. 215, p. 112121, 2021.