Sudan University of Science & Technology

College of graduated Studies

Exploration of Singlet Oxygen Emitted After Irradiation of Dibenzocyanine 45, MethyleneBlue and Rhodamine 6G Dyes Using Lasers with Different Wavelengths

إختبار إمكا نية إنتاج الأكسجين الأحادي من إنبعاث صبغات الدايبنزوسيانين45 و الميثيلين بلو والرودامين 6ج باستخدام ليزرات مختلفة الأطوال الموجية

A thesis submitted as a partial fulfillment of requirements for the degree of master in laser application in physics

By:

Ahmed Abubaker Mohamed Taher

Supervised by:

Prof. Dr. Nafie A. Almuslet

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بسم الله الرحمن الرحيم

قال تعالى:

(وَلاَ تَقْفُ مَا لَيْسَ لَكَ بِهِ عِلْمٌ إِنَّ السَّمْعَ وَالْبَصَرَ وَالْفُؤَادَ كُلُّ أُولَئِكَ كَانَ عَنْهُ مَسْؤُولاً)

صدق الله العظيم

سورة الإسراء، [الآية :36]

DEDICATION

This work is Dedicated to :

My parents

My family

my friends

and all who supported me

ahmed

ACKNOWLEDGEMENT

First of all thanks to God for guiding me to conduct this study.I like to express my deep and great gratitude to my supervisor, prof Dr.Nafie A.Almuslet ,For his guidance and support from the beginning to the final level, He enabled me to develop my understanding of the subject . Without his help, this work would be difficult, if not impossible, to be accomplish. Also I am heartily thankful to Dr. Abdu AL Fatah. And Mr Abdu Elsakhy Mohammed-Elneelin University.

My Thanks are also extending to all my friends for their invaluable support.

aAhmed

ABSTRACT

The aim of this research was to explore the singlet oxygen emitted after irradiation Dibenzocyanine 45, Methylene Blue and Rhodamine 6G dyes after irradiation using two lasers with different wavelengths, so that the singlet oxygen can be used in photodynamic therapy (PDT).

Three samples of DDTTC 45 were prepared by dissolved in propanol, methanol and acetone respectively.

Two samples of Methylene blue prepared by dissolved in methanol and ethanol. One sample of Rhodamin 6G was dissolved in methanol.

UV visible spectrometer was used to record the absorption spectra for the solvents and dyes. Two laser sources were used to irradiate the samples. Firstly diode laser (532nm) with output powers of 100 mW, secondly the diode laser (671nm) with output power of 100 mW. USP2000 spectrometer connected to PC, was used to collect and record the emission spectra after different exposure times.

The results showed that the most efficient sample to produce the singlet oxygen was the DDTTC 45 dissolved in acetone after irradiation by the diode laser 671 nm with exposure time of six minutes which indicated a peak of singlet oxygen at (703 nm). It is worth to mention that, no evidence was noticed for a singlet oxygen production from the other samples.

المستخلص

ان الهدف من هذا البحث هو التحقق من امكانية انتاج الاكسجين الاحادي من انبعاث صبغات الدايبنزوسيانين45 ، الميثيلين بلو وصبغة الرودامين 6G بعد تشعيعها باستخدام ليزرات مختلفة الاطوال الموجية لغرض الاستخدام في العلاج الضوئي (PDT).

حُضرت ثلاثة عينات من صبغة الدايبنزوسيانين بإذابة كل عينة في محلول من المحاليل الثلاث بروبانول، إيثانول واستون. وكذلك حَضرت عينتين من صبغة الميثيلين بلو بإذابة كل عينة في محلول من المحلولين الإيثانول والميثانول.كما حضرت عينة واحدة من صبغة الرودامين 6G مذابة في محلول الميثانول.سجلت اطياف الامتصاص لكل من المحاليل والصبغات باستخدام جهاز (UV-vis spectrometer).

تم استخدام ليزرين لتشعيع العينات المذكورة أعلاه, أولا ليزر الثنائي بطول موجي 532nm وبقدرة 100ملي واط و ثانيا ليزر الثنائي بطول موجي 671nm وبقدرة 100ملي واط ،أستخدم مطياف (USB 2000) لتسجيل الانبعاث للعينات بعد تشعيعها بأزمان مختلفة.

اوضحت النتائج ان افضل العينات من حيث الكفاءه في انتاج الاوكسجين الاحادي هي عينة الدايبنزو سيانين المذابة في محلول الاستون بعد ان تم تشعيعها بليزر الثنائي 671nm بزمن تعريض قدره ست دقائق حيث ظهرت قمة انبعاث عند (703nm) وهي تعود للاكسجين الاحادي.

الجدير بالذكر انه لم تظهر في بقية النتائج اي دليل على انتاج الاوكسجين الاحادي من العينات الاخرى.

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BASIC CONCEPTS OF LASER SPECTROSCOPY

Chapter One

Basic Concepts of Laser Spectroscopy

1.1 Introduction:

Most of the knowledge about the structure of atoms and molecules is based on spectroscopic investigations. Thus spectroscopy has made an outstanding contribution to the present state of atomic and molecular physics, to chemistry and to molecular biology. Information on molecular structure and on the interaction of molecules with their surroundings may be derived in various ways from the absorption or emission spectra generated when electromagnetic radiation interacts with matter[1].

Most types of laser are an inherently pure source of light; they emit near-monochromatic light with a very well defined range of wavelengths. By careful design of the laser components, the purity of the laser light (measured as the "linewidth") can be improved more than the purity of any other light source. This makes the laser a very useful source for spectroscopy. The high intensity of light that can be achieved in a small well collimated beam can also be used to induce a nonlinear optical effect in a sample, which makes techniques such as Raman spectroscopy possible. Other spectroscopic techniques based on lasers can be used to make extremely sensitive detectors of various molecules, able to measure molecular concentrations in the parts-per-10^{12} [2].

For branches of spectroscopy other than Raman spectroscopy most laser sources may appear to have a great disadvantage, that of non-tunability. In regions of the spectrum, particularly the infrared where tunable lasers are not readily available, ways have been devised for tuning, that is, shifting, the atomic or molecular energy levels concerned until the transition being studied moves into coincidence with the laser radiation. This may be achieved by applying an electric field to the sample, and the technique is called laser Stark

spectroscopy. The corresponding technique using a magnetic field is that of laser magnetic resonance (or laser Zeeman) spectroscopy.

From 1960 onwards, the increasing availability of intense, mono-chromatic laser sources provided a tremendous impetus to a wide range of spectroscopic investigations. The most immediately obvious application of early, essentially non-tunable, lasers was to all types of Raman spectroscopy in the gas, liquid or solid phase Laser radiation is very much more intense, and the linewidth much smaller, than that from, for example, a mercury arc, which was commonly used as a Raman source before 1960. A wide variety of ingenious techniques have been devised using laser sources [3].

1.2 Absorption spectroscopy:

1.2.1 Absorption spectra:

It s important to determine the position and nature of the energy levels of the electronic states involve the luminescence excitation and emission process because these energy levels are characteristic properties of the compounds of interest ,in general the simplest method of studying the energy levels of the excited state is absorption spectroscopy.

Absorption lines are typically classified by the nature of the quantum mechanical change induced in the molecule or atom. Rotational lines, for instance, occur when the rotational state of a molecule is changed. Rotational lines are typically found in the microwave spectral region. Vibrational lines correspond to changes in the vibrational state of the molecule and are typically found in the infrared region. Electronic lines correspond to a change in the electronic state of an atom or molecule and are typically found in the visible and ultraviolet region. X-ray absorptions are associated with the excitation of inner shell electrons in atoms. These changes can also be combined (e.g. rotation-vibration transitions), leading to new absorption lines at the combined energy of the two changes.

The energy associated with the quantum mechanical change primarily determines the frequency of the absorption line but the frequency can be shifted by several types of interactions. Electric and magnetic fields can cause a shift. Interactions with neighboring molecules can cause shifts. For instance, absorption lines of the gas phase molecule can shift significantly when that molecule is in a liquid or solid phase and interacting more strongly with neighboring molecules.

Observed absorption lines always have a width and shape that is determined by the instrument used for the observation, the material absorbing the radiation and the physical environment of that material. It is common for lines to have the shape of a Gaussian or Lorentzian distribution. It is also common for a line to be characterized solely by its intensity and width instead of the entire shape being characterized. The integrated intensity obtained by integrating the area under the absorption line is proportional to the amount of the absorbing substance present. The intensity is also related to the temperature of the substance and the quantum mechanical interaction between the radiation and the absorber. This interaction is quantified by the transition moment and depends on the particular lower state the transition starts from and the upper state it is connected to [4,5].

The width of absorption lines may be determined by the spectrometer used to record it. A spectrometer has an inherent limit on how narrow a line it can resolve and so the observed width may be at this limit. If the width is larger than the resolution limit, then it is primarily determined by the environment of the absorber. A liquid or solid absorber, in which neighboring molecules strongly interact with one another, tends to have broader absorption lines than a gas. Increasing the temperature or pressure of the absorbing material will also tend to increase the line width. It is also common for several neighboring transitions to be close enough to one another that their lines overlap and the resulting overall line is therefore broader.

Absorption spectroscopy is useful in chemical analysis because of its specificity and its quantitative nature. The specificity of absorption spectra allows compounds to be distinguished from one another in a mixture. An absorption spectrum can be quantitatively related to the amount of material presented by applied the Beer-Lambert law. [4].

1.3 Emission spectroscopy:

1.3.1 Emission spectra:

In physics, emission is the process by which a higher energy quantum mechanical state of a particle becomes converted to a lower one through the emission of a photon, resulting in the production of light. The frequency of light emitted is a function of the energy of the transition. Since energy must be conserved, the energy difference between the two states equals the energy carried off by the photon. The energy states of the transitions can lead to emissions over a very large range of frequencies. For example, visible light is emitted by the coupling of electronic states in atoms and molecules (then the phenomenon is called fluorescence or phosphorescence). On the other hand, nuclear shell transitions can emit high energy gamma rays, while nuclear spin transitions emit low energy radio waves.

The remittance of an object quantifies how much light is emitted by this certain objects. This may be related to other properties of the object through the Stefan–Boltzmann law. For most substances, the amount of emission varies with the temperature and the spectroscopic composition of the object, leading to the appearance of emission lines. Precise measurements at many wavelengths allow the identification of a substance via emission spectroscopy [5].

This form of spectroscopy examines the wavelength of photons emitted when an atom or molecules drops from a higher electronic state to a lower one and it is the structure of the sample that that dictates the wavelength of the photons the sample can absorb and emit. The mechanism by which the atoms

or molecules are excited are classifies this type of spectroscopy further. For examples if electromagnetic radiation is used, fluorescence spectroscopy can result.

Protons can produce particle-induced x-ray emission, and if heat is applied to a sample causing an increase in collisions between particles flame emission spectra can be detected [3].

Emission of radiation is typically described using semi-classical quantum mechanics: the particle's energy levels and spacing s are determined from quantum mechanics, and light is treated as an oscillating electric field that can drive a transition if it is in resonance with the system's natural frequency.

Emission spectroscopy is a spectroscopic technique which examines the wavelengths of photons emitted by atoms or molecules during their transition from an excited state to a lower energy state. Each element emits a characteristic set of discrete wavelengths according to its electronic structure, and by observing these wavelengths the elemental composition of the sample can be determined. Emission spectroscopy developed in the late 19th century and efforts in theoretical explanation of atomic emission spectra eventually led to quantum mechanics.

There are many ways in which atoms can be brought to an excited state. Interaction with electromagnetic radiation is used in fluorescence spectroscopy, protons or other heavier particles in Particle-Induced X-ray Emission and electrons or X-ray photons in Energy-dispersive X-ray spectroscopy or X-ray fluorescence. The simplest method is to heat the sample to a high temperature, after which the excitations are produced by collisions between the sample atoms. This method is used in flame emission spectroscopy, and it was also the method used by Anders Jonas Ångström when he discovered the phenomenon of discrete emission lines in 1850 [4].

Although the emission lines are caused by a transition between quantized energy states and may at first look very sharp, they do have a finite width, i.e.

they are composed of more than one wavelength of light. This spectral line broadening has many different causes [5].

Emission spectroscopy is often referred to as optical emission spectroscopy, due to the light nature of what is being emitted, Figure (1.1) shows the absorption and emission processes [6].

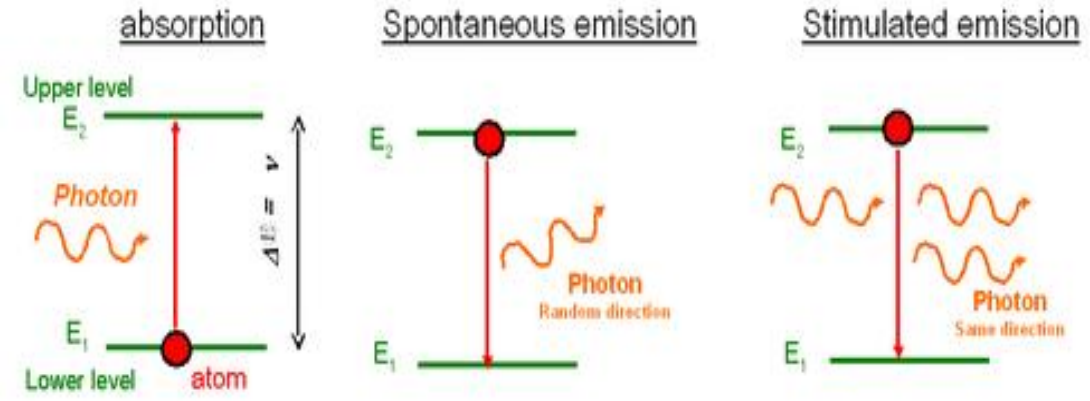

Figure (1.1): Illustration of absorption and emission processes.

The spectral distribution of the radiant flux from a source is called its emission spectrum. The radiation has a continuous spectral distribution described by its spectral energy density .Discrete emission spectra where the radiant flux has distinct maxima at certain frequencies ν_{ik}, generated by transitions of atoms or molecules between tow bound states, a higher energy state (excited state) E_k and a lower state (ground state) E_i,with the relation :

$$h\nu_{ik} = E_k - E_i \qquad (1\text{-}1)$$

The energy intervals (energy difference between neighboring peaks in absorption spectrum observed in the spectrum will be equal to the vibrational energy gabs.

Energy gabs in the spectrum are calculated according to the formula:

$$\Delta E = h\nu_k - h\nu_i = \frac{hc}{\lambda_k - \lambda_i} \qquad (1\text{-}2)$$

In spectrograph the entrance slit is imaged into the focal plane of the camera lens. Because of the dispersive elements in the spectrograph, the position of this image depends on the wavelengths of the incident radiation.

In a discrete spectrum each wavelength λ_{ik} produces a separate line in the imaging plane, provided the spectrograph has a sufficiently high high resolving power. Discrete spectra are therefore also called line spectra, as opposed to continuous power [6]

1.3.2 Types of emission spectra:

They are three types:

(a) Continuous spectra are elicited by glowing solid bodies; no distinguishable lines are formed.

(b) Band spectra are generated by molecules; broad bands are formed.

(c) Line spectra are generated by excited atoms; discrete, distinct lines are formed [7].

1.4 Principles of luminescence spectroscopy:

Luminescence is a type of optical spectroscopy in which molecule is promoted to an electrically excited state by absorption of ultraviolet, visible, or near infrared radiation. The excited molecule then decays back to the ground state, or to a lowering excited electronic state, by emission of light. The emission light is detected Photoluminescence processes are subdivided into fluorescence and phosphorescence. Phosphorescence is a light emission process in which the excited and the ground states have different spin multiplicities. In organic molecule whose ground state is a singlet, there are several energetically accessible triplet excited states (two unpaired spins).Following excitation into the manifold of singlet excited states by absorption. Molecule may undergo non radioactive decay (inter system crossing) to the manifold of triplet states The triplet state may emit a photon as the molecule decays back to the ground state (phosphorescence) .

Most optical luminescence based medical diagnoses involve fluorescence spectroscopy [8].

The general level of an organic dye is shown in figure (1.2) in the singlet state of the molecule; the spin of the excited electron is the anti parallel to the spin of the remaining one. In the triplet state, the spins are parallel. In the diagram, s1and s_2 are singlet states, T1and T_2 are triplet states and S_0 is the ground state [8, 9].

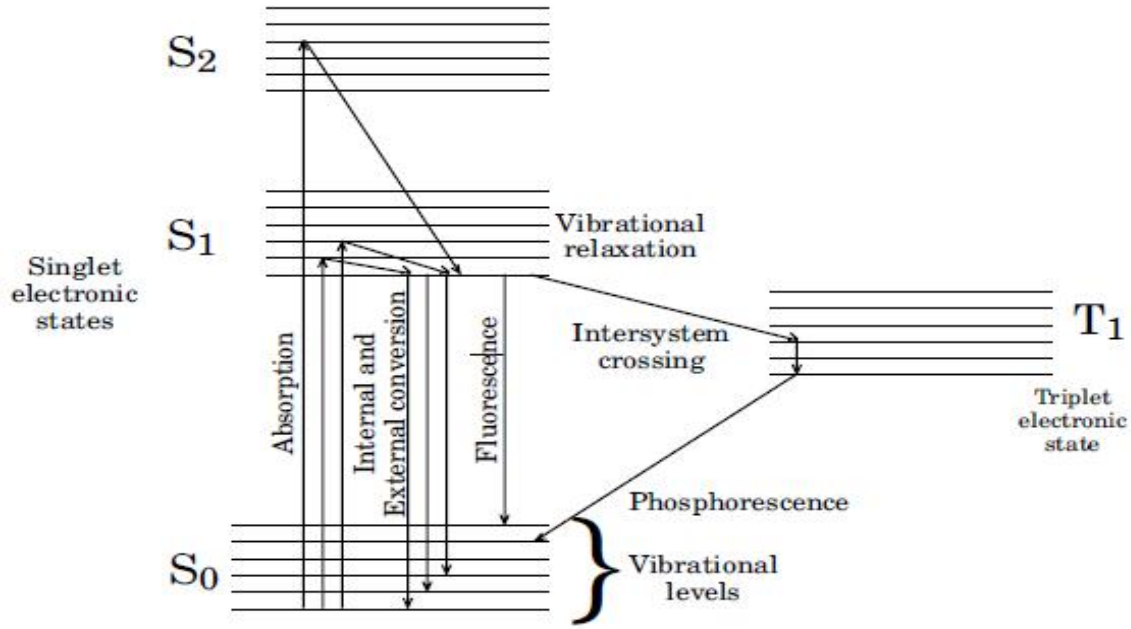

Figure (1.2) Jaboloniski diagram.

1.4.1 Photophysical basis of luminescence:

Luminescence is the emission of light in the visible or ultraviolet region where the sample is not being excited by heating. In luminescence, the light is emitted as the electronically excited molecule returns to the ground state. The excited state can be generated by a variety of techniques; Thus luminescence is divided into categories depending on the mechanism by which the excited state was formed [8].

In chemiluminescence, the excited state is generated by a chemical reaction; in photoluminescence, the transition to the excited state is the result of absorption of electromagnetic radiation. The usefulness of fluorescence spectroscopy stems from two main factors. First, the majority of molecules do

not exhibit fluorescence. As a result, in most samples, the background fluorescence will be very small, and therefore the technique can detect Specific molecules with high sensitivity. Second, the spectral properties of the fluorescence emission are usually highly sensitive to local environment, while for the study of small molecules this is not especially useful, because it merely measures changes in the solvent, for fluorophores in macromolecules, the local Environment is generally different in different locations within the macromolecule, and therefore fluorescence allows direct monitoring of changes in conformation or interactions with other molecules. A number of changes in electronic and vibrational states may occur during absorption and emission processes. Most processes in physics and chemistry, the overall kinetics of the process can be important in determining which processes will be favored. The Jablonski diagram shown in figure (1.2) is useful for illustrating these processes; a full consideration of relative likelihoods of different processes involves an understanding of the time scales involved and of the factors that influence the relative probabilities of the different transitions that return the molecule to the ground state [9].

The absorption of a photon, with concomitant change in electronic state (the upward pointing arrows in the Jablonski diagram above) is very fast. In most cases, the result is a molecule that is in an excited vibrational state as well as an excited electronic state. Vibrational relaxation is fast and usually occurs via collisions. Because vibrational relaxation within the same electronic state is faster than electronic relaxation from the S_1 to the S_0 state, vibrational relaxation generally occurs before the return to the ground (S_0) state.

For most molecules, the ground state is a singlet state. During absorption and emission processes, singlet-to-singlet transitions are more likely than singlet-to triplet Transitions. Depending on the energy of the absorbed photon, the molecule may suffer transition from the ground state to different excited states (S_1, S_2, or higher) [9].

However, in the vast majority of molecules, fluorescence only occurs as a process of transition from S_1 to S_0. This means that, even if the molecule was excited to S_2 or higher, it will generally drop to S_1 by non-radiative processes before fluorescing.

This has obvious consequences for the wavelength range over which the

Fluorescence emission will be observed. The rate constant for fluorescence is a numerical value related to the likelihood of fluorescence emission.

As shown in the Jablonski diagram above, non-radiative processes compete with fluorescence.

If the S1 state has a long lifetime, the molecule may undergo intersystem crossing, which is transition to the triplet state (this is a forbidden process, but may occur at appreciable rates for some molecules; the process is favored because the T_1 state is lower in energy than the S_1 state. Return to the ground state via Phosphorescence is very slow compared to the other processes.

Because triplet-to-singlet transition is also a forbidden process. While

Phosphorescence involves photon emission, the triplet-to-singlet transition may also occur by non-radiative processes (especially in solution.) In solution, phosphorescence is rarely observed unless oxygen (an effective quenching agent for both triplet and singlet states) is removed from the solution. In most biochemical experiments, only fluorescence is useful, because the fluorescent lifetime is short enough to allow fluorescence to occur before quenching. Even if phosphorescence does occur, the rate constant for the process is very small; only the rate constant for intersystem crossing usually matters for consideration of Fluorescence [9, 10]. . Internal conversion is a non-radiative process that allows the molecule to return to the ground state via vibrational relaxation or other relaxation processes within the molecule. Internal conversion is temperature dependent, which complicates the use of fluorescence to monitor other temperature dependent processes (especially thermal unfolding of proteins).

External conversion is a non-radiative process that allows the molecule to return to the ground state as a result of collisions with solvent molecules.

The rate constant for internal conversion is abbreviated.

Quenching is related to external conversion; in quenching, solute molecules remove the extra energy from the excited state.The high concentration of quenching agent required for most types of quenching [10].

The rate for the process depends on both the rate constant and on the quencher concentration. Fluorescence quenching can be an experimentally useful phenomenon, and is discussed in more detail below.

The last type of non-radiative process is photodecomposition, in which the excited state undergoes a chemical change to a different molecule, and never returns to the ground state of interest. The rate constant for photodecomposition is abbreviated The process with the largest rate of relaxation will predominate, but if several processes have similar rates, the processes will compete. The quantum yield the fraction of absorbed light that is re emitted as fluorescent photons [11].

1.5 Laser Induced Fluorescence (LIF):

Laser-induced fluorescence (LIF) is the optical emission from molecules that have been excited to higher energy levels by absorption of electromagnetic radiation specially a laser beam**.** This laser beam is used to excite the specie (a molecule or atom) of interest. For this, the laser has to be selected or tuned so that its wavelength matches an absorption band of the specie (discrete for atoms, broadband for molecules). By absorbing the laser beam photons, the specie transition from a ground electronic state to a short-lived excited electronic state. In order to return to a stable ground state the molecule can release the extra energy through:

- Laser induced emission, a weak phenomenon that only lasts as long as the excitation.

- Thermal transfer to other molecules / atoms via collisions. This is usually called quenching as it competes with the fluorescence.
- Emit a photon, the fluorescence.
- Intersystem crossing This is a forbidden transition (in quantum theory this means its probability of occuring is very small) that requires very tightly controlled conditions to occur and leads to phosphorensce, another luminescence process which is very slow (compared to fluorescence).

The fluorescence lifetime is dependent on the specie but is usually in the order of tens or hundreds of nanoseconds for useful species. Due to collisions with other molecules / atoms, the states involved in the fluorescence usually differs from the states involved in the absorption. As a result, the fluorescence wavelength differs from the laser wavelength. It is usually shifted towards the red. This is a useful property that allows for easy separation of the laser and fluorescence light with filters.

The fluorescence intensity is directly proportional to the number of molecules / atoms in the excited state. Therefore it provides information on the specie concentration. However the excited state population is temperature dependent .Furthermore the fluorescence competes with quenching which, as it is due to collisions, is dependent on pressure, temperature, and concentration of other species. As a result, laser induced is usually used to provide qualitative or semi-quantitative concentration species distribution [26].

1.6 Raman spectroscopy:

Raman spectroscopy provides information about molecular vibrations that can be used for sample identification and quantitation. The technique involves shining a monochromatic light source (i.e. laser) on a sample and detecting the scattered light as shown in figure (1.3) . The majority of the scattered light is of the same frequency as the excitation source,this is known as Rayleigh or elastic scattering. A very small amount of the scattered light (10^{-5}% of the

incident light intensity) is shifted in energy from the laser frequency due to interactions between the incident electromagnetic waves and the vibrational energy levels of the molecules in the sample[27].

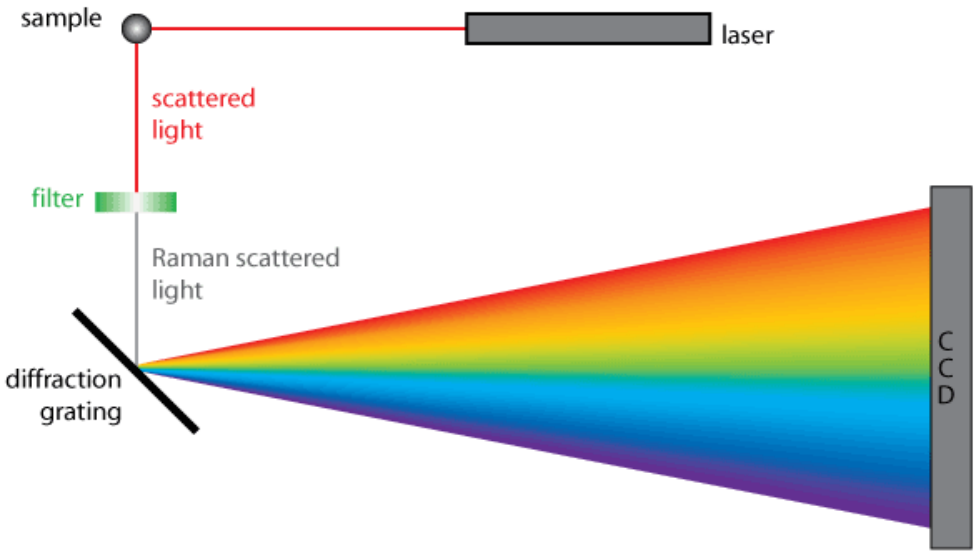

Figure (1.3) Raman experimental setup

1.6.1 Stokes and anti-Stokes scattering:

Energy-level diagram showing the states involved in Raman signal. The line thickness is roughly proportional to the signal strength from the different transitions; figure (1.4) shows the energy level of Raman signal.

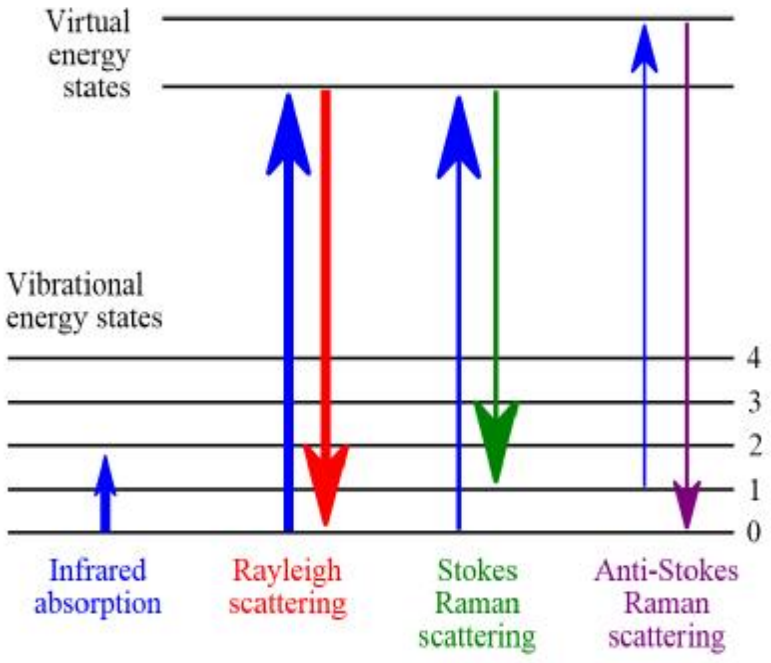

Figure (1.4) Energy-level diagram of Raman signal

The difference in intensity of the Stokes and anti-Stokes components is due to the different number of molecules in each state initially. The population follows the Boltzmann distribution. Therefore there are exponentially fewer molecules that start out in the higher energy vibrational state. Since that gives rise to the anti-Stokes scattering, this is much less intense. The disparity depends on the spacing of the energy levels. So for the less widely spaced rotational levels, the Stokes and anti-Stokes scattering are of similar magnitude. For the vibrational levels, which are spaced further apart, the anti-Stokes signal is significantly weaker than the Stokes signal.The disparity is also reduced with increased temperature, and the difference can be used as a measure of temperature [27].

1.6.2 Medical applications of Raman spectroscopy

There are several Raman active biological molecules in tissue that give distinctive peaks in the spectrum giving structural and environmental information about the tissue. The changes in tissue that occur as a result of a disease yield a characteristic Raman spectrum that can be used for diagnosis. Thus, everything from precancerous tissues to benign abnormalities can in principle be determined and detected.

Several in vivo tests have to be done, to fully evaluate the actual usefulness of Raman spectroscopy. Raman spectroscopy has been studied extensively for tissue diagnosis on skin, breast, esophagus, cervix, lung, throat, etc. Besides Raman spectroscopy, several other optical techniques are being developed for the same applications, such as diffuse use reactance spectroscopy, fluorescence spectroscopy, photo acoustic and diffuse optical tomography. The main advantage of Raman spectroscopy is that tissue consists of many Raman active molecules such as in Skin cancer diagnostics, and Breast cancer diagnostics which have distinctive spectral signatures [28].

1.7 Laser-induced breakdown spectroscopy (LIBS):

Laser-induced breakdown spectroscopy (LIBS) is a type of atomic emission spectroscopy which uses a highly energetic laser pulse as the excitation source. [1, 2]. The laser is focused to form plasma, which atomizes and excites samples. In principle, LIBS can analyze any matter regardless of its physical state, be it solid, liquid or gas. Because all elements emit light of characteristic frequencies when excited to sufficiently high temperatures, LIBS can (in principle) detect all elements, limited only by the power of the laser as well as the sensitivity and wavelength range of the spectrograph & detector. If the constituents of a material to be analyzed are known, LIBS may be used to evaluate the relative abundance of each constituent element, or to monitor the presence of impurities. In practice, detection limits are a function of the plasma excitation temperature , the light collection window, and the line strength of the viewed transition. LIBS makes use of optical emission spectrometry and is to this extent very similar to arc/spark emission spectroscopy [29].

1.8 Physical properties of laser dyes:

Organic dyes are characterized by a strong absorption band in the visible region of the electromagnetic spectrum. Such property is found only in organic compounds which contain an extended system of conjugated bonds, whereas the light absorption of dyes cannot be derived rigorously from their molecular structure owing to the complexity of the quantum mechanics [14].

1. 8. 1 Dye life times:

An annoying characteristic of organic dyes is that the dyes have limited productive life time's .the factors that limited the life time of dyes are thought to be the chemical and photo chemical degradation of the dye in solution.

The life time of the gain of a dye is often specified in terms of watt-hours, based on empirical data. This power –life time product is a measure of the pump-laser energy that has been used to excite the dye and /or actual laser-

induced photochemistry in the dye solution .the chemistry of the dyes can be quiet complicated as is evidenced by the fact that the life time of the dyes, gain can even be affected by the type of metal plumbing components used in the dye circulating system.

The life times of the typical CW dyes range from 75 watt to several 1000 W.h (Rh6G) .Obviously the predicted life times of typical performance and can not be dependent up on without further specification of the conditions. For example, the life times usually assume atypical

Dye –pumping system with approximately one liter of dye solutions [12.15].

1.9 Photodynamic therapy (PDT):

Sometimes called photo chemotherapy, is a form of phototherapy using nontoxic light-sensitive compounds organic dyes that are exposed selectively to light, whereupon they become toxic to targeted malignant and other diseased cells. PDT has proven ability to kill microbial cells, including bacteria, fungi and viruses. PDT is popularly used in treating acne. It is used clinically to treat a wide range of medical conditions, including wet age-related macular degeneration and malignant cancers, and is recognised as a treatment strategy which is both minimally invasive and minimally toxic.

Most modern PDT applications involve three key components. a photosensitizer, a light source and tissue oxygen. The combination of these three components leads to the chemical destruction of any tissues which have either selectively taken up the photosensitizer or have been locally exposed to light. The wavelength of the light source needs to be appropriate for exciting the photosensitizer to produce reactive oxygen species. These reactive oxygen species generated through PDT are free radicals generated through electron abstraction or transfer from a substrate molecule and highly reactive state of oxygen known as singlet oxygen [25].

1.9.1 The photosensitizers:

A sensitizer in chemoluminescence is a chemical compound, capable of light emission after it has received energy from a molecule, which became excited previously in the chemical reaction. Only photosensitizers that undergo efficient intersystem crossing to the excited triplet state and whose triplet state is relatively long lived (to allow time for collision with oxygen) and have few other competing pathways will produce high yields of singlet oxygen. Most photosensitizers in clinical use have triplet quantum yields in the range of 40 to 60%, with the singlet oxygen yield being slightly lower. Competing processes include loss of energy by deactivation to ground state by fluorescence or internal conversion (loss of energy to the environment). However, while a high yield of singlet oxygen is desirable, it is by no means sufficient for a photosensitizer to be clinically useful.

1.10 Principles and Mechanisms of production of singlet oxygen in Photodynamic Therapy (PDT):

Type I reaction involves electron/hydrogen transfer directly from the excited photosensitizer to another molecule via electron/hydrogen abstraction. In this reaction free radicals are formed. These radicals then react rapidly, usually with oxygen, resulting in the production of highly reactive oxygen species. These react in turn with tissue and causes irreversible damages. Type II reaction produces the electronically excited and highly reactive state of oxygen known as singlet oxygen. Direct interaction of the excited triplet state photosensitizer with molecular oxygen (which unusually has a triplet ground state) results in the photosensitizer returning to its singlet ground state and the formation of singlet oxygen. In figure (1.4) Initial events of Type I and II reactions involve photoinduced electron transfer (PET) and excitation energy transfer (EET), respectively. While S_0, S_1 and T_3 represent the ground, singlet and triplet states of the photosensitizer, R represents a biomolecule including oxygen [30].

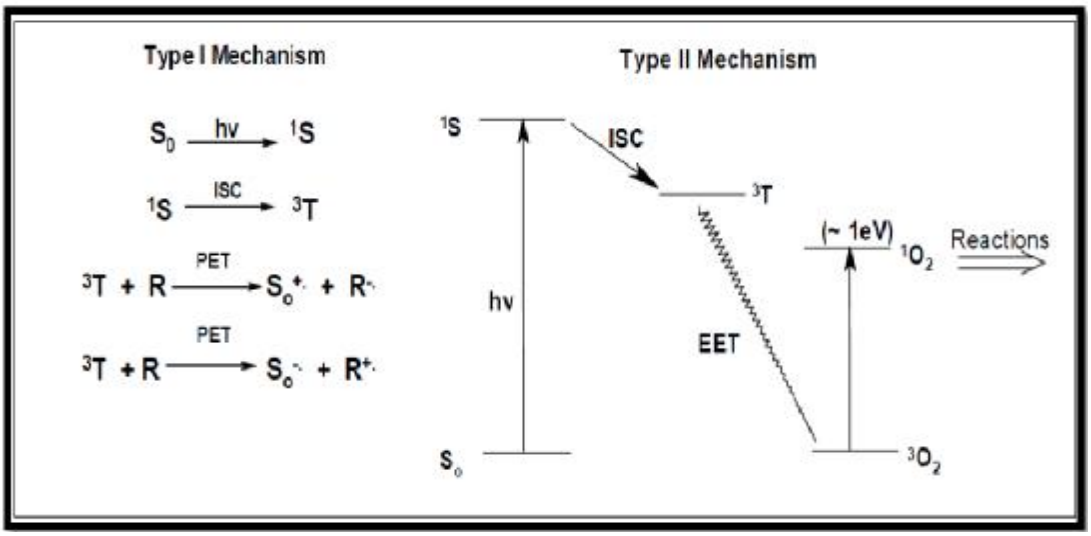

Figure (1.5): The Type I and Type II reactions of a photosensitizer

In PDT, it is difficult to distinguish between the two reactions mechanisms. There is probably a contribution of both type I and II processes, indicating the mechanism of damage is dependent on oxygen and photosensitizer concentration. The oxygen radicals produced are very reactive, and attack cellular targets. The diffusion distance of singlet oxygen in biological tissue has been estimated in the order of 0.01 μm, corresponding to a lifetime of about 0.01 - 0.04 μs. The relative involvement of either Type I or II processes in the PDT action is influenced by factors such as the biological condition of the target, the type of photosensitiser used and its binding site within the tissue. For example, it has been suggested that hypoxic conditions and/or high photosensitiser concentrations might favour Type I reactions, whereas high oxygen concentrations lead to domination of Type II reactions .a photosensitizing compound combined with optical radiation, the response may be of different types, the magnitude and type of reaction is determined by a number of parameters, among others: light intensity, irradiation time, the total dose of light, normally equal to the intensity x time, concentration of the photosensitizer, the time of contact between the sensitizer and the cell, the chemical structure of the sensitizer, the transport of the sensitizer to the cell, and uptake in the cell, the type of cell, the physical and chemical characteristics of the environment and extra- and intra-cellular the sub-cellular localization of the sensitize [8].

1.11 Literature Review:

Evaluation of four new carbocyanine dyes for photodynamic therapy with lasers was done by Lipshutz GS, Castro DJ, Saxton RE, Haugland RP, Soudant J. Laryngoscope in 1994. The search for improved photosensitizers for laser phototherapy of malignancies has led to the examination of a new group of carbocyanine dyes as effective fluorochromes. In this study, four carbocyanine dyes with different absorption maxima of 483 nm [DiOC6(3)], 545.5 nm (DiIC5(3)], 556.6 nm [DiSC5(3)], and 651.0 nm [DiSC3(5)] were tested in vitro. The kinetics of uptake and toxicity of these four dyes were assessed for P3 human squamous cell carcinoma, HT29 colon carcinoma, M26 melanoma, and TE671 fibrosarcoma cell lines at 15, 30, 45, 60, and 180 minutes after exposure with each dye. After sensitization with DiOC6(3), the P3 and M26 cell lines were also tested for phototherapy by treatment with 488-nm light from an argon laser. The results showed that these four carbocyanine dyes had rapid and significant uptake by the carcinoma cell lines with no toxicity at concentrations < 0.1 micrograms/mL. Nontoxic DiOC6(3) levels in sensitized tumor cells after laser phototherapy resulted in approximately 85% inhibition of P3 and approximately 95% inhibition of M26 cell lines by MTT assays. The results suggest that these carbocyanine dyes can be used for tumor photosensitization and wavelength-matched laser photodynamic therapy. Further in vivo studies will be necessary to define the clinical potential of carbocyanine dyes as tumor-targeting agents for phototherapy of cancer.

SOROKIN .P,P LANKARD,JR HAMOND E,C MORUZZV,L have studied the Stimulated emission spectra of two organic dyes, chloro-aluminum phthalocyanine (CAP) and 3,3′-diethylthiatricarbocyanine iodide (DTTC) Giant-pulse ruby laser excitation was used in both cases. An end pumping configuration employed with DTTC resulted in narrow beam divergences and high conversion efficiencies. For CAP, the oscillating transition is one which terminates on an excited vibrational level of the ground electronic state. For

DTTC, stimulated emission at the lowest concentrations occurs at the peak of the Franck–Condon-shifted fluorescence band but moves to longer wavelengths as the concentration is increased. The transient behavior of the CAP laser, pumped in a transverse geometry, was observed and compared with computer solutions of the rate equations. Polarization measurements of the laser beams were also made. An analysis is given of requirements for achieving optimal pumping by means of flash lamps [17].

In 2011 Nafie. A. Almuslet and Nazic. M. Hassan made a spectroscopic and photophysical study for the photosensitizer dye Phenoxazone 9 Three light sources (coherent and incoherent) were used to irradiate the dye for different exposure time and the emission spectra indicated the existence of singlet oxygen, They concluded that it is necessary to characterize photosensitizer during photodynamic reaction and to describe the ability of it to produce singlet oxygen which has therapeutic effect of cancer when irradiated the photosensitizer by a number of light sources [16].

1.12 Aim of the Work:

The main aim of this work is the exploration of the singlet oxygen emitted after irradiation of a number of dyes namely; the dibenzocyanine 45(DDTTC45), Rhodamine 6G and Methylene blue using lasers with different wavelengths. This can be used in photodynamic therapy (PDT)

CHAPTER TWO

THE EXPERIMENTAL PART

Chapter Two

The Experimental Part

2.1 Introduction:

In this chapter all the materials, equipments used in this work and the experimental technique are presented. The Experimental part of this study was designed to analysis emission spectra recorded after irradiation of Dibenzocyanine 45 (DDTTC), Rohdamin 6G and Methylene blue dyes by using lasers with different wavelengths.

2.2 The Materials:

Organic dyes and different solvents were used to prepare the samples. These are:

2.2.1 Dibenzocyanine 45 (DDTTC 45):

The dibenzocyanine 45 is belonging to the family of cyanine dyes. Cyanine is a non-systematic name of a synthetic dye family belonging to the polymethine group. The family of cyanine dyes includes Cy2, Cy3, Cy5, Cy7 and their derivatives, which the numbers are based on the partially saturated indole nitrogen heterocyclic nucleus with two aromatic units being connected via a polyalkene bridge of varying carbon number. Cyanines have many uses as fluorescent dyes, particularly in biomedical imaging. Depending on the structure, they cover the spectrum from infrared to ultraviolet. Cyanines are utilized to increase the sensitivity range of photographic emulsions, such as increasing the range of wavelengths which will form an image on film. Cyanines are mostly green or light blue in color, and are chemically unstable. These dyes molecules display very strong electronic absorption bands in the visible spectral range. These dye molecules assume nearly planar geometry with abundant π electrons which provides possibility of obtaining a well organized crystal structure. Such π -stacking interaction are common for many organic conductors and provide efficient pathways for shuttling

electrons through the resulting macrostructures. Figure (2.1) shows the chemical structure of Dibenzocyanine 45 (DDTTC) [22,23].

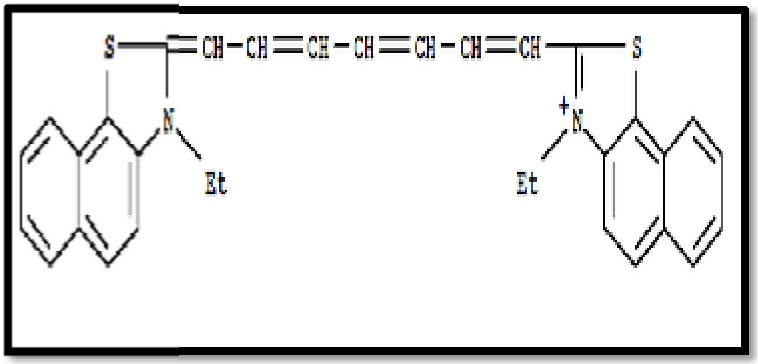

Figure (2.1): Chemical structure of Dibenzocyanine 45 (DDTTC).

Typical characteristics and physical properties of the Dibenzocyanin**e** 45 are listed in Table (2.1)

Table (2.1): Characteristics and physical properties of DDTTC 45

Constitution	Dibenzocyanin 45(DDTTC) thiatricarbocyanine iodid
Chemical Formula	C_{33}-H_{29}-I-N_2-S_2
Molecular Weight (MW)	544.43
Chemical Abstracts Service Registry Number (CAS) NO	20682-18-2
Absorption Maximum	798 nm
Appearance	bronze colored, crystalline brown solid
Molar absorptive	19.6 x 104 L M^{-1}1 cm^{-1}

It can be dissolved in ethanol, propanole and acetone, while it is less dissolved in methanol and it is unable to dissolved in water and chloroform[15]**.**

2.2.2 Rhodamine 6G:

Rhodamine 6G is a highly fluorescent Rhodamine family dye. It is often used as a tracer dye within water to determine the rate and direction of flow and transport. Rhodamine dyes fluoresce and can thus be detected easily and inexpensively with instruments called fluorometers. Rhodamine dyes are used extensively in biotechnology applications such as fluorescence microscopy, flow cytometry, fluorescence correlation spectroscopy.Rhodamine 6G

Chloride powder emits yellow light under green laser. Rhodamine 6G is also used as a laser dye. The dye has a remarkably high photostability, high fluorescence quantum yield and its lasing range has close proximity to its absorption maximum (approximately 530 nm). The lasing range of the dye is 555 to 585 nm with a maximum at 566 nm [17,18].

Rhodamine 6G usually comes in three different forms. Rhodamine 6G chloride is a bronze/red powder with the chemical formula $C_{27}H_{29}ClN_2O_3$. Although highly soluble, this formulation is very corrosive to all metals except stainless steel. Other formulation are less soluble, but also less corrosive. Rhodamine 6G Perchlorate, ($C_{27}H_{29}ClN_2O_7$), comes in the form of red crystals, while rhodamine 6G tetrafluoroborate, ($C_{27}H_{29}BF_4N_2O_3$), appears as maroon crystals. Figure (2.2) shows the chemical structure of Rhoadmine 6G [19, 20].

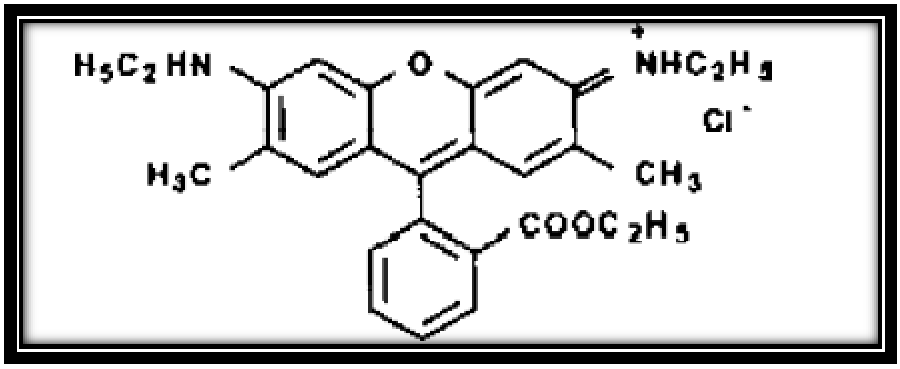

Figure (2.2): Chemical structure of Rhoadmine 6G.

Typical characteristics and physical properties of Rhodamine 6G are listed in Table (2.2).

Table (2.2): Characteristics and physical properties of Rhodamine 6G

Constitution	Benzoic Acid,rhodamine6G
Chemical Formula	$C_{28}H_{31}N_2O_3Cl$
Molecular Weight (MW)	479.02
Chemical Abstracts Service Registry Number (CAS) NO	989-38-8
Absorption Maximum	530 nm
Appearance	red, crystalline solid
Molar absorptivity	10.50×10^4 L mol^{-1} cm^{-1}

2.2.3 Methylene Blue:

Methylene blue is a heterocyclic aromatic chemical compound with the molecular formula $C_{16}H_{18}N_3SCl$. It has many uses in a range of different fields, such as biology and chemistry. At room temperature it appears as a solid, odorless, dark green powder, that yields a blue solution when dissolved in water. The hydrated form has 3 molecules of water per molecule, another histology stain, new methylene blue, nor with the methyl violets often used as pH indicators, Figure (2.3) shows the Chemical structure of Methylene Blue [21].

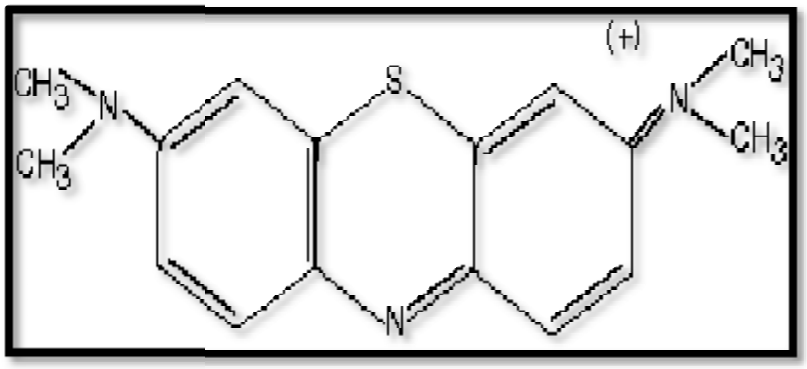

Figure (2.3): Chemical structure of Methylene Blue

Typical characteristics and physical properties of Methylene Blue are listed in Table (2.3) [15].

Table (2.3): Characteristics and physical properties of Methylene Blue.

Constitution	Swiss blue
Chemical Formula	$C_{16}H_{18}ClN_3S$
Molecular Weight (MW)	319.85
Chemical Abstracts Service Registry Number (CAS) NO	20682-18-2
Absorption Maximum	668 and 609 nm.
Appearance	crystalline blue solid
Molar absorptivity	19.6 x 104 L M^{-1}1 cm^{-1}

2.2.4 The solvents

The solvent in which the dye is dissolved plays an important role when defining physical properties and potential hazards. Most dyes are polar molecules, and excitation into their lowest-lying single tstate is accompanied by an increase in the dipole moment. Accordingly, solvent polarity plays an important role in shifting the wavelength. In majority of circumstances, increasing solvent polarity will shift the gain curve toward longer wavelength. In the case of more polar dyes, the shift can be as high as 20-60 nm.

Solvents such as water, methanol and ethanol, which would appear to be optimal for many dyes, are often not useful solvents for near-IR and IR dyes because of the presence of hydroxyl group overtones in this spectral range [12]. The solvents used in this work were:

2.2.4.1 Ethanol:

Ethanol, also known as ethyl alcohol or grain alcohol, is a flammable, colorless, slightly toxic chemical compound. Its molecular formula is C_2H_6O. At the molecular level, liquid ethanol consists of hydrogen –bonded pairs of ethanol molecules. Ethanol is used as a solvent in dissolving dyes. Ethanol can dissolve both polar and non-polar substances. Organic solids of low molecular weight are usually soluble in ethanol. Among ionic compounds many mono-valent salts are at least somewhat soluble in ethanol, with salts of large, polarizable ions being more soluble than salts of smaller ions. Most salts of polyvalent are organic in soluble in ethanol [21].

2.2.4.2 Acetone:

Acetone is the organic compound with the formula $(CH_3)_2CO$. This colorless, mobile, flammable liquid is the simplest example of the ketones. Acetone is miscible with water and serves as an important solvent in its own right, typically as the solvent of choice for cleaning purposes in the laboratory. It is a common building block in organic chemistry [21].

2.2.4.3 Propanol:

Propanol is a primary alcohol with the molecular formula of $CH_3CH_2CH_2OH$. It is also known as 1-propanol, 1-propyl alcohol, n-propyl alcohol, n-propanol, or simply propanol. It is used as a solvent in the pharmaceutical industry, and for resins and cellulose esters. It is formed naturally in small amounts during many fermentation processes [21].

2.2.4.4 Methanol:

Methyl Alcohol, or Methanol, is colorless hygroscopic liquid usually containing 0.01 - 0.04 percent water. It is highly inflammable and toxic. Methanol is a polar, protic solvent frequently used to dissolve dyes like Coumarins, Rhodamines, and Cyanines. Its excellent optical transparency makes it the ideal solvent for pumped dye lasers [21].

Table (2-4) lists the characteristics and physical properties of the used solvents.

Table (2.4): Characteristics and physical properties of the used solvents

Property	**Ethanol**	**Propanol**	**Acetone**	**Methanol**
Molecular weight (g/mol)	46.07	60.1	58.08	32.04
Freezing point (°C)	-114.1	-97.7	-94.7	-97.7
Boiling point (°C)	78.3	97.1	56.53	64.7
Flash point(°C)	12	15	17	11
Density (g/cm^3)	0.7936 0.7894 0.785	0.8034	0.7925	0.796115 0.791320 0.786625
Refractive Index	1.3614 1.3594	1.3284 1.3265	1.35900	1.328420 1.326525
Dielectric constant	24.55	32.7	68.32	32.7
Ionization potential (eV)	10.49	10.84	10.22	10.84
Solubility	water, organic solvents	water, organic solvents	Water, Ethanol, Toluene	water, organic solvents

2.2.4.5 Choice of Solvent :

The followings are the criteria for choosing appropriate solvents for dyes

a) The solvent must be transparent at the pump wavelength and the emission wavelength of the dye.

b) The solvent must be photochemically stable when exposed to the light. In particular, solvents containing chlorine, such as chloroform, and secondary alcohols, such as isopropanol, are not useful as solvents for dyes because of their low photochemical stability.

c) Some solvents are often not useful for near-IR and IR dyes due to the presence of hydroxyl group overtones in this spectral region [12].

2.3 The Equipments:

The equipments used in this study were:

2.3.1 UV – VIS 1240 Spectrophotometer:

This device was used to measure the absorption and the transmission of the dyes before use. The UV-VIS spectrophotometer was supplied from SHIMADZU Company (Japan). It contains a cell (quartz curvette with optical length or thickness of 10 mm supplied from Hellma company (Germany) as a sample holder. The UV 1240 comes standard with a spectrum mode that allows for full spectral data acquisition over the wavelength range from 190 nm to 1100 nm. Upon completion of the spectral scan, the peaks and valleys can be marked within a few seconds; The optical specifications of the UV – VIS 1240 spectrophotometer are listed in table (2-5). The standard peak function allows for clear and accurate detection of the most sensitive wavelengths [22].

Table (2.5): The optical specifications of the UV – VIS 1240 spectrophotometer.

Wavelength range	190 nm to 1100 nm
Display wavelength	0.1nm step (1nm step in spectrum mode)
Light source	Auto adjustment for maximum sensitivity correction with the computer memory halogen lamp and deuterium lamp.
Monochrometer	Incorporates aberration-correcting concave blazed holographic grating
Measurement method	Single beam measurement Silicon Detector
Detector	Silicon photodiode

The UV 1240 spectrophotometer can be connected to PC via the standard RS_232C port. The UV Data manager software designed to help, organize and store data files in memory of computer [22].

2.3.2 The USB 2000 Spectrometer:

Emission spectra were recorded by USB2000 spectrometer and corresponding software. The USB 2000 is a small footprint Fiber Optics Spectrophotometer; it automatically reads the wavelength calibration coefficients of the spectrometer and configures the operating software.

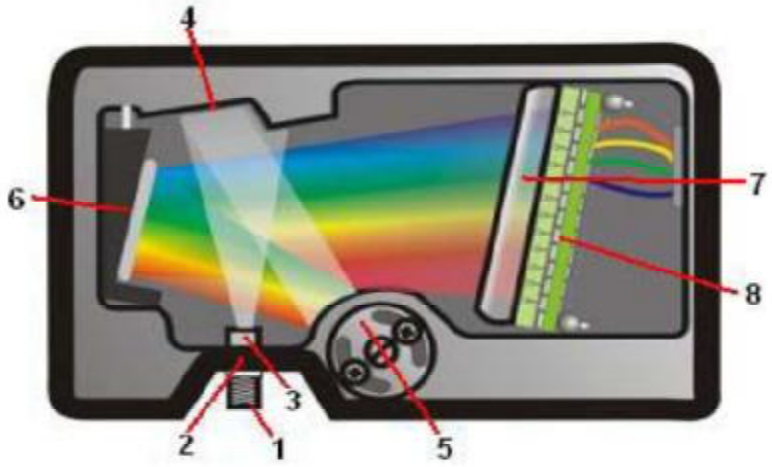

Figure (2.4): USB2000 spectrometer and its components.

This device was supplied from ocean company module USB2E7524; it's a modular design user-configuration wavelength. USB 2000 can detect wavelength from 400-1100 nm. USB 2000 is used to recording the signal as wavelength in (nm) against output intensity in arbitral units. Light source and sample holder are connected to spectrometer via 400 μm fiber; The USB2000 has both USB and serial port connectors, enabling the user to connect the spectrometer to PC. Ocean Optics optical fibers are silica-core and silica-clad fibers. The standard length of a fiber assembly is two meters, and assemblies are available in diameters ranging from 4 μm to 1500 μm [23].

The components of the spectrometer, shown in figure (2.6) are:

1. SMA Connector: The SMA connector secures the input fiber to the spectrometer. Light from the input fiber enters the optical bench through this connector.
2. Slit**:** The slit is a dark piece of material containing a rectangular aperture, which is mounted directly behind the SMA connector. The size of the aperture regulates the amount of light that enters the optical bench and controls spectral resolution.
3. Filter: The filter is a device that restricts optical radiation to pre-determined wavelength regions.
4. Collimating Mirror**:** The collimating mirror focuses light entering the optical bench towards the grating of the spectrometer.
5. Grating**:** The grating diffracts light from the collimating mirror and directs the diffracted light onto the focusing .
6. Focusing Mirror: The focusing mirror receives light reflected from the grating and focuses the light onto the detector.
7. L2 detector collection lens: The L2 detector collection lens (optional) is attached to the CCD detector. It focuses light from a tall slit onto the shorter CCD detector elements. The L2 detector dollection lens should be used with large diameter slits or in applications with low light levels.

8. CCD detector (UV or VIS): The CCD detector collects the light received from the focusing mirror or L2 detector collection lens and converts the optical signal to a digital signal. The spectrometer then transmits the digital signal to the OOIBase32 application.

OOIBase32 is operating software for all ocean optics spectrometers; OOIBase32 is a user-customizable, advanced acquisition and display program that provides a real-time interface to a variety of signal-processing functions. With OOIBase32, user has the ability to perform spectroscopic measurements (such as absorbance, reflectance, and emission), control all system parameters, collect and display data in real time, and perform reference monitoring and time acquisition experiments Table (2.6) lists the optical specifications of the USB 2000 spectrometer [23].

Table (2.6): The optical specifications of the USB 2000 spectrometer.

Wavelength optimization	200 - 1100 nm
Numerical aperture	0.22+/- 0.2
Fiber core	Pure silica
Cladding	Doped fused silica
Fiber profile	Step index multimode
Jacketing	Silicon Monocoil

2.4 Laser Sources:

Two diode lasers with different wavelengthes were used in this study to irradiate the dyes with different irradiation times. These lasers are:

2.4.1 The red diode laser 671 nm:

It is a semiconductor light source emitting coherent light. When a light-emitting diode is forward biased (switched on), electrons are able to recombine with electron holes within the device, releasing energy in the form of photons. The laser used here was manufactured by (Roithner Laser TechnikGmbh), it is a CW laser with 671 nm wavelength and 100 mW . Figure (2.5) shows the red diode laser [24].

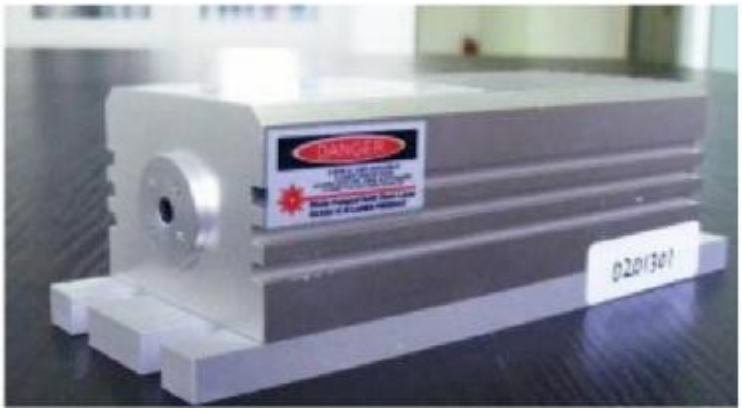

Figure (2.5) Red diode laser 671 nm

The specifications of the used red diode laser are listed in table (2.7).

Table (2.7): the specifications of the red diode laser 671nm.

Wavelength	671 nm ± 1nm
Output power	100 mW
Transverse mode	TEM_{00}
Operating mode	CW
Warm-up time	< 10 minutes
M^2 Factor	<1.2
Beam divergence (full angle)	<1.5 mrad
Beam diameter (at the aperture)	< ~21.0 mm
Polarization ratoe	>100 : 1 (0^0 or 90^0)
Pointing stability after warming up	<0.05
Operating temperature	10 – 35 C^0
Power supply (90-260 VAC)	PSU FAD (included)
Expected time	1000 hour

2.4.2 The green diode laser 532 nm:

The green diode laser used here has wavelength of 532 nm and output power of 100 mW. Figure (2.6) shows the green diode laser and table (2.8) lists the specifications of this laser [24].

Figure (2.6): Green Diode Laser (532 nm)

Table (2.8): Specifications of the green diode laser

CW output power	100 mW
Wavelength	532 nm
Operating mode	CW
Power stability (rms, over 1 hour)	< 10%
Beam mode	TEM_{00}
Beam diameter (at the aperture)	< 1.5 mm
Beam divergence (full angle)	<1.5 mrad
Operating current	< 300 mA(1-20 MW) <650 mA(50 – 100 MW)
Optimum operating tempreature	20-30 C^0
Input voltage	APC(complite driver unit included)

2.5 Experimental procedure and setup:

The experiments were carried out as follows:

A simple method was used to prepare the samples by dissolving them in different solvents, by taking 70mg from the dyes DTTC 45 , Rhodamine 6G, methyline blue).each 70 mg of these dyes was dissolved in 50 ml of the solvents as follows:

1. DDTTC45 was dissolved in propanol, methanol and acetone respectively.
2. Mthylene blue was dissolved in ethanol and methanol. While Rodamin6G was dissolved in propanol.

Firstly the transmissions of the solvents were recorded using uv-vis spectrophotometer to be sure that they are transparent in the range of 190-1100 nm.

Secondly the absorption spectra of the dissolved shambles were recorded by using uv-vis spectrophotometer to ensure that they are transparent in the some region.

Thirdly all the samples were irradiated by the red laser 671nm and the green diode laser 532nm for different time intervals starting from one minute up to seven minutes.

Finally the USB 2000 spectrometer was used to measure the emission spectra of the samples after irradiation. Database was used to identify the emission bands.

2.5.1 Experimental setup:

Each sample from the dissolved dyes was put inside the quartz cuvette. After irradiation by the red and green laser, the emitted light was captured from side, at angle of 90° to laser beam, and was guided by the fiber optic to the detector. For each sample the emission spectrum was recorded for each irradiation time using USB2000 spectrometer. Figures (2.9) and (2.10) show the arrangement of the experimental set up.

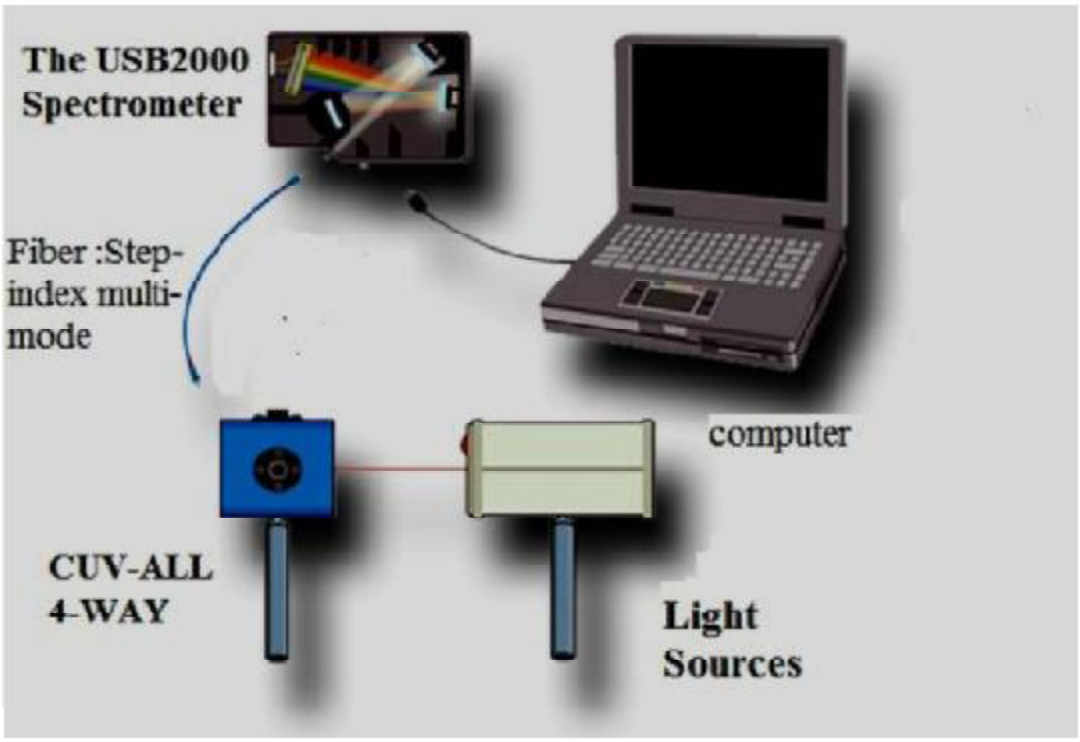

Figure (2.7): The arrangement of the experimental setup

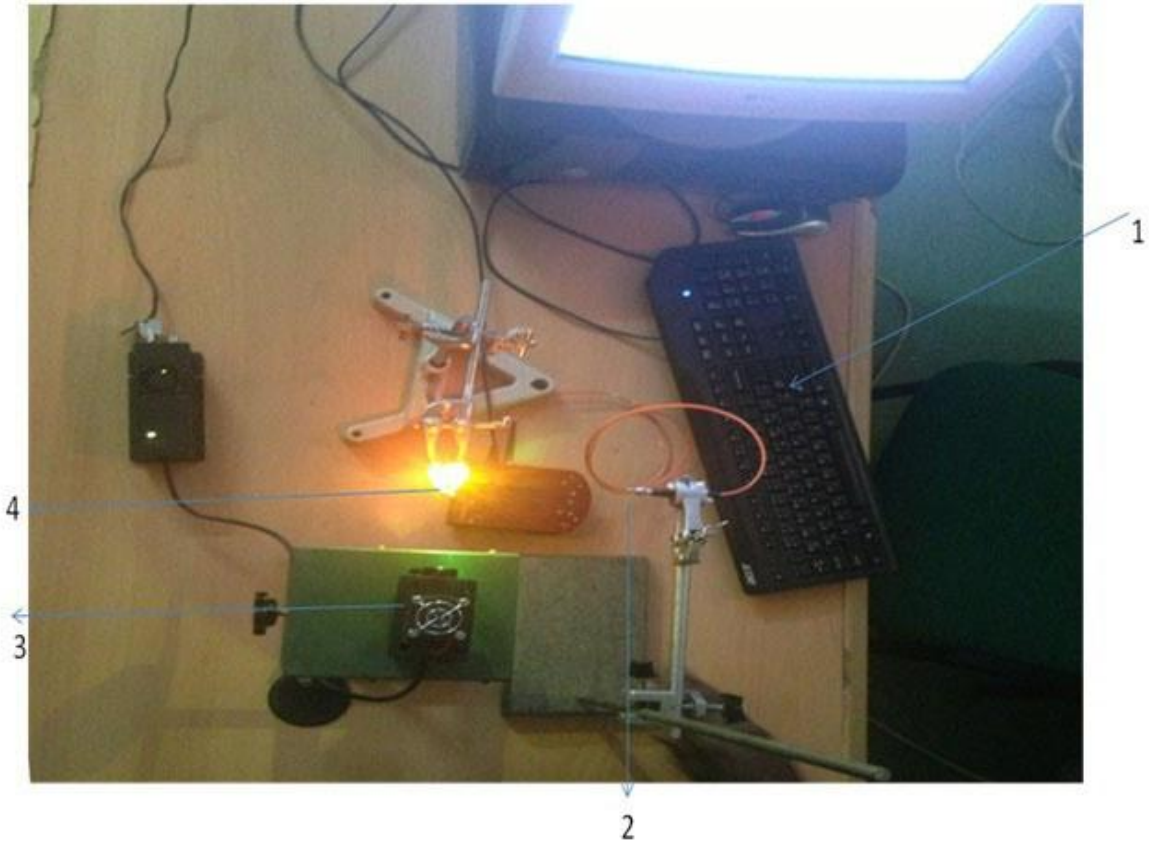

Figure (2.8): graph of the experimental setup.

1. Keyboard
2. USB 2000 spectrometer
3. Green diode laser 532nm
4. The dye sample

Chapter Three
Results and Discussion

Chapter Three

Results and Discussion

3.1 Introduction:

In this chapter the results obtained during this work, the discussion, conclusions and suggested future work are presented. Three types of dyes, namely: (Dibenzocyanine 45), Methylene blue and Rhohdamine 6G were prepared by dissolving them in organic solvents, Ethanol, propanol, Acetone and Methanol, then they were irradiated by two lasers, after the irradiation the emission of the dyes were recorded. The emission results of the dyes were analyzed spectroscopically using two USB 2000 spectrometer and other components.

3.2 Absorption spectra of the solvents:

The solvent must be transparent at the pump wavelength and the emission wavelength of the dye. To identify the transparency of the solvents, the UV – VIS 1240 Spectrophotometer device was used to measure the absorption and the transmission of the used solvents (acetone, ethanol, methanol and propanol). Figures (3.1) shows the absorption spectra of the used solvents ethanol, propanol and acetone while figure (3.2) shows the absorption spectrum of the solvent methanol. .

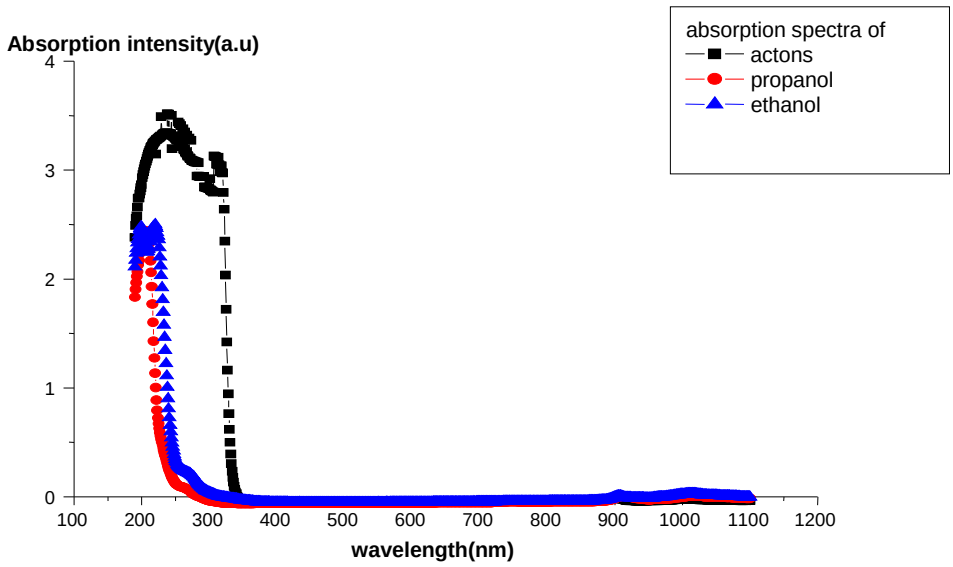

Figure (3.1): Absorption spectra of (Aceton, Ethanol and Propanol)

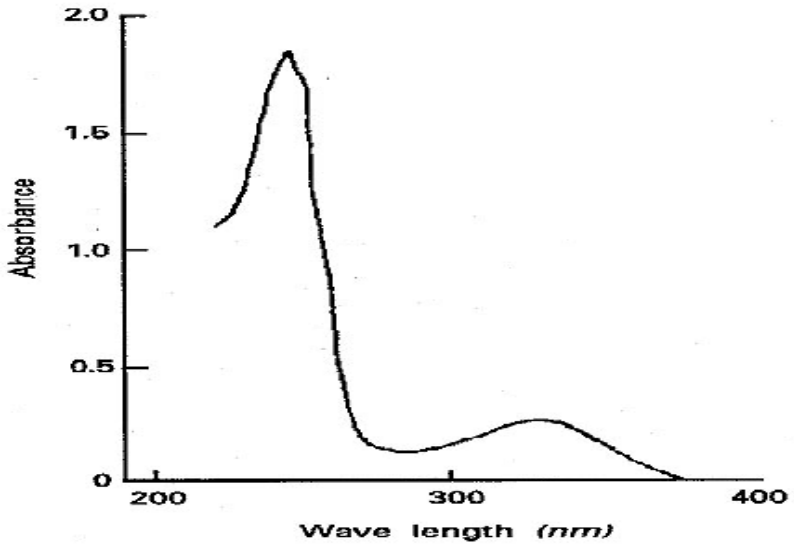

Figure (3.2): Absorption spectrum of the solvent Methanol**.**

3.3 The absorption spectra of the (Dibenzocyanine 45) samples:

The absorption spectra of (DDTTC 45) dissolved in ethanol; propanol, acetone and methanol in the range between 200 nm and 1100 nm are shown in figure (3.3).

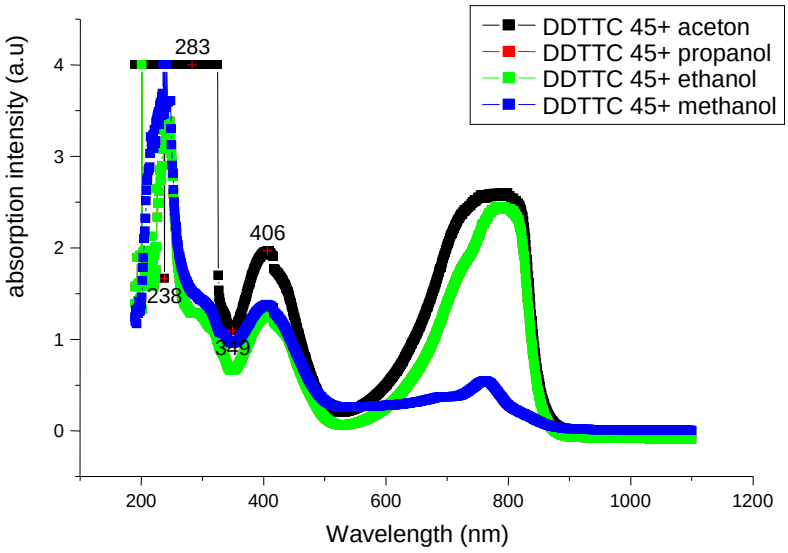

Figure (3.3): Absorption spectra of DDTTC45 dissolved in ethanol, propanol, acetone and methanol

In this figure one can see that (DTTC 45) has strong absorption in the UV and visible regions especially at 283 nm, 349 nm, 406 nm and 780 nm for acetone, propanol, and ethanol respectively **.**The light sources (532 nm and 671 nm) can be used to excite this dye and study its emission.

3.4 Emission Spectra of DDTTC 45 Samples after irradiation by 532 nm:

Figures (3.4) to (3.6) show the emission spectra of DDTTC 45 dissolved in ethanol, propanol and acetone after irradiation by green diode laser 532 nm with 100 mW output power.

Figure (3.4) shows the emission spectra of the DDTTC45 dissolved in ethanol after irradiation for different exposure times starting from 1 minute up to 6 minutes, the step was 1 minute. The emission spectra have two broad bands, first one at 620.20 nm, and the second one at 819.78 nm. Increasing the exposure time led to increase the emission intensity.

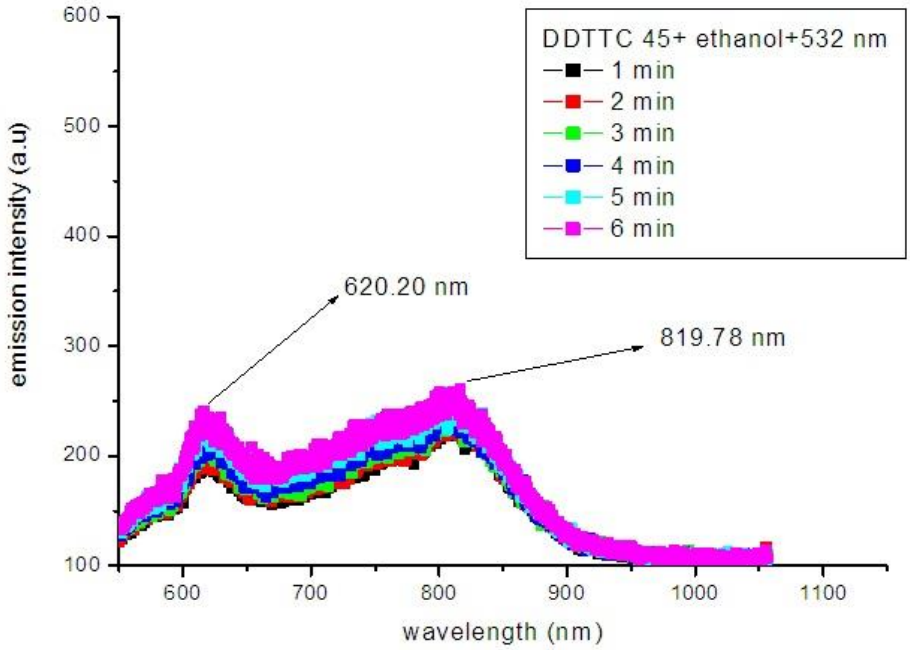

Figure (3.4): emission spectra of DDTTC45 dissolved in ethanol after irradiation by 532 nm for different times.

Figure (3.5) shows the emission spectra of DDTTC 45 dissolved in propanol after irradiation by 532 nm for different exposure times starting from 1 minute up to 5 minutes. The emission spectra indicated two broad bands with different intensities; the first one at 624.96 nm whiles the second one at 830.19 nm. The intensity of the two peaks was increased with increasing the exposure time.

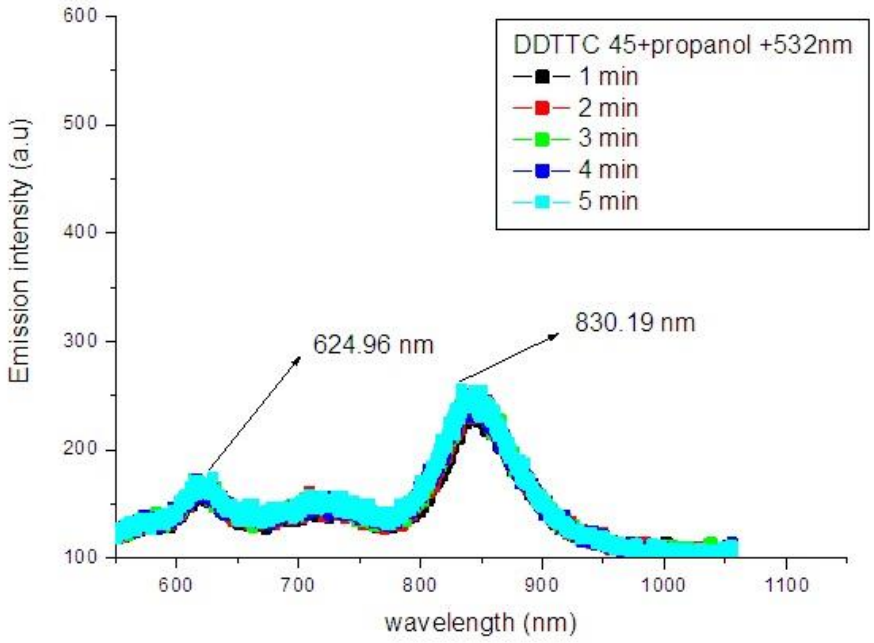

Figure (3.5): Emission spectra of DDTTC 45 dissolved in propanol after irradiation by 532 nm for different times

Figure (3.6) shows the emission spectra of DTTC 45 dissolved in acetone after irradiation by532 nm for different times: times starting from 1 minute up to 6 minutes, the step was 1 minute. Emission spectra showed one broad band at 620.21 nm, increasing the exposure time led to increase the emission intensity.

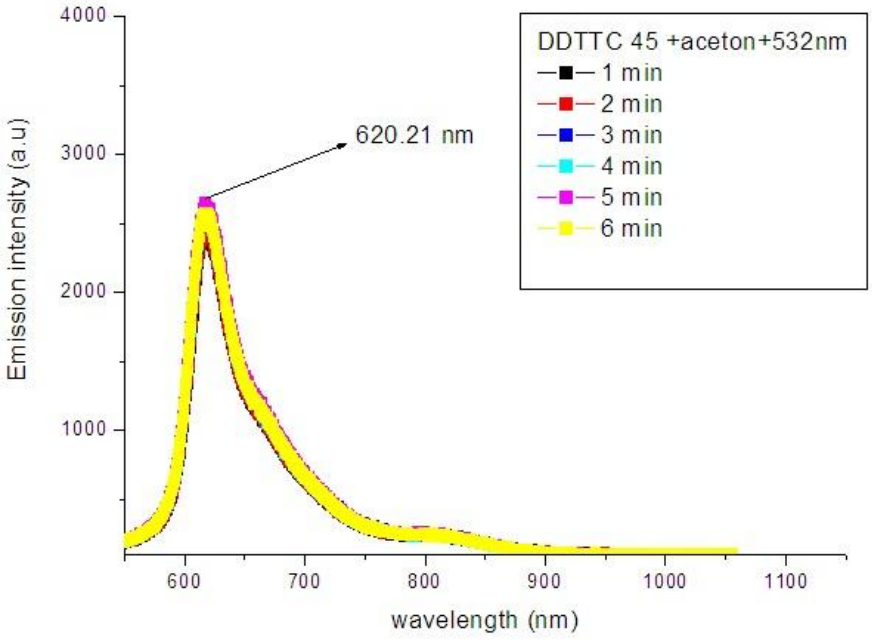

Figure (3.6): Emission spectra of DDTTC45 dissolved in acetone after irradiation by532 nm for different times.

3.5 Emission Spectra of the DDTTC45 Samples after irradiation by 671 nm:

Figures (3.7) to (3.9) show the emission spectra of DDTTC 45 dissolved in ethanol, propanol and acetone after irradiation by red diode laser with 671nm wavelength and 100 mW output power.

Figure (3.7) shows the emission spectra of DDTTC 45 dissolved in ethanol after irradiation by 671nm for different times; starting from 1 minute up to 6 minutes, the step was 1 minute. The emission spectra indicated one broad band at 804.40 nm; Also Increasing the exposure time led to increase the emission intensity.

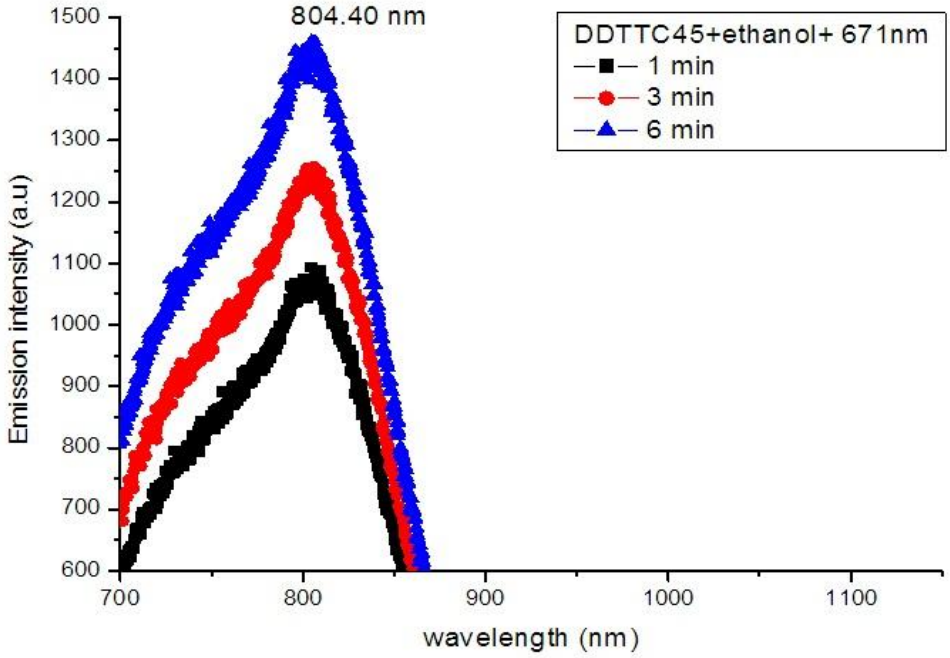

Figure (3.7): Emission spectra of DDTTCe 45 dissolved in ethanol after irradiation by 671 nm for different times

The emission spectra of DDTTC 45 dissolved in propanol after irradiation by 671 nm is shown in figure (3.8); the exposure time was starting from 1minute up to 6 minutes. The emission spectra showed two bands the first one at 719.34 nm and the second one at 844.61 nm increasing the exposure time led to intensity increasing.

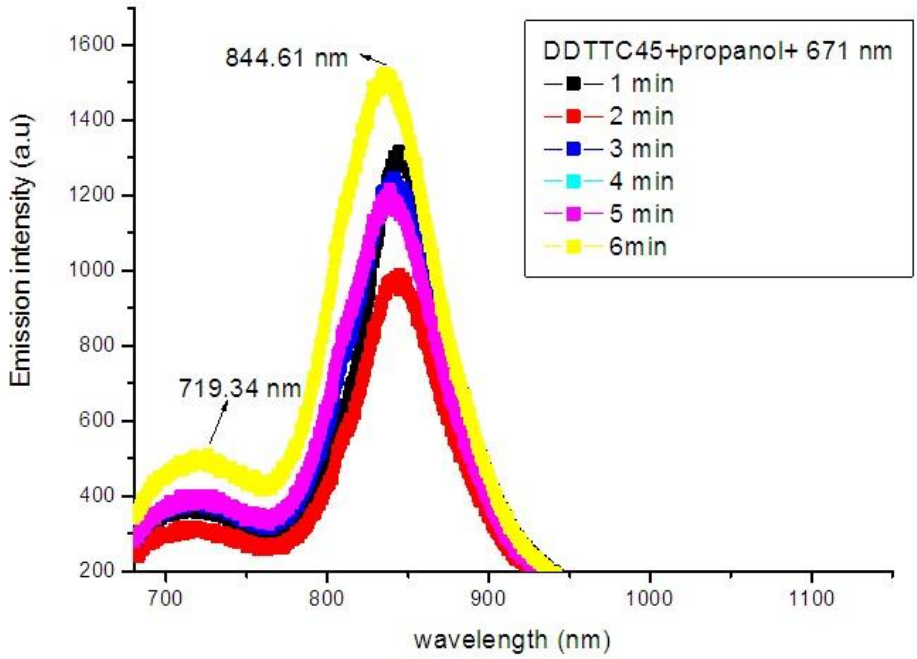

Figure (3.8): Emission spectra of DDTTC 45 dissolved in propanol after irradiation by 671nm for different times.

Figure (3.9) shows the emission spectra of DDTTC 45 dissolved in acetone after irradiation by 671nm for different times: starting from 1 minute up to 6 minutes, one minute each step. The emission spectra indicated one broad band; at 703.23nm indicate the production of singlet oxygen. Increasing the exposure time led to intensity increasing.

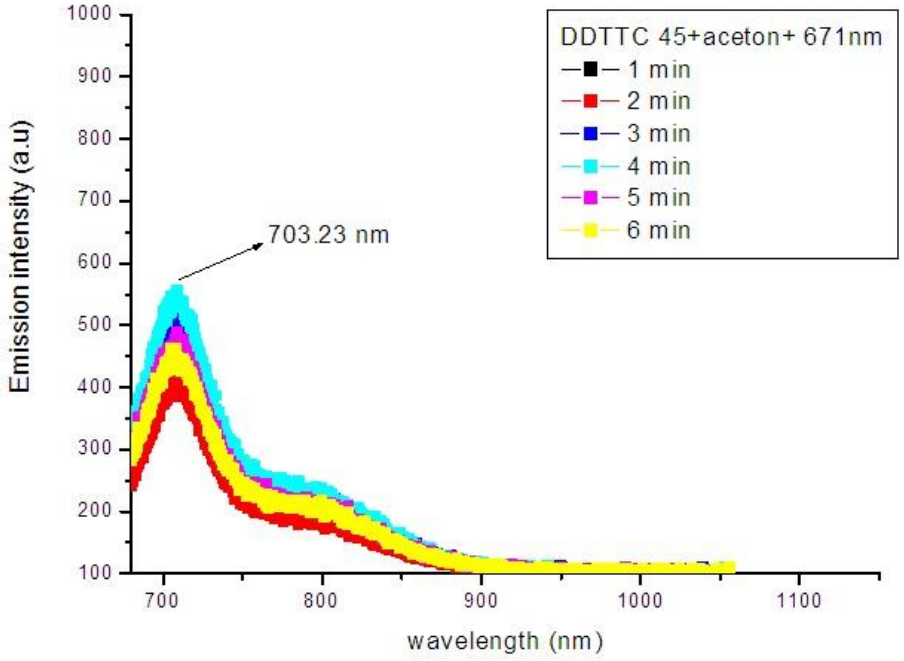

Figure (3.9) emission intensity spectra of DDTTC 45 dissolved in acetone after irradiation by 671nm for different times

3.6 The absorption spectrum of the Methylene Blue:

The absorption spectrum of methylineblue, in the range between 200 nm and 1200 nm is shown in figure (3.10).

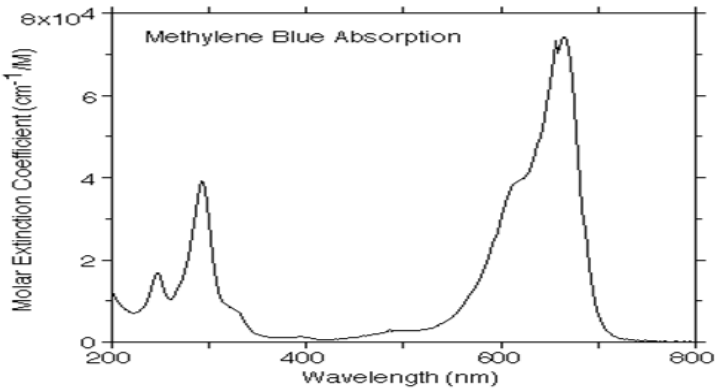

Figure (3.10): Absorption spectrum of Methylene Blue

In this figure one can see that (Methylene blue) has strong absorption in the UV and visible range especially between 600-700 nm.

3.7 Emission spectra of the Methylene Blue samples after irradiation by 532 nm:

Figure (3.11) shows the emission spectra of Methylene Blue dissolved in ethanol after irradiation by 532 nm for different times; starting from 1 minute up to 6 minutes, the step was 1 minute. The emission spectra showed one broad band at 742.67 nm, increasing the exposure time led to increasing the emission intensity.

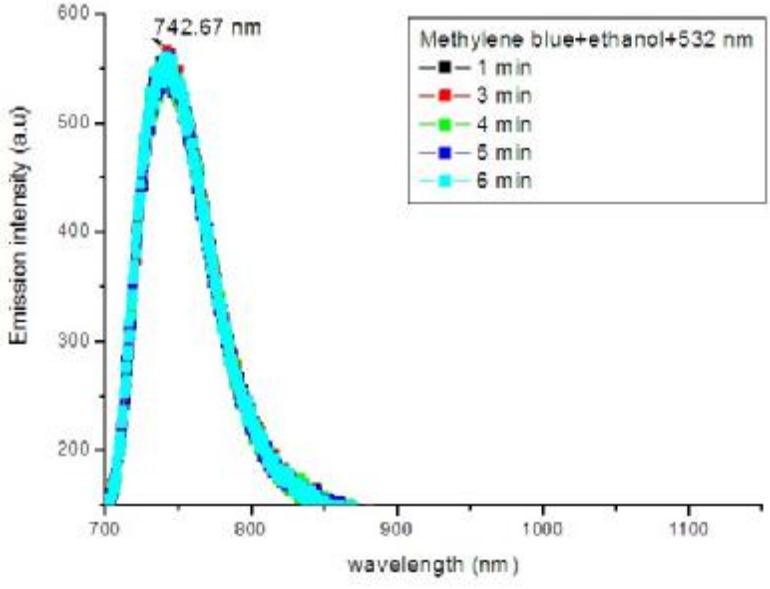

Figure (3.11): Emission spectra of Methylene Blue dissolved in ethanol after irradiation by 532 nm for different times

the emission spectra of Methylene Blue dissolved in methanol is shown in figure (3.12) after irradiation by 532nm for different time; starting from 1 minute up to 7 minutes, the step was 1 minute . The emission spectra indicated the same band at 742.33 nm. Also increasing the exposure time led to intensity increasing.

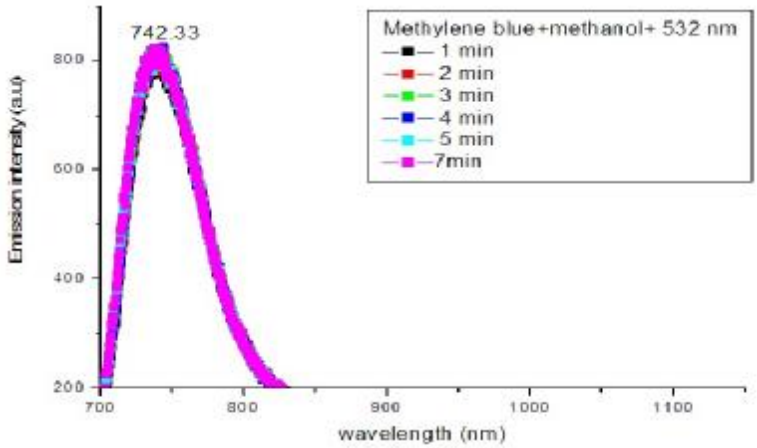

Figure (3.12): Emission spectra of Methylene Blue dissolved in methanol after irradiation by 532nm for different times.

3.8 Emission spectra of Methylene blue after irradiation by 671 nm:

Figure (3.13) shows the emission spectra of Methylene Blue dissolved in methanol after irradiation by 671nm for different times: starting from 1 minute up to 7 minutes, the step was 1 minute. The emission spectra indicate two bands with different intensities; at 748.05 nm and 812.73 nm. The emission intensity increased with increasing the exposure time.

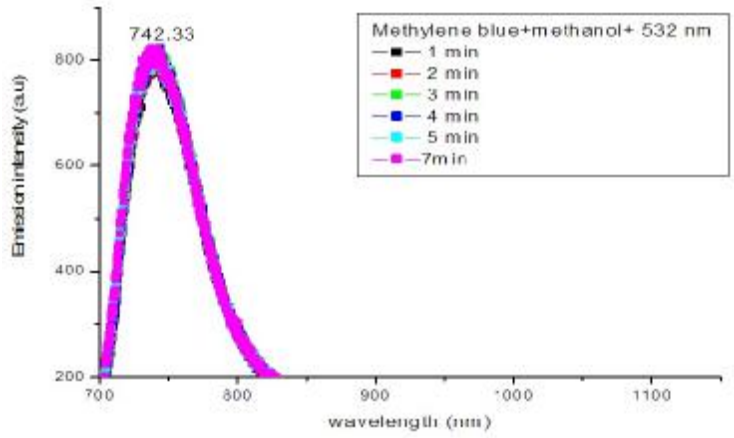

Figure (3.13): Emission spectra of MethyleneBlue dissolved in methanol after irradiation by 671nm for different times.

The emission spectra of Methylene Blue dissolved in ethanol are shown in figure (3.14) where the dye was irradiated by 671 nm for different times; starting from 1 minute up to 6 minutes, the step was 1 minute. The emission spectra indicated two bands with different intensities at 748.51 nm and at 811.04 nm. Increasing the exposure time led to increasing emission intensity.

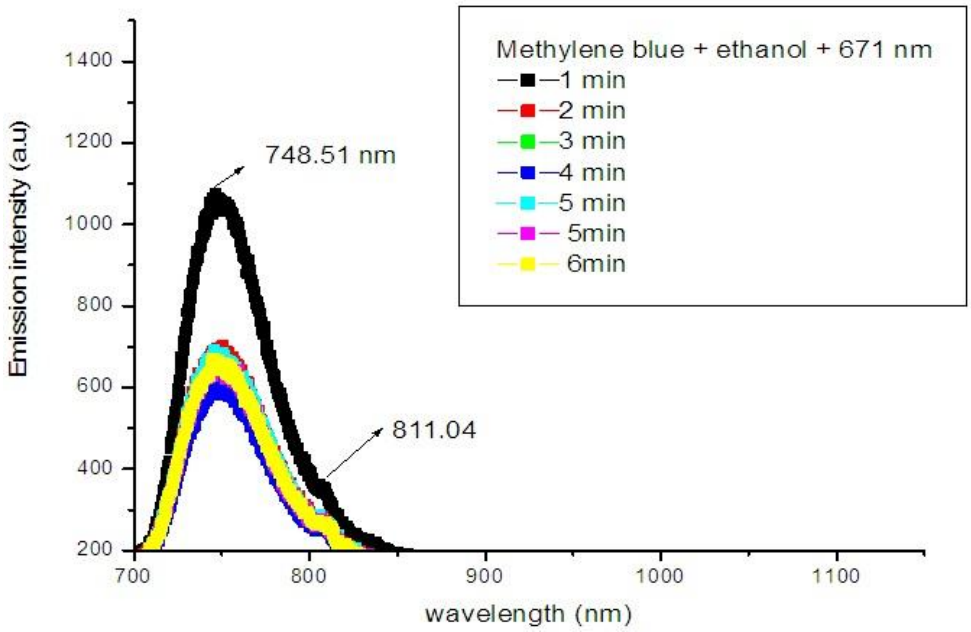

Figure (3.14): Emission spectra of MethyleneBlue dissolved in ethanol after irradiation by 671 nm for different times.

3.9 The absorption spectrum of the of Rhodamine 6G sample:

The absorption spectrum of the Rhodamine 6G dissolved in methanol in the range between 200 nm and 1200 nm is shown in figure (3.15).

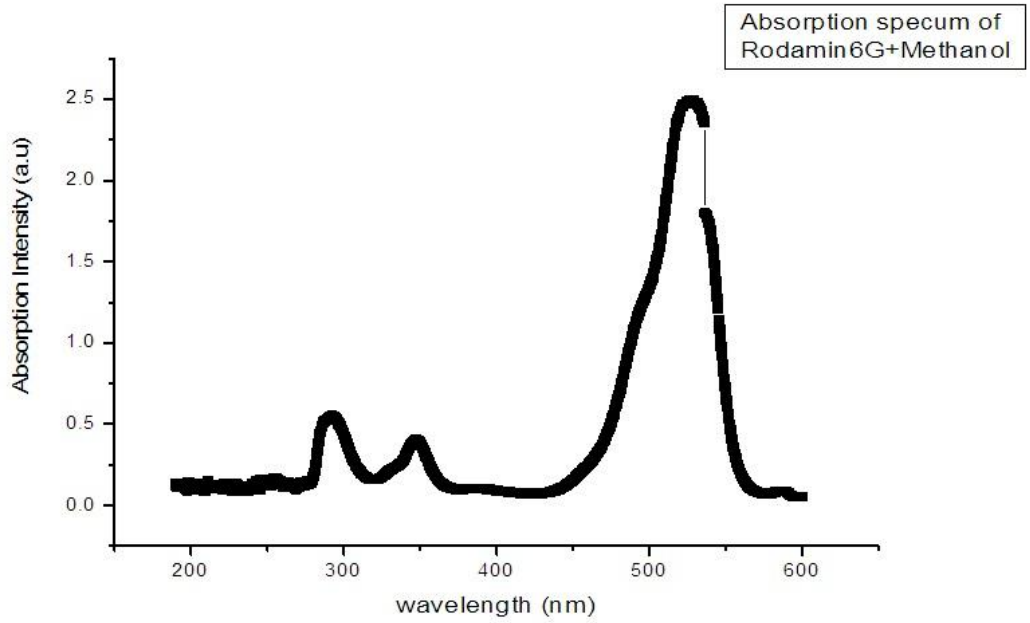

Figure (3.15): Absorption spectrum of Rohdamine 6G dissolved in methanol.

In this figure one can see that (Rohdamine 6G) has strong absorption at 531nm. One can conclude that; the light sources (532nm) can be used to excite this dye In order to study its emission.

3.10 Emission Spectrum of the of Rohdamine 6G samples after irradiation by 532 nm:

Figure (3.16) shows the emission spectra of Rhodamin 6G dissolved in methanol after irradiation by 532 nm for different times; starting from 30 second up to 6 minutes. The emission spectra indicated one broad at 590.49 nm. Increasing the exposure time led to intensity increasing.

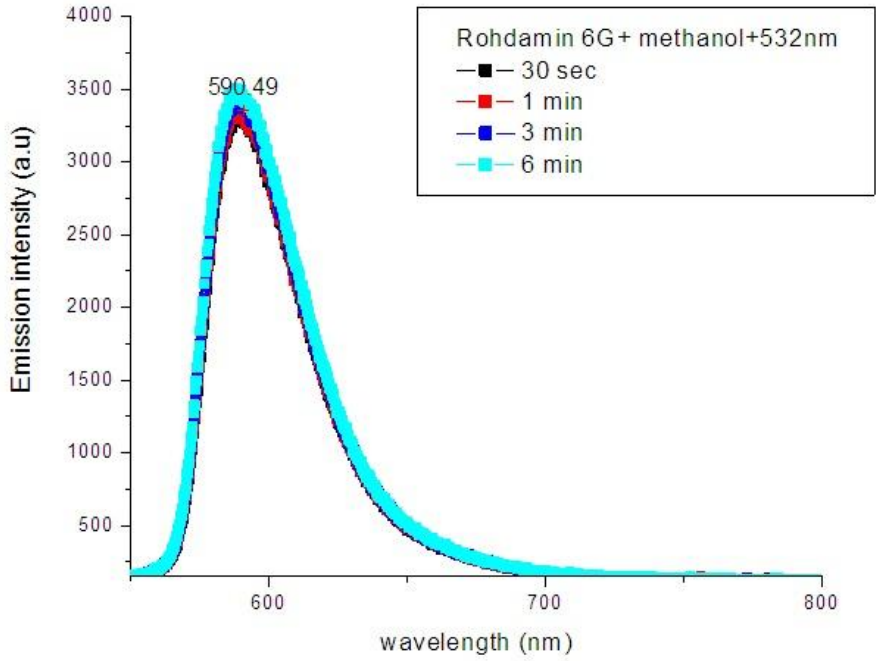

Figure (3.16): Emission spectra of Rhodamin 6G dissolved in methanol after irradiation by 532nm for different times.

Table (3.1) lists the maximum emission intensities recorded in this work which contains dye type, solvents, laser type used for irradiation, the exposure times and the emitted wavelengths.

Table (3.1) the maximum emission intensity recorded in this work.

Type of dye	Solvent	Laser used for irradiation	Exposure time (minutes)	emitted wavelengths (nm)
DDTTC 45	Ethanol	532 nm	6	620.20 819.73
	Propanol		5	624.96 830.19
	Acetone		6	620.21
	Ethanol	671nm	6	804.4
	Propanol		6	719.34 844.61
	Acetone		6	703.23
Methylene Blue	Ethanol	532nm	6	742.67
	Methanol		7	742.33
	Methanol	671nm	7	748.51 812.73
	Ethanol		6	748.06 811.73
Rhodamin 6G	Methanol	532nm	6	590.49

3.11 Discussion:

From the experimental results obtained in this work one can noticed that:

DDTTC 45 was efficient in the production of singlet oxygen, where the $^1O_2$ emission band at 703 nm was detected in the emission spectrum.

The efficient solvent to produce singlet oxygen was the acetone which gave positive results.

The other samples were failed to produce singlet oxygen in the experimental performance concluded in this work.

Using lasers in the visible region as excitation source for these dyes led to an emission in the visible and IR region except for the Rhodamin 6G where the emission was at the visible region.

3.12 Conclusions:

From the experimental results obtained in this work it can be concluded that:

- Increasing the irradiation time led to increase the emission intensity.
- The results showed that, the irradiation by the diode laser 532 nm in all samples led to intensity increasing without fluctuation but, the irradiation by the diode laser l671 nm led to fluctuation in all samples except the DDTTC45 dissolved in ethanol.
- DDTTC 45 is suitable Dye for the production of singlet oxygen.
- The suitable light source in this work to produce singlet oxygen from DDTTC45 was the diode laser 671 nm where it was succeeded in production of singlet oxygen.

3.13 Future work:

The followings can be suggested as future work:

- Study the efficiency of the dyes emission with longer exposure time and determine their stability.
- Usage of different organic and non organic solvents to study their effect on the dyes solubility, and emission.
- Study the emission of other dyes that can be used in medicine as photo-sensitizers for photodynamic therapy (PDT).

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