Sudan University of Science and Technology

Collage of Graduate Studies

Production of Singlet Oxygen From (Eosin blue, Rosa Bengal and Safranin O) Dyes Using Coherent and Incoherent Light Sources

إنتاج الأكسجين الآحادي من صبغات الإيوسين الأزرق، روز البنغال والسفرانين بإستخدام مصادر ضوئية مترابطة وغير مترابطة

A Thesis submitted in Partial Fulfillment of the Requirements for the Degree of Master in Laser Application in physics

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آيـــــــــــة

قال تعالى:

﴿قَـالُـوا سُبْحَـانَـكَ لاَ عِلْمَ لَـنَـا إِلاَّ مَـا عَـلَّـمْـتَـنَـا إِنَّـكَ أَنـتَ الْـعَـلِـيـمُ الْـحَـكِـيـمُ﴾

صدق الله العظيم
سورة البقرة الآية (32)

Dedication

To my father…

Marwa

Acknowledgement

I would like to express my sincere appreciation to my supervisor Prof.Dr. Nafie A. Almuslet for his guidance, continuous support, patience and directing me to this productive study.

I would like to express my gratitude to Alneelain university labs and Sudan university labs for their cooperation and supplies.

My special thanks to my family and my beloved husband for their continuous support, patience and endless encouragement.

Marwa

ABSTRACT

In this work the possibility of production of singlet oxygen was investigated for three dyes, namely (Eosin blue, Rose Bengal and Safranin O) after irradiation by two light sources: the first is green diode laser with wavelength (532 nm) and 100 mW output power and the second is light emitting diode (LED) with wavelength (365 nm) and 1200 mW output power.

The samples were prepared by dissolving Eosin blue in ethanol and then methanol and dissolving Rosa Bengal and Safranin O in distilled water.

UV-VIS spectrometer model 6505 was used to record the absorption spectra of the samples and USB 2000 spectrometer connected to computer was used to record the emission spectra of the samples after different irradiation times starting from 30 seconds up to 120 seconds. The emission spectra were recorded and analyzed.

The obtained results from Rose Bengal and Safranin O dyes both dissolved in distilled water showed indication for production of singlet oxygen ($^1O_2$), due to its emission band at 634 nm which is one of the bands of singlet oxygen ($^1O_2$) after irradiation by green diode laser (532 nm). Therefore, these dyes can be used in the photodynamic therapy (PDT).

Eosin blue dissolved in ethanol and methanol, separately and after irradiation by both sources didn't show any indication for the production of singlet oxygen.

المستخلص

في هذا البحث تم التحقق من إمكانية إنتاج الأكسجين الآحادي من طيف الإنبعاث لثلاثة أصباغ هي (الإيوسين الأزرق ،روز البنغال و السفرانين) بعد أن تم تشعيعهم بمصدرين ضوئيين: الأول هو ليزر الثنائي الأخضر بطول موجي 532 نانوميتر وقدرة 100 ملي واط والثاني هو ثنائي الباعث الضوئي بطول موجي 365 نانوميتر وقدرة 1200 ملي واط.

تم تحضير العينات بإذابة الإيوسين الأزرق في الإيثانول والميثانول وإذابة روز البنغال والسفرانين في الماء المقطر.

تم إستخدام جهاز مطياف نوع (UV-VIS spectrometer) موديل 6505 لتسجيل أطياف الإمتصاص للعينات وتم إستخدام جهاز (USB 2000 spectrometer) متصل مع كمبيوتر لتسجيل طيف الإنبعاث للعينات بعد فترات تشعيع زمنية مختلفة تبدأ من 30 ثانية الى 120 ثانية ، ثم تم تسجيل أطياف الإنبعاُث وتحليلها.

النتائج المتحصلة من صبغة روز البنغال وصبغة السفرانين بعد إذابتهم في الماء المقطر وتشعيعهم بليزر الثنائي الأخضر (532 نانوميتر) أظهرت فعالية في إنتاج الأكسجين الآحادي ($^1O_2$) حيث ظهرت حزمة الإنبعاث للأكسجين الآحادي ($^1O_2$) عند 634 نانوميتر وبالتالي يمكن إستخدام هذه الصبغات في العلاج الضوئي.

لم تظهر صبغة الإيوسين الأزرق بعد إذابتها في الإيثانول والميثانول وتشعيعها بالمصدرين أي فعالية في إنتاج الأكسجين الآحادى.

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CHAPTER ONE

LASER SPECTROSCOPY, BASIC CONCEPTS

1.1 Introduction:

Laser Spectroscopy continues to develop and expand rapidly. Many new ideas and recent realizations of new techniques based on old ideas have contributed to the progress in this field.

There are, firstly, the improvement of frequency-doubling techniques in external cavities, the realization of more reliable cw-parametric oscillators with large output power, and the development of tunable narrow-band UV sources, which have expanded the possible application of coherent light sources in molecular spectroscopy. Furthermore, new sensitive detection techniques for the analysis of small molecular concentrations or for the measurement of weak transitions, such as overtone transition in molecules, could be realized (W. DemtrÖder, 2008).

The impact of lasers on spectroscopy can hardly be overestimated. Lasers represent intense light sources with spectral energy densities which may exceed those of incoherent sources by several orders of magnitude. Furthermore, because of their extremely small bandwidth, single-mode lasers allow a spectral resolution which far exceeds that of conventional spectrometers. Many experiments which could not be done before the application of lasers, because of lack of intensity or insufficient resolution, are readily performed with lasers. The high intensity and spectral monochromacy of lasers have opened a new class of spectroscopic

techniques which allow investigation of structure of atoms and molecules in much more detail (W. DemtrÖder, 2008).

Equipped with a monochromatic, intense and often tunable light source branches such as nonlinear and high-resolution spectroscopy rapidly evolved. Ultra-fast pulsed lasers nowadays allow time-resolved spectroscopy on the atto second time-scale and frequency combs using ultra-stable lasers locked to weak but narrow molecular lines serve as frequency standards. It became customary to distinguish between the term *spectroscopy*, which is associated with the study of spectra of atoms and molecules in general, and *spectrometry*, which refers to the use of spectroscopic information to assess atomic and molecular number densities, i.e. concentrations.

Most of the optical spectroscopic or spectrometric methods are based upon the principles of either absorption, emission, fluorescence, ionization or scattering. A variety of techniques is regularly applied to solid, liquid and gaseous samples, exploiting a manifold of physical phenomena such as saturation and polarization or the photo-acoustic- and Raman-effect. They utilize a substantial part of the electromagnetic spectrum, restricted only by the lack of suitable light sources or optical components in some wavelength regions (F. Schmidt, 2007).

1.2 Thesis objectives:

This thesis is predominantly focused on the detection of singlet oxygen in the emission spectra of some dyes namely;(Eosin blue, Rose Bengal and Safranin O) irradiated with different sources, the produced singlet oxygen can be used in photodynamic therapy (**PDT**).

1.3 Thesis structure :

This thesis consists of three chapters; chapter one contains the thesis objectives as well as basic concepts of laser spectroscopy, absorption and emission spectroscopy, laser absorption spectroscopy, laser in emission spectroscopy, definition and mechanism of photodynamic therapy, photosensitizers and literature review. Chapter two describes the experimental part (materials, setup and methods). Finally, chapter three contains the results, the discussion of the results, conclusions and recommendations.

1.4 Absorption spectroscopy:

1.4.1 Types of spectra:

Spectra are broadly classified into two groups: emission spectra and absorption spectra.

Absorption spectroscopy refer to the spectroscopic technique that measure the absorption of radiation, as a function of frequency or wavelength, due to its interaction with samples. The sample absorbs energy, i.e. photons, from the radiating source. The intensity of the absorption varies as a function of frequency, and this variation is the absorption spectrum.

A material's absorption spectrum is the fraction of incident radiation absorbed by the material over range of frequencies. The absorption spectrum is primarily determined by the atomic and molecular composition of the material. Radiation is more likely to be absorbed at frequencies that match the energy difference between two quantum mechanical states of the molecules. The absorption that occurs between two states is referred as an absorption line and a spectrum is typically composed of many lines. The

frequencies where absorption lines occur, as well as their relative intensities, primarily depend on the interactions between molecules in the sample, the crystal structure in solids, and on several environmental factors (e.g., temperature, pressure, electromagnetic field). The lines also have a width and shape that are primarily determined by the spectral density or the density states of the system. The width and shape of absorption lines are determined by the instrument used for the observation, the material absorbing the radiation and the physical environment of that material. It is also common for a line to be described solely by its intensity and width instead of the entire shape being characterized.(J. Hollas, 2004).

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. The 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. The width of the absorption lines may be determined by the spectrometer used to record it. Increasing the temperature or pressure of the absorbing material also tend to increase the linewidth. 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 yet.

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, making absorption spectroscopy useful in wide variety of applications. For instance, Infrared gas analyzer can be used to identify the presence of

pollutants in the air, distinguishing the pollutant from nitrogen, oxygen, water and other expected constituents. The specificity also allow unknown samples to be identified by comparing a measured spectrum with library of reference spectra. In many cases, it is possible to determine qualitative information about sample even if it is not in a library(J. Ingle et al, 1988).

An absorption spectrum can be quantitatively related to the amount of the material present using Beer-Lambert law.

1.4.2 Beer-Lambert Law

The Lambert-Beer law describes the effect of the absorption process, when light passes through some materials. It connects the expected decrease in the transmitted light (absorbance) with the properties of the material. Figure (1.1) shows a basic setup for absorption measurements using Lambert-Beer law.

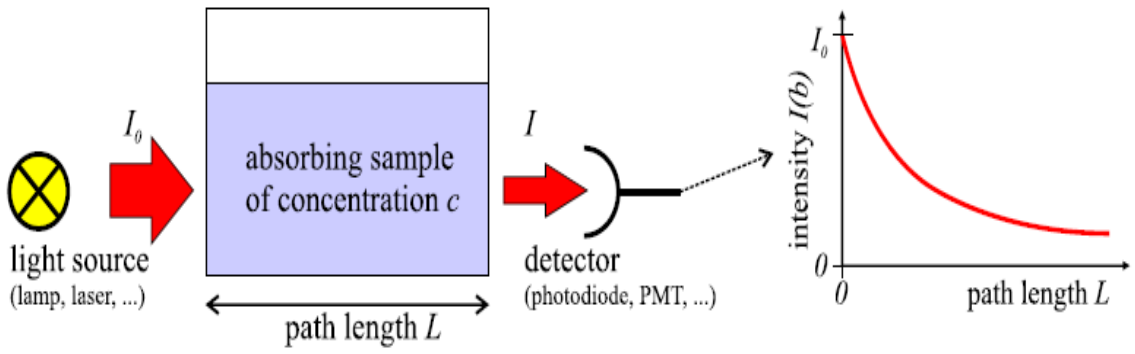


Fig. (1.1): Setup of an Absorption Measurement (K. Tóth et al, 2001).

The law may be written in terms of the absorbance A which is defined as the logarithmic relative decrease of intensity:

$$A := \log_{10} (I_0 / I) \qquad (1\text{-}1)$$

Where I_0 and I are the intensities before and after the sample, respectively. The absorption is also called optical density (OD), so if a solution in a cuvette has OD = 1, this states that only 10% of the light pass (i.e. 90% are absorbed).

Lambert-Beer's law states that:

$$A = \varepsilon(\lambda) . L . c \qquad (1\text{-}2)$$

Where c is the sample concentration and L is the optical path length. The wavelength dependent coefficient ε (λ) is called molar absorptivity and is given in units of $M^{-1} cm^{-1}$.

Using equation (1-2) one can obtain the concentration of a sample in solution by measuring I and I_0 for a given path length L and absorptivity ε (λ). If ε (λ) is not known it can be obtained by plotting the absorbance A against a series of concentrations c, 2c, 3c… the resulting linear graph has a slope of L. ε (λ).

The absorptivity may also be used to identify different components in the sample, such as DNA, proteins or dyes. This is usually done in an absorption spectrometer (depicted in Fig. 1.2) which measures I_0 in a reference sample to get rid of any influence by the solvent. Thus the measurement of the absorption is absolute, independent of the spectrometer, being the comparison of two measured intensities (K. TÓth et al, 2001).

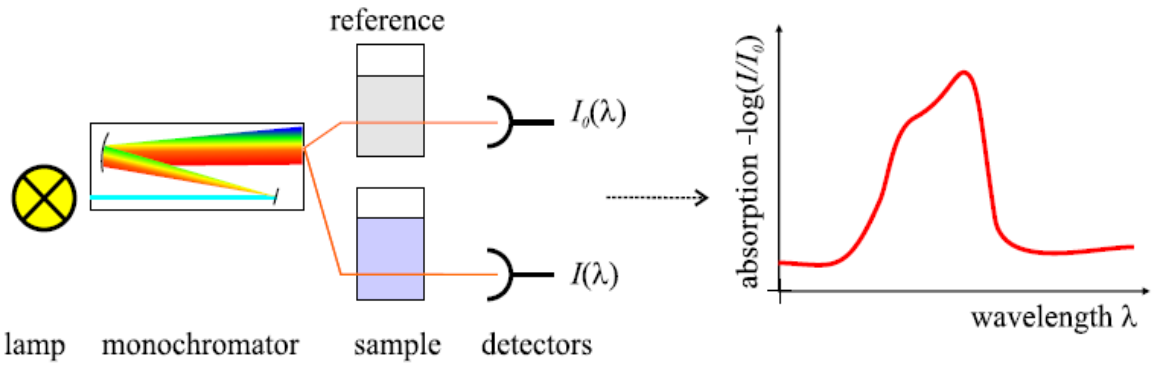


Fig. (1.2): absorption spectrometer (K. Tóth et al, 2001).

1.4.3 Laser Absorption Spectroscopy:

Laser-based absorption spectroscopy (LAS) is a powerful technique for qualitative and quantitative studies of atoms and molecules. An important field of use of LAS is the detection of species in trace concentrations, which has applications not only in physics and chemistry but also in biology and medicine, encompassing environmental monitoring, regulation of industrial processes and breath analysis. Although a large number of molecular species can successfully be detected with established LAS techniques, there are some applications that require higher sensitivity, selectivity and accuracy, yet robust and compact instrumentation (F. Schmidt, 2007).

1.5 Emission spectroscopy:

In emission spectroscopy, energy acquired by an atom can be re-emitted as radiation which is collected and analyzed by a spectrometer. From quantum theory, we know that electrons occupy discrete energy levels. Atoms, which are characterized by the energetic configurations of these electrons, emit light whenever electrons fall from a higher excited energy level to a lower level. The radiative process was originated from various reactions, such as

excitation from the ground state by electron impact and de-excitation of the excited state by spontaneous emission of a photon. Therefore, numerous parameters can be analyzed by means of optical emission spectroscopy (OES) (N. Nayan et al, 2009).

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. There are many ways in which atoms can brought to an excited state (D. Ostlie et al, 2007).

1.5.1 Types of emission spectra:

Emission spectra are of three kinds (a) continuous spectra, (b) band spectra and (c) line spectra.

Continuous spectra: Solids emit continuous spectra when they are heated until they glow. Continuous spectrum is due to excitation of the atoms or the molecules of the substance.

Band spectra: The band spectrum consists of number of bands of different colors separated by dark regions. The bands are sharply defined at one edge called the head of the band and shade off gradually at the other edge. Band spectrum is emitted by substances in the molecular state when the thermal excitement of the substance is not quite sufficient to break the molecules into continuous atoms.

Line spectra: A line spectrum consists of bright lines in different regions of the visible spectrum against a dark background. All the lines do not have the same intensity. The number of lines, their nature and arrangement depends on the nature of the substance excited. Line spectra are emitted by vapours elements. No two elements do ever produce similar line spectra (S. Lakshmi et al, 2012).

1.6 Lasers in emission spectroscopy:

1.6.1 Laser Induced Fluorescence

Fluorescence is the result of a three-stage process in the electron shell of certain molecules called fluorophores. The three processes are excitation, non-radiating transitions and fluorescence emission.

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 species (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 species (discrete for atom, broadband for molecules) (K.TÖth et al, 2001).

Upon excitation of fluorophores with light of suitable wavelength (which can be considered as an instantaneous process occurring at timescales of $\leq 10^{-15}$s) the fluorophore generally resides in one of the many vibrational levels of an excited singlet state (see Jablonski diagram shown in Figure 1.3). The probability of finding the molecule in one of the possible excited singlet states, S_n, depends on the transition probabilities and the excitation wavelength. In other words, the occupation of singlet states is controlled by

the interaction of the electron involved in the transition with the electric field of the excitation light. Upon excitation to higher excited singlet states, molecules relax through internal conversion to higher vibrational levels of the first excited singlet state, S_1, within 10^{-11}–10^{-14}s. Molecules residing in higher vibrational levels will quickly (10^{-10}–10^{-12}s) fall to the lowest vibrational level of this state via vibrational relaxation by losing energy to other molecules through collisions. From the lowest lying vibrational level of the first excited singlet state, the molecule can lose energy via radiationless internal conversion followed by vibrational relaxation. Depending on the molecular structure, radiative depopulation of S1 might occur by spontaneous emission of a fluorescence photon (M. Sauer et al, 2010).

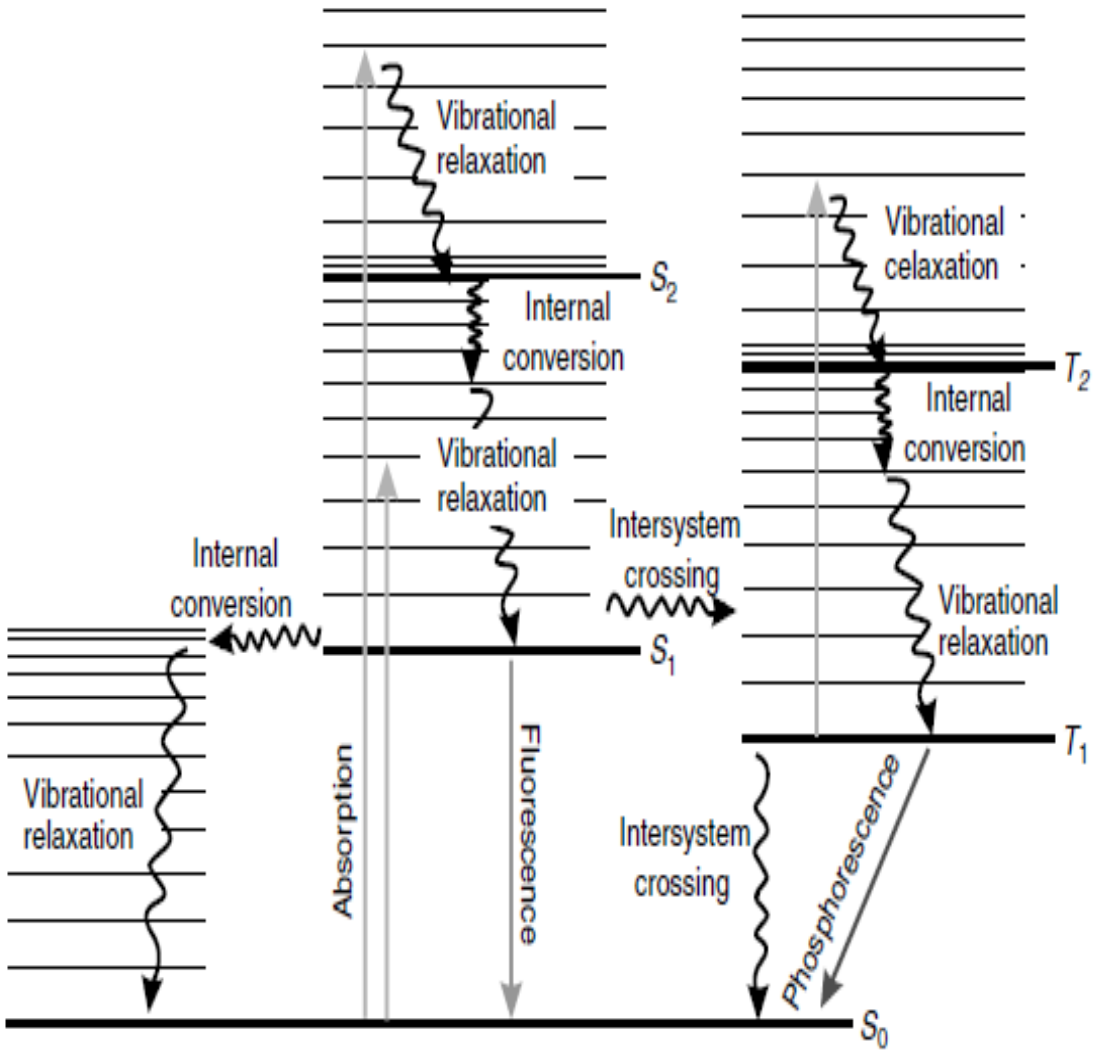


Figure (1.3): Jablonski diagram shows Transitions giving rise to absorption and fluorescence emission spectra (M. Sauer et al, 2010).

Laser-induced fluorescence (LIF) has a large range of applications in spectroscopy. First, LIF serves as a sensitive monitor for the absorption of laser photons in fluorescence excitation spectroscopy. In this case, the undispersed total fluorescence from the excited level is generally monitored. Second, it is well suited to gain information on molecular states if the

fluorescence spectrum excited by a laser on a selected absorption transition is dispersed by a monochromator. A third aspect of LIF is the spectroscopic study of collision processes. Another aspect of LIF concerns its application to the determination of the internal-state distribution in molecular reaction products of chemical reactions (W. Demtröder, 2008).

1.6.2 Laser Induced Breakdown Spectroscopy (LIBS):

(LIBS) is a type of atomic emission spectroscopy which uses a highly energetic laser pulse as the excitation source. The laser is focused to form a plasma, which atomizes and excites samples. The formation of the plasma only begins when the focused laser achieves a certain threshold for optical breakdown, which generally depends on the environment and the target material (P. Singh et al, 2007). Plasma light emissions can provide 'spectral signatures' of chemical composition of many different kinds of materials in solid, liquid, or gas state. LIBS can provide an easy, fast, and situ chemical analysis with a reasonable precision, detection limit, and cost. Additionally, as there is no need for sample preparations, it could be considered as 'put and play' technique suitable for a wide range of applications (F. Anabitarate et al, 2012).

Considerable progress has been made during the last few years on very different and versatile applications of LIBS, including remote material assessment in nuclear power stations, geological analysis in space exploration, diagnostics of archaeological objects, metal diffusion in solar cells, and so forth. Today LIBS is considered as an effective technique when a fast and whole chemical analysis at atomic level is required.

The main devices involved in a LIBS analysis are shown in Figure (1.4), a high –energy pulsed laser (usually in the nanosecond range) is directed to the sample. This light energy vaporizes the sample and induces the plasma.

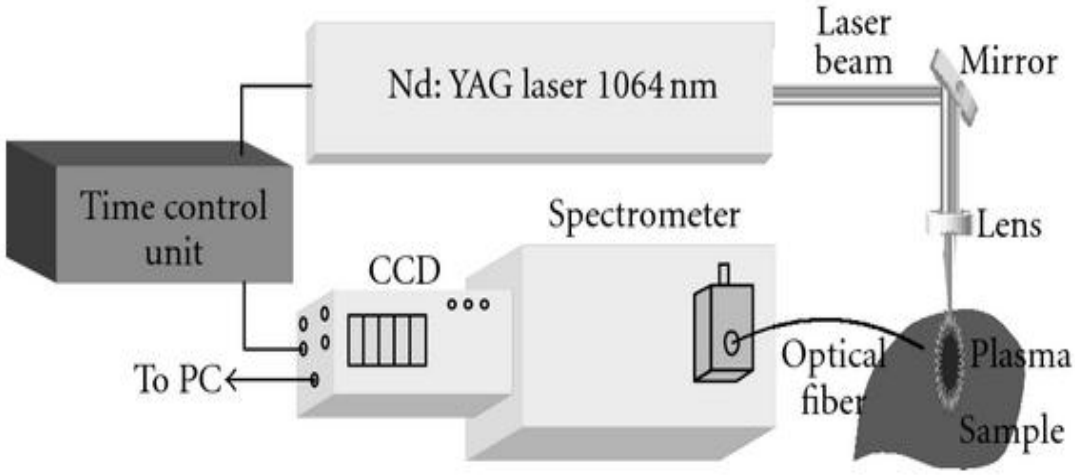


Figure (1.4): Typical LIBS set-up (F. Anabitarate et al, 2012).

The spectrometer is in charge of diffracting the light collected, with a more or less complex optical system, in order to obtain the spectral signature. Then, the light is detected by using devices such as a photomultiplier tube (PMT), a photodiode array (PDA), or a charge-coupled device (CCD). Finally, the acquired spectrum is processed by a computer for further analysis.

LIBS set-ups need an accurate time control to avoid some plasma life stages and to improve the spectral signature. The choice of the laser combined with the set spectrometer-detector and time control, adapted to environmental conditions, can determine the success or failure of experiment (F. Anabitarate et al, 2012).

The main device of LIBS is the laser. It generates the energy to induce the plasma features. The main parameters related to the laser are the pulse time, the energy per pulse, the wavelength, and the number of pulses per burst. Obviously, each application works better with a combination of these parameters. Non-second-pulsed lasers are the most of this common for LIBS.

The most common laser used in LIBS is pulsed Nd: YAG laser. This kind of laser provides a compact, reliable, and easy way to produce plasmas in LIBS experiments. Other kinds of lasers can be used in LIBS, such as CO_2 or excimer lasers to work in far IR or UV ranges, respectively (F. Anabitarate et al, 2012).

LIBS has numerous advantages compared to the other techniques. This technique doesnot require any special sample preparation, and requires only a small volume or mass of material to be tested. It has a high spatial resolution on the target material, typically less than 100 μm, which is limited by the size of the focused laser being used. In addition, it is relatively simple to implement, not very expensive, yields a real-time response, and can be performed by nonexperts (Q. Mohaidat, 2011).

1.7 Laser Raman Spectroscopy:

Raman Effect was first discovered by C.V. Raman and K. F Krishnan in 1928. In 1930, C.V. Raman was awarded the Noble Prize in physics for this discovery.

Raman spectroscopy is a form of molecular spectroscopy that involves the scattering of electromagnetic radiation by atoms or molecules. It probe the vibrational, rotational, and other low-frequency modes of molecules, the Raman signal is observed as inelastically scattered light (T. Thompson, 2008).

Raman from particle point of view can be visualized by a quantum energy diagram (see fig. 1.5). We'll start first with Rayleigh scattering. When incident light hits a molecules, an electron in a ground vibrational state is promoted to a virtual state. It then relaxes and returns to the same vibrational state from which it started. This is denoted by ν_0, which means there has been no change in the frequency; this is elastic scattering(T. Thompson, 2008). .Figure (1.5)shows the principles of Raman spectroscopy.

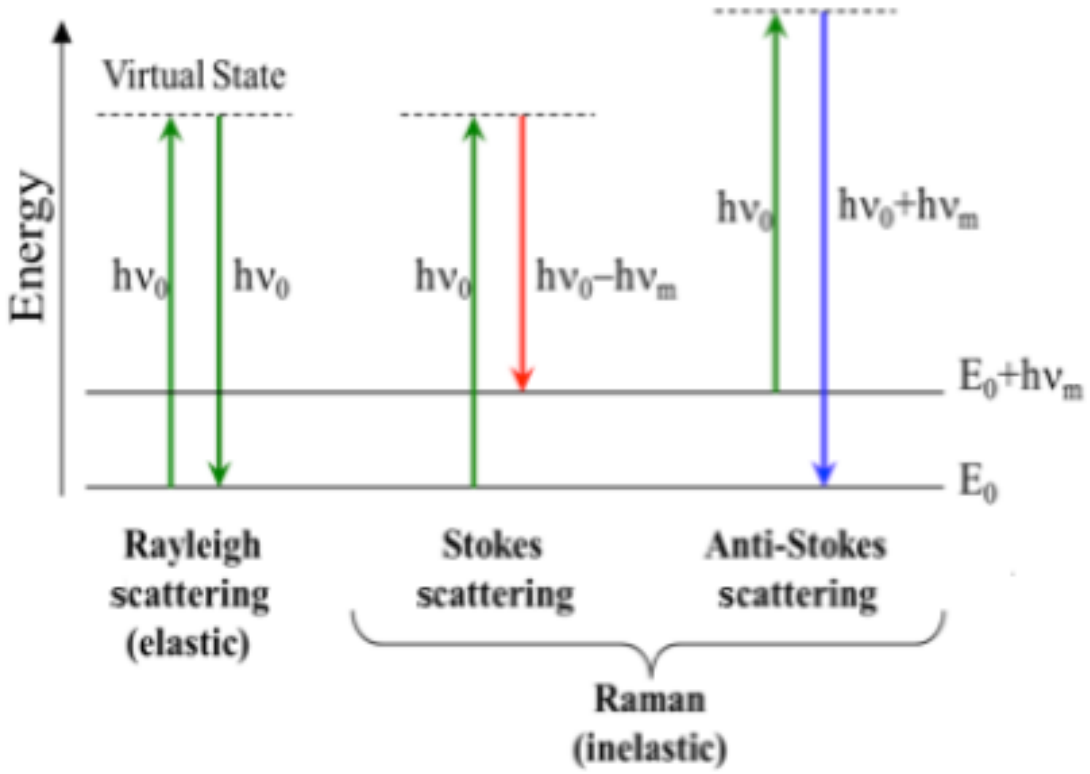


Figure (1.5): schematic diagram of the principles of Raman spectroscopy.

Raman is a nondestructive technique and typically requires little to no sample preparation. The Raman analysis also can be performed directly through transparent containers, including plastic bags, glasses, jars, cuvettes, and so on. Furthermore Raman can be used for both qualitative and quantitative analysis, and Raman technique is highly selective, meaning that it is able to differentiate molecules in chemical species that are very similar.
Raman also has the advantage of fast analysis times. A typical analysis can take just a few seconds, unlike FTIR spectroscopy, Raman in insensitive aqueous absorption bands (T. Thompson, 2008).

1.8 Photodynamic Therapy (PDT):

PDT, sometimes called photo chemotherapy, is a form of phototherapy using nontoxic light-sensitive organic dyes that are exposed selectively to light, whereupon they become toxic to targeted microbial cells, including bacteria, fungi and viruses. It is used clinically to treat a wide range of medical conditions, including wet ager-elated macular degeneration and malignant cancers, and is recognized as a treatment strategy which is both minimally invasive and minimally toxic (D. Tuanvo, 2003).
Photodynamic therapy (PDT) as a cancer treatment modality relies on the simultaneous presence of three components; light, photosensitizer and oxygen. Once excited by the light, the photosensitizer can interact with

oxygen, leading to the formation of toxic oxygen species (A. Johansson, 2007).

1.8.1 Mechanism of singlet oxygen production in PDT Action:

It all begins with the photosensitizer absorbing a photon of energy from the light source of the correct wavelength (B. Mayia, 2010). Light with a wavelength tuned to match an absorption band of the photosensitizer excites it from the ground state, S_0, into a higher lying singlet state, S_1. From here, the photosensitizer molecules either relax back down to the ground state or cross into a triplet state, T_1. From the triplet state, the transition back to the ground state is spin-forbidden and hence the lifetime of the excited state is long, allowing the molecule to interact with its surroundings. The two processes that constitute the photodynamic reactions are referred to as Type I and II reactions. Type I reactions, involving electron or hydrogen atom transfer from the triplet state of the photosensitizer to substrates other than oxygen molecules, lead to the formation of highly reactive radicals or radical ions. These radicals most often react with oxygen to form different reactive oxygen species (ROS), followed by the formation of oxygenated products. Type II reactions involve an electron spin exchange between T1 and ground state oxygen molecules $^3O_2$, leading to the formation of highly reactive singlet oxygen,$^1O_2$. Both processes occur simultaneously and are critically dependent on the presence of oxygen.

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 photosensitizer used and its binding site within the tissue. For example, it has been suggested that hypoxic conditions and/or high

photosensitizer concentrations might favour Type I reactions, whereas high oxygen concentrations lead to domination of Type II reactions (A. Johansson, 2007). Figure 1.6 shows type I and type II reactions.

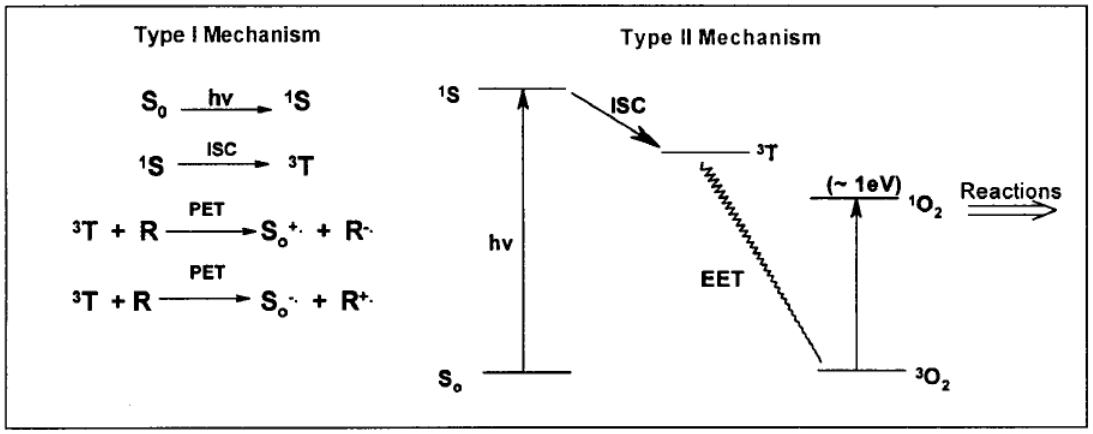


Figure (1.6): The Type I and Type II reactions of a photosensitizer.

PET : photoinduced electron transfer between the excited state and substrate.

EET: electronic excitation energy transfer.

R: substrate.

Chances of occurrence of both Type I and Type II reactions from the singlet state are rather remote due to the fact that this state is usually short-lived and any reaction involving it as a partner should occur within its lifetime (t) which is typically between nanoseconds (ns = 10^{-9} s) and pico seconds (ps = 10^{-12} s). On the other hand, triplet states, which are generated from the singlet precursor, have a better chance of reacting with other substrates because they are long-lived (t ranges from microseconds, μs =10^{-6}s to milliseconds, ms = 10^{-3} s) and there is enough time for the reaction to occur subsequent to mutual diffusion of the reaction partners. The triplets can undergo either electron transfer or energy transfer reactions with the

available substrates. Most available data suggest the involvement of an energy transfer reaction between the triplet PDT photosensitizer and molecular oxygen resulting in the formation of singlet oxygen ($^1O_2$) *via* the Type II mechanism (B. Maiya, 2010).

1.9 Photosensitizers for PDT:

Photosensitizers are compounds that are capable of absorbing light of specific wavelength (chromophores) and transforming it into useful energy (B. Maiya, 2010). A good PDT photosensitizer is expected to fulfill the following minimum criteria. It should:

(a) have a strong absorbance with high extinction coefficient Ɛ at longer wavelengths (600-850 nm) where tissue penetration of light is at a maximum and still energetic enough to produce $^1O_2$,

(b) have excellent photochemical reactivity, with high triplet state yields (Φ_T) and long triplet state lifetimes (Φ_T) and be able to effectively produce $^1O_2$ and other reactive oxygen species,

(c) have minimal dark toxicity and only be cytotoxic in the presence of light,

(d) be preferentially retained by the target tissue,

(e) be rapidly excreted from the body, thus inducing a low systemic toxicity, and

(f) be chemically pure and of known specific composition.

A good PDT photosensitizer should also possess favorable triplet state properties. The effectiveness of photodynamic activity has notable dependence on the triplet state quantum yield and its lifetime. Because longer life times permit diffusion of the reactants (i.e. the triplet sensitizer and molecular oxygen) to form the initial encounter complex which ultimately relaxes to the products (i.e. ground state photosensitizer and $^1O_2$)'.

Finally energy of the first excited triplet state of a photosensitizer should be approximately greater than 86 kJ mol $^{-1}$ to promote a type II mechanism(B. Maiya, 2010).

1.10 Literature Review:

PDT was first developed at the beginning of the twentieth century in Munich when Oscar Raab and his professor Herman Von Tappeiner, in 1900, noticed the effects of photosensitivity on paramecia. Raab observed the rapid death of the protozoon *Paramecium caudatum* after light exposure in the presence of acridine dye. The presence of light, which modified the effect of the dye, led to the identification of a photosensitizer. Subsequently, Professor von Tappeiner went on to carry out other experiments and discovered that the presence of oxygen was necessary in order for the reaction to occur, thus creating the term PDT (M.C. Issa et al, 2010). In 1907, Von Tappeiner and Jodlbauer published a textbook on this therapy, which they referred to as an oxygen dependent photosensitizing process for the treatment of skin tumors and the destruction of infectious particles. They described their experiences with 5% topical eosin and artificial light for the treatment of nonmelanoma skin cancer and for other dermatoses such as lupus vulgaris and condyloma planus. At this time, they speculated that eosin, like acridine, after being incorporated into the cell, would produce a cytotoxic reaction when exposed to an adequate light source in the presence of oxygen (M .C. Issa et al, 2010).

In 1990 Sorokin, P.P et al 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 (F.sch.fr, 1990).

In 1998, the quantum yields of singlet oxygen production by merocyanine 540 have been measured by M. Hoebeke during visible light irradiation performed in methanol and ethanol. These appear to be one hundred times smaller than the quantum yield for Rose Bengal measured under the same conditions. Flash photolysis experiments demonstrate the ability of merocyanine 540 molecules to isomerize under visible light irradiation: the isomerization quantum yields were about 0.65 in both ethanol and methanol. This information combined with the fluorescence quantum yield data account for the low values for singlet oxygen production. The solutions were contained in a quartz cell, and stirred during irradiation ($\lambda > 500$ nm) to ensure good mixing. The fluorescence quantum yield Φ_{fl} of MC540 in methanolic solution was determined (M. Hoebeke et al, 1998).

In 2009 Vikesh Kumar studied the production and chemical property of singlet oxygen and superoxide radical by dyes. This study reported that there

are several low lying singlet oxygen ($^1O_2$) and superoxide radical (O^-_2) which are important in photochemical oxidation. Irradiation with sun light in vitro the photosensitizer like benzanthrone, metanil yellow and p-aminodiphenylamine were found to produce reactive oxygen species such as singlet oxygen ($^1O_2$) and/or superoxide radicals ($^-O_2$) (V.Kumar, 2009).

In 2011 Nafie. A. Almuslet and Nazic. M. Hassan made a spectroscopic and photophysical study for the ability of Phenoxazone 9 to produce $^1O_2$ after irradiation by some monochromatic light sources (coherent and incoherent) for different exposure times and the emission spectra indicated the existence of singlet oxygen, They concluded that it is necessary to characterize the photosensitizer during photodynamic reaction and to describe its ability to produce singlet oxygen which has therapeutic effect of cancer when irradiated the photosensitizer by a number of light sources.(N. M. Hassan, 2011)

In 2012 Fatima Pır Çakmak and Mustafa Özgür Güler synthesized three different photosensitizers (Bodipy dyes group) for PDT action, Process properties are emphasized through the photo physical changes in spectrum. In that work, synthesis, characterization novel water soluble, near IR absorbing Bodipy photosensitizer was used. These dyes were irradiated by LED applied at 660 nm. This photosensitizer is designed to have singlet oxygen generation capability only in cancer tissue as a result of glutathione triggered activation. The absorption and emission spectra were recorded for the three photosensitizers and singlet oxygen generation efficiency has been improved which enable phototoxicity of the method to be accomplished (F.P. Çakmak, 2012).

In 2014 Nafie A. Almuslet and Ahmed A. Mohamed, studied the emission of three dyes (dibenzocyanine 45, methylene blue and Rhodamine 6G) after irradiation by two laser sources. The results showed that the most efficient dye that can produce the singlet oxygen was the DDTTC 45 dissolved in acetone after irradiation by diode laser 671 nm with exposure time of six minutes where the emission spectra indicated the existence of singlet oxygen (A.A.M. Taher, 2014).

In 2016 Nafie A. Almuslet and Nahla E. Ahmed made a spectroscopic study for the production of $^1O_2$ from three dyes (Alcain blue, coumarin and bromocresol) irradiated for different times using different light sources [LED at (365 nm), green diode laser(532 nm) and red diode laser (671 nm)]. It was found that the most efficient dye that can produce the singlet oxygen was Alcian blue dissolved in chloroform after irradiation by green diode laser(532 nm) where a peak of singlet oxygen at (634 nm) with exposure time of 4 minutes was recorded (N.E.A. Mohammed, 2016).

CHAPTER TWO

THE EXPERIMENTAL PART

2.1 Introduction:

In this chapter, materials, equipment used in this work and the experimental technique are presented. The experimental part of this study was designed to analyze emission spectra recorded after irradiation of Eosin blue, Safranin O and Rosa Bengal dyes by coherent and incoherent sources.

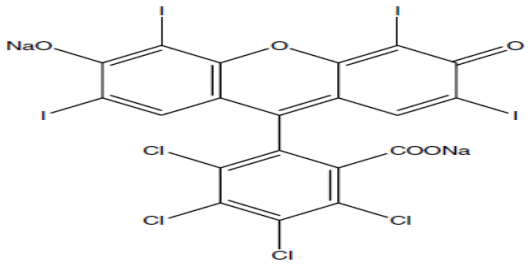
2.2 Materials:

Three organic dyes and different solvents were used to prepare the samples as follows:

2.2.1 Rose Bengal:

Rose Bengal is sodium salt which commonly used in eye drops stain damaged conjunctival and corneal cells. The stain is used in the preparation of Foraminifera for microscopic analysis, allowing the distinction between forms that were alive or dead at the time of collection. A form of Rose Bengal is also being studied as a treatment for certain cancers and skin conditions. The cancer formulation drug, known as PV-10, is currently undergo clinical trials of melanoma and breast cancer. Rosa Bengal was originally prepared in 1882 by Ghnem, as an analogue of fluorescein. Its name derives from rose (flower) and Bengal (region). Rose Bengal is also used in synthetic chemistry to generate singlet oxygen from triplet oxygen. The singlet oxygen can then undergo a variety of useful reactions. Rose Bengal can be used to form many derivatives that have important medical

functions. Figure (2-1) shows the chemical structure of Rose Bengal and table (2-1) list its physical and chemical properties (R. Sabnis, 2010).



Figure(2-1):The chemical structure of Rose Bengal

Table (2-1): The physical and chemical properties of Rose Bengal:

Chemical formula	$C_{20}H_4Cl_4I_4O_5$
Molecular weight	1017.64
Chemical Abstract Service Registry Number (CAS) NO	632-69-9
Absorption Maximum	548 nm
Physical form	Red brown powder
Molecular Mass	973.67
Solubility	Soluble in water, ethanol
Melting point	>200˚C
Chemical/Dye Class	Xanthene

2.2.2 Eosin Blue:

Eosin is a name of several fluorescent acidic compounds which bind to and from salts with basic, or eosinophilic, compounds like proteins containing amino acid residues such as arginine and lysine, and stain them dark red to

pink as a result of actions of bromine on fluorescein. The name Eosin comes from Eos, the Ancient Greek word for 'dawn'. Eosin B (also known as Eosin bluish, Acid red 91,C.I. 45400, Saffrosine, Eosin Scarlet, or imperial red). Figure (2-2) shows the chemical structure of Eosin Blue and table (2-2) lists its physical and chemical properties.

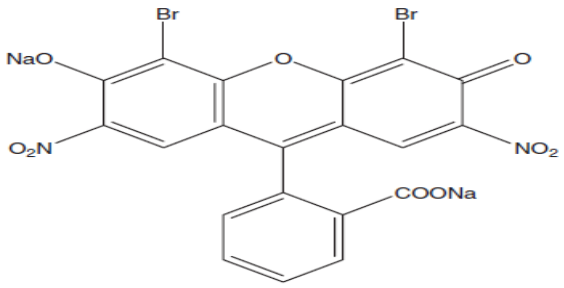


Figure (2-2): Chemical structure of Eosin Blue

Table (2-2): The physical and chemical properties of Eosin blue:

Chemical formula	$C_{20}H_6Br_2Na_2O_9$
Molecular weight	624.06
Chemical Abstract Service Registry Number (CAS) NO	548-24-3
Physical form	Red-brown to green crystals or powder
Solubility	Freely soluble in water; soluble in ethanol
Melting point	295˚c
Absorption (λ max)	514 nm, 395 nm
Emission (λ max)	544 nm
chemical/Dye Class	Xanthene

2.2.2.1 Biological Applications Antimalarial agent; protein assay; detecting enzyme activity; treating cancer, malaria, diabetes, a variety of conditions affecting skin, mouth, digestive tract, urinary tract, reproductive tract, respiratory tract, circulatory system, head, neck, endocrine system, lymphoreticular system; dental materials (R. Sabnis,2010).

2.2.3 Safranin O:

Safranin (also safranin O, Phenazinium, dimethyl or basic red 2) is a biological stain. this is a positively charged molecule (cation) and can be binding with a negative charged cell elements. Safranin technique is a constrast stain and it is used to distinguish a cellular structure previously stained with other dyes. Safranin stain employed in different histological techniques that detecting enterochromaffin cells in gastrointestinal tract. The chemical structure of safranin O is shown in figure (2-3) and its physical and chemical properties are listed in table (2-3).

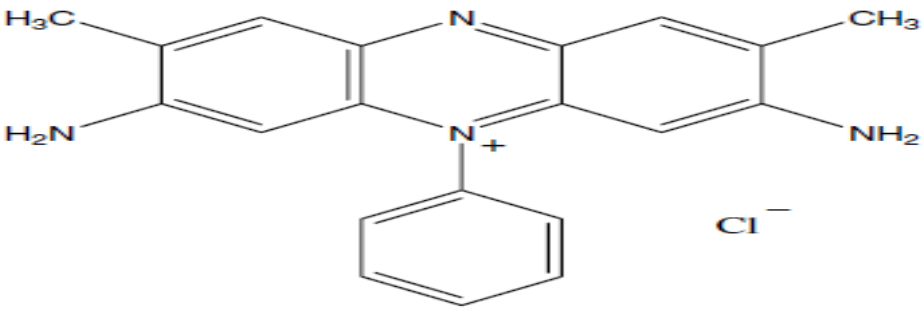


Figure (2-3) chemical structure of Safranin O.

Table (2-3):The physical and chemical properties of Safranin

Chemical formula	$C_{20}H_{19}ClN_4$
Molecular weight	350.84
Chemical Abstract Service Registry Number (CAS) NO	477-73-6
Physical form	Dark red to dark green powder
Solubility	Soluble in water, ethanol, ethylene glycol, methyl cellosolve, pyridine
Melting point	>240˚C (decompose)
Absorption (λ max)	530 nm
Chemical/Dye Class	Phenazine

2.2.3.1 Staining Application: Antigen; bacteria; brain; cellulose; lignin; mitochondria; nucleated and non-nucleated blood cells; nucleic acids; proteins; spinal cord; hairs.

2.2.3.2 Biological Applications: Hematotoxicity assays; measuring membrane potential; detecting microorganisms; treating diabetes-associated pain, mechanical allodynia, oncological diseases; food packaging materials. (R.Sabnis,2010).

- 40 mg of each dye was used to prepare the samples in this work.

2.2.4 The solvents:

2.2.4.1 Ethanol:

Ethanol, also known as ethyl alcohol or grain alcohol,it is a volatile, flammable, colorless liquid, 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 monovalent salts are at least somewhat soluble in ethanol, with salts of large, polarizable ions being more soluble than salts of smaller ions. Most of polyvalent salts are organic and soluble in ethanol (K. E, Adams, T. S, Rans, 2013).

2.2.4.2 Methanol:

Methyl Alcohol, or Methanol, is colorless hygroscopic liquid usually containing 0.01 - 0.04 percent water. It is highly toxic and inflammable.
Methanol is a polar, protic solvent frequently used to dissolve dyes like Coumarins, Rhodamines, and Cyanines. It has excellent optical transparency make it ideal solvent for pumped dye lasers (K.E, Adams, T. S, Rans, 2013).

2.2.4.3 Distilled water:

Distilled water is that has had many of its impurities removed through distillation. It’s also called purified water. Distillation involve boiling water and then condensing the steam into a clean container. Water-soluble dyes are made by dissolving powder dye in distilled water. Table (2-4) lists the characteristics and physical properties of used solvents.

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

Property	Distilled water	Ethanol	Methanol
Molecular weight (g/mol)	18.1528	46.07	32.04
Freezing point (˚ C)	0.0 ˚ C	-114.1	-97.7
Boiling point (˚ C)	99.98˚ C	78.3	64.7
Density	0.9998 g/mol	0.7936 g/cm³	0.796115 g/cm³
Refractive Index	1.333 (20˚ C)	1.3614	1.3284
Viscosity	0.890	1.078	
Solubility	Poorly soluble in haloakanes, aliphatic and aromatic hydrocarbonsethers. Improved solubility incarboxylates,alcohols, ketones, amines. Miscible with methanol, ethanol, isopropanol, acetone, glycerol.	Water, organic solvent	Water, organic solvent

2.3 Equipments:

Equipment used in this study were:

2.3.1 UV-VIS Spectrometer:

Spectrometer deals with visible light, near-ultraviolet and near-infrared. The UV_VIS spectrometer used in this work was supplied from JENWAY Company (Britain), model 6505, equipped with a quartz cuvette having

optical length or thickness of 10 mm. It is come with spectral mode that allows for full spectral data acquisition over wavelength range from 190–1100 nm, accuracy ± 1.0 nm, resolution 0.1 nm, absorbance range from (-0.300 to 3000A), operating temperature 5˚C to 40˚C and maximum humidity of 80%. This device was used to measure the absorption and the transmission of dyes samples before irradiation with the laser sources.

Figure (2.4): The UV_VIS spectrometer.

2.3.2 The USB 2000 Spectrometer:

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. The USB 2000 was used to record the emission spectra with corresponding software. USB 2000 can detect wavelengths from 200-1100 nm. 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. The grating diffracts light from the collimating mirror

and guide the diffracted light onto the focusing mirror. The device used here was supplied from ocean company model USB2E7524. Figure (2-5) shows the main components of USB2000 and its specifications is given in table (2-5).

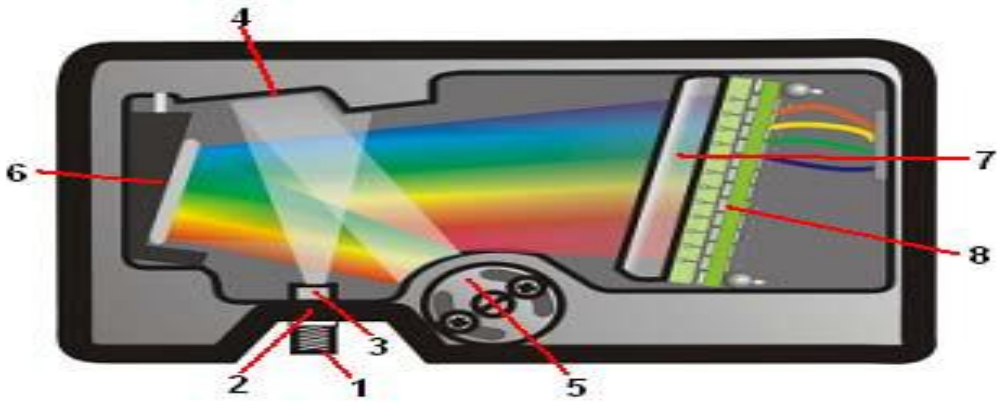


Figure (2.5):The Ocean Optics USB 2000 spectrometer.

1. SMA connecter.
2. Slit.
3. Filter.
4. Collimating mirror.
5. Grating.
6. Focusing mirror.
7. L2 detector collection lens.
8. CCD detector (UV or VIS).

Table (2-5): the specification of USB2000.

Specification	Value
Sensitivity	75 photons per count at 400 nm 41 photons per count at 600 nm
Signal-to-noise ratio	250:1 (at full signal)
Dimensions	148.6 mm × 104.8 mm × 45.1 mm
Resolution	0.1 nm
Fiber optic connector	SMA 905 to single-strand optical fiber (0.22 NA)
Fiber profile	Step index multimode

2.3.3 Light Sources:

2.3.3.1 Green diode laser:

The green diode laser used here has a wavelength of 532 nm and an output power of 100 mW. This laser was supplied from RoithnerLaserTechnikGmbh_ Austria. Figure (2.6) shows the green diode laser and table (2.6) lists its specifications.

Figure (2.6): The green diode laser (532nm).

Table (2.6): 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
Input voltage	APC(complite driver unit included)
Operating current	< 300 mA(1-20 mW) <650 mA(50 – 100 mW)

2.3.3.2: UV Light Emitting Diode (LED):

The UV light emitting diode used in this work has a wavelength of 365nm and output power of 1200 mW. The LED was manufactured by LED ENGIG, the forward current is 700 mA and forward voltage is 16.4 V.Figure (2.7) shows the UV light emitting diode (LED).

Figure (2.7) The UV light emitting diode (365nm).

2.4 The Setup and The Experimental Procedure:

Figure (2-8) shows the arrangement of the setup:

The experiments were carried out as follows :

-Firstly all dyes samples were dissolved in the solvets without purification as follows:

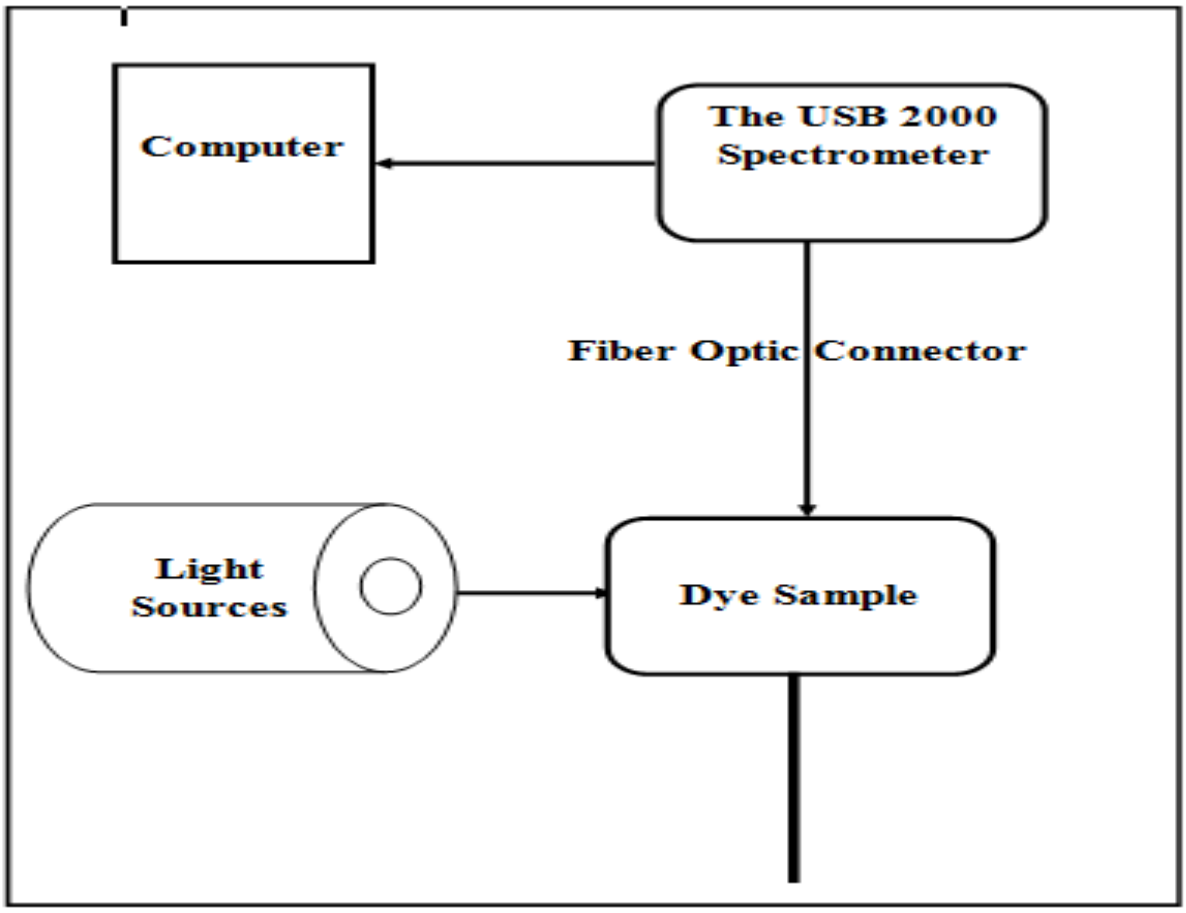
a. 40 mg of Eosin blue was dissolved in 60 ml of Ethanol and then separately in Methanol.
b. 40 mg of Safranin O and Rosa Bengal were dissolved in 60 ml of distilled water each time.

- In the beginning the dye concentration had been tested using 50 mg of each dye dissolved in 60 ml of the solvent then it had been changed to 40 mg of each dye dissolved in 60 ml , which gave a good result of $^1O_2$ production.

-Then the transimation of the solvents alone were recorded by using the UV_VIS spectrometer to make sure that they are transparent in the range 320 to 1100 nm. After that, all samples (dyes + solvents) were poured into quartz cuvette and then was placed inside the UV-VIS spectrometer and the absorption spectra were recorded for all of them.

-Afterward, all samples (Eosin blue, Safranin O and Rosa Bengal) were irradiated by the green diode laser (532nm) and the UV light emitting diode (LED) (365nm), subsequently for different irradiation times starting from 30 seconds up to 120 seconds.

Finally, all the emission spectra were recorded by the USB 2000 spectrometer for all the samples in the range of (200 nm – 1100 nm).



Figure(2.8):The arrangement of the experimental setup

CHAPTER THREE

Results and Discussion

3.1 Introduction:

This chapter presents the results, discussion, conclusions and suggested future work. The results were obtained from three types of dyes (Eosin blue dissolved in ethanol and methanol, Safranin O and Rose Bengal dissolved in distilled water). The absorption of each dye was recorded using UV-VIS spectrometer to identify the portion of the spectrum that dye can absorb, then they were irradiated by green diode laser (532 nm) and monochromatic light emitting diode (365 nm), separately. After irradiation, the production of singlet oxygen was investigated from the emission spectra of each dye, using the USB 2000 spectrometer and other components, after different irradiation times starting from 30 seconds up 120 seconds.

3.2 Absorption spectra of Eosin blue:

The absorption spectra of Eosin blue dissolved in ethanol and methanol is shown in figure (3.1).

In this figure one can see that Eosin blue has strong absorption bands in the visible region peaked at 480 nm and 525 nm when it was dissolved in methanol and ethanol , respectively. There is a shift in the bands due to the differences in the photochemical interaction of the solvents.

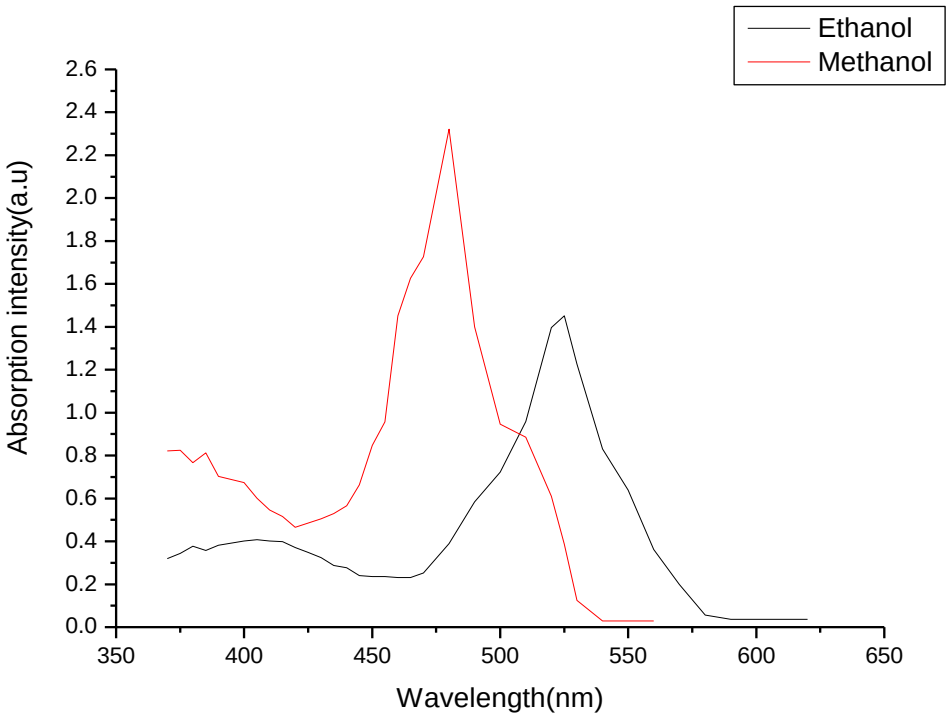


Figure (3.1): the absorption spectra of Eosin blue dissolved in ethanol (black)and methanol (red).

3.3 Emission spectra of Eosin blue after irradiation by green diode laser (532 nm):

Figures(3.2) and (3.3) show the emission spectra of Eosin blue dissolved separately, in ethanol and methanol after irradiation by green diode laser (532 nm) with100 mW output power after different irradiation times starting from 30 seconds up to 120 seconds.

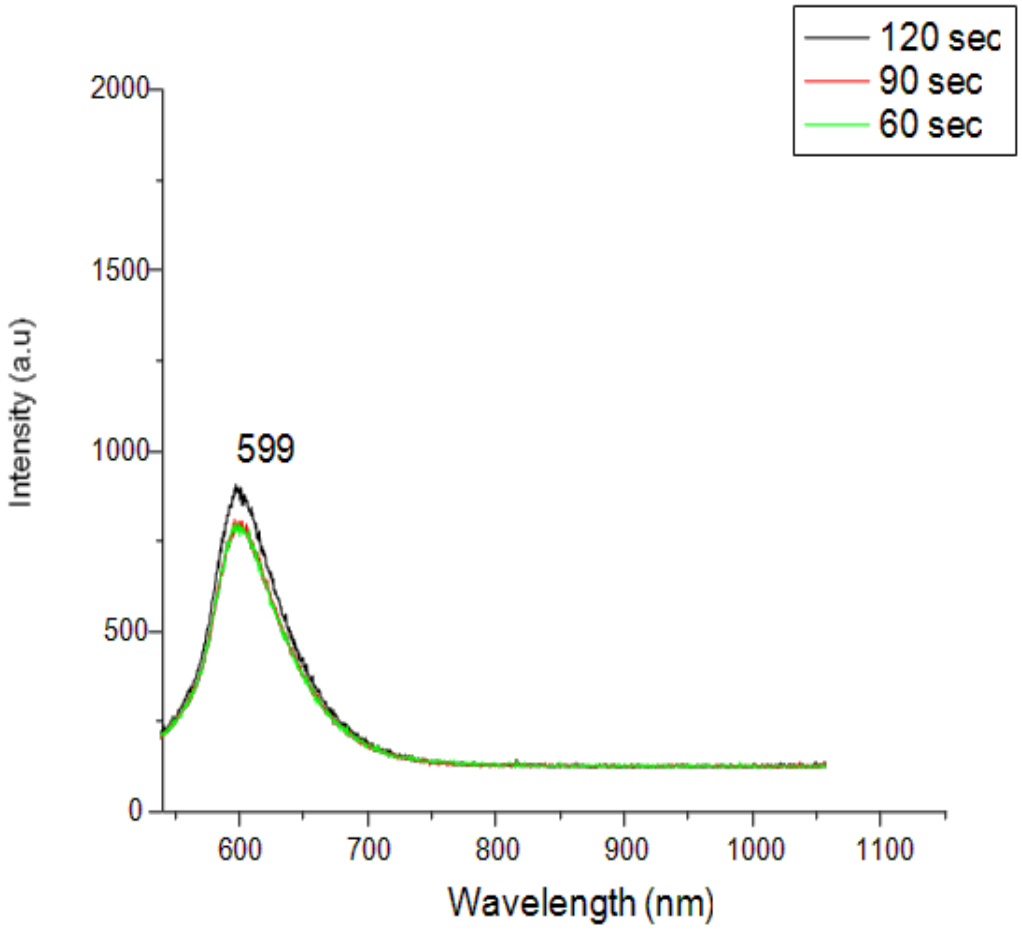


Figure (3.2): Emission spectra of Eosin blue dissolved in Ethanol after irradiation by 532 nm for different times.

Figure (3.2) showed that the emission spectrum of Eosin blue dissolved in ethanol and irradiated by (532 nm) have one band at 599 nm and by increasing the irradiation time, the intensity increases subsequently. This spectrum didn't indicate the production of singlet oxygen because the peaks of singlet oxygen are 634 and 703 nm according to the literature (G. Cosa et al, 2016) and 1270 nm according to other literatures.

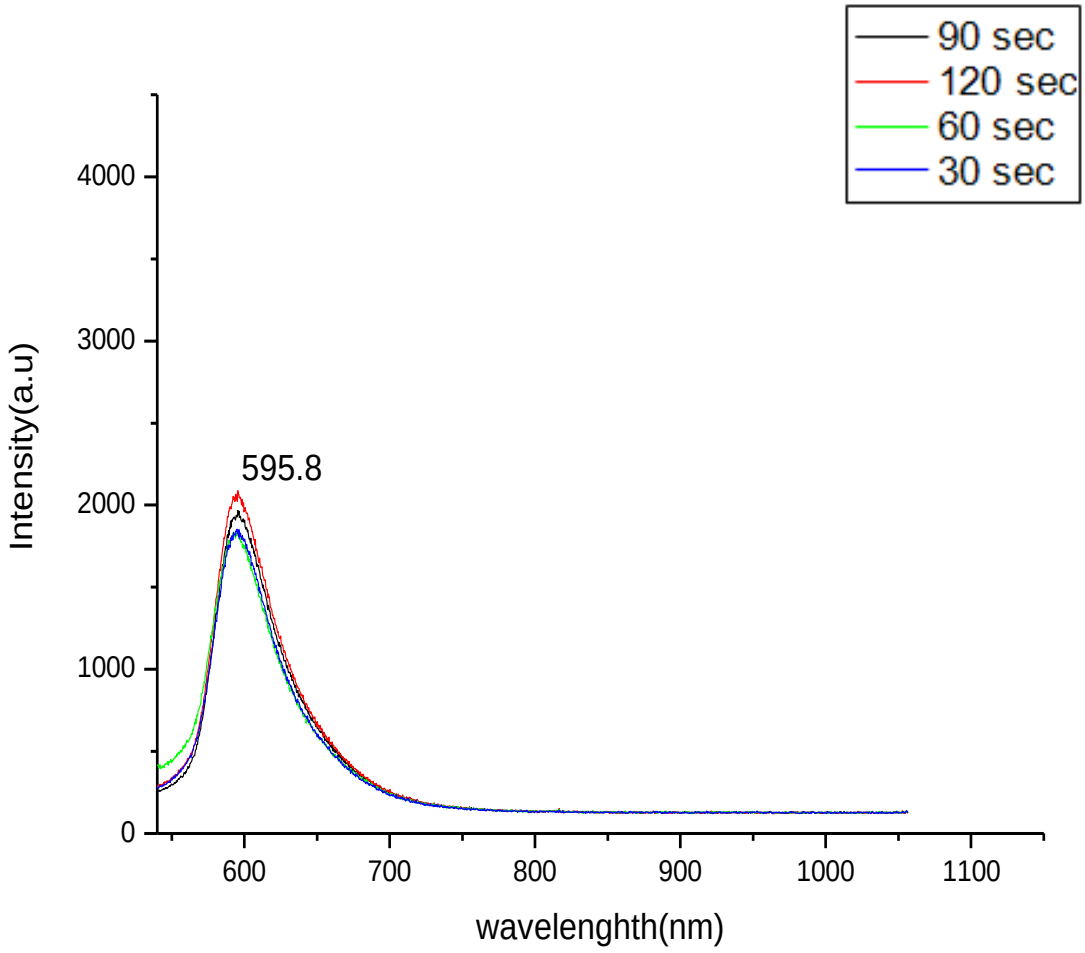


Figure (3.3): Emission spectra of Eosin Blue dissolved in methanol after irradiation by (532 nm) for different times.

In figure (3.3) the emission spectra showed one band at 595.8 nm. The spectra didn't indicate the production of singlet oxygen and the emission intensity increased with increasing the irradiation time.

3.4 Emission spectra of Eosin blue after irradiation by light emitting diode LED (365 nm):

Figures (3.4) and (3.5) show the emission spectra of Eosin blue dissolved in ethanol and methanol, respectively, after irradiation by LED with 365 nm wavelength and 1200 mW output power with different irradiation times starting from 30 seconds up to 120 seconds.

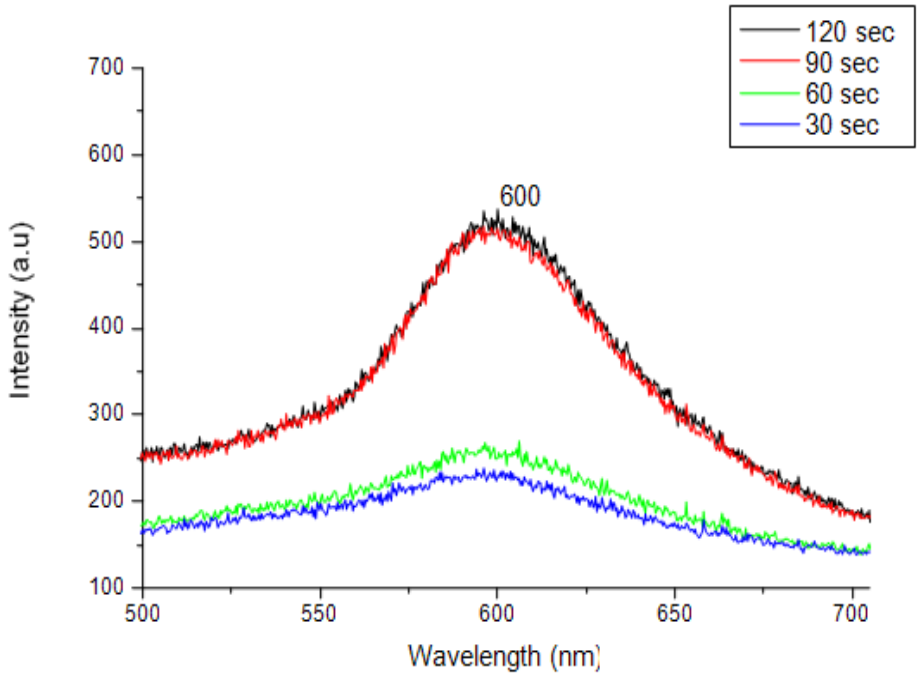


Figure (3.4): Emission spectra of Eosin blue dissolved in ethanol after irradiation by LED (365nm) for different times.

Figure (3.4) showed that the emission spectra of Eosin blue dissolved in ethanol and irradiated by LED (365 nm) have a broad band at 600 nm and by increasing the exposure time the intensity increases ,subsequently. These spectra didn't indicate the production of singlet oxygen.

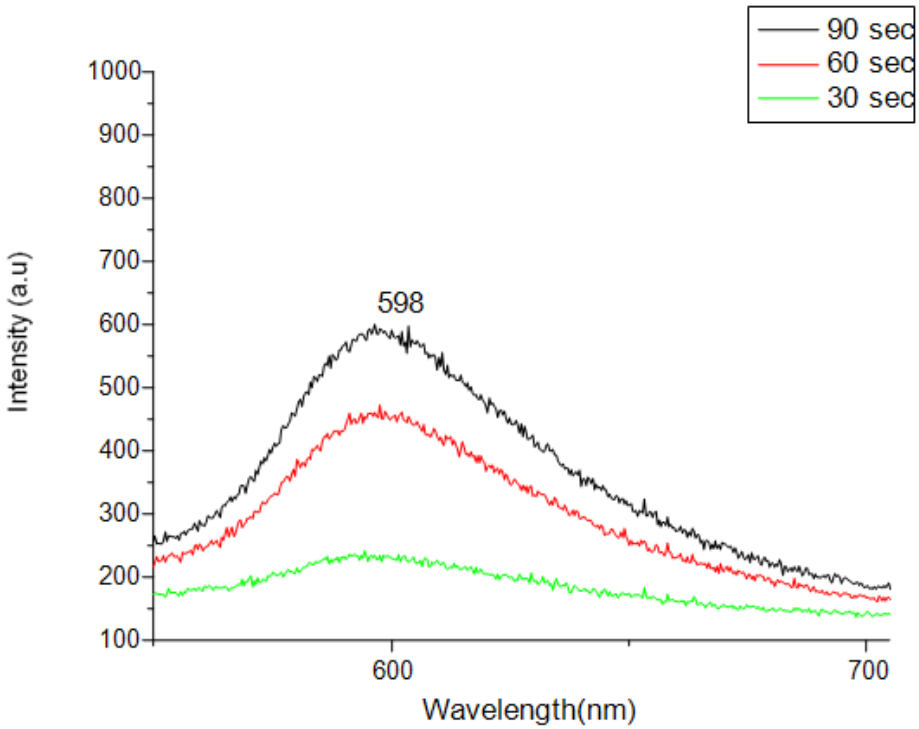


Figure (3.5): Emission spectra of Eosin blue dissolved in methanol after irradiation by LED (365nm) for different times.

In figure (3.5) one can see that the emission spectra of Eosin blue dissolved in methanol and irradiated by 365 nm have one broad band at 598 nm. These spectra didn't indicate the production of singlet oxygen; and increasing the exposure time led to increase the intensity.

3.5 Absorption spectrum of Safranin O:

The absorption spectrum of Safranin O dissolved in distilled water is shown in figure (3.6).

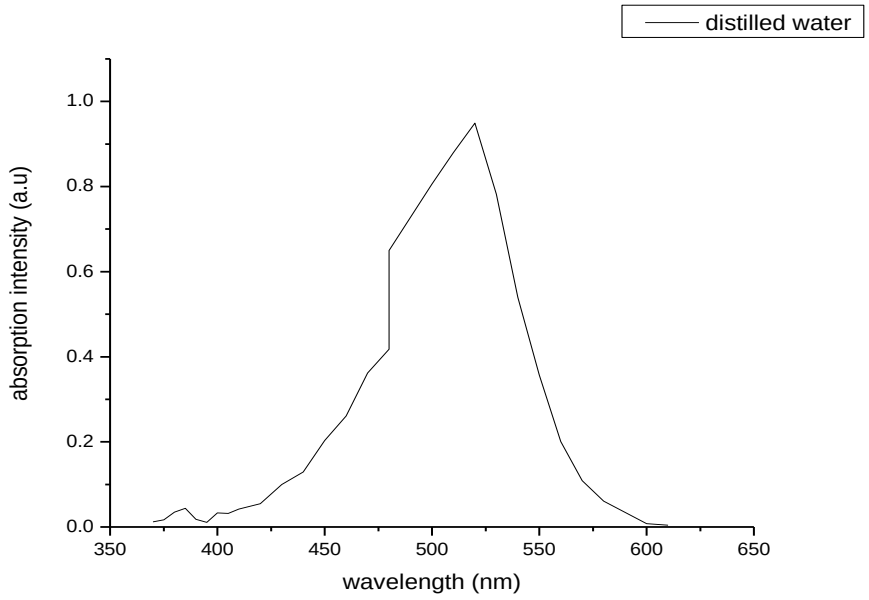


Figure (3.6): Absorption spectrum of Safranin O dissolved in distilled water.

In this figure one can see that Safranin O has strong absorption band in the visible region at 520 nm. The light emitting diode (365 nm) and green diode laser were used to excite this dye and then recording its emission spectra.

3.6 Emission spectra of Safranin O samples after irradiation by green diode laser (532 nm):

Figure(3.7) shows the emission spectra of Safranin O dissolved in distilled water after irradiation by green diode laser (532 nm) with 100 mW output power after different irradiation times starting from 30 seconds up to 120 seconds. The emission spectra showed one band at 634 nm. This band indicated the production of singlet oxygen where it has strong emission band at 634 nm. The intensity of this band increased with increasing the irradiation time.

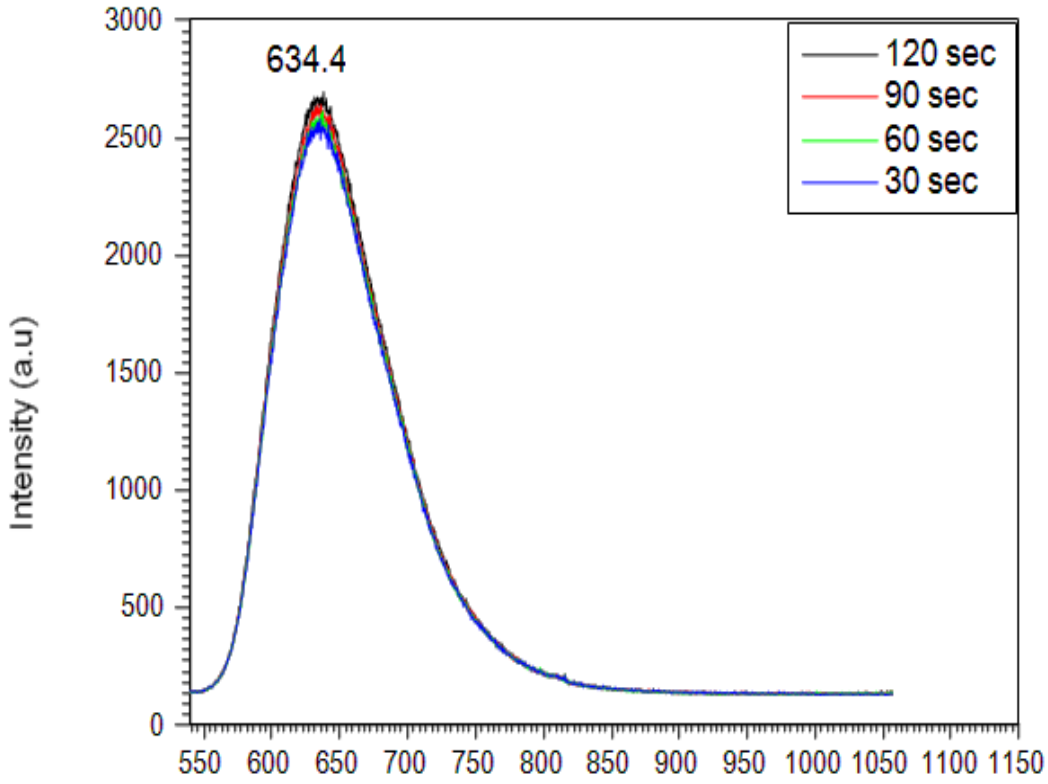


Figure (3.7): Emission spectra of Safranin O dissolved in distilled water after irradiation by (532 nm) for different times.

3.7 The emission spectra of Safranin O samples after irradiation by light emitting diode LED (365 nm):

Figure (3.8) shows the emission spectra of Safranin O dissolved in distilled water after irradiation by light emitting diode (365 nm) with 1200 mW output power after different irradiation times starting form 30 seconds up to 120 seconds. The emission spectra showed one band at 589 nm. The emission spectra didn't indicatethe production of singlet oxygen.

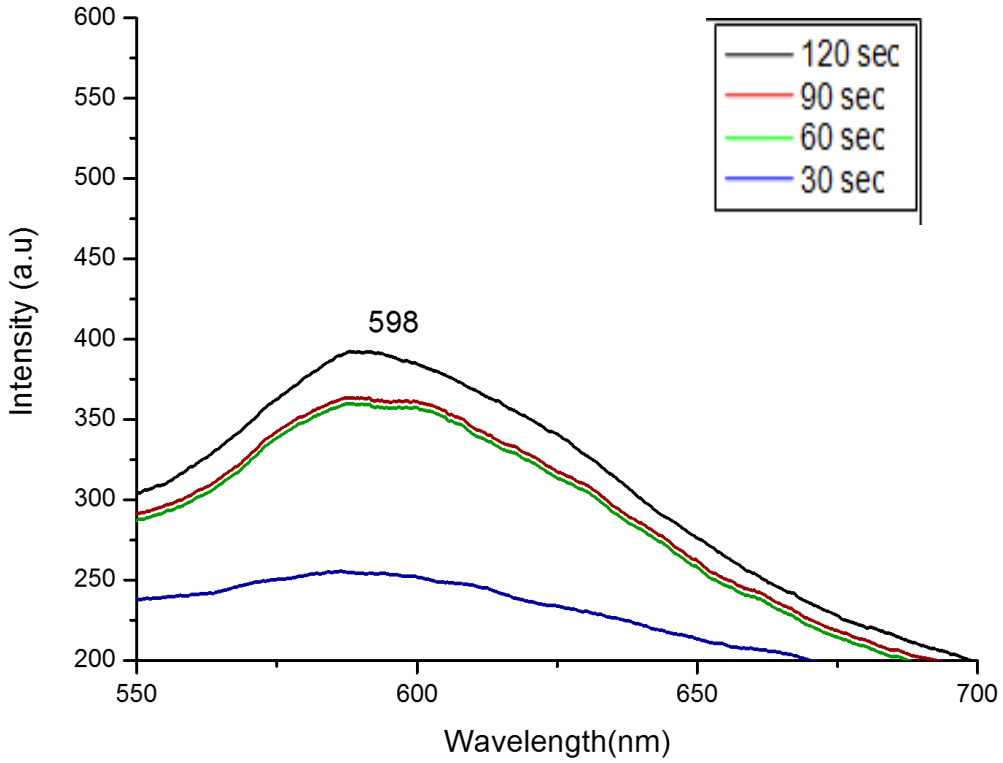


Figure (3.8): Emission spectra of Safranin O dissolved in distilled water after irradiation by LED (365 nm) for different times.

3.8 Absorption spectrum of Rose Bengal:

The absorption spectrum of Rose Bengal dissolved in distilled water is shown in figure (3.9).

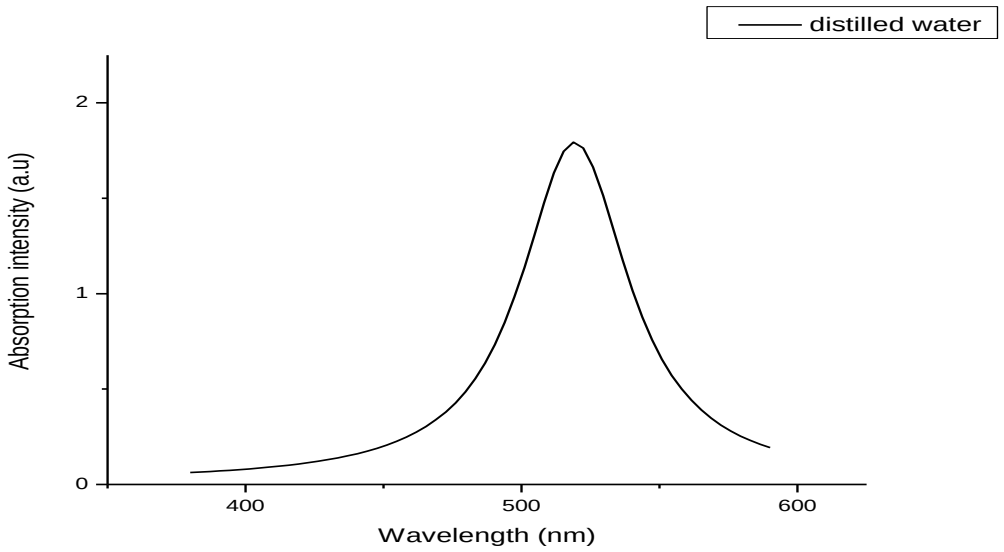


Figure (3.9): The absorption spectrum of Rose Bengal dissolved in distilled water.

This figure shows that Rose Bengal has a strong absorption band in the visible region at 520 nm. The light emitting diode (365 nm) and green diode laser (532 nm) were used to produce the emission spectra for this dye.

3.9 The emission spectra of Rose Bengal after irradiation by green diode laser (532 nm):

Figure(3.10) shows the emission spectra of Rose Bengal dissolved in distilled water after irradiation by green diode laser (532 nm) with 100 mW output power after different irradiation times starting for 30 seconds up to 120 seconds. The emission spectra showed one band at 633.81 nm ≈ 634 nm. The spectra indicate the production of singlet oxygen where it is known that singlet oxygen has an emission band at 634 nm. Increasing the exposure time increased the production of excited molecules then increased the emission intensity.

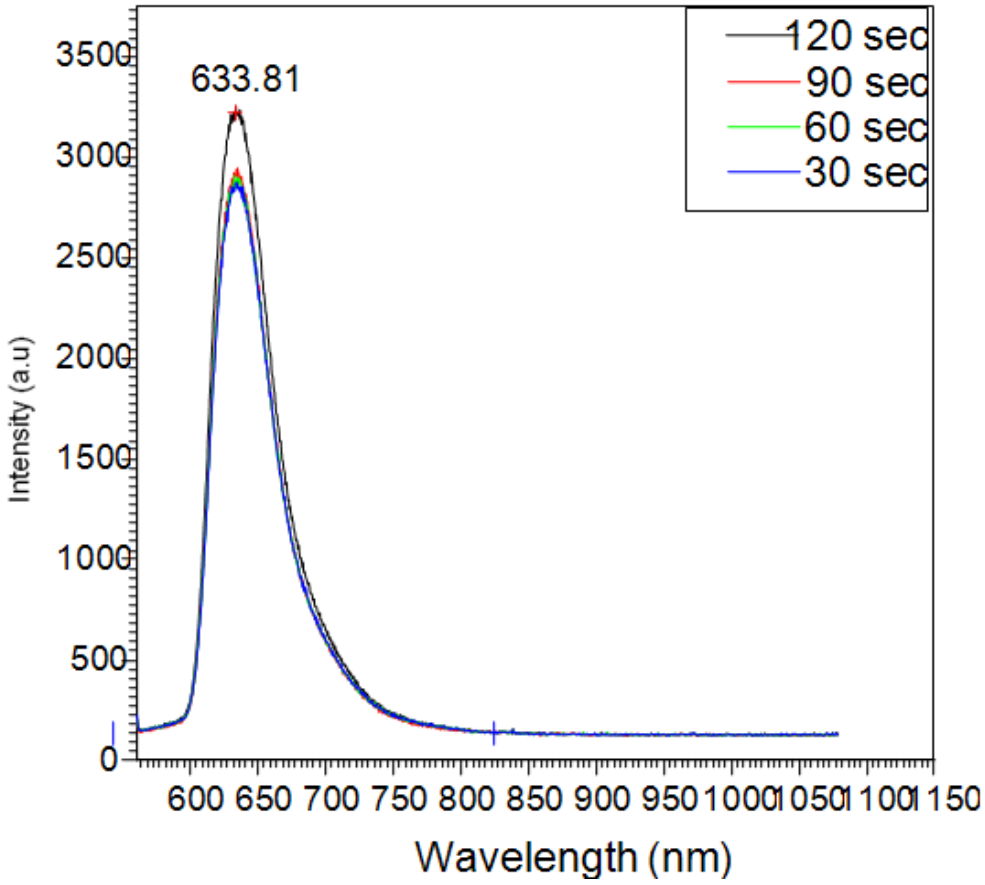


Figure (3.10): Emission spectra of Rose Bengal dissolved in distilled water after irradiation by (532 nm) for different times.

3.10 The emission spectra of Rose Bengal after irradiation by light emitting diode LED (365 nm):

Figure (3.11) shows the emission spectra of Rose Bengal dissolved in distilled water after irradiation by light emitting diode (365 nm) with 1200 mW output power for different irradiation times starting form 30 seconds up to 120 seconds. The emission spectra showed one band at 583 nm. The emission spectra didn't indicate the production of singlet oxygen.

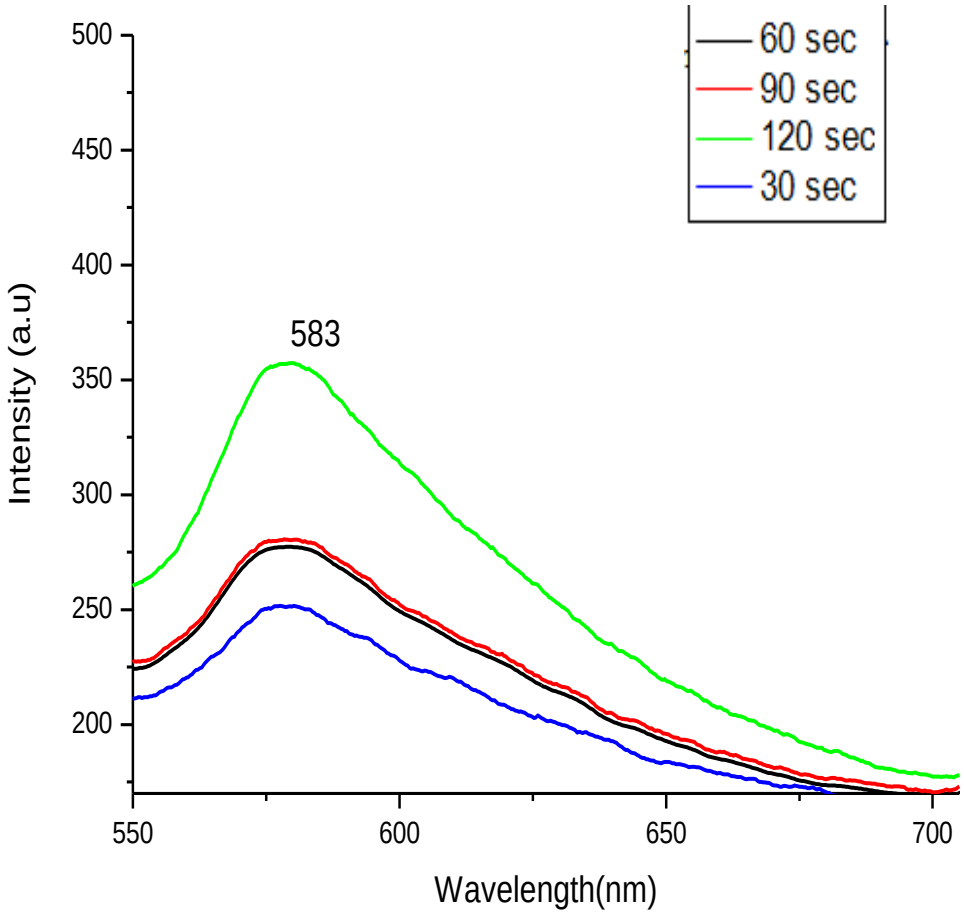


Figure (3.11): Emission spectra of Rose Bengal dissolved in distilled water after irradiation by LED (365 nm) for different times.

It has been observed that decreasing the concentration below 50 mg for each 60ml gave shifting in the emission spectra toward the peak of singlet oxygen (at 634 nm in this work) which increases the probability of having a singlet oxygen, whereas 40mg were dissolved in 60 ml of the solvent for each dye in this work, while increasing the concentration above this limit gave a shift toward shorter wavelengths away of the singlet oxygen peak.

Table (3-1) lists the data obtained from the emission of Eosin Blue, Safranin O and Rose Bengal in different solvents after irradiation by different sources for different times.

Table (3.1): Data obtained from emission of Eosin Blue, Safranin O and Rose Bengal in different solvents after irradiation by different light sources for different times.

Type of dye	Solvent	Excitation source(nm)	Emission band (nm)	Production of singlet oxygen
Eosin Blue	Ethanol	532	599	No
		365	600	No
	Methanol	532	595.8	No
		365	598	No
Safranin O	Distilled water	532	634.4	Yes
		365	589	No
Rose Bengal	Distilled water	532	633.81	Yes
		365	583	No

3.11 Discussion:

The majority of photosensitizers available for PDT utilize Type II photodynamic processes, i.e., The photodynamic effect is achieved through the production of singlet oxygen. The process begins with the absorption of a photon by photosensitizers in its ground state S_0, promoting it to an excited state. The excitation energy of an S_1 state may be released by one of different processes, with relative probabilities that depend on the molecular structure and the environment. (a) The photosensitizr molecules can return to its ground state by emission of a fluorescence photon, which can be used for fluorescence detection. Or alternatively, (b) the molecules may transfered to a triplet state by energy transfer, a process known as intersystem crossing (ISC). A high inter-system-crossing yield is an essential feature of a good type II photosensitizer. Once in its triplet state, the molecule may undergo a collisional energy transfer with ground state molecular oxygen (type II) or with substrate (type I). In type II interaction, the photosensitizer returns to its ground state, and oxygen promoted from its ground state(a triplet state $^3O_2$) to its excited (singlet $^1O_2$) state. Since the photosensitizer molecule is not consumed in this process, the same photosensitizer molecule may create many singlet oxygen molecules. The production of singlet oxygen caused by the collision energy transfer between the photosensitizer triplet state [T] and molecule oxygen [$^3O_2$] to create singlet oxygen [$^1O_2$] as shown in equation (3-1). While equation (3-2) illustrates that electron transfer may compete with the production of singlet oxygen by energy transfer. The interaction between [T] and [$^3O_2$] in equation (3-2) does not yield singlet oxygen and produces a superoxide radical anion O^-_2 (T. Zhu et al, 2007):

$[T] + [^3O_2] \rightarrow [\ ^1S_0] + [^1O_2]$ _____ (3-1)

$[T] + [^3O_2] \rightarrow [\ S^+] + [O^-_2]$ _____ (3-2)

$^1S_0$ is the ground state, where S^+ is the oxidized photosenitiser free radical and O^-_2 is the anion radical superoxide (the univalent anion, O^-_2 , obtained from molecular oxygen by adding an electron).

From the way of production of singlet oxygen above, one conclude that Eosin Blue didn't indicate the production of singlet oxygen for one of two diffrent reasons either (a)when the dye molecules absorbed the photon energy get excited from S_0 to S_1 and then returned to its ground state S_0 by emission of fluorescence; thus, didn't undergo intersystem crossing to triplet state,which is necessary for $^1O_2$ production or (b) the dye molecule in the triplet state [T] undergo electron transfer instead of energy transfer to the ground state oxygen [$^3O_2$] which produce superoxide instead of singlet oxygen. By the same way, one can explain that for Safranin O and Rose Bengal the energy transfer occurred from T to $^3O_2$, which gave positive results and so singlet oxygen has produced.

Most compounds that form triplet states that are able to produce radicals or reactive oxygen species have a tricyclic or heterocyclic structures with the presence of heavy atoms which increase the probability of forming triplet state and therefore induce singlet oxygen reaction (K. Berg, 2011). The excitation energy to the higher energy excited state of these dye sensitizers within the range (2.5 - 3.5eV)(M. Lazár, 2009). In this work both green diode laser with 532 nm and photon energy equivalent to 2.5305 eV and LED with 365 nm and photon energy equivalent to 3.4968 eV can provide the enough photon energy for the excitation of the sensitizer form the ground

state to singlet excited state. In the excited state the photon energy and the coherent of the light source have an effect in dissociation a tricyclic or hetrocyclic structure with the presence of heavy atoms resulting from the main reactants, which increase the probability of forming triplet state; therefore production of singlet oxygen, or vise versa.

The photosensitizer; basically, is a chemical interacted material when exposes to light. The primary act of photons absorption elevates the molecules in the ground state to the excited state, chemical interaction occurs with light and lead to the dissociation of the photosensitizer components to other resulting components and when increasing the irradiation times, the dissociated components absorb a higher number of photons which in turn increase the intensity.

The emission bands of the three dyes (Eosin blue, Rose Bengal and Safranin O) have red shift from the absorption band. The peak of the emission band of Eosin Blue in methanol showed a blue shift from that in ethanol with both coherent (532 nm) and incoherent (365 nm) sources and this because of the differences in the photochemical interaction of the solvents.

3.12 Conclusions:

From the obtained results one conclude that:

* Eosin Blue dissolved in ethanol and methanol didn't produce singlet oxygen $^1O_2$ after irradiation by green diode laser (532nm) and LED (365 nm).

* Among the three dyes (Eosin Blue, Rose Bengal and Safranin O), Rosa Bengal and Safranin O, dissolved in distilled water, can produce singlet oxygen $^1O_2$ after irradiation by green diode laser (532 nm) and therefore can be used in photodynamic therapy (PDT).

3.13 Recommendations:

The followings can be suggested as future work:

- Studying the production of singlet oxygen from emission spectra of dyes with different concentration, it had better to be less than 50 mg/ml of the dye.
- Usage Prophyins and their derivatives in singlet oxygen production because they are ideal candidates for this goal. In addition, other types of dyes can be tested.

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