Sudan University of Science and Technology College of Graduate Studies

Usage of laser emission spectroscopy to produce singlet oxygen from Methylene blue, Eosin yellow and Fluorscin.

استخدام مطيافية الانبعاث بالليزر لانتاج االاوكسجين الاحادي من المثالين الازرق, الايوسين الاصفر و الفلورسين

A thesis Submitted for Partial Fulfillment for the Requirements of Master Degree in Laser Application in Physics

By:

Ibrahim Slah Mohammed Osman

Supervised by

Prof. Dr. Nafie A. Almuslet

May 2017

الآية

قال تعالى((قل لو كان البحر مدادا لكلمات ربي لنفد البحر قبا أن تنفد كلمات ربي ولو جئنا بمثله مددا))

الكهف-الايه*109*

Dedication

To whom gives without return giving as an inexhaustible treasure, as flower not withering and spring not drawing, to whom laying the thorns on my path to guide me to the way of science, to the teacher of my life, to the great hart **my father.**

To the pure white hart, to the symbol of love, to the beautiful woman **my mother.**

To those whom among them I feel power, to those whom are a part of me and I am a part of them, to whom I consider as a gift from god **my brothers.**

Acknowledgement

I would like to thank my supervisor **Professor NafieA. AL-Muslet** for supervising this research and give us help provided us with necessary information to complete this work.

Also my thanks are extended to each hand has a virtue to complete this research specially **Teacher Abd Alhamed** and Ms. **Sara Mahmoud**.

Abstract

In this work the emission of singlet molecular oxygen at room temperature was determined by using spectroscopic method.

The dyes were prepared from (Methylene blue, Eosin yellow and Fluorscin dissolving in methanol ethanol).Then UV visible spectrometer was used to record the absorption spectra for the solvents and the dye samples. Then the samples were irradiated by different light sources to firstly (LED (365 nm with output power 1200 mW), secondly by the green diode laser (532 nm) with output power 100m W. Thirdly the red diode laser (671 nm).

USB 2000 spectrometer was used to record the emission spectra of the samples with different times start from 30sec to 120 sec.

The result showed when Methylene blue dissolved in methanol and ethanol after irradiated by red diode laser (671 nm) have efficiency to produce singlet oxygen where the peak at 703 nm and Eosin yellow dissolved in methanol and ethanol after irradiated by LED (365 nm) also have efficiency to produce singlet oxygen where the peak at 577 nm.

Where singlet oxygen formation can be used in photodynamic therapy to treatment same skin diseases and destroy cancer tumor.

المستخلص

فـي هذا العمل تمت دراسة انبعاث الاوكسجين الأحادي في درجة حراره الغرفه باستخدام الطريقه المطيافيه. تم تجهيز الاصباغ (الميثالين الازرق, الايوسين الاصفر والفولرسن) باذابتها في الايثانول و الميثانول ثم استخدم المطايف في المدي المرئي وفوق البنفسجيه لتحديد طيف الامتصاص للمذيبات والاصباغ.

من ثم تم تشعيع الاصباغ بمصادر ضوئيه مختلفه وهي:

اولا: الثنائي الباعث للضوء ذو الطول الموجي 365 نانومتر و قدره خرج 1200ملي وات.

ثانيا :الليزر الثنائي ذو الطول الموجي 532 نانومتر وقدره خرج 50 ملي وات.

ثالثا: الليزر الثنائي ذو الطول الموجي 671 نانو متروقدره خرج 100 ملي وات.

باستخدام USB 2000 spectrometer تم تحديد طيف الانبعاث في فترات زمنيه مختلفه ابتدا من 30 ثانيه الي 120 ثانيه .

من النتائج تم استنتاج ان الميثالين الازرق المذابه في الايثانول والميثانول التي تم تشعيعها Diode laser ذو الطول الموجي 671 نانو متر لها كفاءه عاليه في انتاج الاوكسجن الاحاديه في 703 نانومتر وايضا الايوسين الاصفر المذابه في الايثانول و المثانول والتي تم تشعيعها الثنائي الباعث للضوء في 577 نانومتر. الاوكسجين الاحاديه الناتجه تستخدم في العلاج الضوئي مثل علاج بعض الامراض الجلديه واستئصال الاورام السرطانيه.

Contents

List of figures

List of table

CHAPTER ONE

BASIC CONCEPS

1.1 Introduction:

Light is the principal source of information for most humans. Everything we see is due to light interacting with objects around us before entering our eyes and being interpreted by our brain to form images that we can analyze and respond to. On the smallest scale possible, light can be described as small energy packets, or quanta, called photons. A photon is a massless elementary particle with zero electrical charge and described by its polarization and its wave vector which specifies the wavelength and direction of propagation of the photon. (B. Thorsted, 2015)

The term spectroscopy was first coined in 1666, when Sir Isaac Newton demonstrated the dispersal of white light through a prism into different colors. Contemporaries thought they were looking at ghosts and called themselves ghost watchers. Ghost in Latin is spectrum, while watcher in Greek is scopos hence the term spectroscopy. (A. Hind, 2011)

1.2 The study objectives:

The main objective of this study is to obtain the emission spectra of singlet oxygen that are produced from (Methylene blue, Eosin yellow and Fluorscin dyes) dissolved in ethanol and methanol after they were irradiated by (green diode laser 532nm, red diode laser 671nm and LED 365nm).

1.3 Thesis structure:

This thesis contains three chapters; chapter one contains the study objectives, concepts of spectroscopy, types of laser spectroscopy (laser absorption spectroscopy (diract and indiract), scattering spectctroy (elastic(Raylegh

scattering spectctroy) and inelastic (Raman spectctroy)) laser emission spectroscopy (laser induce fluorcence and laser induce breakdown spectctroy)). Also contians the definition of photodynamic tharepy and PDT action in the body. In addition, photosinsitizer definition, light sources in PDT, singlet oxygen and its geanration beside the literature review. Chapter two describes the experimental part (materials and methods). Finally, chapter three contains the results and the discussion of the results, conclusion and recommendations.

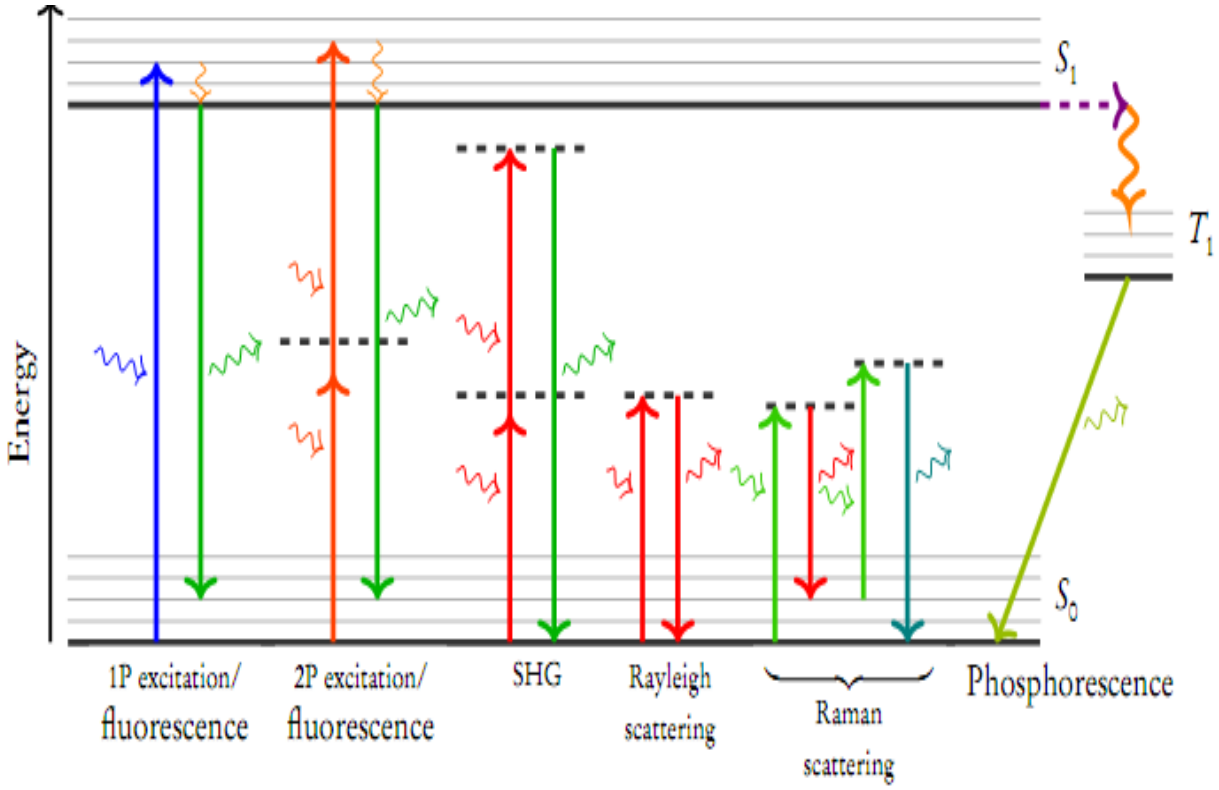

Fig. (1.1): Jablonski diagram of different photon-matter interactions. (B. Thorsted, 2015)

1.4 Spectroscopy:

Spectroscopy is basically the measurement of the interaction of light with various materials. By analyzing the amount of light absorbed or emitted by a sample, we can determine what it's made of and how much of it there is. (A. Hind, 2011)

Generally any molecules composed of number atoms. They have unique energy levels called electronic energy levels (did not find tow materials have same energy levels).

Each electronic state is split into multiple sublevels representing the vibrational levels of the molecule and vibrational levels split into multiple rotation levels.

Electronic states of molecules can be grouped into two broad categories, singlet states and triplet states. A singlet state is one in which all of the electrons in the molecule have their spins paired. Triplet states are those in which one set of electron spin have become unpaired.

When a photon with energy hŭ hits an atom or molecule an interaction between the two will occur. Fundamentally, there are four types of interactions between light and matter: reflection, refraction, absorption and emission. Depending on the properties of the molecule and photon, one or more of these interactions are possible. In Figure (1.1) a few examples of absorption interactions between photons and matter are presented. Rayleigh and Raman scattering are examples of inelastic (emitted photon is identical to absorbed) and elastic scattering (energy of emitted photon is not equal to that of absorbed), respectively. Second-harmonic generation (SHG) is a frequency-doubling phenomenon where two identical photons are combined to form a new photon with twice the energy and half the wavelength. (B. Thorsted, 2015)

1.4.1 Absorption spectroscopy:

In absorption spectroscopy, though the mechanism of absorption of energy is different in the ultraviolet, infrared and nuclear magnetic resonance regions. The energy required for the transition from a state of lower energy to state of higher energy is exactly equivalent to the energy of electromagnetic radiation that causes transition called absorption. (S. Kumar, 2006)

During absorption, the intensity of an incident electromagnetic wave is attenuated in passing through a medium. Absorption is due to a partial

conversion of light energy into heat motion or certain vibrations of molecules of the absorbing material. The terms transparent and opaque are relative, since they certainly are wavelength dependent. (M. Niemz, 1996)

As light passes through a sample, its power decreases as some of it is absorbed. This attenuation of radiation is described quantitatively by two separate, but related terms: transmittance and absorbance. Transmittance is the ratio of the source radiation's power exiting the sample, P_T, to that incident on the sample, P_0.

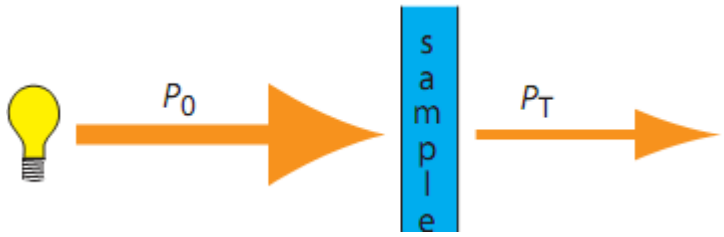

Fig. (1.2): Schematic diagram showing the attenuation of radiation passing through a sample.

1.4.2 Emission spectroscopy:

Since the early 1800s, scientists realized that elements emitted specific colors of light. As atomic theory developed spectroscopies learned that those colors wavelengths or frequencies were a unique signature for each atom and ion. Hence spectra became fingerprints of the emitting atomic species. This is the basis for spectrochemical analysis using atoms. (A. David, & I. Radziemski, 2006)

Emission spectroscopy is spectroscopic technique which examines the wavelengths of photons emitted by atoms or molecules during their transition from an excited state to lower energy state, each element emits a characteristic set of discrete wavelengths according to its electronic structure ,and observing these wavelengths the elemental composition of the sample can be determined.

All other forms of light emission are called Luminescence. (E. Perkin, 2000)

Luminescence is most conveniently defined as the radiation emitted by a molecule, or an atom, after it had absorbed energy to go to an exited state.

Luminescence processes can be interpreted only in terms of the exited state from which luminescence emission occurs and its relationship to the ground state of the molecule. Although the simple picture of photon absorption by a molecule subsequent by a reemission of a photon to give luminescence seems to be quite straightforward, there are non radiative processes which precede and/or compete with photon emission. The main types of luminescence consist of fluorescence and phosphorescence.

Absorption of an ultraviolet or visible photon promotes a valence electron from its ground state to an excited state with conservation of the electron's spin. For example, pair of electrons occupying the same electronic ground state have opposite spins and are said to be in a singlet spin state. The excited states are not stable and will not stay indefinitely. If we observe a molecule in the excited state, at some random moment it will spontaneously return to the ground state. This return process is called decay, deactivation or relaxation. Under some special conditions, the energy absorbed during the excitation process is released during the relaxation in the form of a photon. This type of relaxation is called emission. Emission of a photon from a singlet excited state to a singlet ground state, or between any two energy levels with the same spin, is called fluorescence. The probability of a fluorescent transition is very high, and the average lifetime of the electron in the excited state is only 10^{-5}–10^{-8} s. Fluorescence, therefore, decays rapidly after the excitation source is removed. (J Hodak, et. al, 2008)

Phosphorescence in the production of excited states by promotion of an electron into a higher orbital, the direction of the spin of the electron is preserved. Since most molecules have an even number of electrons and these are normally

arranged in pairs of opposite spin, the promotion of an electron does not disturb this parity.

However, it is possible for the spin of the promoted electron to be reversed so that it is no longer paired and the molecule has two independent electrons of the same spin in different orbitals. Quantum theory predicts that such a molecule can exist in three forms of very slightly differing, but normally indistinguishable energy, and the molecule is said to exist in a triplet state. The indirect process of conversion from the excited state produced by absorption of energy, the singlet state, to a triplet state, is known as intersystem crossing and can occur in many substances when the lowest vibrational level of the excited singlet state, S_1, has the same energy level as an upper vibrational level of the triplet state. The transition from an excited triplet state to the ground state with the emission of phosphorescence requires at least 10^{-4} seconds and may take as long as 10^{2} seconds. This delay was once used as the characterization of phosphorescence, but a more precise definition requires that phosphorescence be derived from transitions directly from the triplet state to the ground state .(E. Perkin, 2000)

1.5 Laser spectroscopy:

The availability of laser sources has substantially changed the field of spectroscopy. But lasers have been employed in many more innovative approaches to spectroscopic investigation. The resolution of a tunable laser system is far better than that of the best conventional dispersive spectrometers. The combination of narrow spectral line width, high radiance, and tenability available with lasers has led to great improvement in resolution for absorption spectroscopy. Laser spectroscopy has been carried out in the visible, near infrared, and near ultraviolet regions of the spectrum for many years using tunable dye lasers. (J. Ready, 1997)

1.6 Types of laser spectroscopy:

Laser analytical techniques can be classified on the basis of the physical process caused by the interaction of laser radiation with matter. The primary process is absorption of photons by atoms or molecules. If the absorption lines are strong and the number density of the absorbing species is sufficient, the difference between incident and transmitted energies may be measurable for a single pass of the laser beam through the sample .In this case the signal can be measured by a simple light detector Such as a photodiode. For weaker absorption lines and lower number densities of the absorbing species multi pass schemes are used. Another set of analytical techniques (optocalorimetric methods) is based on indirect measurement of the energy absorbed by the sample. Absorbed energy is transformed into kinetic energy of the sample particles followed by an increase in the local temperature of the sample. The resultant changes in pressure and refractive index can be measured by optoacoustic methods (absorption spectroscopy).

The absorption of laser photon led to excitation of atoms or molecules. In many cases, radiative decay of the excited state is very efficient, i.e. Quantum yields of luminescence or fluorescence are very high. Measurement of the intensity of emitted photons as a function of analyte concentration is a widespread analytical method with conventional light sources (emission spectroscopy).

Also laser may be scattering when photon energy does not corresponding to energy band gap of molecules (scattering spectroscopy).

The use of intense laser sources greatly increased the sensitivity and selectivity of traditional luminescence methods (M. Bolshov & Y. kuritsyn, 2001)

1.6.1Laser absorption spectroscopy:

Laser absorption spectroscopy done by two ways;

1.6.1.1 Direct laser absorption spectroscopy:

The first and perhaps most obvious type of laser spectroscopy is absorption spectroscopy. The laser beam is transmitted through the sample the spectrum of which is desired, and the wavelength of the laser light is tuned through the region of interest. There is no need for gratings, prisms, or any of the other dispersive elements used in conventional spectrometers. (J. Ready, 1997)

The amount of absorption, which is related to the concentration of the absorber (analyte), is determined by comparing the incident power to the one measured after transmission through the sample. (F. Schmidt, 2007)

The relationship between the ratio of transmitted to incident light and the thermophysical properties of the absorbing gas by Beer's Law (I. Schultz, 2014) shown in Eq. (1.1).

$$\left(\frac{I_t}{I_0}\right)_\nu = \exp\left(-\alpha_\nu\right) \tag{1.1}$$

When the radiation passes through a solution, the amount of light absorbed or transmitted is an exponential function of the molecular concentration of the solute and also a function of length of the path of radiation through the sample. Therefore,

$$\text{Log}\,(I_o / I) = \varepsilon\, c\, l \tag{1.2}$$

I_o: Intensity of the incident light (or the light intensity passing through a reference cell).

I: Intensity of light transmitted through the sample solution.

c: Concentration of the solute in mol L^{-1}

l: Path length of the sample in cm.

ε : Molar absorptivity or the molar extinction coefficient of the substance whose light absorption is under investigation.

The ratio I / I_o is known as transmittance T and the logarithm of the inverse ratio I_o / I is known as the absorbance A.

Therefore

$$- \text{Log} (I / I_o) = - \log T = \varepsilon c l \quad (1.3)$$

$$\text{Log} (I_o / I) = A = \varepsilon c l \quad (1.4)$$

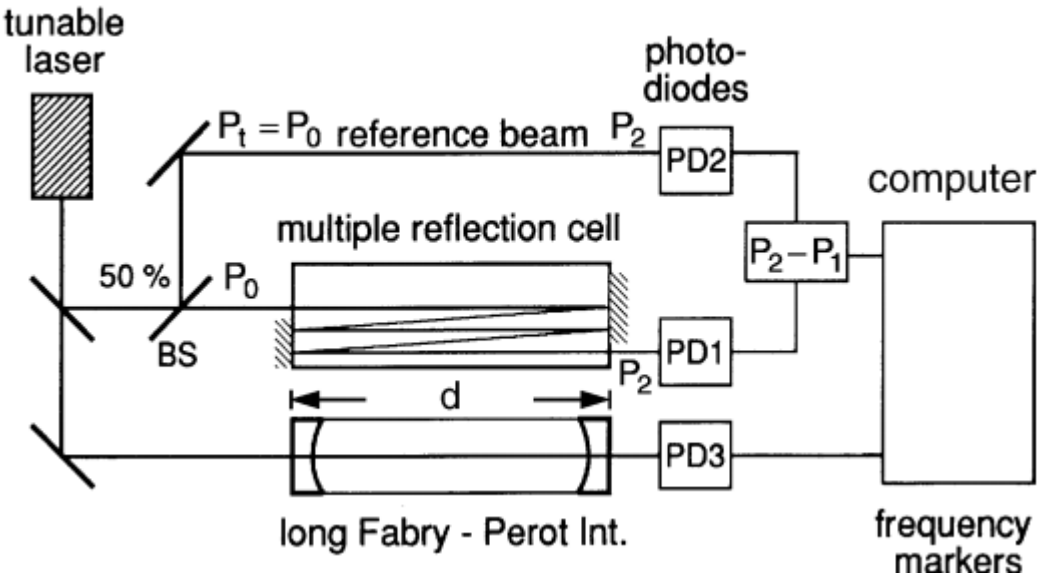

Fig. (1.3): Absorption spectroscopy with tunable single-mode laser. (W. Demtroder, 2008)

1.6.1.2 Indirect absorption (Photoacoustic Spectroscopy):

Photoacoustic spectroscopy is a sensitive technique for measuring small absorptions that is mainly applied when minute concentrations of molecular

species have to be detected in the presence of other components at higher pressure.

The laser beam is sent through the absorber cell as shown in Figure (1.3). If the laser is tuned to the absorbing molecular transition $E_i \rightarrow E_k$, part of the molecules in the lower level E_i will be excited into the upper level E_k. By collision with other atoms or molecules in the cell, these excited molecules may transfer their excitation energy ($E_k - E_i$) completely or partly into translational, rotational or vibrational energy of the collision partners. At thermal equilibrium, this energy is randomly distributed onto all degrees of freedom, causing an increase of thermal energy and with it a rise in temperature and pressure at a constant density in the cell.

When the laser beam is chopped at frequencies $\Omega < 1/T$, where T is the mean relaxation time of the excited molecules, periodic pressure variations appear in the absorption cell, which can be detected with a sensitive microphone placed inside the cell. The output signal S [Volt] of the microphone is proportional to the pressure change Δp induced by the absorbed radiation power $\Delta W/\Delta t$ (W. Demtroder, 2008)

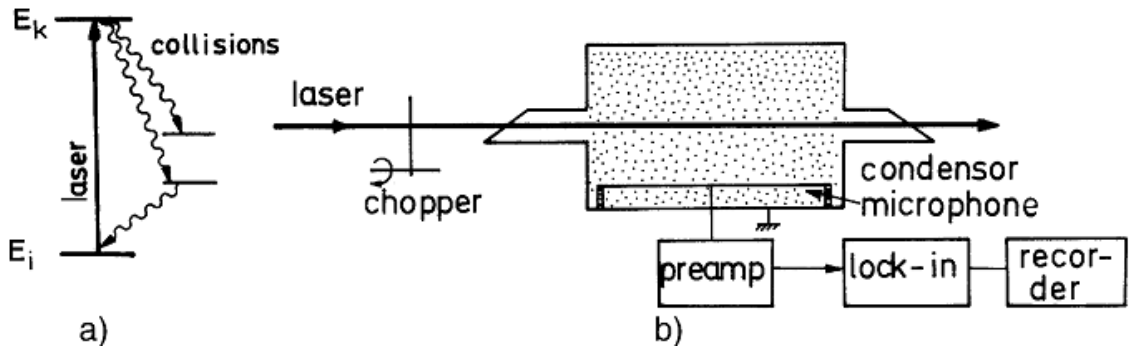

Fig. (1.4): Photoacoustic spectroscopy: (a) level scheme (b) Schematic experimental arrangement. (W. Demtroder, 2008)

1.6.2 Laser in emission spectroscopy:

Laser emission spectroscopy has two mine types (laser Induce Fluorescence and laser induce breakdown).

1.6.2.1 Laser Induce Fluorescence (LIF):

Laser induce Fluorescence is special type of molecular emission and is techniques that assay the state of a biological system by studying its interactions with fluorescent probe molecules. This interaction is monitored by measuring the changes in the fluorescent probe optical properties. (P. So, & C. Dong Y, 2002) laser induce Fluorescence spectroscopy is versatile technique in which molecular species are irradiated with laser radiation in a specific wavelength range (normally in the ultraviolet or visible for electronic spectroscopy) that is in resonance with the differences in molecular energy levels. Such resonantly tuned radiation has a fairly good probability of induce transition to excited state of molecules, which me be followed by relaxation of molecules to ground electronic state by spontaneous emission of photon whose energy corresponds to separation in molecular energy levels. laser usually in LIF, frequency doubled tunable dye laser. (P. Misra, et. al.1993)

Fluorescence emitted from the first electronic excited level after absorption of laser. Fluorescence signal is detected by sensitive detector which always sets at 90° to the incident beam.

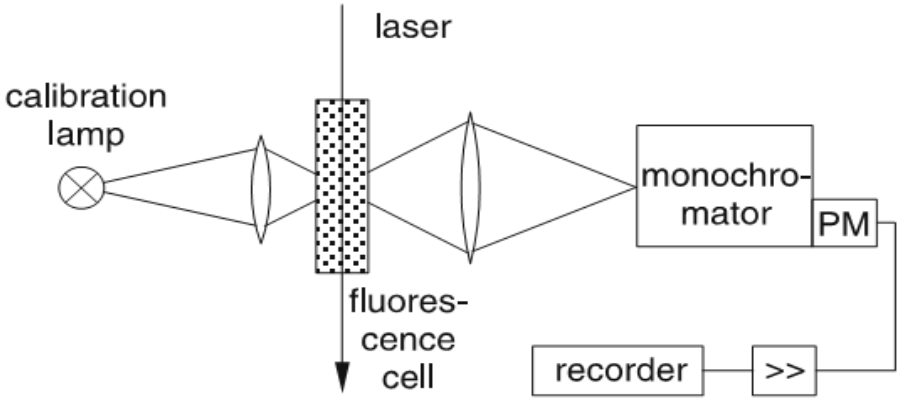

Fig. (1.5): Experimental arrangement for measuring LIF spectra.

1.6.2.2 Laser induce break down spectroscopy (LIBS):

Laser induced breakdown spectroscopy (LIBS), also sometimes called laser induced plasma spectroscopy (LIPS) or laser spark spectroscopy (LSS). (A. David & l. RadziemskI, 2006)

Laser induced breakdown spectroscopy (LIBS) is an analytical technique that allows for the determination of a sample's elemental composition based on laser ablation followed by atomic, ionic, and molecular emission processes coming from elements transferred into the plasma as a result of laser induced breakdown. In LIBS , a plasma is generated by introduction of a high power laser on to the target surface(solid sample), while the atomic emission signal from the plasma is detected for the elemental analysis of solid samples. Since the power density of the incoming laser beam needs to be higher than some threshold value to generate the laser induced plasma, laser used for LIBS studies are usually high power pulsed and narrow pulse width.(IB .Gornushkin, et.al. 2004)

A portion of the plasma light is collected and a spectrometer disperses the light emitted by excited atomic and ionic species in the plasma, a detector records the emission signals, and electronics take over to digitize and display the results. (A. David & l. RadziemskI, 2006)

Plasma light emissions can provide spectral signatures of chemical composition of many different kinds of materials in solid, liquid or gas state.

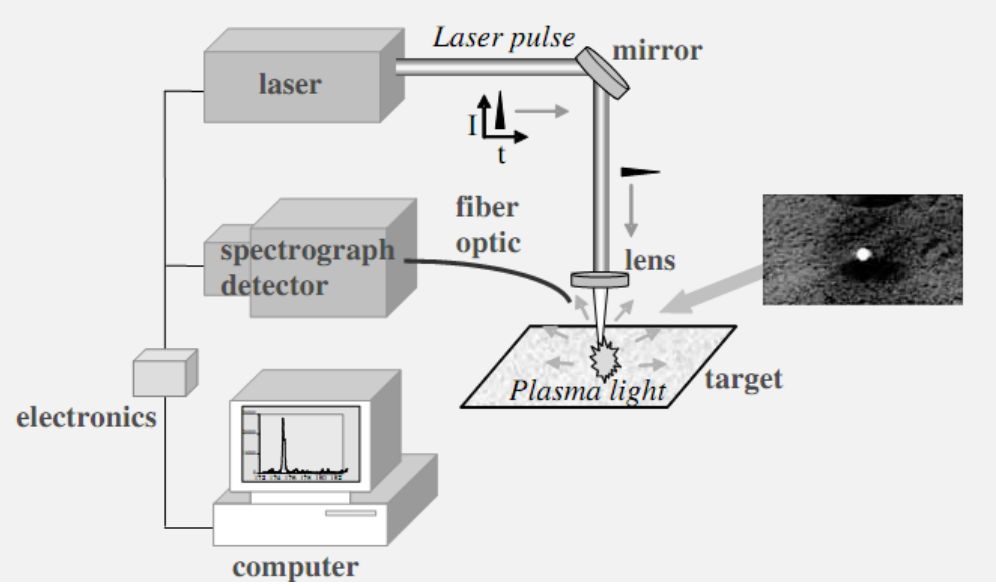

Fig. (1.6): Laser-induced breakdown spectroscopy.

1.6.3 Laser scattering spectroscopy:

Scattering takes place at frequencies not corresponding to those natural frequencies of particles. A scattering is divided in two types' elastic and inelastic scattering which can distinguish between them, depending on whether part of the incident photon energy is converted during the process of scattering.

Elastic scattering; where incident and scattered photons have the same energy (Rayleigh scattering). Its only restriction is that the scattering particles be smaller than the wavelength of incident radiation. Scattering is inversely proportional to the fourth power of wavelength. The latter statement is also known as Rayleigh's law.

$$I_s = \frac{1}{\lambda^4} \tag{1.5}$$

Another type of elastic scattering is Mie scattering but differ from Rayleigh scattering is a weaker dependence on wavelength ($\sim \lambda^{-x}$ with $0.4 \leq x \leq 0.5$)

compared to Rayleigh scattering ($\sim \lambda^{-4}$). Second, Mie scattering preferably takes place in the forward direction, whereas Rayleigh scattering is proportional to $1 + \cos2(\theta)$ according forward and backward scattered intensities are the same.

$$Is(\theta) = \frac{1+\cos2(\theta)}{\lambda^4} \quad (1.6)$$

Inelastic scattering**;** (Brillouin and Raman scattering) is another type of scattering.

Which scattered photon does not have same energy of incident photon Brillouin scattering is one type of inelastic scattering it arises from acoustic waves propagating through a medium, thereby inducing inhomogeneities of the refractive index.

Brillouin scattering of light to higher (or lower) frequencies occurs because scattering particles are moving toward (or away from) the light source. It can thus be regarded as an optical Doppler effect in which the frequency of photons is shifted up or down. (M. Niemz, 1996)

The Raman effect involves scattering of light by molecules of gases, liquids, or solids. The Raman effect consists of the appearance of extra spectral lines near the wavelength of the incident light. The Raman lines in the scattered light are weaker than the light at the original wavelength. The Raman-shifted lines occur both at longer and shorter wavelengths than the original light; the lines at the shorter wavelengths are usually very weak.

The Raman spectrum is characteristic of the scattering molecule. The Raman lines occur at frequencies $v \pm \upsilon$ where v is the original frequency and υ are the frequencies corresponding to quanta of molecular vibrations or rotations. If the scattered frequency is lower than the original frequency (longer wavelength), the incident light has excited a molecular vibration or rotation and the optical photon has decreased energy. This situation is called Stokes scattering. If the frequency of the scattered light is higher than the incident light (shorter wavelength), the

light has gained energy from the vibrational or rotational quanta. This is called anti-Stokes scattering. (J. Ready, 1997)

1.6.3.1 Elastic scattering spectroscopy (ESS):

ESS is a point measurement technique that, when performed using appropriate optical geometry, is sensitive to the morphologic changes at that cellular and subcellular level. These include size and hyper chromaticity of cell nuclei, nuclear crowding, changes in the size of mitochondria and other cellular organelles. ESS spectra depend on the scattering efficiency of the cellular and subcellular organelles at each wavelength. Therefore, normal and abnormal tissues generate different spectral signatures, which represent the optical equivalent of histologic appearances.

The equipment used for ESS has been consists of an optical biopsy box that contains the power supply, a pulsed xenon arc lamp and a spectrometer, an optical probe that contains the two fibers for transmitting and receiving light, and a laptop computer for spectral analysis.

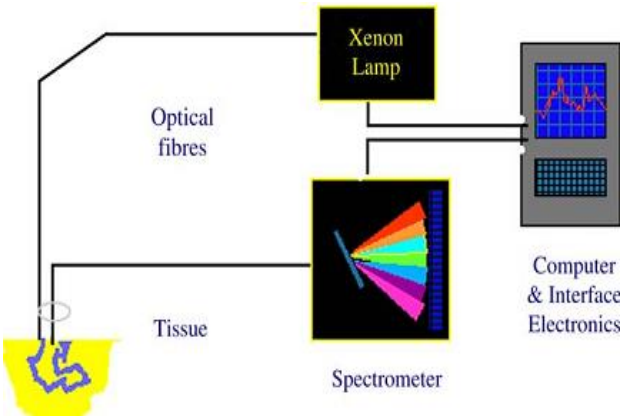

Fig. (1.7): Schematic diagram of the elastic scattering spectroscopy.

1.6.3.2 Raman spectroscopy:

Raman spectroscopy has long been used for qualitative analysis and for identification of characteristic localized units of structure within molecules. The

availability of laser sources has provided an important new device for use in Raman spectroscopy. The narrow line width and high radiance of laser sources make it much easier to identify the scattered light and to determine the amount of the wavelength shift. These same properties permit higher resolution of Raman spectra than was possible with conventional sources. The variety of available laser wavelengths makes it possible to carry out Raman spectroscopy while avoiding interfering absorption bands of the molecule being studied. With a tunable laser, the excitation frequency can be tuned to a resonant frequency of the molecule to produce a larger Raman signal. Although a tunable laser is not necessary for Raman spectroscopy, the use of a tunable source can greatly enhance the Raman signal The Raman spectroscopic technique is applicable to very small samples, because Raman spectroscopy requires a sample only of sufficient size to fill the focused beam of an argon laser, a volume about 10μm in diameter. (J. Ready, 1997)

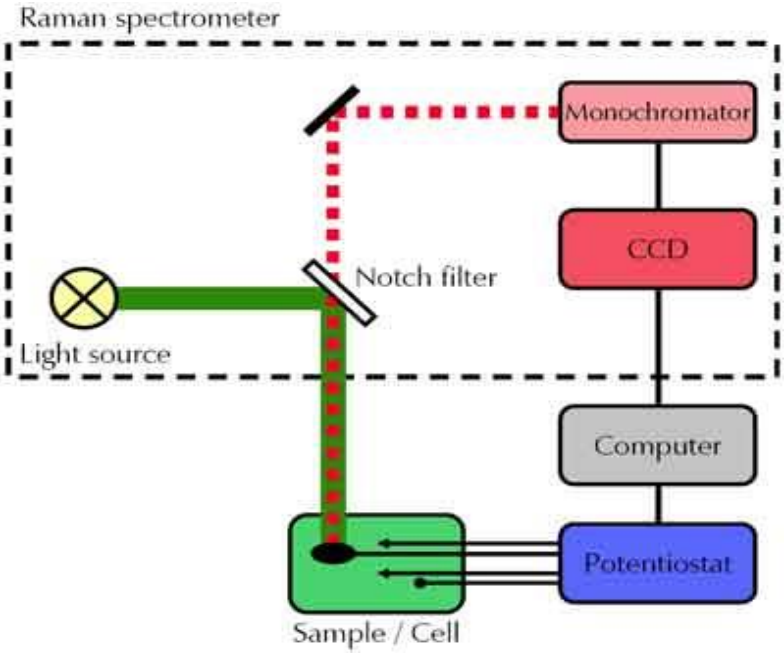

Fig. (1.8): Experiment arrangement for Raman spectroscopy.

One important variation of Raman spectroscopy Coherent anti-Stokes Raman scattering, or CARS as it is usually known, depends on the general phenomenon of wave mixing, as occurs, for example, in a frequency doubling crystal. In that case three waves mixing occurs involving two incident waves of wavenumber υ and the outgoing wave of wavenumber 2υ.

In CARS, radiation from two lasers of wavenumbers υ_1 and υ_2, where $\upsilon_1 > \upsilon_2$, fall on the sample. As a result of four-wave mixing, radiation of wavenumber $\upsilon_3$is produced where

$$\upsilon_3 = 2\upsilon_1 - \upsilon_2 = \upsilon_1 + (\upsilon_1 - \upsilon_2) \qquad (2.7)$$

The wave mixing is much more efficient when $\upsilon_1 - \upsilon_2 = \upsilon i$, where υ_i is the wavenumber of a Raman-active vibrational or rotational transition of the sample. The scattered radiation υ_3 is to high wavenumber of υ_1 (i.e. on the anti-Stokes side) and is coherent, unlike spontaneous Raman scattering: hence the name CARS. As a consequence of the coherence of the scattering and the very high conversion efficiency to υ_3, the CARS radiation forms a collimated, laser-like beam. (J. Hollas, 2004)

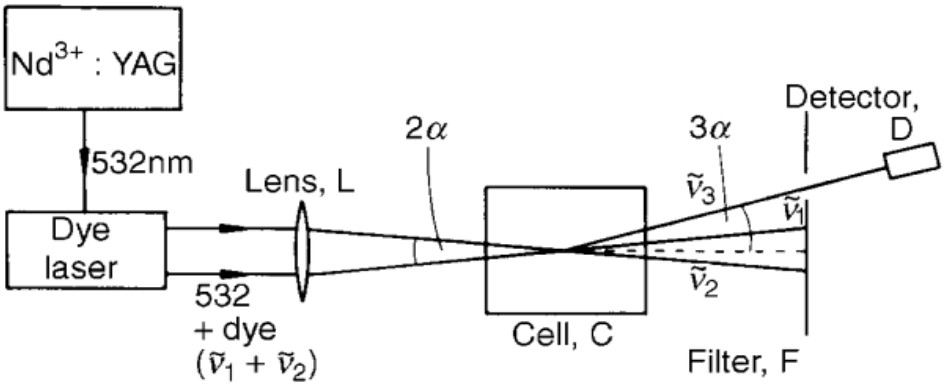

Fig. (1.9): Experimental arrangement for coherent anti-Stokes Raman scattering. (J. Hollas, 2004)

1.6.3.3 Rayleigh scattering spectroscopy:

Rayleigh scattering is a powerful diagnostic tool for the study of gases and is particularly useful for aiding in the understanding of complex flow fields and combustion phenomena. Although the mechanism associated with the scattering,

induced electric dipole radiation, is conceptually straightforward, the features of the scattering are complex because of the anisotropy of molecules, collective scattering from many molecules and inelastic scattering associated with rotational and vibrational transitions. These effects cause the scattered signal to be depolarized and to have spectral features that reflect the pressure, temperature and internal energy states of the gas. The very small scattering cross section makes molecular Rayleigh scattering particularly susceptible to background interference. Scattering from very small particles also falls into the Rayleigh range and may dominate the scattering from molecules if the particle density is high. This particle scattering can be used to enhance flow visualization and velocity measurements, or it may be removed by spectral filtering. New approaches to spectral filtering are now being applied to both Rayleigh molecular scattering and Rayleigh particle scattering to extract quantitative information about complex gas flow fields. (R. Miles,et.al. 2001)

1.7 Photodynamic therapy:

Light is a form of energy. Molecules of certain chemical compounds (photosensitizers) have the ability to absorb a photon of visible light and then transfer most of their absorbed energy to a molecule of oxygen. This causes a transient increase in the chemical reactivity of the oxygen molecule, and converts it into a relatively strong oxidizing agent known as singlet oxygen Figure (1.10).

PDT makes use of light-induced singlet oxygen to kill cells by causing lethal oxidative damage to biologically important structures.

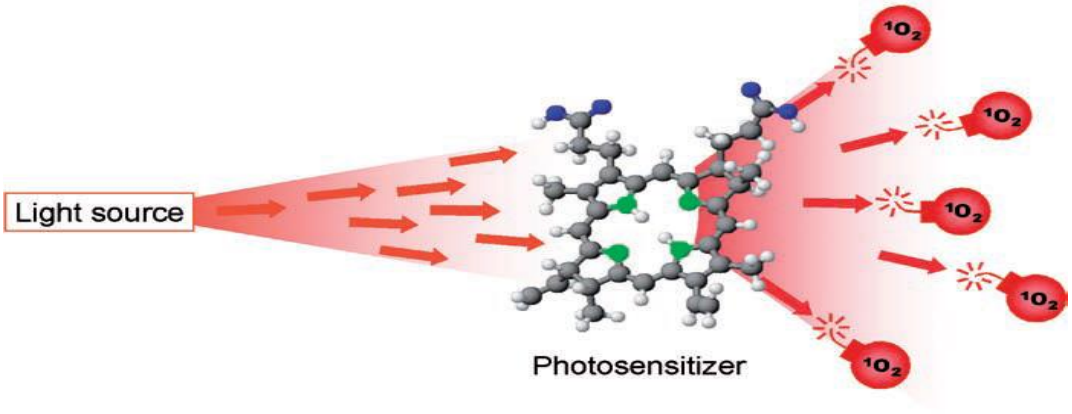

Fig. (1.10): A photosensitizer molecule absorbs light of appropriate wavelength and can excite multiple oxygen molecules to a biologically reactive state ($^1O_2$). (R. Pottier, 2006)

In principle, PDT is a simple adaptation of chemotherapy. A photosensitizer, light and oxygen work together to cause death (by necrosis or apoptosis) of the diseased tissue. The photosensitizer itself is non-toxic, but it can transfer the energy of absorbed light to molecular oxygen and thus produce chemically aggressive oxygen. The activating light by itself (visible or near infrared (IR) radiation) is harmless too.

The intriguing potential of PDT lies in its ability to act selectively on the target tissue without the need for precise light targeting. In addition, PDT can be performed repetitively without the accumulation of serious side effects, and it can be combined with most other treatment modalities. (R. Pottier, 2006)

PDT represents the method of choice for treatment of age-related macular degeneration and is appreciated as minimally invasive therapeutic procedure to treat skin, oesophageal, head and neck, lung, and bladder cancers with high cure rates. (M. Elsaie, 2013)

PDT utilizes photo reactive drugs, or photosensitizers, which are selectively retained by tumor cells and other hyper proliferating tissues .In tissue the photosensitizer alone will not cause damage to the cells , but when activated by exposure to specific wavelengths of light, several photochemical reactions occur,

one of which is thought to be the production of singlet oxygen. These excited oxygen species initiate a free radical chain reaction leading to vascular thrombosis, disruption of cellular organelles, and tumor necrosis .The principal components needed for effective PDT therapy include a blood supply to deliver adequate concentrations of photosensitizer and oxygen to the target tissue and an effective delivery system to provide the activating light to the compound.

Cellular death is accomplished at a subcellular level through the activation of pathways that produce toxic effects within the cell. These effects may include cell membrane disruption, lipoprotein breakdown. Photodynamic therapy allows patients to be treated on an outpatient basis and can be performed in a cost-effective manner. **(**M. Kaplan, & R. Somers, 1998**)**

1.7.1 Photodynamic Action in the Body:

During PDT, a sensitizer can be administered intravenously, intraperitoneally, or topically, and it selectively localizes in a tumor due to physiological differences in the tumor and healthy tissues. Localization into cancer cells and achieving a maximum tumor-to-normal cell concentration ratio can take 3 to 96 h, depending on the photosensitizer and the tumor. Following localization, fluorescence from the sensitizer can be used to diagnose and detect the tumor. Irradiation at a wavelength specific to the photosensitizer produces singlet oxygen, which reacts with and destroys the tumor as shown in Figure (1.10) Chances of skin photosensitivity are high, even though the dye has greater affinity for tumor tissue. This effect requires patients to limit sunlight exposure to eyes and skin up to thirty days or longer following treatment depending on the sensitizer. (A Ormond &. H. freeman, 2013)

Tumoricidal effect of PDT is triggered by a direct damage of malignant cells producing cell death by necrosis and/or apoptosis. PDT also affects tumor vasculature, causing the shutdown of vessels with subsequent depriving of the

tumor with oxygen and nutrients. Finally, PDT can immune stimulate or immuneosupress the immune system.

Biological tissue is very sensitive to $^1O_2$and is quickly destroyed in its presence. In recent years there has been a heightened interesting utilizing compound that can photosensitize the formation of $^1O_2$ for use in PDT. Singlet oxygen is very reactive towards biological tissue and will quickly react with unsaturated organic compounds and fatty acids. Cell membranes such as plasma mitochondrial and nuclear membranes are rapidly destroyed by $^1O_2$.

Photodynamic therapy has great potential to treat a variety of different types of neoplastic diseases such as skin, head and neck, esophageal, and lung cancer. In PDT a photosensitizer (PS) is administered topically or by intravenous injection to a patient. This is pictorially represented in Figure 1. Initially inert, the PS travels throughout the body and is taken up and retained by the tumor. In some circumstances the PS has a lower affinity for healthy cells and is removed from the body through the liver and kidneys.

The sensitizer is then activated by exposing the treatment area to a wavelength of light were the PS undergoes excitation from the ground state, S0, to the first excited state, S_1. The S_1state is short lived, and the PS quickly undergoes intersystem crossing from the S1 to the triplet state T_1. Collision interaction between the PS T_1 and ground state $^3O_2$ result in the formation of $^1O_2$. The use of PDT allows the possibility to reduce or completely remove a cancerous tumor, in some cases, without the need for surgery, chemotherapy, or radiation.

Other advantages count fast healing rates, modest scar formation, and the possibility for re-treatment several times if necessary. (S. Andrasik, 2007)

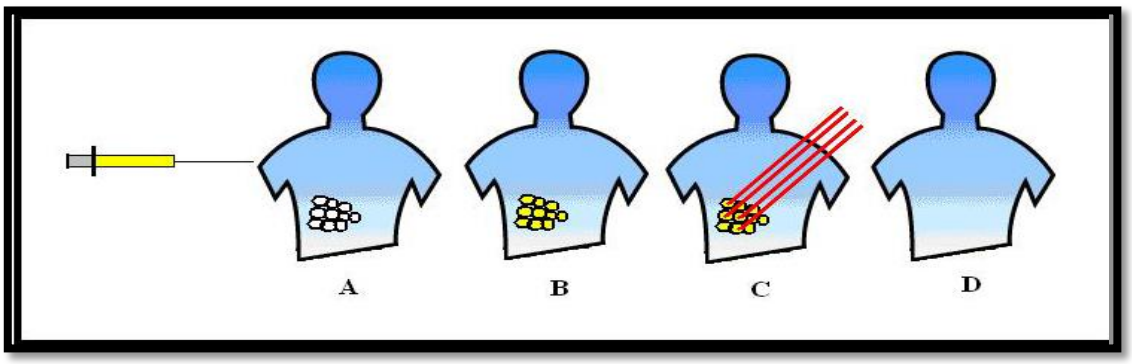

Fig. (1.11): Graphical representation of PDT. (A) Injection of PS into patient, (B) localization of PS in tumor, (C) activation of PS with light, (D) tumor eradicate
(S.Andrasik. 2007)

1.8 Photosensitizers:

The half-life of a molecule of photosensitizer that has absorbed the energy of a photon of light is very short, as is the half-life of the resulting singlet oxygen. (P. Donat& Giulio, 2006)

Photosensitizers (PS) are natural or synthetic chromophore containing compounds that absorb light at a specific wavelength and can induce chemical or physical changes of another chemical entity to generate reactive oxygen species (ROS). An ideal PDT photosensitizer possesses the following characteristics:

(1) It is readily available in pure form with known chemical composition and low dark toxicity.

(2) The PS is stable and soluble in aqueous media.

(3) It may be rapidly excreted from the body to reduce systemic toxicity at the end of treatment.

(4) It exhibits high quantum yield in cell inactivation, which is frequently mediated by singlet oxygen ($^1O_2$).

(5) High molar absorption coefficient in the 600–1000 nm range because endogenous molecules such as hemoglobin and melanin have strong absorption below 600 nm, thereby limiting the effective penetration depth of light in target

tissues. Furthermore, water absorbs many photons above 1000 nm and reduces availability towards chromophores and longer wavelengths are energetically inadequate for the production of $^1O_2$.

(6) There should be maximum quantum yield of triplet formation and the triplet state should extensively exist in order to rapidly react with neighboring target molecules. (T. Peng & H. Tsai, 2015)

1.9 Light Source in PDT:

Light source is another important parameter in PDT and include laser (argon laser and argon-pumped dye laser, metal vapor- sources/lamps (quartz halogen lamps, metal halide lamps, xenon arc lamps, phosphor-coated sodium lamp, fluorescent lamps, etc.). The efficacy of PDT depends on factors such as wavelength, tissue penetration, dose, pulsing, position, state of the cells tissues and absorption properties of the photosensitizers The principle requirement is ample light illumination at the appropriate wavelength which can sensitive PSs and penetrate deep into the target/tumor tissues. Since PDT is most effective toward tumors that can be reached directly by light or light delivery devices such as optic fibers, highest PDT efficacy is attained when light delivery is homogenous and adequate throughout the target tumor tissue volume debele. (T. Peng & H. Tsai, 2015)

1.10 Singlet oxygen:

Ground state molecular oxygen, O_2, is essential for life in all aerobic organisms on earth. It is highly abundant throughout the universe and is the most common component of the earth's crust. It can undergo excitation in a photosensitized process to become a powerful oxidant where it can undergo destructive reactions with organic compounds. Excited state $O_2$is commonly referred to as singlet oxygen ($^1O_2$) and its photosensitized production has given rise to a fascinating field of medicine known as photodynamic therapy (PDT). (S. Andrasik, 2007.)

Singlet oxygen ($^1O_2$) is an electronically excited state of molecular oxygen which is extremely reactive. It attacks and oxidizes proteins, lipids and nucleic acids, and consequently it is an important reactive oxygen species (ROS) in biological systems. It is less stable than triplet oxygen ($^3O_2$). (A. Telgy, 2014)

1.10.1 Generation of singlet oxygen:

The formation of $^1O_2$ can occur by a number of different pathways such as thermally, chemically, or by means of a photosensitizer.

Cyclic organic peroxides can readily undergo thermal decomposition resulting in $^1O_2$ formation.

Khan and Kasha, who performed some of the earliest direct spectroscopic studies on $^1O_2$, used the chemical reaction between sodium hypochlorite and hydrogen peroxide to form $^1O_2$. Singlet oxygen can also be generated in an energy transfer process between a photosensitizer and $^3O_2$. (S. Andrasik, 2007)

Photosensitized generation is a simple and controllable method for the production of $^1O_2$, requiring only oxygen, light of an appropriate wavelength and a photosensitizer capable of absorbing and using that energy to excite oxygen to its singlet state. Sensitizer excitation is generally achieved via a one photon transition ($h\breve{\upsilon}$) between the ground state S_o and a singlet excited state S_n. Relaxation of the S_n state yields the lowest excited singlet state of the sensitizer S_1. Intersystem crossing generates the sensitizer triplet state, T_1. The lifetime of the T_1 state is longer (ms) than that of the S_1 state (ns) allowing this excited state to react in one of two ways, defined as Types I and II mechanisms. A Type I mechanism involves hydrogen-atom abstraction or electron transfer between the excited sensitizer and a substrate, yielding free radicals. These radicals can react with oxygen to form an active oxygen species such as the superoxide radical anion. In a Type II mechanism, singlet oxygen is generated via an energy

transfer process during a collision of the excited sensitizer with triplet oxygen. See Scheme. (M. Derosa. 2002)

Generally collision of two singlet oxygen molecules (O_2 (b ^1S)) may result in collision induced emission (CIE) when two oxygen molecules simultaneously transit to the ground O_2(X, ^3T) state with emission of one photon in the visible spectral region. In particular, one can observe the following transitions. (M Zagidullin, et.al. 2014)

$$O_2(a, v=0) + O_2(a, v=0) \rightarrow O_2(X, v=0) + O_2(X, v=0) + h\upsilon\ (634nm)$$

$$O_2(a, v=0) + O_2(a, v=0) \rightarrow O_2(X, v=0) + O_2(X, v=1) + h\upsilon\ (703nm)$$

$$O_2(a, v=0) + O_2(a, v=1) \rightarrow O_2(X, v=0) + O_2(X, v=0) + h\upsilon\ (579nm)$$

$$O_2(a, v=0) + O_2(b, v=0) \rightarrow O_2(X, v=0) + O_2(X, v=0) + h\upsilon\ (479nm)$$

$$O_2(a, v=0) + O_2(b, v=0) \rightarrow O_2(X, v=0) + O_2(X, v=1) + h\upsilon\ (514nm)$$

$$O_2(a, v=0) \rightarrow O_2(X, v=0) + h\upsilon\ (1268nm)$$

$$O_2(b, v=0) \rightarrow O_2(X, v=0) + h\upsilon\ (762nm)$$

$$O_2(b, v=1) \rightarrow O_2(X, v=1) + h\upsilon\ (771nm)$$

$$O_2(b, v=1) \rightarrow O_2(X, v=0) + h\upsilon\ (688nm)$$

$$O_2(b, v=0) \rightarrow O_2(X, v=1) + h\upsilon\ (864nm)$$

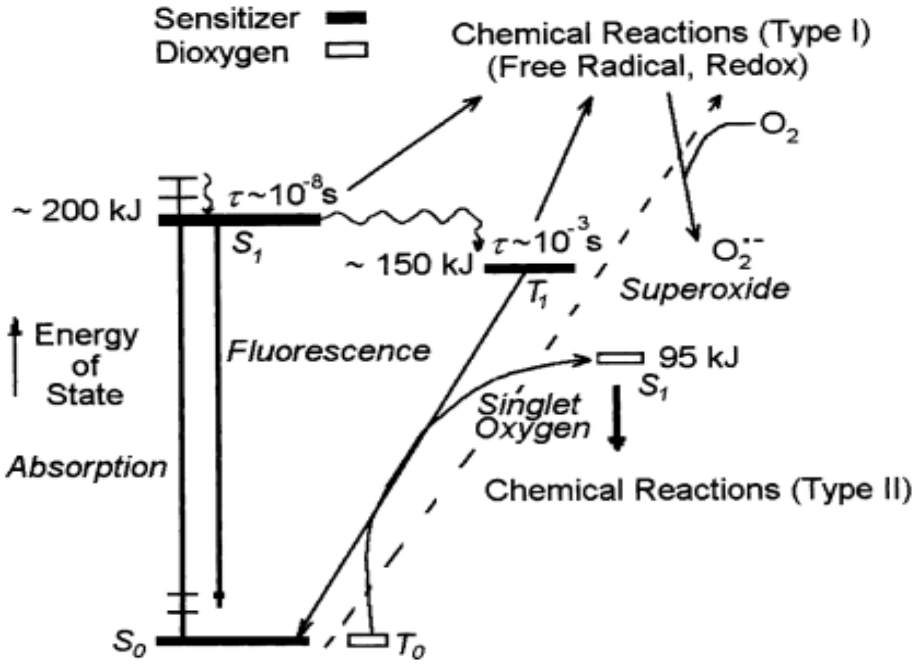

Fig. (1.12): Generation of excited photosensitizer states and reactive dioxygen species.

(M. Derosa. 2002)

Table (1.1): Kinetics of photosensitization (S: photosensitizer, RH: substrate with H-bond, CAR: carotenoid). (M. Niemz, 1996)

Excitation • Singlet state absorption 1	 $S + h\nu \Rightarrow {}^1S^*$
Decays • Radiative singlet decay 1 • Nonradiative singlet decay 1 • Intersystem crossing 1 • Radiative triplet decay 3 • Nonradiative triplet decay 3	 $S^* \Rightarrow {}^1S + h\nu$ (fluorescence) $S^* \Rightarrow {}^1S$ ${}^1S^* \Rightarrow {}^3S^*$ ${}^3S^* \Rightarrow {}^1S + h\nu$ (phosphorescence) ${}^3S^* \Rightarrow {}^1S$
Type I reactions • Hydrogen transfer • Electron transfer • Formation of hydrogen dioxide • Formation of superoxide anion	 ${}^3S^* + RH \Rightarrow SH\bullet + R\bullet$ ${}^3S^* + RH \Rightarrow S^{\bullet-} + RH^{\bullet+}$ $SH^\bullet + {}^3O_2 \Rightarrow {}^1S + HO^\bullet_2$ $S^{\bullet-} + {}^3O_2 \Rightarrow {}^1S + O^\bullet_2$
Type II reactions • Intramolecular exchange 3 • Cellular oxidation 1	 $S^* + {}^3O_2 \Rightarrow {}^1S + {}^1O^*_2$ $O^*_2 + \text{cell} \Rightarrow \text{cell ox}$
Carotenoid protection • Singlet oxygen extinction 1 • Deactivation 3	 ${}^1O^*_2 + {}^1CAR \Rightarrow {}^3O_2 + {}^3CAR^*$ $CAR^* \Rightarrow {}^1CAR + \text{heat}$

1.11 Literature Review:

Between the years of 1933-1938, Kautsky and Bruijn devised a series of clever experiments to prove diffusible singlet oxygen, and not a bound oxygen complex, was the mechanism by which dye-sensitized photooxidation reactions occurred in these experiments, they physically separated the sensitizer and acceptor compounds by absorbing them individually onto silica gel and then

coated it onto a glass tube. Oxidized product formation was monitored by observing a color change of the acceptor compound after exposing the tube with an arc lamp at different oxygen pressures. The results from the experiments unequivocally demonstrated that the dye sensitized photooxidation of the substrate occurred by means of diffusion of oxygen. (S.Andrasik. 2007)

In 1960, while investigating a method to improve the measurement of emission spectra, Seliger reported on a sharp chemiluminescence band at 634 nm from a reaction between sodium hypochlorite and hydrogen peroxide. Intrigued by Seliger's results, Khan and Kasha followed up on this observation and in 1963 assigned the emission at 634 nm to the $^1\Sigma g^+ \rightarrow {}^3\Sigma g^-$ transition by singlet oxygen. In 1970, Andrews and Abrahamson reported for the first time the emission of singlet oxygen at 762 nm for the $^1\Sigma g^+ \rightarrow {}^3\Sigma g^-$ transition by photosensitization using gaseous O_2 and fluoronaphthalene. (S.Andrasik. 2007)

In 25 may 1999 F. Stracke, Ma. Heupel, E. Thiel was investigated the formation of singlet molecular oxygen $^1O_2$ photosensitized by rhodamine dyes, by measuring its IR phosphorescence. The quantum yield for triplet population and the triplet lifetime of the investigated dyes is measured by using a laser-scanning-microscopy technique. It results that the formation of $^1O_2$ can be prevented effectively by quenching of the S_1 or T state of the photosensitizer. The presence of the paramagnetic $^3O_2$ leads to an increased S_1!T intersystem crossing rate of the photosensitizers and therefore to a reinforced formation of singlet molecular oxygen. It is found for rhodamine 6G as well as for rose bengal that in air-saturated acetonitrile nearly the half of the excited dye triplets are quenched by molecular oxygen. The $^1O_2$ concentration can be significantly reduced by decreasing the $^3O_2$ concentration below its air saturated level. (F. Stracke, 1999)

In February 2002 Maria C. DeRosa, Robert J. Crutchley study of singlet molecular oxygen production and reactivity has emerged as a rich and diverse area with implications in fields ranging from polymer science to cancer therapy he address the photophysical properties of singlet oxygen and of the photosensitizers used in its generation. Photosensitizers based on organic molecules and coordination compounds are examined and compared. Recent advances in the photosensitized production of singlet oxygen and its uses in photochemistry and photobiology are highlighted. (M. Derosa. 2002)

In 2006, Belfield et al. reported, for the first time, quantitative determination of singlet oxygen upon 2PE of a PS in solution, using an indirect chemical quenching procedure to calculate the $^1O_2$ quantum yield.

In 28 February 2013 Xavier Ragàs, Xin He, Montserrat Agut, Mónica Roxo-Rosa, António Rocha Gonsalves , Arménio C. Serra and Santi Nonell. They have examined the production and fate of singlet oxygen in Escherichia coli upon photosensitization with three structurally-different cationic photosensitizers, namely New Methylene Blue N (NMB), a member of the phenothiazine family, ACS268, a hydrophobic porphyrin with asingle cationic alkyl chain, and zinc(II) tetra methylte trapyridinoporphyrazinium salt, a phthalocyanine-like photosensitizer with four positive charges on the macrocycle core. The kinetics of singlet oxygen production and decay indicate different localization for the three photosensitizers, whereby NMB appears to localize in an aqueous-like microenvironment, whereas ACS268 localizes in an oxygen-shielded site, highly reactive towards singlet oxygen. The tetracationic zinc (II) tetrapyridinoporphyrazine is extensively aggregated in N the bacteria and fails to produce any detectable singlet oxygen. (X. Ragas,et. al, 2013)

May 17, 2013 M. V. Zagidullina, b, M. I. Svistuna, N. A. Khvatova, and A. S. Insapovb Collision induced emission of singlet oxygen molecules is studied using spectrometers calibrated for absolute spectral sensitivity. The collision induced emission rate constants at wavelengths of 479, 514, 577, 634, and 703 nm are determined within the temperature range 90–315 K. It is found that the intensities of the emission bands increase with decreasing temperature below 100 K. They discussed the interrelation between the collision induced emission rate constants and the intensities of the collision induced absorption bands. (M Zagidullin, et.al. 2014)

In 2014 D. Kuznetsov, Y. Myagchenko, O Slobodyanyuk studied the correlation between the photoluminescence intensity of a dye-sensitizer (methyl pheophorbide-a, MPP-a) and the efficiency of the singlet oxygen generation. The excitation spectrum of emission at 1270 nm has one strong band at 403 nm that coincides with the sort band of MPP-a and only a weak peak at 667 nm, which practically coincides with the strongest band of the MPP-a excitation spectrum. Therefore, the choice of an excitation wavelength for the efficient generation should be based on direct measurements of the excitation spectra of the sensitized 1emission. (Y Kuznetsov et. al. 2014,)

In 2014 Nafie A. Almuslet and Ahmed A. Mohamed, made a spectroscopic and photo physical study for the emission of three dyes (dibenzocyanine 45, methylene Blue and Rhodamine 6G).They irradiated these dyes for different exposure time by two lasersources. 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 and the emission spectra indicated the existence of singlet oxygen.

In 2016 Nafie A. Almuslet and Nahla E. Ahmed. Studied the emission of some dyes (Alcain blue, Coumarinand bromocresol) dissolved in (Propanol, Ethanol

and chloroform) after irradiated by different light sources (LED (365 nm) with output power 1200 mW, green diode laser (532 nm) with output power 100 mW and red diode laser (671 nm) with output power 100mW). They found the most efficient sample to produce singlet oxygen, was the Alcian blue dissolved in chloroform after irradiation by the green diode laser (532 nm) where a peak of singlet oxygen at (634 nm). (A. Nafie & E. Nahla, 2016)

CHAPTER TWO

THE EXCREMENTAL PART

2.1 Introduction:

This chapter presents the equipments, materials and the experimental procedure. All the items related to the experimental part are presented in details.

2.2 Equipment:

2.2.1. USB 2000 spectrometer:

This device is versatile general purpose UV, Vis and NIR spectrometer for absorption, transmission, reflection or emission. The USB2000+ Miniature fiber optic spectrometer is a unique combination of technologies and a powerful analog to digital (A/D) converter.USB 2000 use in this work was supplied from ocean optics company (U.S.A) it is a simple optical instrument based on a diffraction grating and a one dimensional CCD detector array. The spectrometer box is shown in Figure 2.1. Light enters via a slit located at the bottom of a threaded receptacle which can be used to connect an optical fiber that is terminated with a SMA plug. This instrument achieves a spectral resolution of about 0.5 nm between wavelengths of 370 to 680nm. The spectrograph is based on a Czerny-Turner optical design, which has no moving parts. (J. Graham, 2009)

Fig. (2.1): The ocean optics USB 2000 spectrometer.

Table (2.1): The specification of USB 2000.

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 x 104.8 mm x 45.1 mm
Detector	2048-element linear silicon CCD array
Detector range	200-1100 nm
Gratings	14 gratings available
Optical resolution	Depends on grating and size of entrance aperture
Fiber optic connector	SMA 905 to single-strand optical fiber (0.22 NA)

2.2.2 UV-visible spectrophotometers:

A spectrophotometer is an instrument for measuring the transmittance or absorbance of a sample as a function of the wavelength of electromagnetic radiation. The key components of a spectrophotometer are:

- Source that generates a broad band of electromagnetic radiation. The ideal light source would yield a constant intensity over all wavelengths with low noise and long term stability. Unfortunately however, such a source does not exist. Two sources are commonly used in UV-visible spectrophotometers one in UV and the other in Visible.
- Dispersion device that selects from the broadband radiation of the source a particular wavelength (or more correctly, a waveband) two types of dispersion devices, prisms and holographic gratings are commonly used in UV-visible spectrophotometers.
- Sample area.

- Detectors convert a light signal into an electrical signal. Ideally, it should give a linear response over a wide range with low noise and high sensitivity. Spectrophotometers normally contain either a photomultiplier tube detector or a photodiode detector.
- Other optical components, such as lenses, mirrors or relay light through the instrument. (T. Owen , 2000)

The UV-visible spectrophotometer used in this work is model 6305 and table (2.2) specification of it.

Table (2.2): The specification of UV-visible spectrophotometers.

Specification	Value
Range	198 to 1000nm
Resolution	1nm
Accuracy	± 2nm
Stability	1%/hr after 20 minute warm up
Beam height	15mm
Light source	Xenon flash lamp module with power <50W
Weight	6kg

2.2.3 Diode lasers:

Semiconductor lasers are unique when compared to other types of lasers. Diode laser very small and operate relatively at low power input and they are very efficient.

Diode laser is a semiconductor device which directly converts electrical energy into laser light. They also operate in different away in that they require merging different materials, and laser action occurs in the interface between those two

materials, one of the materials has an excess of electron (n type) and other materials (p type) has deficit of electrons or excess of holes (missing electrons).When forward bias voltage is placed across this junction electrons are forced into the region from n type material and holes are forced into junction from the p type materials. These electrons with negative charge and holes with positive charge are attracted each other and when they collide they neutralize each other and in the process emit recombination radiation. Electrons in the n type material exist in high energy (conduction band) than the holes (valence band). this energy difference designated as the band gab of the material .different materials combinations have different bandgaps and thus emit different wavelengths of light. (M. Niemz, 1996)

Usually operates in the near-infrared and into the visible region of the spectrum. The two ends of the laser diode are polished to increase internal reflection. (J. Hollas, 2004.)

Diode laser emitter might produce at most a few Watts of output power.

In this work were used diode lasers emit in 532nm (50mW) and 671nm (100mW) their specifications are listed at Table (2.2) and (2.3) respectively.

Table (2.3): Specifications of green laser (532nm).

Wavelength	532nm
Beam Size	1.2mm
Beam Divergence	<2.0 mrad
Mode	TEM_{00}
Operating Voltage	(4 -6)V
Operating Current (25^{o}C)	< 300 mA DC
Operating Temperature	10^{o}C to + 30^{o}C
Output Power Options	50 Mw
Weight	40g
Lifetime MTTF	5000 hours
Operation mode	CW

Table (2.4): Specifications of red laser (671nm).

Power	100Mw
Wavelength	671nm
Beam Size	0.75 ± 0.15mm
Spatial Mode	TEM_{00}
Bandwidth	30 GHz
Noise bandwidth	0.1Hz - 10kHz
Coherence length	< 1.0cm
Beam angle	3< 2 mrad
Operating temperature	15 - 40^{o}C
Head weight	0.7 kg

2.2.4 Light emitting diode (LED):

A light emitting diode is a p-n junction which depending on the semiconductor structure, could theoretically be designed to emit monochromatic wavelengths throughout the entire electromagnetic spectrum. (J. Heathcote, 2010)

When a light-emitting diode is forward biased (switched on) electrons are able to recombine with holes within the device, releasing energy in the form of photons. This effect is called electroluminescence and the color of the light (corresponding to the energy of the photon) is determined by the energy gap of the semiconductor.

LEDs present many advantages over incandescent light sources including lower energy consumption, longer lifetime, improved robustness, smaller size, faster switching, greater durability and reliability.

Table (2.5): Specifications for monochromatic LED (365nm).

LED Light Wavelength	365 nm
Typical peak Irradiance	9,500 mW/cm^2
Continuous operation without additional cooling	Max 10 minutes
Electrical power input	5 W

2.3 Materials:

2.3.1 Ethanol (ethyl alcohol or grain alcohol):

This is a clear colorless liquid with a characteristic agreeable odor. Ethanol CH_3CH_2OH is an alcohol, a group of chemical compounds whose molecules contain a hydroxyl group, –OH, bonded to a carbon atom. Ethanol melts at –114.1°C, boils at 78.5°C and has a density of 0.789 g/mL at 20°C. Its low freezing point has made it useful as the fluid in thermometers for temperatures below –40°C, the freezing point of mercury and for other low-temperature

purposes such as for antifreeze in automobile radiators. Ethanol has been made since ancient times by the fermentation of sugars. All beverage ethanol and more than half of industrial ethanol is still made by this process. Simple sugars are the raw material. Zymase, an enzyme from yeast, changes the simple sugars into ethanol and carbon dioxide. (Shakhashiri, 2009)

2.3.2 Methanol:

Methanol is a chemical with the formula CH_3OH (often abbreviated MeOH) and is very toxic to the humans. Methanol acquired the name wood alcohol because it was once produced chiefly as a byproduct of the destructive distillation of wood. Industrial methanol is produced in a catalytic process directly from carbon monoxide, carbon dioxide and hydrogen. Methanol is the simplest alcohol being only a methyl group linked to a hydroxyl group. It is a light, volatile and colorless flammable liquid.(Aurangzeb, et. al. 2017)

2.3.3 Methylene Blue (Methylthionine Chloride):

Methylene Blue is a heterocyclic aromatic chemical compound with molecular formula ($C_{16}H_{18}ClN_3S\ 3H_2O$).

Methylene blue (MB) is a cationic thiazine dye with the chemical name tetramethylthionine chloride. It has a characteristic deep blue color in the oxidized state, but the reduced form, leukomethylene blue (LMB) is colorless. Methylene blue has been widely used in a variety of clinical settings to identify anatomic. (J. Cragan, 1999)

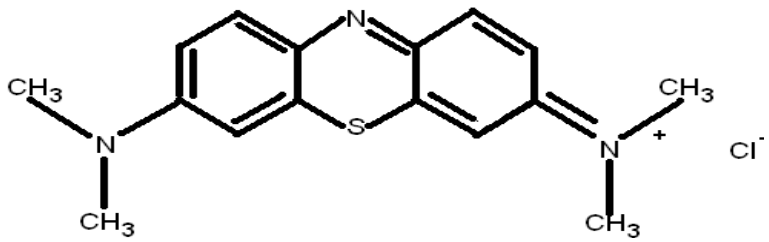

Fig. (2.2): Shows Chemical structure of Methylene blue.

2.3.4 Eosin yellow:

Eosin is an orange pink dye with chemical formula $C_{20}H_8O_{11}Br_4$ used in textile dying and ink manufacturing. Figure (2.3) shows Chemical structures for Eosin yellow and Table (2.7) the physical properties for Eosin yellow.

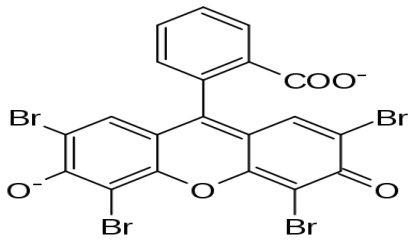

Fig. (2.3): Shows Chemical structures for Eosin yellow.

Table (2.6): The physical properties, Eosin yellow.

Chemical formula	Powder: $C_{20}H_6Br_4Na_2O_5$ Solution: $C_{20}H_{14}O_5Br_4Na_2$
Chemical group	Stains
Atomic weight	691.86
Weight per litter	Solution: 1.02kg
Appearance	Red crystals with bluish tinge or brownish red powder. Red aqueous solution
Solubility/miscibility	Water

2.3.5 Fluorscin:

This matter has a very high molar absorptivity in the visible region, a large fluorescence quantum yield and a high photostability which makes it very useful in applications where a high sensitivity is needed. Fluorscin in queues solution occurs in cationic, neutral, anionic and dianionic. Form making its absorption and fluoresce properties strongly PH dependent. Figure (2.4) shows the reaction

to produce fluorscin and Table (2.8) shows technical information for Fluorscin. (Sjoback. R., 1995)

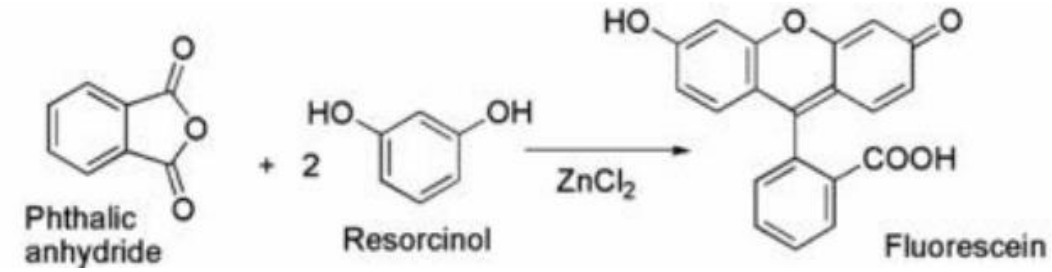

Fig. (2.4): Reaction to produce Fluorscin.

Table (2.7): The technical information of Fluorscin.

Chemical Name	Fluorscin
Molecular Formula	$C_{20}H_{12}O_5$
% Carbon	72.28%
% Oxygen	24.07%
% Hydrogen	3.64%
Molecular Weight	332.30
Solubility	Insoluble in water
Melting Point	314 - 316^{o} C
Appearance	Yellowish to red powder

2.4 The Experimental procedure:

The absorption spectra of solvent were recorded using the UV-VIS spectrophotometer to be sure that they are transparent in the range 190 nm to 1100 nm. To prepare the samples; 5 mg of each dye (Methylene blue, Eosin yellow and Fluorscin) was dissolved in 50 ml of the different solvents as follows:

1-Methylene blue was dissolved in Ethanol and methanol.

2-Eosin yellow was dissolved in Ethanol and methanol.

3-Fluorscint was dissolved in Ethanol.

By above steps samples were ready for irradiation as follows:

Eosin yellow and Fluorscin was irradiated by green laser (532nm) and monochromatic LED (365nm). Methylene blue was irradiated by red laser (671nm).

The emission spectrum of each sample was recorded by the USB 2000 spectrophotometer in the rang (400-1100) nm; Computer and software's were used as data acquisition (OOIBase32, UV Data Manager besides Origin lab). The experimental arrangement is shown in Figure (2.5).

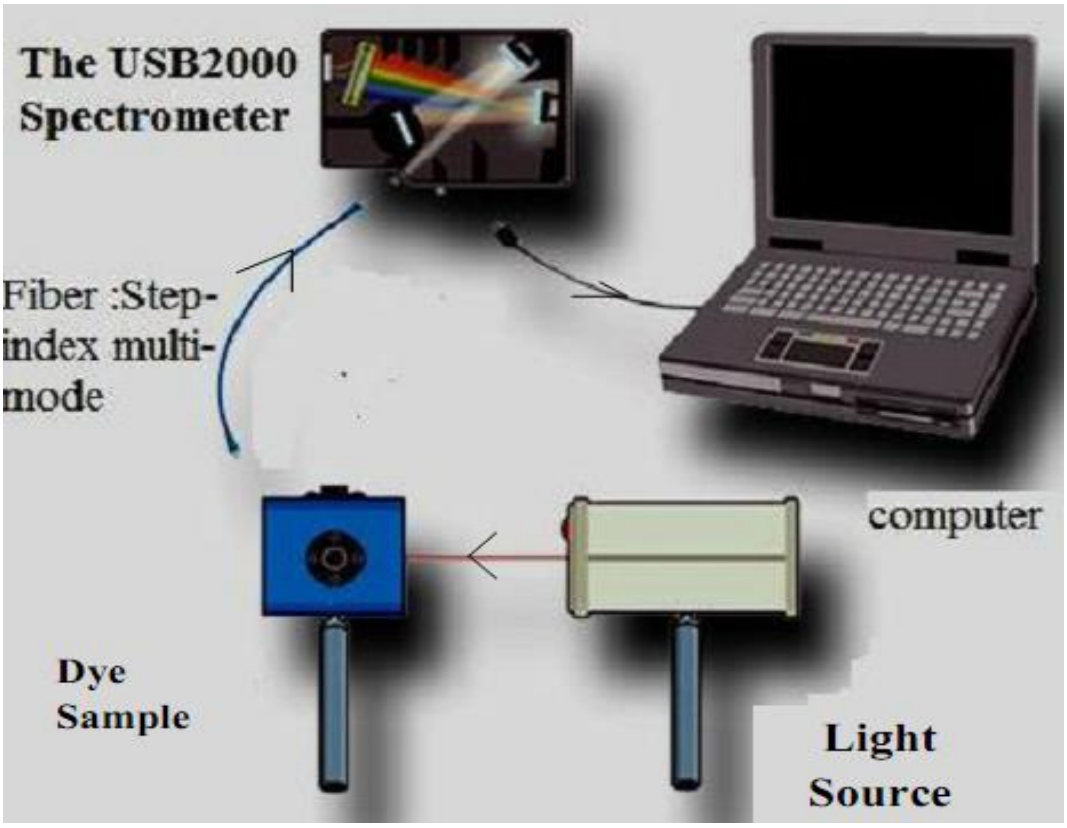

Figure (2.5): The arrangement of the experimental setup. (Nafie A. Almuslet & Ahmed A. Mohamed, 2014)

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 Introduction:

This chapter presents the results of investigation of singlet oxygen emission from various dyes that can be used for different purposes including photodynamic therapy. The results were obtained from samples (Methylene blue, Fluorescein and Eosin yellow) dissolved in Ethanol and Methanol after irradiated with different light sources. Firstly the absorption spectrum of each dye was recorded using UV-Vis spectrophotometer before the irradiation to determine the exact portion of the spectrum that the dye absorbs. Then diode lasers (532 nm, 67 nm) and LED 365 nm were used to irradiate the samples at different times begin from 30 sec to 120 sec. Then the emission spectra were recorded.

3.2 Results:

3.2.1 The Absorption spectra of Methylene blue dissolved in ethanol and methanol:

The absorption spectra of Methylene blue dissolved in ethanol and methanol are recorded by the UV-VIS spectrometer shown in Figure (3.1).

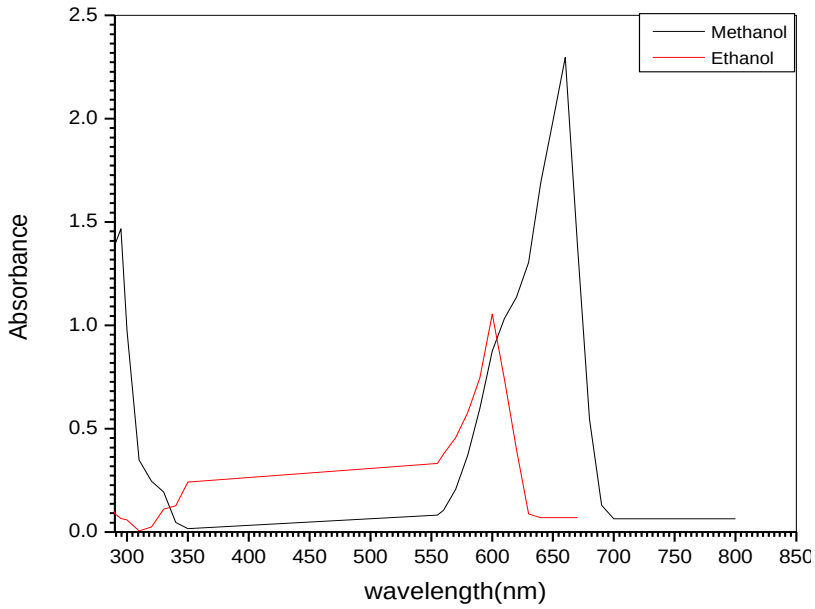

Fig. (3.1): The absorption spectra of Methylene blue dissolved in, ethanol and methanol.

In Figure (3.1) one can see that Methylene blue has strong absorption peaks in the wavelength 290 and 659nm. There is a shift in the peaks; this shift is attributed to the difference in the optical characteristics for solvents and the dissimilarity in the photochemical interaction according to solvent.

3.2.2 The emission spectra of Methylene blue dissolved in ethanol, after irradiation by red laser (671nm):

The emission spectra of Methylene blue dissolved in ethanol, after irradiations by red laser (671nm) for different times are shown in Figure (3.2).

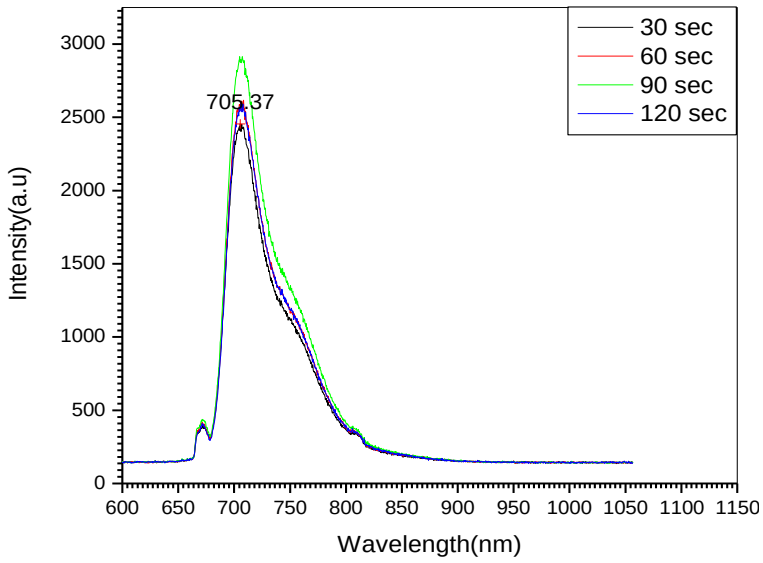

Fig. (3.2): Emission spectra of Methylene blue + Ethanol after irradiation by Red laser (671nm) for different times.

From figure (3.2) we found that the emission spectra of Methylene blue dissolved in ethanol after irradiation by Red laser (671nm) have one broad band at 705.37nm which is fingerprint of singlet oxygen Increasing exposure time led to increase peaks intensity**.** . (R Boodaghians, 1982)

3.2.3 The emission spectra of Methylene blue dissolved in methanol after irradiated by diode laser (671nm):

The emission spectra of Methylene blue dissolved in methanol, after irradiation by red laser (671nm) for different times, are shown in Figure (3.3).

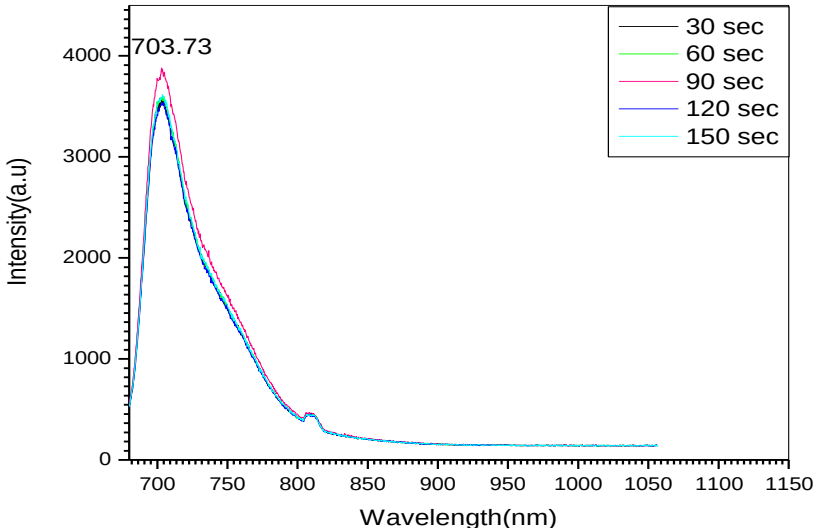

Fig. (3.3): Emission spectra of Methylene blue + Methanol after irradiation by red laser (671nm) for different times.

The emission spectra in Figure (3.3) have one peak at (703.73nm) which is attributed to produce singlet oxygen (M Zagidullin, et.al. 2014). Increasing exposure time led to increase intensities**.** The shift in the singlet oxygen emission between the two cases is because of the difference in the solvent type**.**

3.2.4 The absorption spectra of Eosin yellow dissolved in ethanol and methanol:

The absorption spectra of Eosin yellow dissolved in ethanol and methanol are shown in Figure (3.4) which was recorded by the UV-VIS spectrometer. One can see that Eosin yellow has strong absorption peaks at 312 and 526nm. There is a shift of 19nmbetween tow cases (ethanol and methanol) in the spectra; this shift is attributed to the difference in the optical characteristics of the solvents and the dissimilarity in the photochemical interaction.

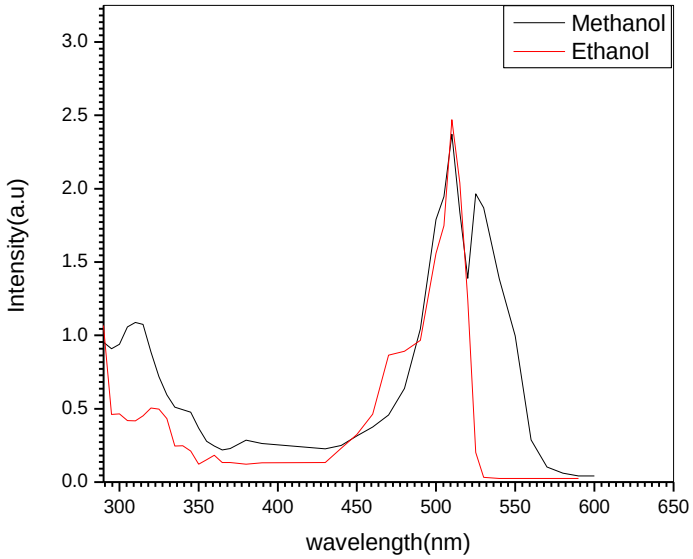

Fig. (3.4): The absorption spectra of Eosin yellow dissolved in, ethanol and methanol.

3.2.5 The emission spectra of Eosin yellow dissolved in methanol, after excitation by diode laser (532nm):

The emission spectra of Eosin yellow dissolved in methanol after excitation by diode laser (532nm) with different times are shown in Figure (3.5).

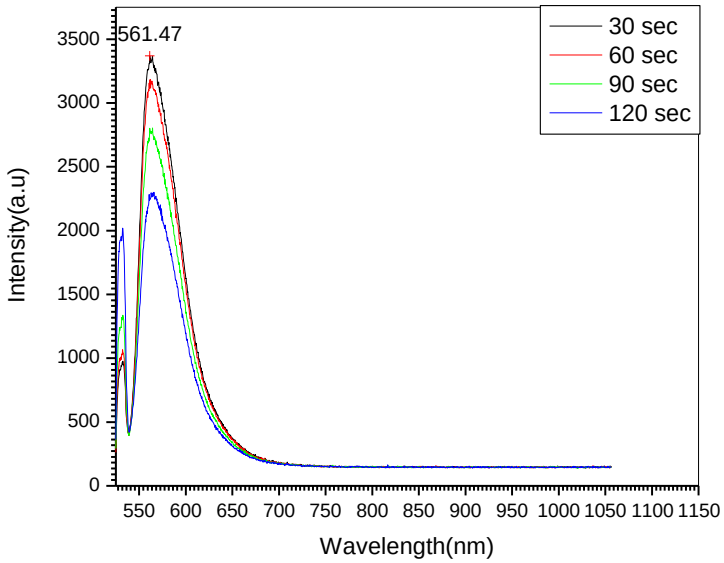

Fig. (3.5): Emission spectra of Eosin yellow + Methanol; after irradiation by green diode laser (532nm) for different times.

From Figure (3.5) it was found that the emission spectra of eosin yellow dissolved in methanol after irradiation by Green laser (532nm) have one broad band at 561.47nm. That means it does not produce singlet oxygen. Increasing exposure time led to decrease intensities**.**

3.2.6 The emission spectra of Eosin yellow dissolved in ethanol, after excitation by diode laser (532nm):

The emission spectra of Eosin yellow dissolved in ethanol, after excitation by diode laser (532nm) with different times, are shown in Figure (3.6).

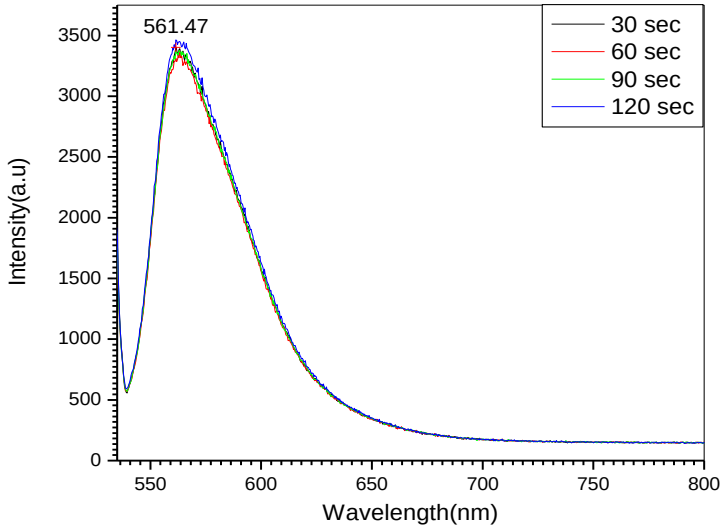

Fig. (3.6): Emission spectra of Eosin yellow + Ethanol; after irradiation by greendiode laser (532nm) for different times.

From Figure (3.6) shows that; the emission spectra of eosin yellow dissolved in methanol after irradiation by diode laser (532nm) have one broad band at 561.47nm. That means it does not produce singlet oxygen in this cases.

3.2.7 The emission spectra of Eosin yellow dissolved in ethanol, after irradiation by monochromatic LED (365nm)

The emission spectra of Eosin yellow dissolved in ethanol, after irradiation by monochromatic LED (365nm) for different times are shown in Figure (3.7).

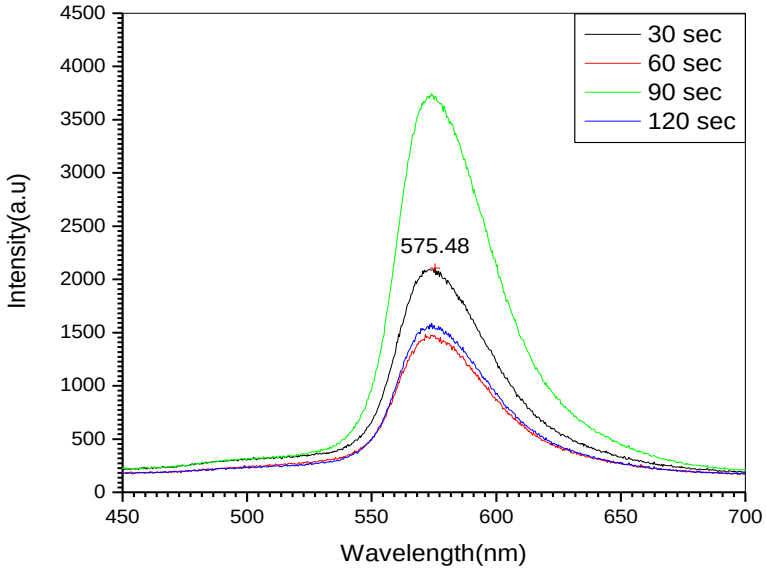

Fig. (3.7): Emission spectra of Eosin yellow + Ethanol after irradiation by LED (365 nm) for different times.

This figure shows that; the emission spectra of eosin yellow dissolved in methanol after irradiation by LED (365nm) have one broad band at 575.48nm that means it can produce singlet oxygen (R Boodaghians, 1982) . Increasing exposure time led to decrease intensities.

3.2.8 The emission spectra of irradiation of Eosin yellow dissolved in Methanol, after irradiation by monochromatic LED (365nm):

The emission spectra of Eosin yellow dissolved in Methanol, after irradiation by monochromatic LED (365nm) for different times are shown in Figure(3.8).

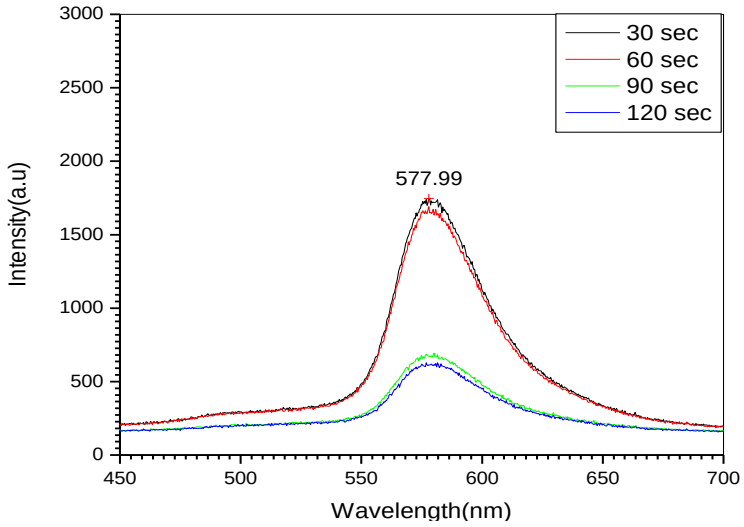

Fig. (3.8): Emission spectra of Eosin yellow + Methanol after irradiation by LED (365 nm) for different times.

From figure (3.8) one can see that, the emission spectra of eosin yellow dissolved in methanol after irradiation by LED (365nm) have one broad band at 577.99nm, which is one of emission peaks of singlet oxygen. (M Zagidullin, et.al. 2014). That means that this dye can produce singlet oxygen. Increasing exposure time led to decrease intensities**.**

3.2.9 The absorption spectra of Fluorescein dissolved in ethanol:

The absorption spectra of Fluorescein dissolved in ethanol are shown in Figure (3.9) which was recorded by the UV-VIS spectrometer.

From Figure (3.9) one can see that Fluorescein has strong absorption peaks at 325.3 and 510.2nm.

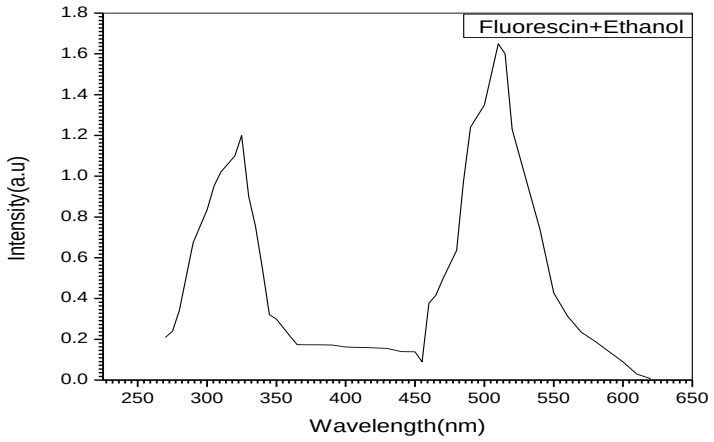

Fig. (3.9): The absorption spectra of Fluorescein dissolved in, ethanol.

3.2.10 The emission spectra of Fluorescein dissolved in ethanol after irradiation by green laser (532nm):

The emission spectra of Fluorescein dissolved in ethanol, after irradiation by green laser (532nm) for different times are shows in Figure (3.10).

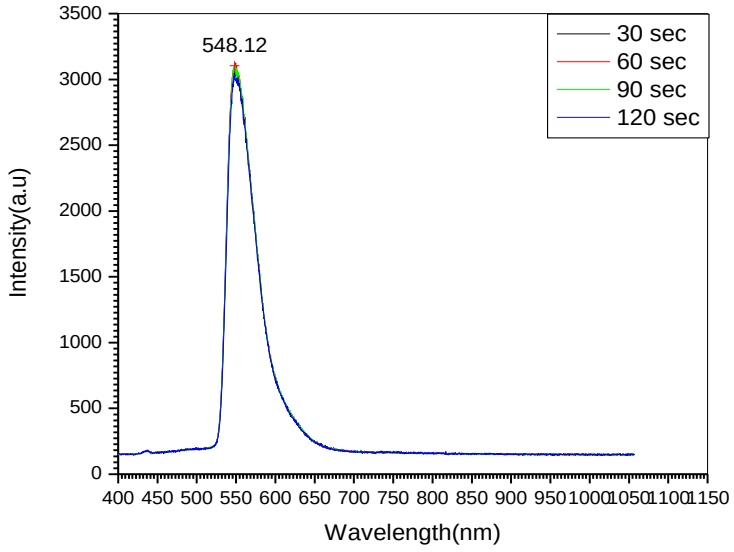

Fig. (3.10): Emission spectra of Fluoresceint + Ethanol after irradiation by green laser (532nm) for different times.

From Figure (3.10) it is obvious that the emission spectra of Florescent dissolved in ethanol irradiated by green laser (532nm) emit one broad band at 548.12nm. That means it does not produce singlet oxygen.

3.2.11 The emission spectra of Fluorescein dissolved in ethanol, after isrradiation by monochromatic LED (365nm):

The emission spectra of Fluorescein dissolved in ethanol, after irradiation by monochromatic LED (365nm) for different times are shown in Figure (3.11).

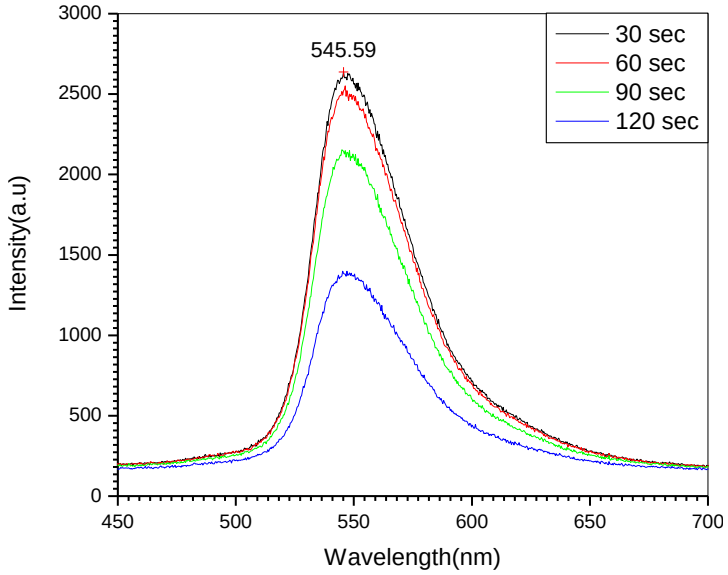

Fig. (3.11): Emission spectra of Fluorescein + Ethanol after irradiation by LED (365 nm) for different times.

There is wide peak at 545.59nm which is not related to the emission of singlet oxygen**.**

3.3 Discussion:

From Figure (3.1) one can see that the absorption spectrum of Methylene blue dissolved in ethanol and methanol is the range between (290-659nm) and the

maximum absorption is at 659nm.

From Figure (3.2) and Figure (3.3) the emission peaks of this sample, dissolved in ethanol and methanol and irradiated by red diode laser 671nm, are at 705.37 nm and 703.73nm, respectively. This proves the methylene blue is efficient in the production of singlet oxygen, where the $^1O_2$ emission band at 703nm was detected in the emission spectra. The efficient solvent to produce singlet oxygen was methanol which gave positive results.

From Figure (3.4) it can see the absorption spectrum of Eosin yellow dissolved in ethanol and methanol in the range between (312-550nm) and the maximum absorption is at 526nm.

Figures (3.5) and (3.6) showed that the emission peak of Eosin yellow dissolved in methanol and ethanol after irradiation by green laser (532nm), is at 561.47nm.

In Figure (3.7) and Figure (3.8) the emission peaks of Eosin yellow dissolved in methanol and ethanol after irradiation by monochromatic LED (365nm), are at 577.99 and 575.48nm, respectively. This that prove the Eosin yellow is efficient to produce singlet oxygen when dissolved in methanol, where the $^1O_2$ emission band at 577nm for$^1 O_2$ was detected in emission spectra.

From Figure (3.9) one can see that the absorption spectrum of Fluorescein dissolved in ethanol has strong absorption at (325.3 and 510.2nm).

In Figure (3.10) and Figure (3.11) the emission peaks of Fluorescein dissolved in ethanol after irradiation by monochromatic LED (365nm) and green laser (532nm) are at 545.59 and 548.12nm, respectively. That means it does not produce singlet oxygen.

The shift in the singlet oxygen emission due to difference in the solvent type**.**

3. 4 Conclusions:

From the results obtained in this work one can conclude that:

- The Methylene blue and Eosin yellow are appropriate dye for the production of singlet oxygen.
- The appropriate light source to produce singlet oxygen from Methylene Blue was the diode laser 671nm where it was succeeded in the production of singlet oxygen at (703nm).
- The appropriate light source for the production of singlet oxygen from Eosin yellow was the monochromatic LED (365nm) at (577nm).

3.5 Recommendations:

- Studying the emission of other types of photosensitizers such as porphyrings, phthalocyanines and transition metal complexes.
- Using anther sources to irradiated photosensitizer such as UV laser.
- Cooling the photosensitizer before irradiated.

References

Andrasik,s j., (2007). Singlet oxygen generation using new fluorine based photosensitizer unded one and two photon excitation

Aurangzeb Afzal, D., Fatima, S .and Ahmed, A.M.,(2017). METHANOL POISONING.

Bolshov, M. A. and kuritsyn, Y.A, (2001). laser analytic spectroscopy. Ullmann's Encyclopedia ofIndustrial Chemistry.

Boodaghians, R., Borrell, P. M. Borrell, P and Grant, K. R, (1982) *Intensities of Hot Bands in the Dimol Emissions of Singlet Molecular Oxygen*, O_2 .Journal of the chemical society, Faraday Transactions 2: Molecular and chemical physics 78 (8), pp.1195-1209.

Cragan, J.D., (1999). Teratogen update; Methylene Blue. *Teratology, 60(1), pp 42-48.

David, A.C. and RadziemskI, l. J. (2006) Handbook of Laser-Induced Breakdown Spectroscopy.Cambidge University, 25.

Debele,T.A.,Peng,S and Tsai,H.C., (2015). Drug Carrier for Photodynamic Cancer Therapy. International journal of molecular sciences, 16(9), pp 22094-22136.

Demtroder,W., (2008). Laser spectroscopy (Vol. 2).Berlin, Heidelberg, New York ; Springer.

Derosa, M.C.and Crutchley,R.J., 2002 .hotosensitized singlet oxygen and its applications. Coordination Chemistry Reviews, 233, pp351-371.

Dhar,A.,Johnson, K.S.,Novelli.M.R.,Bown, S .G.,Bigio,I.J.,lovat, L.B.,and Bloom,S.L., 2006. Elastic scattering spectroscopy for the diagnosis of colonic lesion; initial resultsof a novel optical biopsy technique.Gastrointestinal endoscopy, 63(2),p 257-261.

. Perkin .E., 2000 An Introduction to Fluorescence Spectroscopy PerkinElmer Publishers, Inc.

Elsaie, M., L., 2013. Photodynamic therapy new research. Nova Science Publishers, Inc.

Gornushkin IB, Amponsah-Manager K, Smith BW, Omenetto N, and Winefordner JD. 2004. Microchip Laser Induced Breakdown Spectroscopy: a Preliminary Feasibility Investigation. *Applied Spectroscopy,* 58(7), 762–769.

Graham, J.R., 2009. UCB, the USB 2000 Spectrometer.

Hind, A., 2011 "Agilent 101An Introduction to Optical Spectroscopy"

Hodak,J.,Harvey,D. and Valeur,B., 2008. Introduction to Fluorescence and Phosphorescence Spectroscopy for chemistry BSAC program.

Hollas, J.M., 2004. Modern spectroscopy. John Wiley&Sons.

Kaplan, M.J., Somers, R.J., Greenberg, 1998, Photodynamic Therapy in the Management of Metastatic Cutaneous Adenocarcinomas: Case Reports From Phase 1/2 Studies Using Tin Ethyl Etiopurpurin (SnET2).Journal of surgical oncology ,67(2) pp121-125.

Kumar. S. 2006. Spectroscopy of Organic Compounds. Cosmic rays, 10, p 4.

Kuznetsov, Y. Myagchenko, O Slobodyanyuk., 2014. The Excitation Spectra of Singlet Oxygen photoluminescence of methyl pheophorbide-a, Taras Shevchenko National University of Kyiv.

Miles, R. B., Lempert W.R and Forkey ,J.N., 2001. laser Rayleigh scattering . measurement science and Technology, 12(5),pR33.

Misra, P., Zhu, X., Hsueh, C.Y.and Halpem JB., 1993. Laser excitation and Emission Spectroscopy of the Methoxy Radical in Supersonic Jet. Chemical physics.

Nafie A. Almuslet and Nahla E. Ahmed., 2016. Usage of different lasers to produce emission of light from some dyes.

Niemz, M.H., 1996. Laser tissue interactions; fundamental and applications. Springer Science &Business Media.

Ormond, A.B. and freeman, H. S., 2013. Dye Sensitizers for Photodynamic Therapy. Materials,6(3),pp817-840

Owen T., Fundamentals of modern UV-visible spectroscopy (2000).Agilent technologies, 73.

Perkin E, Inc. 2000., An Introduction to Phosphorescence Spectroscopy.

Pottier,R.H., 2006., Photodynamic Therapy with ALA A Clinical Handbook(vol.6) .Royal Socisty of Chemistry.

Ragas, X., He, X., Agut, M., Rosa.,Serra, A.C. and Nonell, S., 2013. Singlet Oxygen in Antimicrobial Photodynamic Therapy Photosensitizer Dependent Production and Decay in E. coli. Molecules, 18(3), pp2712-2725.

Ready, J. F, 1997. Industrial applications of lasers. Academic press.

Schmidt, F., 2007, Laser-based Absorption Spectrometry ;development of NICE-OHMS towards ultra-sensitive trace species detection (Doctoral dissertation, Fysik).

Schultz,I., 2014 .Practical applications of laser absorption spectroscopy for aeroengine theting.Doctrol dissertation, STANFORD UNIVERSITY.

Sjoback,R., Nygren,J.and Kubista,M., 1995. Absorption and fluorescence properties of fluorescin. spectrochimical Acta Part A; molecular and biomolecular spectroscopy,51(6),ppL7-L21.

So, P.T. and Dong CY., 2002. Fluorescence spectrophotometry, eLS.

Telgy,.A., 2014 Singlet oxygen production by PSII under light stress;mechanism and the role of B carotene. Plant and Cell Physiolog,55(7). pp1216-1223

Thorsted, B, and Brewer, J.R., 2015. Characterization of Fluorophores forTwo-Photon and STED Microscopy inTissue (Doctoral dissertation syddansk Universitet)

Vogel, 1956. Practical Organic Chemistry, 3 Edition, , pp 985

Xavier Ragàs, Xin He, Montserrat Agut, Mónica Roxo-Rosa, António Rocha Gonsalves , Arménio C. Serra and Santi Nonell. 2013.

Zagidullin,M., Svistun,M., Khvatov,N and Insapov, A., 2014. Collision Induced Emission of Singlet Oxygen in the Visible Spectral Region at Temperatures of 90–315 K.Optical spectroscopy, 116(4).