Sudan Universityof Science and Technology
College of Graduate Studies

Usage of different lasers to produce emission of light from some dyes

استخدام ليزرات مختلفه لانتاج إنبعاث ضوئي من عدة صبغات

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

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الآية

"اللهُ نُورُ السَّمَاوَاتِ وَالأَرْض مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ المِصْبَاحُ فِي زُجَاجَةٍالزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِنْ شَجَرَةٍ مُبَارَكَةٍ زَيْتُونَةٍ لَا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ نُورٌ عَلَى نُورٍ يَهْدِي اللهُ لِنُورِهِ مَنْ يَشَاءُ وَيَضْرِبُ اللهُ الأَمْثَالَ لِلنَّاس وَاللهُ بِكُلِّ شَيْءٍ عَلِيمٌ"

(سورة النور الايه(35)

DEDICATION

To my parents

who introduced me to the joy of reading from

birth enabling such a study to take place today ,

my husband (ABU MAZIN),

my son (MAZIN),

myfamily,

my friends,

and all persons who support me

Acknowledgement

I would like to thank my supervisor ***ProfessorNafieA. AL-Muslet****for supervising this research and for his continuous guidance during the course.*

Also my thanks are extended to all staff in ***Al-NeelainUniversity****School of Physics and Applied Physics for their kindness and support specially Teacher* ***Abdulsakhei*** *thanks also Mr****AhamedabuBakar for supporting me****.*

Contents

List of figures

List of Tables

ABSTRACT

In this work, the emission of some dyes were studied after excitation by different lasers. The emitted light can be used for different applications including Photodynamic Therapy (PDT). The dyes were preparedfrom (Alcain blue, Coumarinand bromocresol) dissolved in (Propanol, Ethanol and chloroform).

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; firstly LED (365 nm) with output power 1200 mW, secondly by the green diode laser (532 nm) with output power 100 mW. Thirdly the red diode laser (671 nm) with output power 100mW. USB 2000 spectrometer wasused to record the emission spectra of the samples with different times. From the obtained results it was concluded that the most efficient sample to produce singlet oxygen, which is used for PDT, 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) was recorded. Some recommendations were suggested, as future work, in the end of this work.

المستخلص

في هذا العمل تمت دراسة انبعاث عدة صبغات بعد تشعيعها باستخدام ليزرات مختلفة الاطوال الموجية لغرض استخدامها في عدة تطبيقات منضمنها الاستخدام في العلاج الضوئي. حُضرت العينات باذابة كل من صبغة الكومرين والالكاين بلو والبروموكريسول فى كل من المحاليل (الايثانول , البروبانول والكلورفورم) .

ثم سجلت اطياف الامتصاص لكل العينات باستخدام جهاز (UV spectrophmeter) ثم شععت العينات المذكورة كما يلي: اولا بالثنائى الباعث الضوئى بطول موجي365 nmوبقدرة 1200 ملى واط ثم ثانيا, ليزر الثنائى بطول موجى671 nm وقدرة 100ملى واطوثالثا بليزر الثنائى بطول موجى532nm وقدرة 100ملى واط.استخدم مطياف(USB2000 spectrometer) لتسجيل الانبعاث للعينات. أوضحت النتائج ان افضل العينات كفاءة فى انتاج الاوكسجين الاحادى المستخدم لاغراض العلاج هى عينه الالكاين بلو المذابه فى الكلورفورم بعد ان تم تشعيعها بليزر الثنائي532 nm حيث ظهرت قمة الانبعاث عند634 nm والتى تعود للاوكسجين الأحادي . وفى نهايه البحث تم اقتراح عدة أعمال مستقبلية مواصلة لهذا العمل.

CHAPTER ONE

LASER SPECTROSCOPY, BASIC CONCEPTS

1.1Introduction:

Most of the knowledge about the structure of atoms and molecules is based on spectroscopic investigations. Thus spectroscopy has made an outstanding contribution to the present state of atomic and molecular physics, to chemistry and to molecular biology. Information on molecular structure and on the interaction of molecules with their surrounding may be derived in various ways from the absorption or emission spectra generated when electromagnetic radiation interacts with matter (D. wolfgang, 2002)

Most types of laser are an inherently pure source of light; they emit near-monochromatic light with a very well defined range of wavelengths .By careful design of the laser components, the purity of the laser light (measured as the "linewidth") can be improved more than the purity of any other light source. This makes the laser a very useful source for spectroscopy. The high intensity of light that can be achieved in a small well collimated beam can also be used to induce a nonlinear optical effect in a sample, which makes technique based on lasers can be used to make extremely sensitive detectors of various molecules , able to measure molecular concentrations in the parts per – 10^{12} (G. Gauglitz& T. Dinch, 2003)

For branches of spectroscopy other than Raman spectroscopy most laser sources may appear to have a great disadvantage, that of no tenability in regions of the spectrum, particularly the infrared where tunable lasers are not readily available, ways have been devised for tuning, that is, shifting, the atomic or molecular energy levels concerned until the transition being studied moves into

coincidence with the laser radiation. This may be achieved by applying an electric field to the sample, and the technique is called laser Stark spectroscopy. The corresponding technique using a magnetic field is that of laser magnetic resonance (or laser Zeeman) spectroscopy.From 1960 onwards, the increasing availability of intense, monochromatic laser provided a tremendous impetus to a wide range of spectroscopic investigations. The most immediately obvious application of early, essentially non-tunable, lasers was to all types of Raman spectroscopy in the gas, liquid or solid phase. Laser radiation is very much more intense, and the line width much smaller, than that from, for example, a mercury arc, which was commonly used as a Raman source before 1960. Awide variety of ingenious techniques have been devised using laser sources (W. Brian, 2002)

1.2 Aim of this work:

This work aimed to study the emission of some dyes after excitation by different lasers and to investigate the possibility of singlet oxygen production from number of dyesnamely;(Alcian blue, bromocresol, Coumarin 500).

1.3 Thesis structure:

This thesis contains three chapters; chapter one contains the aim of the work in addition to concepts of laser spectroscopy , absorption and emission spectroscopy, lasers inemission spectroscopy, definition and mechanism of photodynamic therapy , chemical dyesand literature review. Chapter two describes the experimentalpart (materials and methods). Finally, chapter three contains the results and the discussion of the results, conclusionand recommendations.

1.4 Absorption spectroscopy:

Material's absorption spectrum is the fraction of incident radiation absorbed by the material over a range of frequencies. The absorption spectrum is primarily determinedby 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 due to a transition between two states is referred to 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 atomic and molecular structure of the sample (C. Danial, 2002).

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

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

Observed absorption lines always have a width and shape that is determined by the instrument used for the observation, the material absorbing the radiation and the physical environment of that material. It is common for lines to have the shape of a Gaussian orLorentzian distribution. It is also common for a line to be characterized solely by its intensity and width instead of the entire shape being characterized.The integrated intensity obtained by integrating the area under the absorption line is proportional to the amount of the absorbing substance present. The intensity is also related to the temperature of the substance and the quantum mechanical interaction between the radiation and the absorber. This interaction is quantified by the transition moment and depends on the particular lower state the transition starts from and the upper state it is connected to. The width of absorption lines may be determined by the spectrometer used to record it. A spectrometer has an inherent limit on how narrow a line it can resolve and so the observed width may be at this limit. If the width is larger than the resolution limit, then it is primarily determined by the environment of the absorber. A Liquid or solid absorber, in which neighboring molecules strongly interact with one another, tends to have broader absorption lines than a gas (C.Danial, 2002).

1.4.1Beer - Lambert Law

The ideaBeer-Lambert Law is illustrated in Figure (1.1) The Absorbance (or optical density) and Transmission (or Transmittance) of monochromaticlight through a sample can be calculated by measuring thelight intensity entering and exiting the sample.

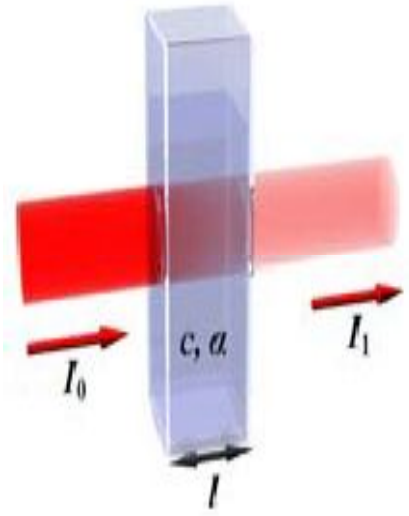


Figure (1.1): monochromatic Light of Intensity 'I_o' passes through asample with concentration 'C'.

Some light energy is absorbed by the sample. The amount of light energy exiting the sample has Intensity 'I' the Beer-Lambert Law is given by the following equations:

Light Absorbance : $$A = \log \frac{I_o}{I} = kcl$$

Light Transmission : $$T = (\frac{I_o}{I}) = 10^{-kcl}$$

The following terms are defined:

• Light Intensity entering a sample is "I_0"

• Light Intensity exiting a sample is "I"

• The Concentration of Sample is "C"

• The length of the light path in glass sample cuvtette is "L"

• "K" is a constant for a particular solution and wavelength.

1.4.2 Laser absorption spectroscopy:

Is refers to techniques that use lasers to assess the concentration or amount of a species in gas phase by absorption spectroscopy (AS). Optical spectroscopic techniques in general and laser-based techniques in particular, have a great

potential for detection and monitoring of constituents in gas phase. They combine a number of important properties, e.g. a high sensitivity and a high selectivity with non-intrusive and remote sensing capabilities. Laser absorption spectroscopy has become the foremost used technique for quantitative assessments of atoms and molecules in gas phase. It is also a widely used technique for a variety of other applications, e.g. within the field of optical frequency metrology or in studies of light matter interactions. The most common technique is tunable diode laser absorption spectroscopy (TDLAS) which has become commercialized and is used for a variety of applications. (G. Kluczynski, 2001)

The most appealing advantages of (LAS) itis ability to provide absolute quantitative assessments of species. Its biggest disadvantage is that it relies on a measurement of a small change in power from a high level; any noise introduced by the light source or the transmission through the optical system will deteriorate the sensitivity of the technique. Direct laser absorption spectrometric (DLAS) techniques are therefore often limited to detection of absorbance ~10^{-3}, which is far away from the theoretical shot noise level, which for a single pass DAS technique is in the 10^{-7} – 10^{-8} range. This detection limit is insufficient for many types of applications. The detection limit can be improved by reducing the noise using transitions with larger transitions strengths or increasing the effective path length. The first can be achieved by the use of a modulation technique, the second can be obtained by using transitions in unconventional wavelength regions, whereas the third by using external cavities (G. Kluczynski, 2001).

1.5 Emission spectroscopy:

Emission is a process by which a substance releases energy in the form of electromagnetic radiation. Emission can occur at any frequency at which absorption can occur, and this allows the absorption lines to be determined from an emission spectrum. The emission spectrum will typically have a quite different intensity pattern from the absorption spectrum, though, so the two are not

equivalent. The absorption spectrum can be calculated from the emission spectrum using appropriate theoretical models and additional information about the quantum mechanical states of the substance. 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 (F.Peter, 2007).

The emission spectrum can be used to determine the composition of a material, since it is different for each element of the periodic table. One example is astronomical spectroscopy: identifying the composition of stars by analyzing the received light. The emission spectrum characteristics of some elements are plainly visible to the naked eye when these elements are heated. For example, when platinum wire is dipped into a strontium nitrate solution and then inserted into a flame, the strontium atoms emit a red color. Similarly, when copper is inserted into a flame, the flame becomes green. These definite characteristics allow elements to be identified by their atomic emission spectrum. Not all emitted lights are perceptible to the naked eye, as the spectrum also includes ultraviolet rays and infrared lighting. An emission is formed when an excited gas is viewed directly through a spectroscope. The simplest method is to heat the sample to a high temperature, although the emission lines are caused by a transition between quantized energy states and may at first look very sharp, the finite width, i.e. they are composed of more than one wavelength of light. This spectral line broadening has many different causes. Emission spectroscopy is often referred to as optical emission spectroscopy, due to the light nature of what is beingemitted (B. Carroll. 2007).

1.5.1 Types of emission spectra:

There are basically three types of emission spectra, they are:

1- Continuous emission spectrum: this spectrum consists of a wide range of unrepeated wavelengths in a definite wavelength range.

2-Band spectra or Line emission spectra: This spectrum consists of sharp lines of definite wavelengths. Line spectra are generated by excited atoms; discrete, distinct lines are formed (poozacreations. BlogSpot, 2015).

1.5.2 Application of emission spectroscopy:

Emission spectroscopy can be applied asa technique to analyze organic matter on irregular and rough surfaces of steel materials easily and accurately and with high sensitivity. Emission spectroscopy provides an effective way to analyze an extremely thin film of organic matter on the surface of steel, that by heating a base material and a material with adhered organic matter on a hot plate, it is possible to easily obtain an emission spectrum, and that when the organic film is extremely thin, the emission intensity is proportional to the thickness.Emission spectroscopy analysis can be used in the atmospheric pressure plasma polishing (APPP).Also the emissionspectroscopy isapplied in medicine includesphotodynamic therapy(has emerged as an alternative to chemotherapy andradiotherapy for the treatment of various diseases including cancer)(F.Peter, 2007).

1.6 Lasers in emission spectroscopy:

1.6.1 Laser induced fluorescence

Fluorescence is simply the property of certain materials to absorb radiation at one wavelength then re-emit that radiation at another wavelength usually longer wavelength, the optical emission from atoms, or molecules that have been excited to higher energy levels by absorption of laser radiation. In this case laser provides a very selective means for populating excited states, which gives more accurate and

sensitive measurements. 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 dispersed by a monochromator*(*D.Wolfgang, 2003).

There are two types of Laser Induced Fluorescence:

1-Laser induced atomic fluorescence

2-Laser induced molecular fluorescence.

Figure (1.2) shows the component of laser induced fluorescence

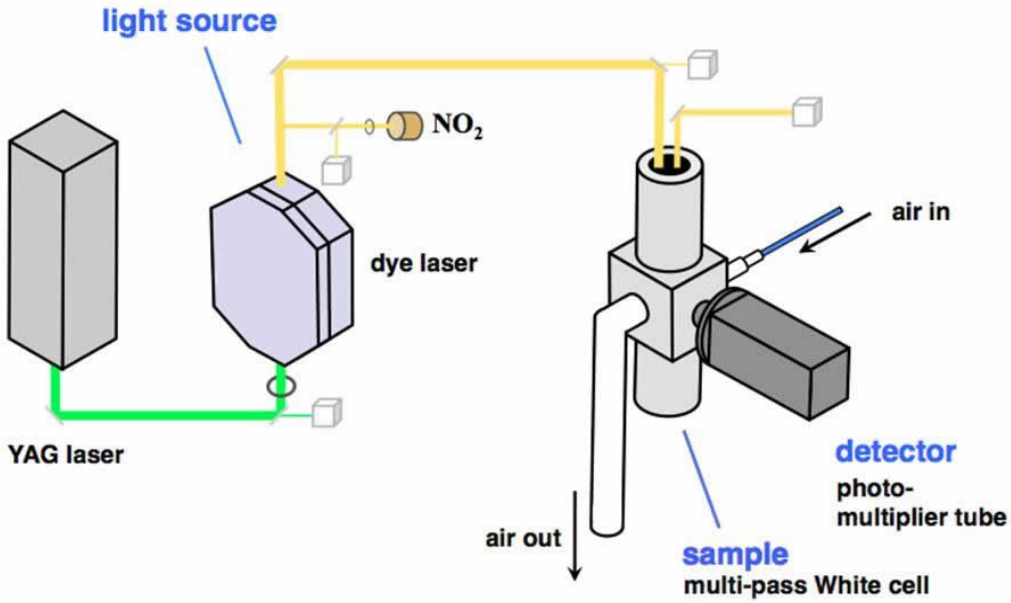


Figure (1.2) the components of the laser induced fluorescence

1.6.2 Laser induced breakdown spectroscopy:

One of the applications of laser includes the Laser Induced Breakdown Spectroscopy, shortly LIBS. LIBSisan atomic emission spectroscopic technique which uses a pulsed laser to form plasma. Plasma is considered to be a cloud of electrons and ions that results from the breakdown.

Laser plasma can be formed on any type of samples: solid, liquid, gas and aerosol. When a high power pulsed laser sent onto the sample, the sample absorbs the energy from the laser; heated up, melts and evaporates. Due to the high peak powers of the laser, temperatures may reach up to 20.000 K. At that moment; the sample atomizes, ionizes and forms the plasma. Plasma is a luminous cloud that has time dependent characteristics. After the laser pulse is off, cooling process starts through the expansion of the plasma with a shockwave in front. During the plasma cooling, irradiative emission of the light at characteristic wavelength of the sample is observed while excited atoms and ions relax back to the ground state. This atomic emission is monitored by a time resolved, gated detector (N. Reinhand, 2012).

Figure (1.3) shows the components of the LIBS technique.

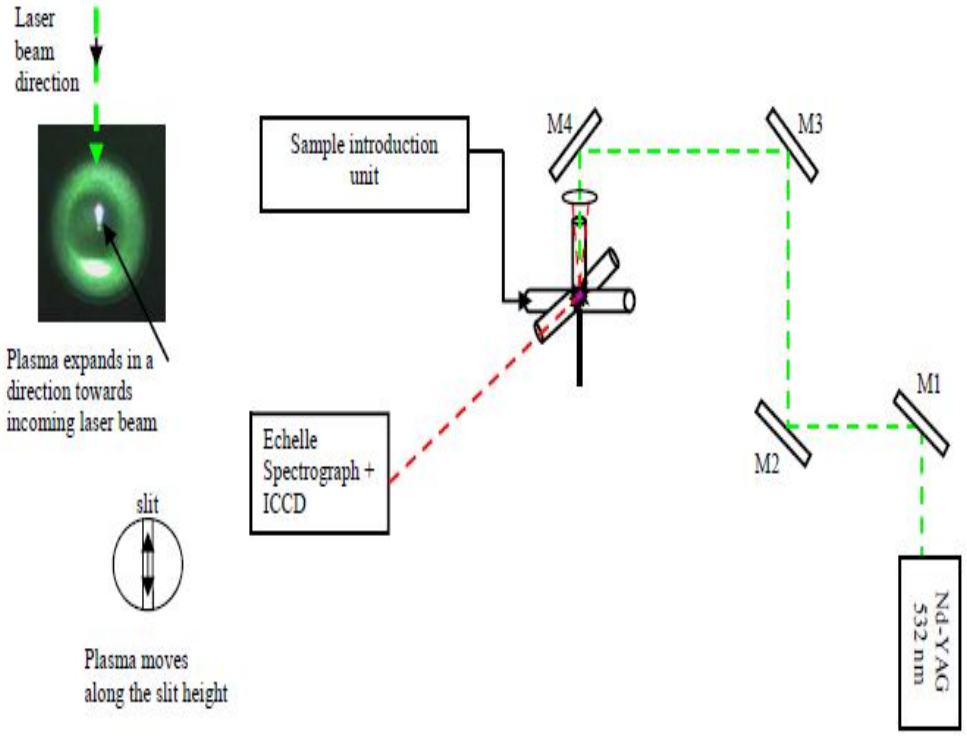


Figure.(1.3) the components of the LIBS technique

1.7 Laser Raman spectroscopy:

Raman spectroscopy is a spectroscopic technique used to observe Vibrational, rotational, and other low-frequency modes in a system. Raman spectroscopy is commonly used in chemistry and physics to provide a fingerprint by which molecules can be identified.It relies on inelastic scattering, or Raman scattering, of monochromatic light, usually from a laser in the visible, near infrared, or near ultraviolet range. The laser light interacts with molecular vibrations, phonons or other excitations in the system, resulting in the energy of the laser photons being shifted up or down. The shift in energy gives information about the vibrational modes in the system. Infrared spectroscopy yields similar, but complementary, information.Typically, a sample is illuminated with a laser beam. Electromagnetic radiation from the illuminated spot is collected with a lens and sent through a monochromator. Elastic scattered radiation at the wavelength corresponding to the laser line (Rayleigh scattering) is filtered out, while the rest of

the collected light is dispersed onto a detector by either a notch filter or a band pass filter.Spontaneous Raman scattering is typically very weak, and as a result the main difficulty of Raman spectroscopy is separating the weak in elastically scattered light from the intense Rayleigh scattered laser light. Historically, Raman spectrometers used holographic gratings and multiple dispersion stages to achieve a high degree of laser rejection. In the past, photomultipliers were the detectors of choice for dispersive Raman setups, which resulted in long acquisition times. However, modern instrumentation almost universally employs notch or edge filters for laser rejection and spectrographs axial transmissive (AT), Czerny–Turner (CT) monochromator, or FT (Fourier transform spectroscopy based), and CCD detectors (S.Ewen, 2005). Figure (1.4)shows a simple diagram illustratingRayleigh and Raman scattering.

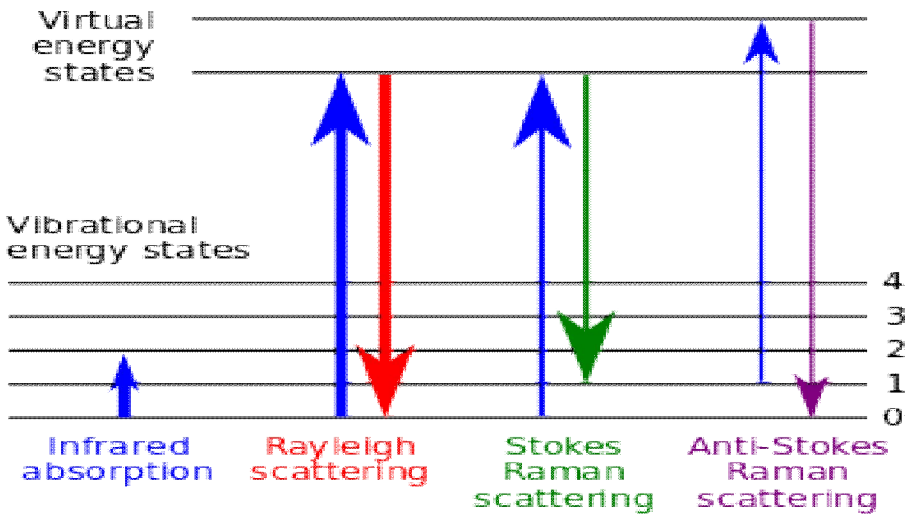


Figure (1-4): the energy levelsdiagram of Raman effect

1.8 Chemical Dyes:

Chemical dyes are large organic molecules with molecular weights of a few hundred. These organic molecules are dissolved in a suitable liquid solvent (such as ethanol, methanol, or an ethanol-water mixture). Organic dyes are characterized by strong absorption band in the visible region of the electromagnetic spectrum. Such property is found only in organic compounds which contain an extended system of conjugated bonds, whereas the light absorption of dyes cannot be derived rigorously from their molecular structure owing to the complexity of the quantum mechanics (J. Duarte &W.Loyldwillman, 2004).

An annoying characteristic of organic dyes is that the dyes have limited productive life times. The factors that limited the life time of dyes are thought to be the chemical and photo chemical degradation of the dye in solution. The life time of the gain of a dye is often specified in terms of watt-hours, based onempirical data. This power –life time product is measure of the pump-laser energy that has been used to excite the dye and /or actual laser induced photochemistry in the dye solution(J. Duarte&W.Loyldwillman, 2004).

Dyes are used in different types of industries include paper and pulp, adhesives, art supplies, beverages, ceramics, construction, cosmetics, food, glass, paints, polymers, soap, wax biomedicine. Dyes are also used by industries for inks and tinting. Today, various dyes are manufactured to meet the requirements of each type of industries. Dyes are available in various forms. Examples are dry powders, granules, pastes, liquids, pellets, and chips. There has also been an increasing usage of dyes in the medicine sector. Here, dyes act as an important ingredient in many of the medical tests conducted on humans. These dyes, in coordination with latest diagnosis equipment, allow flawless handling and high accuracy in tests that are carried out on patients (W.Suetaka, 1990).

1.9 Photodynamic therapy:

Over the last years, photodynamic therapy (PDT) has emerged as an alternative to chemotherapy and radiotherapy for the treatment of various diseases including cancer. It involves the use of light, photosensitizer (PS) and oxygen. The excitation of photosensitizes (PSs) with light of an appropriate wavelength leads to energy or electron transfer to neighboring oxygen or substrateMolecules. Reactive Oxygen Species (ROS) and singlet oxygen ($^1O_2$) which are commonly accepted to be the main cytotoxic species are formed and lead to the destruction of cancer cells by both apoptosis and necrosis. The efficacy of PDT depends on the photo sensitizer's ability to produce ROS and $^1O_2$, oxygen availability, light dose and photosensitizer concentration in theTreated area (A. Johansson, 2007).
Figure (1.5) shows the stages of photodynamic therapy.

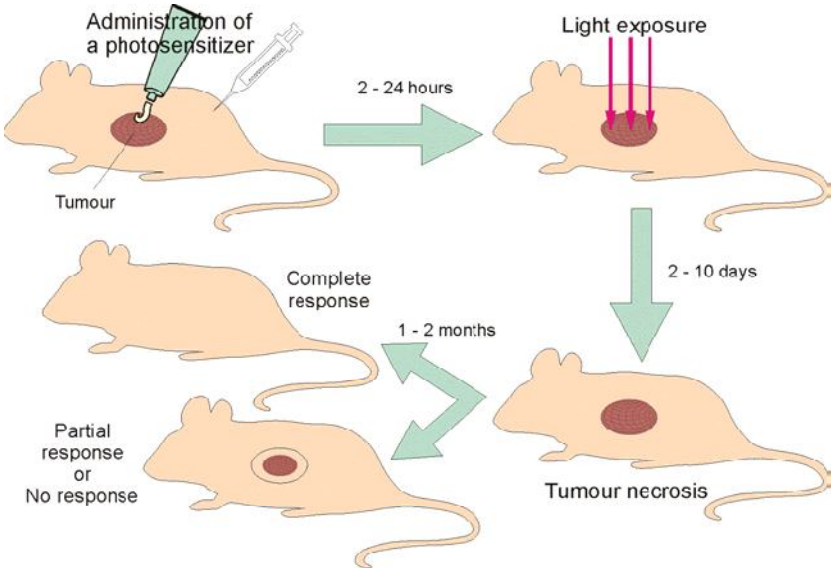


Figure (1.5): The Stages of Photodynamic Therapy

The precise mechanisms of PDT are not yet fully understood but two general mechanisms of photo induced damage in biomolecules have been proposed: Type I and type II. Type I reaction involves electron/hydrogen transfer directly from the excited photosensitizer to another molecule via electron/hydrogen abstraction. In this reaction free radicals are formed. These radicals then react rapidly, usually

with oxygen, resulting in the production of highly reactive oxygen species. These react in turn with tissue and causes irreversible damages. Type II reaction produces the electronically excited and highly reactive state of oxygen known as singlet oxygen. Direct interaction of the excited triplet state photosensitizer with molecular oxygen (which unusually has a triplet ground state) results in the photosensitizer returning to its singlet ground state and the formation of singlet oxygen(Y, Nazila .2006).

In figure (1.6) Initial events of type I and II reactions involve photo induced electron transfer (PET) and excitation energy transfer (EET), respectively. While S_0, ^1S and ^3T represent the ground, singlet and triplet states of the photosensitizer, R represents a biomolecule including oxygen.

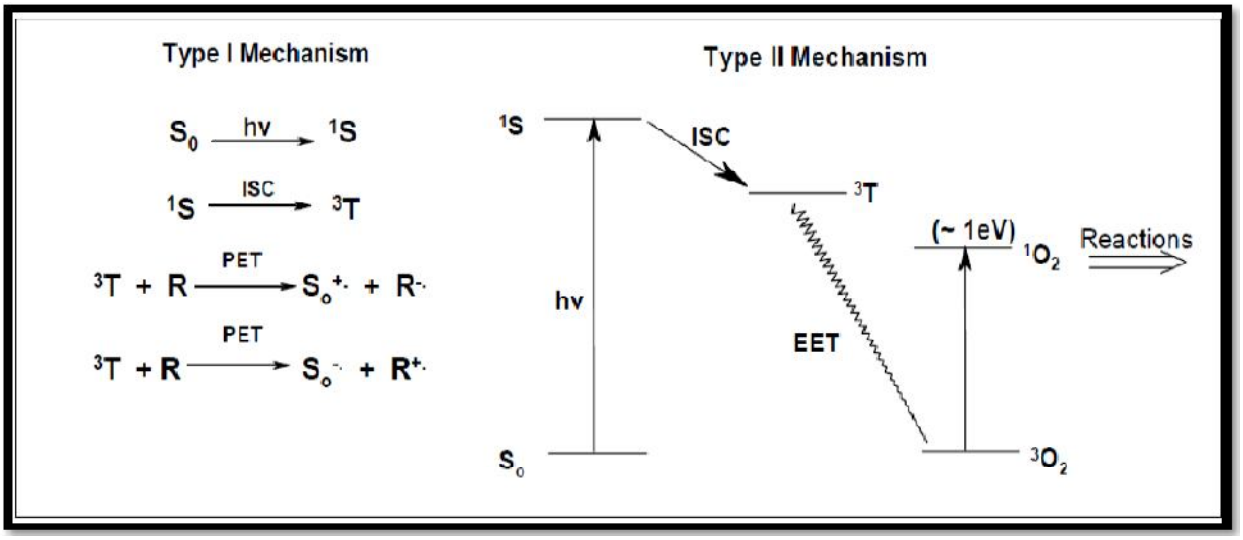


Figure (1.6) Type I and type II reactions of photosensitizer

1.10 Literature Review:

In 1998 A. BISWASstudied the Time- resolved spectroscopy of laser emission from dye – doped droplets.Micrometer –sized droplets of Rhodamine6G solution in water and ethanol were irradiated by high-intensity nanosecond pulses from a frequency-doubled Nd:YAG laser. Coupling of the spontaneous fluorescence emission with natural resonant modes of the spherical droplets resulted in stimulated emission with each droplet behaving like laser cavity. Spectral observations suggested that droplet lasing emission is supported by resonance of a single mode order.The emission exhibited faster rise times and was shorter lined than corresponding bulk-liquid fluorescencelasing droplets was generally initiated almost simultaneously with elastic scattering, unlike Raman scattering, which is significantly delayed.

In 2002 P.Sorokin studied the stimulated emission spectra of two organic dyes,chloro-aluminum phthalocyanine (CAP) and 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 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.

In 2005 V. Masilamani studied the Dual amplified spontaneous emission from coumarin dye. The dual amplified spontaneous emission observed from solutions of 7-amino-4-methyl coumarin dye (coumarin 440) in certain solvents such as n-

butyl acetate, dioxane etc. when exposed to high power nitrogen laser excitation. The results suggest that twisted intramolecular charge transfer coumarinphotoisomers which form exciplexes with the solvent molecules have enough gain to produce amplified spontaneous emission even where there is apparently no detectable fluorescence.

VikeshKumar in 2009 studied the Production and Chemical Property of singlet oxygen and superoxide radical by dyestuffs. 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 photo sensitizer like benzanthrone, met nil yellow and p amino diphenylamine were found to produce reactive oxygen species such as singlet oxygen ($^1O_2$) and/or superoxide radicals.

In 2014 NafieA.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.

CHAPTER TWO

THE EXPERIMENTAL PART

2.1 Introduction:

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

2.2 The Materials

Organic dyes and different solvents were used to prepare the sample.

2.2.1 Alcian blue 8GX:

Alcian blue is any member of a family of polyvalent basic dyes, of which the Alcian blue 8G, has been historically the most common and the most reliable member. It is used to stain acidic polysaccharides such as glycosaminoglycans in cartilages and other body structures, some types of mucopolysaccharides, sialylatedglycocalyx of cells etc. For many of these targets it is one of the most widely used cationic dyes for both light and electron microscopy. Use of Alcain blue has historically been a popular staining method in histology especially for light microscopy in paraffin embedded sections and in semi thin resin sections. In addition to its wide use as a stain Alcain blue has also been used in other diverse applications e.g. gelling agent for lubricating fluids, modifiers for electrodes, charged coating agents etc(J. Kiernan, 2006).

The Physical and chemical properties of Alcian blue 8GX are listed in Table (2-1).

Table (2-1) The Physical and chemical Properties of Alcian blue 8GX

Molecular Weight	1298.86432 g/mol
Chemical formula	$C_{56}H_{68}CL_4CuN_{16}S_4$
Molar mass	1,298.86 $g.mol^{-1}$
Hydrogen Bond Donor Count	0
Hydrogen Bond Acceptor Count	12
Exact Mass	1297.271628 g/mol
Heavy Atom Count	81
Formal Charge	0

2-2-2 Bromocresol purple:

Bromocresol purple is a pH indicator and is usually prepared as a 0.04% aqueous solution. Bromocresol purple (pH indicator)besides its primary function as an indicator, bromocresol purple is used in medical laboratories to measure albumin, and as an addition to acid stop baths used in photographic processing as an indicator that the bath has reached neutral pH and needs to be replaced (J. Durate,1990). The Physical and chemical Properties of Bromocresol purple are listed in Table (2-2).

Table (2.2) The Physical and chemical Properties of Bromocresol purple

Chemical formula	$C_{21}H_{16}Br_2O_5S$
Molar mass	$240.22mol^{-1}$
Molecular Weight	540.22174 g/mol
Hydrogen Bond Donor Count	2
Hydrogen Bond Acceptor Count	5
Exact Mass	539.906472 g/mol
Heavy Atom Count	28
Formal Charge	0

2-2-3 Coumarin 500:

Coumarin500 is a fragrant organic chemical compound in the benzopyrone chemical class, which is a colorless Coumarin compounds represent an important type of naturally occurring and synthetic oxygen-containing heterocyclic with typical benzopyrone framework. This type of special benzopyrone structure enables its derivatives readily interact with a diversity of enzymes and receptors in organisms through weak bond interactions, thereby exhibit wide potentiality as medicinal drugs. So far, some Comorian 500 -based drugs such as anticoagulant and ant neurodegenerative agents have been extensively used in clinic. Coumarin 500-containing supramolecular medicinal agents as a new increasing expansion of supramolecular chemistry in pharmaceutical science have also been actively investigated in recent years.

Coumarin 500-derived artificial ion receptors, fluorescent probes and biological stains are growing quickly and have a variety of potential applications in monitoring timely enzyme activity, complex biological events as well as accurate

pharmacological and pharmacokinetic properties(Hawaii, 2015).The physical and chemical properties of Coumarin are listed in table (2.3)

Table (2-3) the Physical and chemical Properties of Coumarin 500.

Chemical formula	$C_9H_6O_2$
Molar mass	146.1427 g/mol
Molecular Weight	146.14274 g/mol
Hydrogen Bond Donor Count	0
Hydrogen Bond Acceptor Count	12
Exact Mass	1295.274578 g/mol
Heavy Atom Count	11
Formal Charge	0

2-2-4 The solvents:

2.2.4.1 Ethanol:

Ethanol, also known as ethyl alcohol or grain alcohol, is a flammable, colorless, slightly toxic chemical compound. Its molecular formula is C_2H_6O. At the molecular level, liquid ethanol consists of hydrogen –bonded pairs of ethanol molecules. Ethanol is used as a solvent in dissolving laser 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 salts of polyvalent are

oractically insoluble in ethanol. And like the methanol, it should be placed in suitable containers appropriately labeled (B.Ulrich, 2000).

2.2.4.2 Propanol:

Propanol is a colorless, mobile, neutral liquid of average volatility and has a characteristic alcoholic dour. It is freely miscible with water and all common solvents such as alcohols, ketenes, aldehydes, ethers, glycols, aromatic and aliphatic hydrocarbons. Propanol is used as a solvent and an intermediate. It shows fewer tendencies to absorb water than lower alcohols, and has a considerably milder and more pleasant odor than higher alcohols. The main application as a solvent is in flexographic and other printing inks.
In the coatings industry, Propanol is a useful medium-volatility alcohol for improving the drying characteristics, e. g. of alkyd resins, electrode position, paints and baking finishes (B.Ulrich, 2000).

2.2.4.3 Chloroform:

Chloroform is the organic compound with formula $CHCl_3$. The colorless, sweet-smelling, dense liquid is a trihalomethane, and is considered somewhat hazardous. Several million tons are produced annually as a precursor to Teflon and refrigerants, but its use for refrigerants is being phased out. Chloroform is a common solvent in the laboratory because it is relatively unreactive, miscible with most organic liquids, and conveniently volatile (B.Ulrich, 2000).
Table (2.4) lists the Characteristics and Physical Properties of Solvents used in this work

Table (2.4): The Characteristics and Physical Properties of Solvents:

Solvent properties	Ethanol	Propanol	Chloroform
Molecular weight (g/mol)	46.07	60.1	119.38
Freezing point(c◦)	-114.1	-97.7	
Boiling point (c◦)	78.3	97.1	61.2
Density (g/cm^3)	0.7936	0.8034	1.483
Refractive index	1.3614	1.3284	1.4459
Viscosity	1.078	1.938	

2.3 The Equipments:

The equipments used in this study were:

2.3.1 UV – VIS 1240 Spectrophotometer:

This device was used to measure the absorption and the transmission of the dyes before use. The UV-VIS spectrophotometer was supplied from SHIMADZU Company (Japan). It contains a cell (quartz cuvette with optical length or thickness of 10 mm supplied fromHellma Company (Germany) as a sample holder. The UV 1240 comes standard with a spectrum mode that allows for full spectral data acquisition over the wavelength range of 190 nm to 1100 nm. Upon completion of the spectral scan, the peaks and valleys can be marked within a few seconds.

Optical specifications of UV – VIS 1240 Spectrophotometer are listed in Table (2-5). The standard peak pick function allows for clear and accurate detection of the most sensitive wavelengths(Shimadzu.com,2015)

Table (2.5): The optical specifications of the UV-VIS 1240 Spectrophotometer

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

2.3.2 The USB2000 Spectrometer:

Emission spectra were recorded by USB2000 Spectrometer and corresponding software, The USB 2000 is a small footprint Fiber Optics Spectrophotometer; it automatically reads the wavelength calibration coefficients of the spectrometer and configures the operating software. This device was supplied from ocean company module USB2E7524; it's a modular design user-configuration wavelength. USB 2000 can detect wavelength from 200-1100 nm, USB 2000 is used to recording the signal as wavelength in (nm) against output intensity in arbitral units. Light source and sample holder are connected to the

spectrometer via 400 μm fiber; The USB2000 has both USB and serial port connectors, enabling the user to connect the spectrometer to PC (R.Graham, 2009). Figure (2.1) exposed USB2000 spectrometer with components.

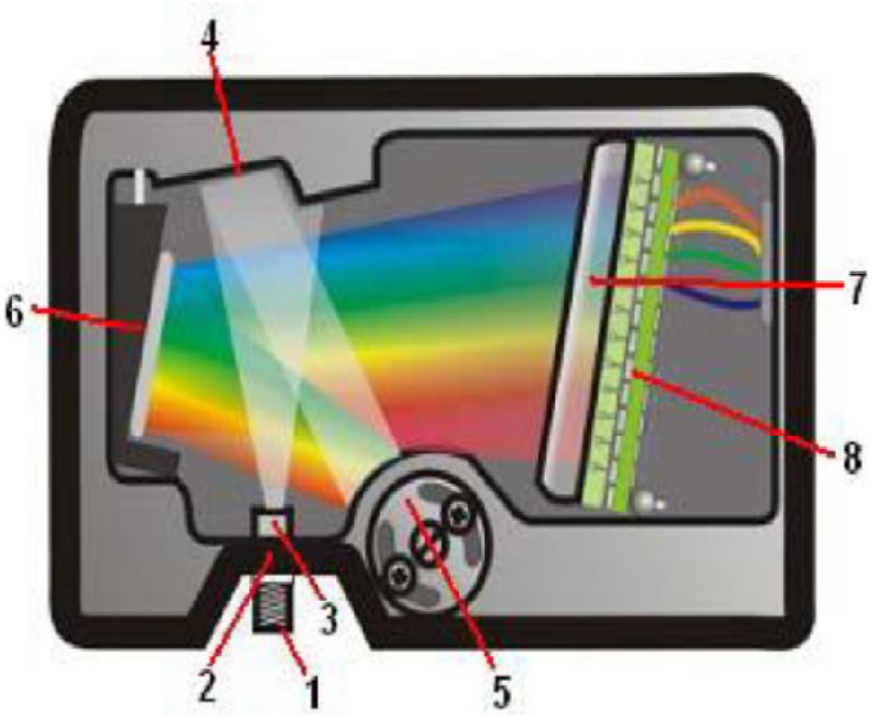


Figure (2.1) a schematic of the USB 2000 spectrometer

The components of the spectrometer are shown in figure (2.1)

1. SMA Connector

2. Slit.

3. Filter

4. Collimating mirror

5. Grating

6. Focusing Mirror

7. L2 Detector Collection Lens

8. CCD Detector (UV or VIS)

2.3.3 Light Sources:

2.3.3.1The green diode laser 532 nm:

The green diode laser used here was supplied form(RoithnerlaserTechnikGmbh - Austria) and it has wavelength of 532 nm and output power of 100 mW.Table (2.7)below lists the specifications of this laser.

Table (2-7): the specifications of the green diode laser 532nm.

CW out power	100 mW
Wavelength	532 nm
Operating mode	CW
Power stability (rms,over 1hour)	<10%
Beam mode	TEM_{00}
Beam diameter (at the aperture0	<1.5 mm
Beam divergence(full angle)	<1.5mrad

2.3.3.2 The red diode laser 671 nm:

The red diode laser used here was manufactured by (RoithnerlaserTechnikGmbh - Austria), it is CW laser with 671nm wavelength and output power of 100 mW.Table (2-8) lists the specifications of the red diode laser 671nm.

Table (2-8): the specifications of the red diode laser 671nm:

Out Put power	100mW
Wavelength	671nm
Operating mode	CW
Transverse mode	TEM_{00}
Beam diameter(at the aperture)	<21.0mm
Beam divergence(full angle)	<1.5mrad

2.3.3.3 UV Light Emitting Diode (LED):

The LED used in this work is monochromatic LEDemits wavelength of 365 nm and output power of 1200 mW. It was manufactured by LED ENGIN, the forward current is 700 mA and forward voltage is 16.4 V.

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 (Alcain blue 8xG, Comorian, brome cresol purple) was dissolved in 50 ml of the different solvents as follows:

1-ALcain blue was dissolved in Propanol, Ethanol, and chloroform

2-bromocreasol purple was dissolved in Propanol, Ethanol, and chloroform

3-coumarain 500 was dissolved in Ethanol, chloroform and Propanol

By this step nine samples were ready for irradiation. Each one of the samples was irradiated bythe three light sources.

Each time the emission was recorded by the USB 2000 spectrophotometer in the rang (400nm-1100 nm); Computer and software's were used as data acquisition (OOIBase32, UV Data Manager besides Origin lab). The experimental apparatus used in this work are shown in figure (2.2).

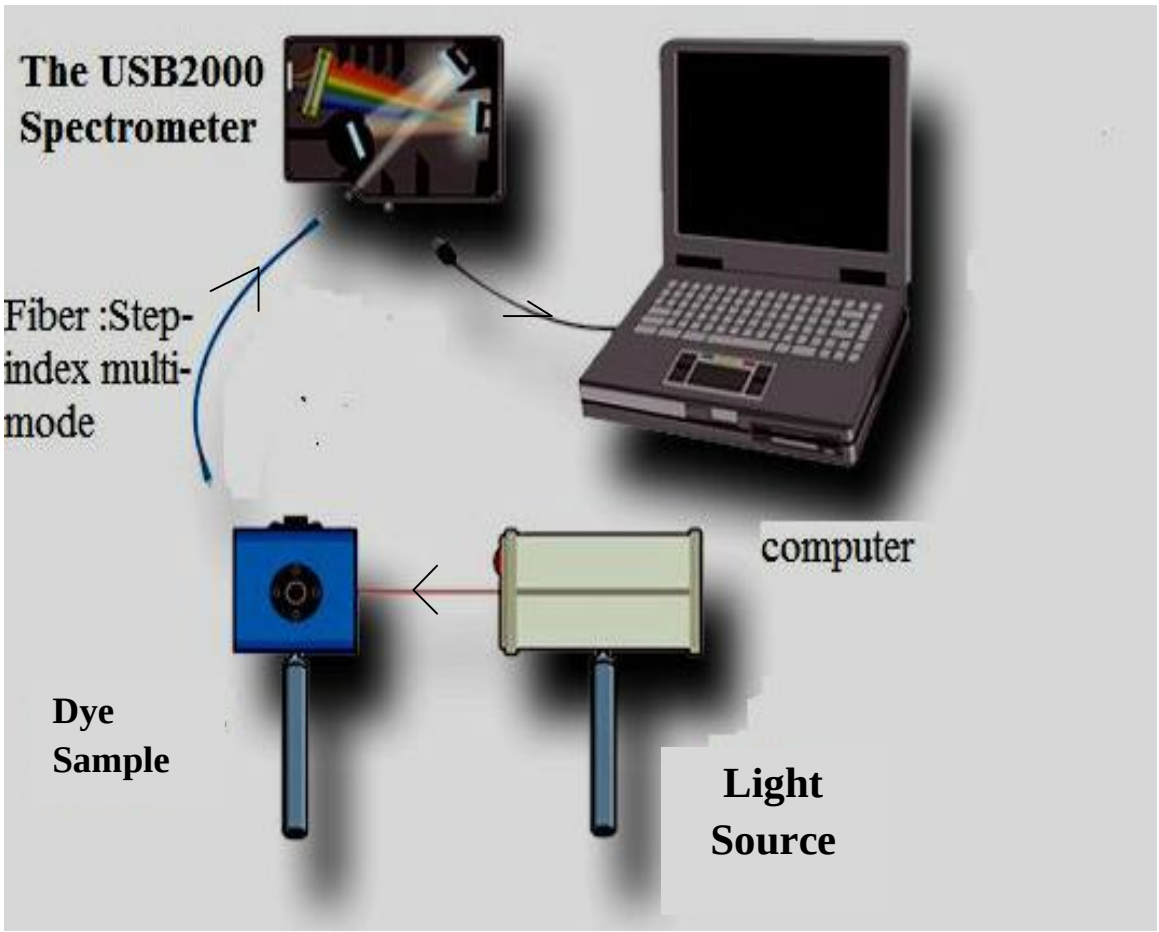


Figure (2.2) the arrangement of the experimental setup

CHAPTER THREE

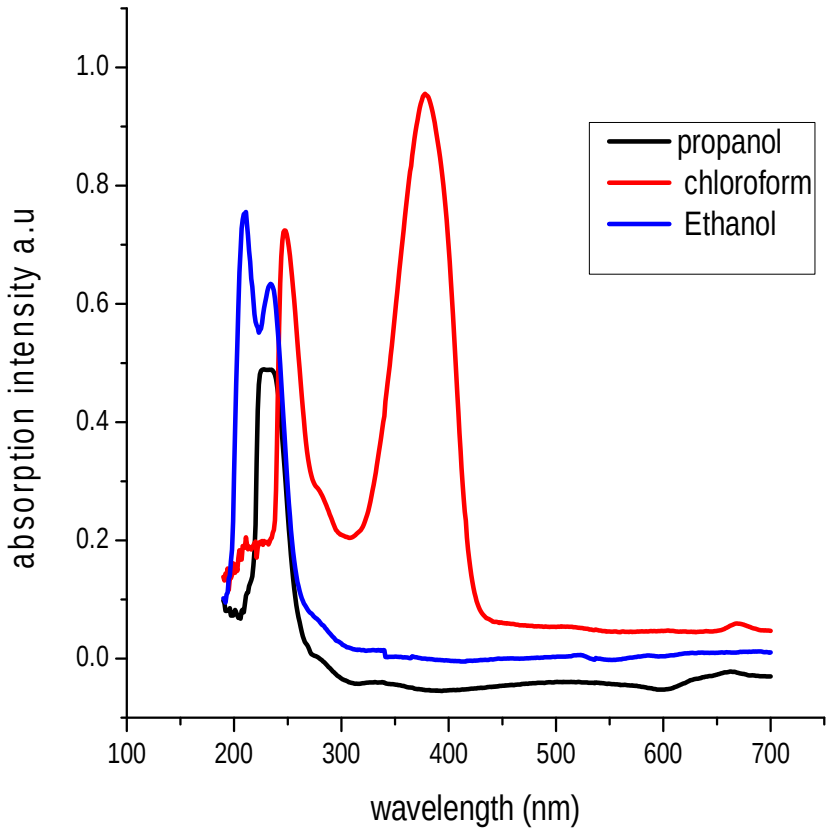
RESULTS and DISCUSSION

3-1 Introduction:

This chapter presents the results of investigation of emission for various dyes that are suitable to be used for different purposes including photodynamictherapy. The results were obtained from samples (Alcian Blue 8GX, Coumarin 500 and bromocresol Purple) dissolved in three solvents (ethanol, Propanol, and chloroform) irradiated with different light sources. The absorption of each dye was recorded using UV-Vis spectrophotometer before the irradiation to determine the exact portion of the spectrum that the dye absorb, then diode lasers (532 nm , 671 nm) and LED 365 nm were used to irradiate the samples and the emission spectra were recorded during irradiation in different times (1, 2 3 and 4 minutes).

3-2TheAbsorption spectra of Alcianbluedissolved in ethanol, Propanol, and chloroform:

The absorption spectra ofAlcian blue dissolved in ethanol, Propanol, and chloroform are shown in figure (3.1), which was recorded by the UV-VIS spectrometer.



. **Figure (3.1):**

The absorption spectra of Alcian blue dissolved in Propanol, ethanol and chloroform

In figure (3.1) one can see thatAlcian blue has strong absorption in the range (200 - 420) nm.There is a shift (20.234 nm) in the spectra; this shift is attributed to the difference in the optical characteristics for solvents and the dissimilar in the photochemical interaction according to solvent.

3-3TheEmission Spectra of Alcian blue 8GX dissolved in chloroform after irradiation by (365 nm and 532 nm):

The emission spectra of irradiation of Alcian blue dissolved in chloroform, after irradiation by monochromatic LED (365 nm) for different times are shown in Figure. (3.2).

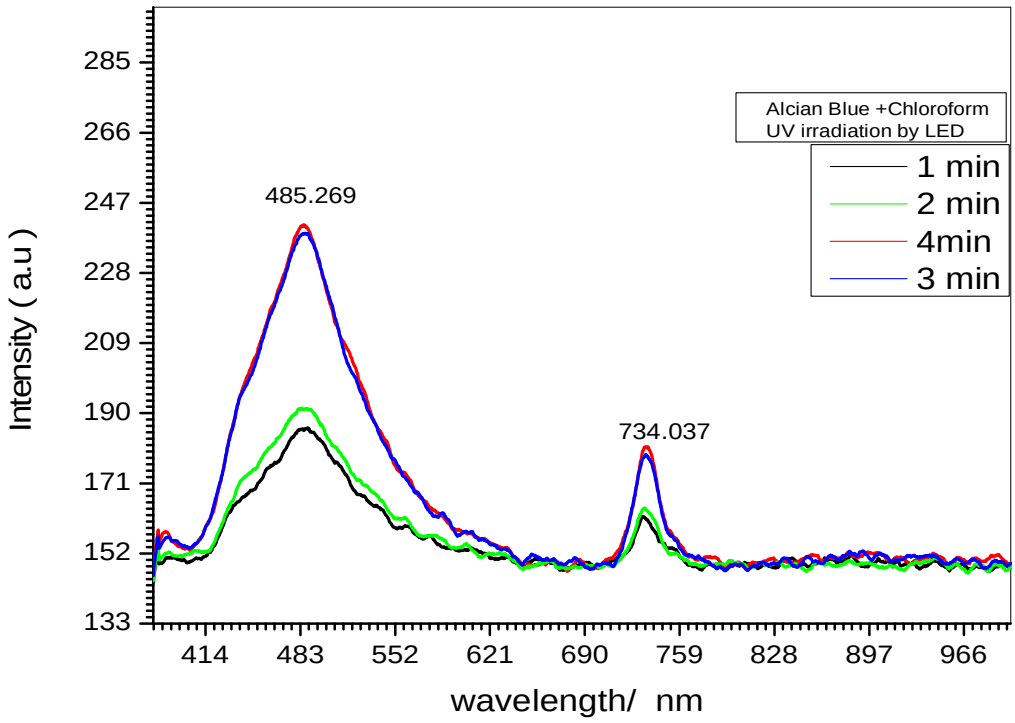


Figure. (3.2): Emission spectra of Alcian blue + Chloroform after irradiationby LED (365 nm) for different times

From figure (3.2) it was found that; the emission spectra of Alcian Blue dissolved in chloroform and irradiated by (365 nm) , have two broad bands, the first one is at (485.269 nm) and the second is at (734.037 nm). This sample didn't produce the singlet oxygen that havetwo emission bands at 703 nm and 634 nm. Increasing the exposure time led to increase the bands intensities.

The emission spectra of Alcian blue dissolved in chloroform, after excitation by (532 nm) with different times, are shown in Figure (3.3)

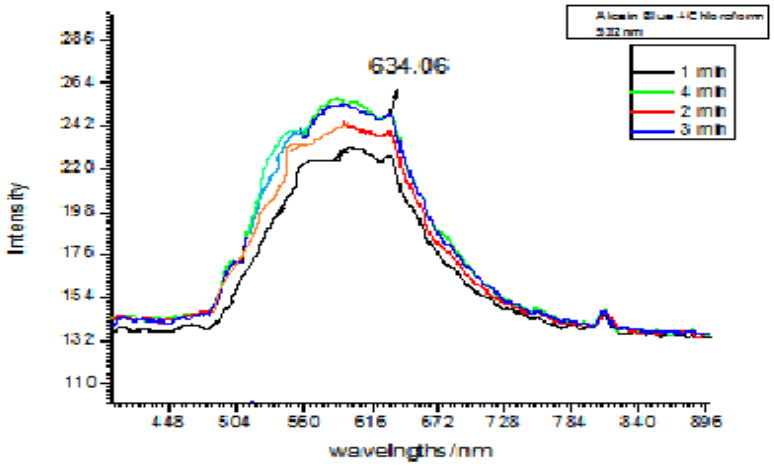


Figure.(3.3): Emission spectra of Alcian blue + Chloroform after irradiationby green diode laser (532nm) for different times

The emission spectra have two peaks at 551.669 nm and 634.06 nm.This p (634.06 nm) indicates the production of singlet oxygen.

3-4Theemission spectra of Alcian blue dissolved in Ethanol after irradiation by LED (365 nm)

Figure (3-4) shows the emission spectra of Alcian blue dissolved in Ethanol after irradiation by monochromatic LED (365 nm) for different times.

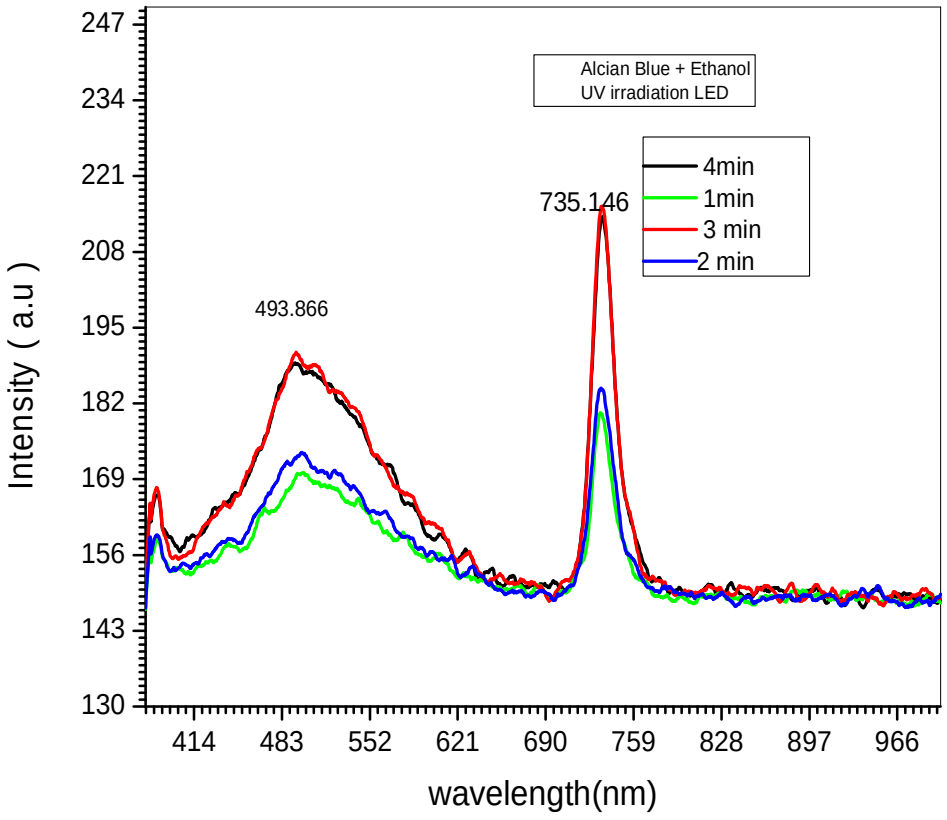


Figure. (3.4): the emission spectra of Alcian blue + Ethanol after irradiation by 365 nm for different times.

The emission spectra in the figure (3-4) shows two bands at (493.866 nm and 735.146 nm). Increasing the exposure time led to increase the bands intensities.

Table (3.1) the emission bands of Alcian blue dissolved in chloroform after excitationby differentligth sources.

Dye	solvent	Excitation source (nm)	Emission λ_{max}(nm)
Alcian blue	chloroform	365	485.269
		532	634.06

Table (3.2):the wavelengths of the emissionpeaks of Alcian blue dissolved in different solvents after excitation by 365 nm.

Dye	Solvent	Excitation source	Emission $\lambda_{max(nm)}$
Alcian blue	Ethanol	365 nm	493.866
	Propanol		485.269

3.5Theabsorption spectra of bromocresol purpledissolved in chloroform, propanol and ethanol:

Figure (3-5) shows the absorption spectra of bromocresol purple dissolved in chloroform, ethanol and Propanolare shown in figure (3.5), which was recorded by the UV-VIS spectrophotometer.

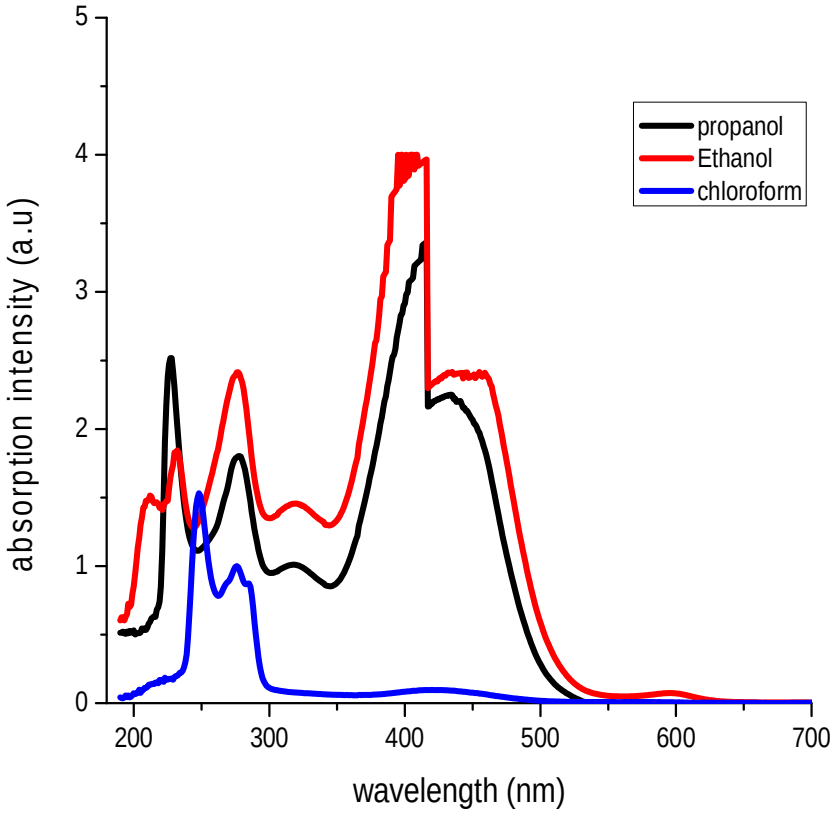


Figure (3.5): The absorption spectra of bromocresoldissolved in Propanol, ethanol and chloroform

In this figure one can see thatbromocresol has strong absorption in the UV (200nm - 350 nm) and visible range (400 nm - 550 nm). There is a shift in the spectra; this shift is attributed to the difference in the optical characteristics for solvents and the dissimilar in the photochemical interaction according to solvent.

3.6 The emission spectra ofbromocresol purple dissolved in chloroform after irradiation by monochromatic LED (365 nm):

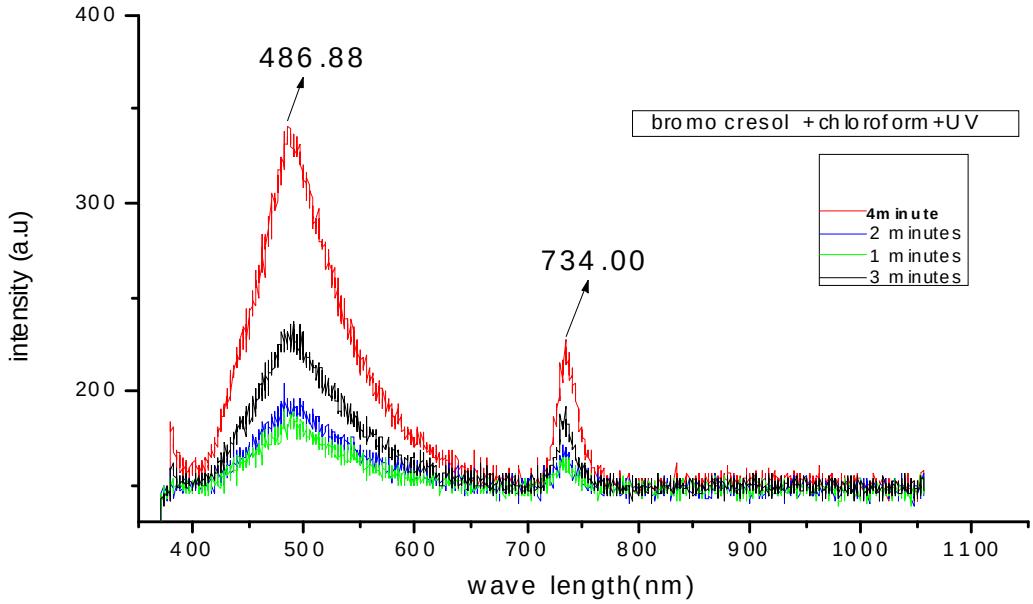


Figure (3.6) Emission spectra of bromocresol+ Chloroform after irradiationby 365nm for different times

Figure (3.6)shows the emission spectra of bromocresol dissolved in chloroform after irradiation by 365 nm for different exposure times (1, 2, 3and 4 min). The emission spectra have two bands at 486.88 nm and 734.00nm. Increasing the exposure time led to the increase in bands intensities.

3-7Theemission spectra of bromocresolpurpledissolved in ethanol after irradiation by 532 nm:

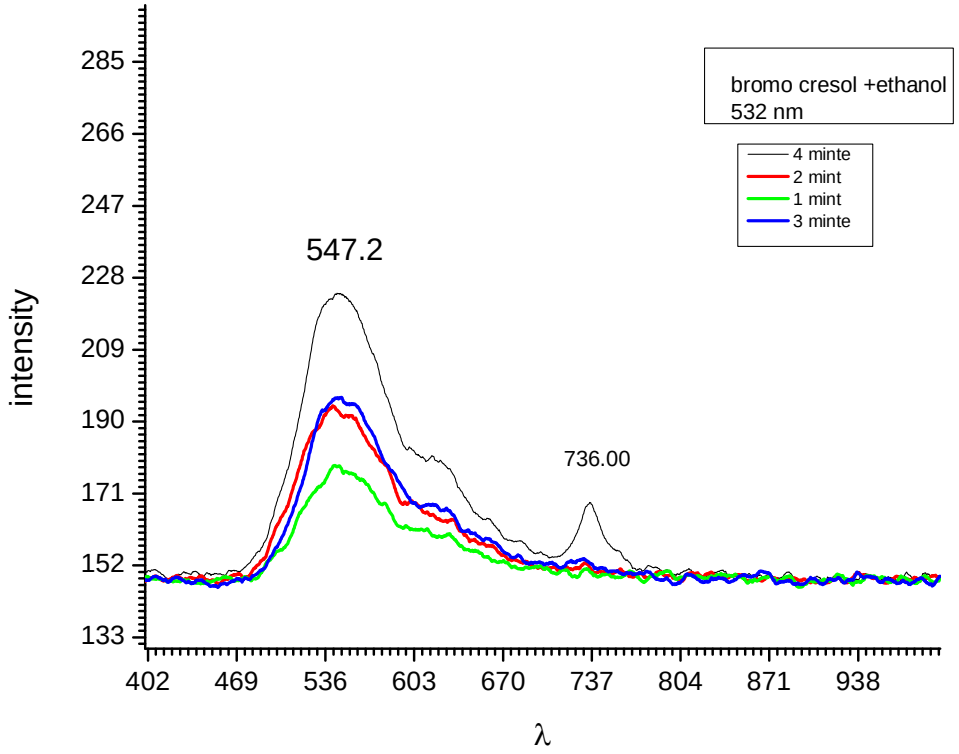


Figure (3-7)theemission spectra of brome cresol dissolved in ethanol after irradiation by 532 nm for different times.

In figure (3.7)it was found that; the emission spectra of bromocresoldissolved in ethanol andirradiated by (532 nm), have two bands, the first one is at (736.00 nm) and the second is at (547.2 nm). Increasing the exposure time led to the increase in bands intensities.

(3.8)The emission spectra of bromocresol purple dissolved in ethanol after irradiation by 365 nm:

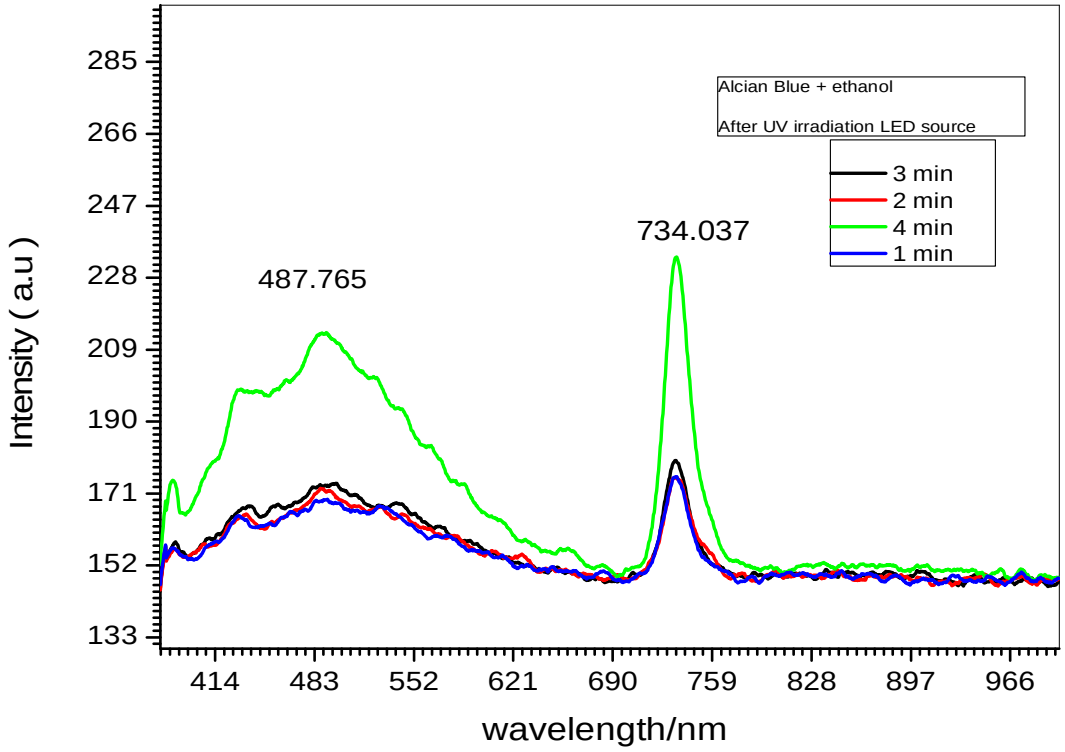


Figure (3-8) theemission spectra of brome cresol dissolved in ethanol after irradiation by 365 nm for different times.

In figure (3.8)itcan be seen that; the emission spectra of bromocresoldissolved in ethanol andirradiated by (365 nm),contain two bands, the first one is at (734.037 nm) and the second is at (487.765 nm. Increasing theexposure time led to the increase in bands intensities.

3.9 The emission spectra of bromocresol dissolved in Propanol after irradiation by monochromatic LED (365nm):

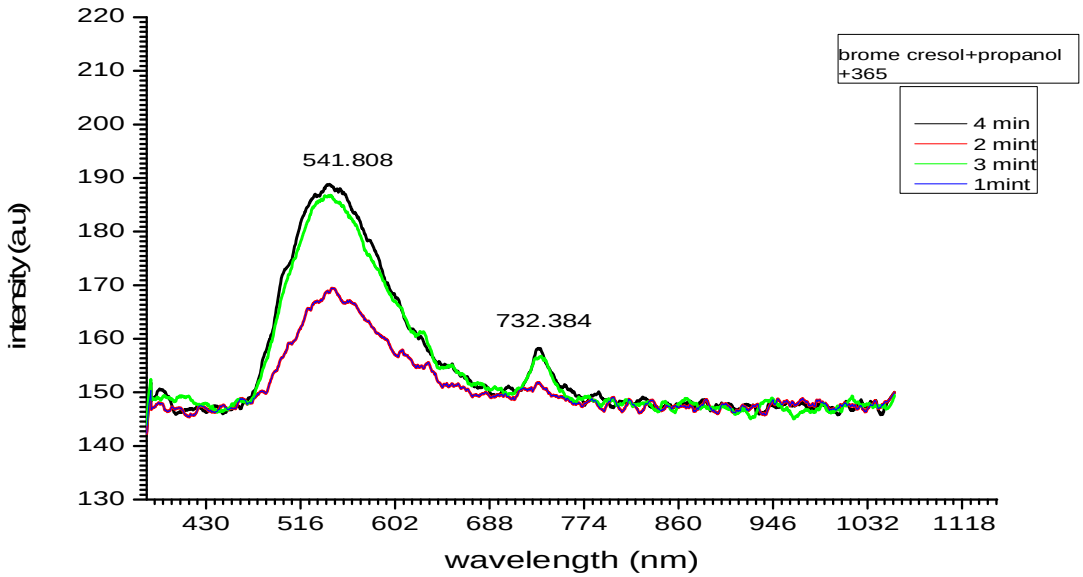


Figure (3.9) Emission spectra of bromocresol dissolved in Propanol after irradiationby 365nm for different time.

From figure (3.9) it can be noticed that the emission spectra of bromocresol dissolved in Propanol andirradiated by 365nm,have two bands, the first one at 541.808 nm and the second isat732.384nm. Increasing the exposure time led also to the increase in bands intensities.

Table (3.3): the emission peaks of the bromocresol purple dissolved in different solvents after excitation by365 nm.

Dye	solvent	Excitation source	Emission $\lambda_{max(nm)}$
Bromocresol	chloroform	365 nm	486.88
	Ethanol		487765
	Propanol		541.808

Table (3.4): theemissionbandsofbromocresol purple dissolved ethanol after excitation by differentligth sources.

Dye	solvent	Excitation source (nm)	Emission $\lambda_{max(nm)}$
Bromocresol	Ethanol	532	547.200
		634	487.765

3.10The absorption spectra of the Coumarin500 dissolved in ethanol, Propanol and chloroform:

The absorption spectra of Coumarin 500 dissolved in ethanol, Propanol and chloroform are shown in figures (3.10), which were recorded by the UV-VIS spectrophotometer.

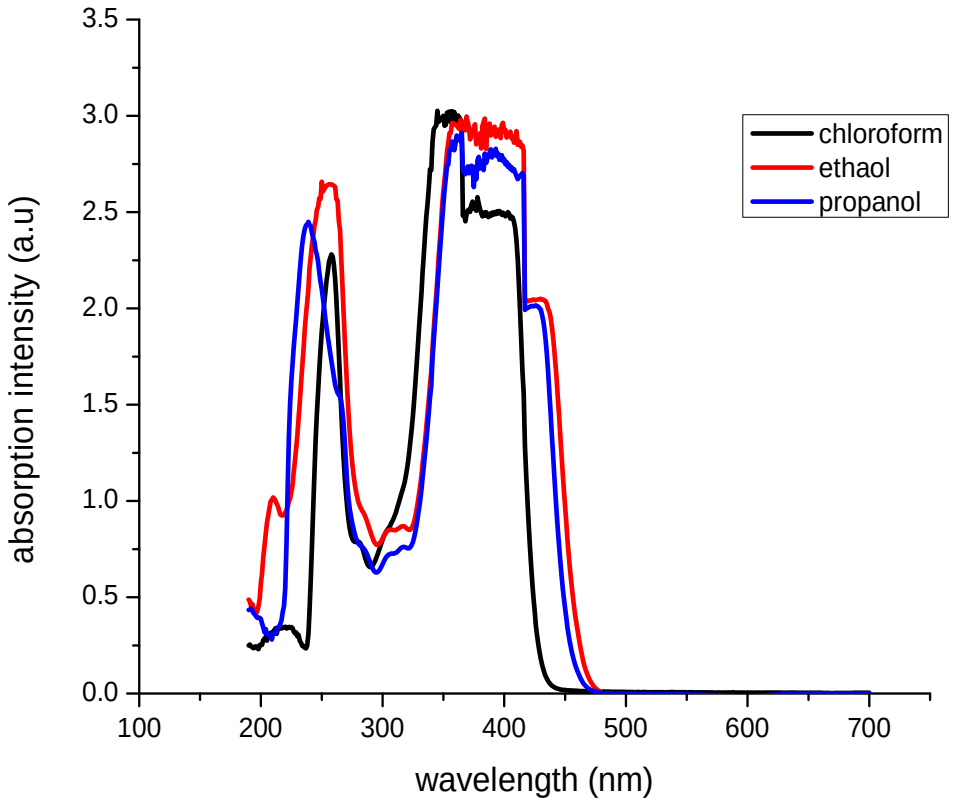


Figure. (3.10) the Absorption spectrum of Coumarin dissolved in chloroform,Propanol and ethanol

In this figure one can see thatCoumarin 500 has strong absorption in the UV (200 - 350) nm and visible range (400 - 450) nm.

3.11 The emission spectra of Coumarin 500 dissolved in chloroform after irradiation by 532nm:

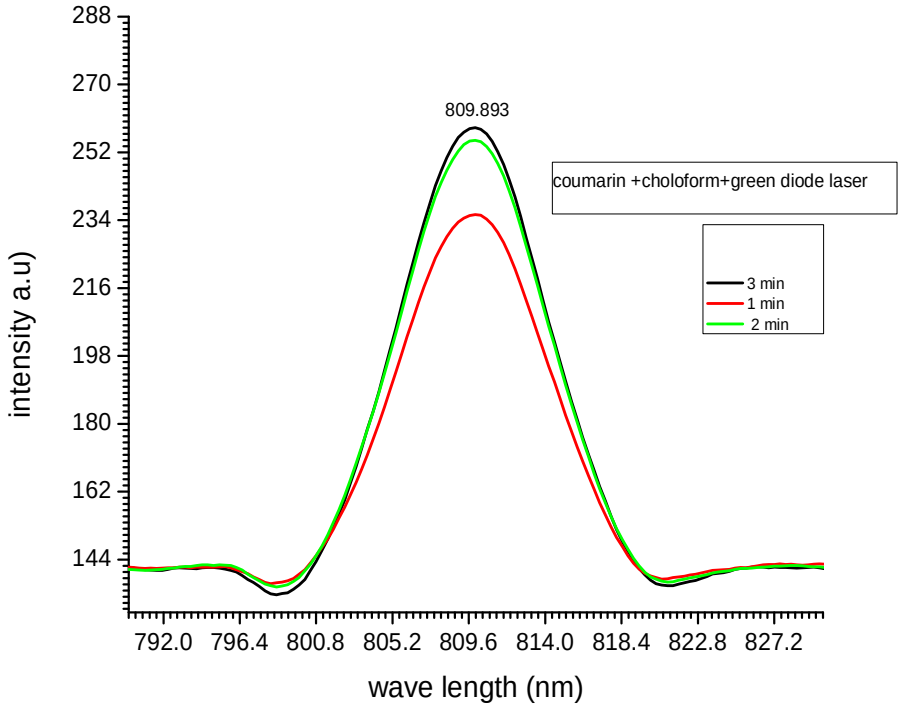


Figure (3.11) emission spectra of Coumarin dissolved in chloroform after irradiationby 532 nm for different times.

In figure (3.11) it can be seen that the emission spectra of Coumarindissolved in chloroform,and irradiated by 532 nm,have bandat 809.893 nm. Increasing the exposure time led also to an increase in the band intensity.

3.12The emission spectra of Coumarin 500 dissolved in chloroform after irradiation by 671 nm:

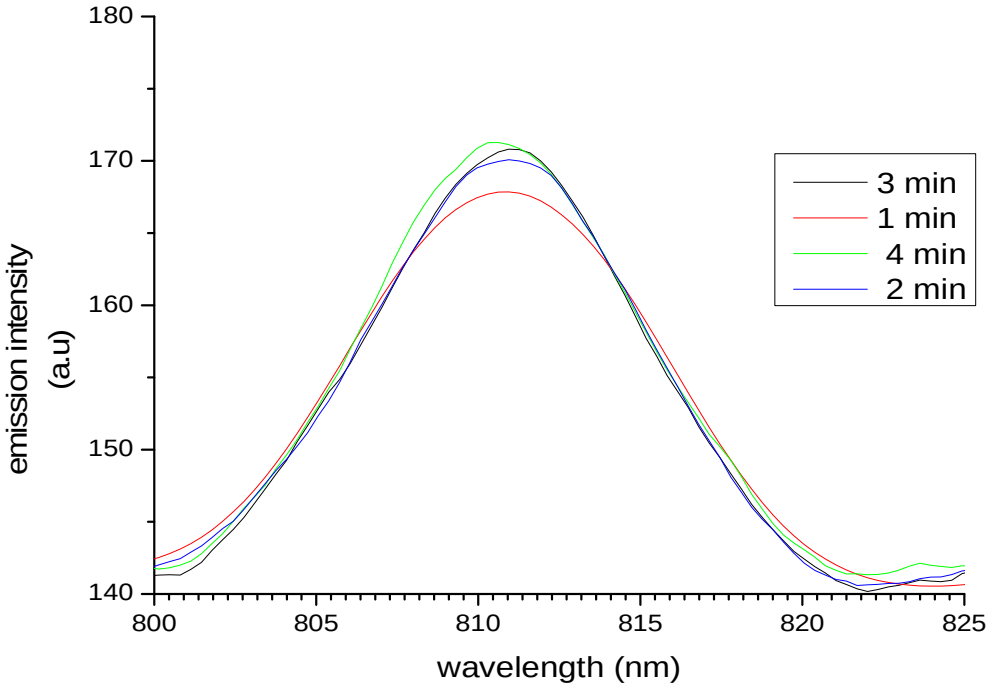


Figure (3.12): the emission spectra of Coumarin 500 dissolved in chloroform after irradiation by 671 nm for different times.

Figure (3.12) it can be noticed that the Emission spectra of Coumarin 500 dissolved in chloroform and irradiated by diode laser 671 nm have broad band at 811.254 nm. Increasing the exposure time led also to an increase intheband intensity.

3.13 The emission spectra of Coumarin dissolved in Propanol after irradiationby monochromatic LED (365 nm):

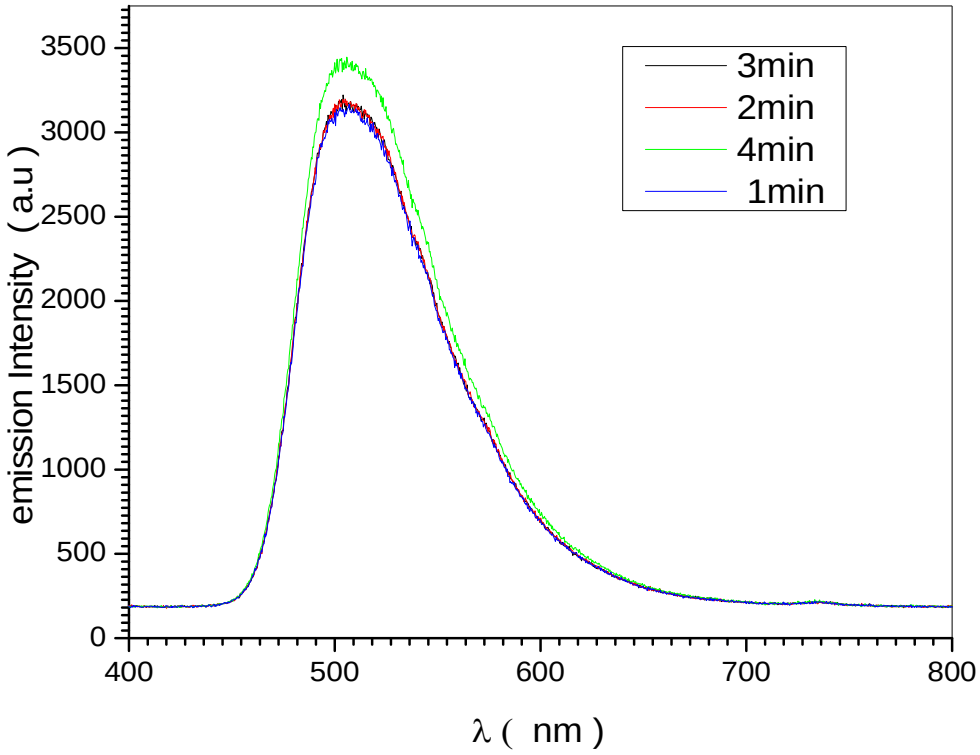


Figure (3.13) the emission spectra of Coumarin 500 dissolved in Propanol after irradiationby LED for different times

From figure (3.13) it was found that theemission spectra of Coumarindissolved in Propanol and irradiated by 365nm have broad band at 502.275nm . Increasing the exposure time led also to an increase in theband intensity.

Table (3.5): the emission bands of Comorian 500 dissolved in chloroform after excitation by different light sources

Dye	solvent	Excitation source (nm)	Emission $\lambda_{max(nm)}$
Coumarin	chloroform	532	809.893
		671	811.254

3.14 The Discussion:

From figure (3.1)and figure (3.2) one can see that the absorption spectrum of Alcian blue dissolved in chloroform in the ranged between (200-420) nm and the maximum absorption is at 400 nm.The emission peaks of this sample, after irradiation by 365 nm, are at 485.269 nm and 734.037nm.Figure (3.3) proved thatAlcian blue was efficient in the production of singlet oxygen, where the $^1O_2$ emission band at 634 nm was detected in the emission spectra. The efficient solvent to produce singlet oxygenwas chloroform which gave positive results. From figure (3.4) it was found that the maximum of the emission peak of Alcian blue dissolved in ethanol, after irradiation by 365 nm, is at 493.866 nm. In figures (3.5) and (3.6) the emission peak of bromocresol purple dissolved in chloroform after irradiation by (365 nm),is at 486.88 nm.

Infigure (3.7) the absorption spectrum of bromocresol dissolved in ethanol located in the rang (200-550) nm shows that the maximum absorptionis at 450 nm, while the emission peak after irradiation by 532 nm, isat 547.200 nm.Figure (3.9) shows that the maximum of the emission peak of bromocresol purple dissolved in propanol after irradiation by 365 nmis at 541.808 nm, while the maximum absorption is at 400 nm.

From figure (3.10) and (3.11) it can be noticed that the absorption spectrum of Coumarin dissolved in chloroform is located in the range (200-450) nm and the maximum absorptions are at 250 nmand 400 nm, while the emissionpeakof this sample after irradiation by 532 nm at 809.893 nm.

In figure (3-12),the maximum emission peak of Coumarindissolved in chloroform after irradiation by 671 nm at 811.254 nm, while the maximum absorption is at

400 nm.

In all cases, theincreasing of exposure time led to the increase in the emission band intensities. Tables (3-1), (3-4), (3-5) indicate the difference of emission wavelengthdue tothe different excitation sources. In tables (3-2) and (3-3) the differencein the emission wavelength isdue to photochemical interaction between the solvent and the dye.

3.15 Conclusions:

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

The Alcain blue 8GX is appropriate dye for the production of singlet oxygen.Increasing the irradiation time led to increasing the emission intensity. The appropriate light source to produce singlet oxygen from Alcian Blue 8GX was the diode laser 532 nm where it was succeeded in the production of singlet oxygen at (634nm).

3.16 Recommendations:

The followings can be recommended as future work:

- Studying the emission of other dyes such as Verteporfinthat can be used in medicine as photosensitizes for photodynamic therapy (PDT).

- Using other lasers like UV lasers, as example, to irradiate the dyes studied in this work.

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