Sudan University of Science and Technology
College of Graduate Studies

Analysis of Nigella Sativa Oil for Laser Production of Some Proposed Medical Applications

تحليل زيت الحبة السوداء لإنتاج الليزرلبعض التطبيقات الطبية المقترحة

A dissertation Submitted inPartial Fulfillment of the Requirements of the Degree of Master of Science in Medical Physics.

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:

الآية

قال تعالى:

﴿ لَقَدْ مَنَّ اللَّهُ عَلَى الْمُؤْمِنِينَ إِذْ بَعَثَ فِيهِمْ رَسُولًا مِنْ أَنْفُسِهِمْ يَتْلُو عَلَيْهِمْ آيَاتِهِ وَيُزَكِّيهِمْ وَيُعَلِّمُهُمُ الْكِتَابَ وَالْحِكْمَةَ وَإِنْ كَانُوا مِنْ قَبْلُ لَفِي ضَلَالٍ مُبِينٍ﴾

صدق الله العظيم

سورة آل عمران، الآية (164)

Dedication

I dedicate this work to my mother, who nurtured me, taught me her love and affection, To my father, I have lost since my youth. To my husband, stood beside me and encouraged me. To my children, to my mother Sumaya, always advised and encouraged me.

I also dedicate this dissertation to my brothers; sister for coloring my life, to all who taught me characters, and without their encouragement, this work would not have seen the light.

My deepest thank goes to all my family and friends, I dedicate you all this humble research, asking God to help him.

Acknowledgments

First of all, I would like to thanks Allah for giving me the strength to finish this study.

Special thanks, Dr. Ali Abdel Rahman Saeed Marouf, supervisor of my dissertation for his guidance and assistance throughout the this research .I would like to express my gratitude to the staff of the laser laboratory of El-Nilein University, the laboratory of forensic evidence and staff of Science, Radiology and Institute of Laser, Sudan University of Science and Technology.

Abstract

The present study has been designed to identify the bioactive constituents present in components of NigellaSativa oil using Gas Chromatography-Mass Spectroscopic (GC-MS), to investigate the possibility of producing laser from it using He-Ne laser as an excited source. Preliminary absorption spectrum ranged from 600 to 680 nm, emission spectrum ranged from 650 nm to 700 nm, this range includes the laser wavelengths that used to treat wound healing and wrinkles and 690 nm treat in photodynamic therapy, (GC-MS) analysis of NigellaSativa oil was performed using GC and interpretation on mass spectrum (GC-MS). Totally 7 compounds were identified based on their molecular mass, retention time and peak values, among them, the highest amount were obtained for some compounds such as 9-octadecenoic acid (48.16%) and low peak value such as tetradecanoic acid (myristic acid), isopropyl ester (0.52) .Thus, the results of this study platform of using cumin oil as herbal alternative for various diseases.

المستخلص

يعتبر زيت الحبة السوداء من الزيوت النباتية المهمة منذ العصور القديمة, وقد تم تصميم هذه الدراسة لتحديد النشاط البيولوجي للمركبات الموجودة في زيت الحبة السوداء باستخدام كروماتوغرافيا الغاز الكتلي الطيفي، والتحقق من إمكانية إنتاج الليزر منه باستخدام ليزر الهليوم–نيون كمصدر تشعع. وقد تم الحصول على أطياف الامتصاص من 600 إلى 680 نانو متر، وتراوحت أطياف الانبعاث من 650 نانومتر إلى 700 نانومتر، ويشمل هذا النطاق أطوال الليزر التي تستخدم لعلاج السرطان وعلاج الجروح والتجاعيد، وقد تم تحليل زيت الحبة السوداء باستخدام جهاز كروماتوغرافيا الغاز وتمت قراءة 7 مركبات على أساس الكتلة الجزيئية، زمن الاحتفاظ وقيم الذروة، ووجد أن أعلى نسبة تركيز لهذه المركبات هو مركب 9–أوكتاديكانويك (حمض الالاديك) وأقل نسبة تركيز هو مركب تتراديكانويك (حمض المرستيك)، وبناء على النتائج السابقة فإن هذه الدراسة تقدم مقترحات لاستخدام زيت الحبة السوداء كبديل للأعشاب لمختلف الأمراض.

Table of Contents

Table of figures

List of Tables

Chapter One

Introduction

1.1 Introduction

Plants have been major source of medicine in all cultures from ancient times. In the traditional system, various indigenous plants are being used in the diagnosis, prevention and elimination of physical, mental or social imbalance (Manjunath, 1990). Medicinal plants are the backbone of the traditional medicine (Farnsworth, 1994) and are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga et al., 2005). The drugs are derived from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc; some drugs are prepared from excretory plant product such as gum, resins and latex.

Over the years, interest in natural products has acquired a cyclic phenomenon. In many countries, including India and China, thousands of tribal communities still use folklore medicinal plants for the cure of various diseases. The great interest in the use and importance of medicinal plants in many developing countries has led to intensified efforts on the documentation of ethno medical data of medicinal plants (Dhar et al; 1968; Waller, 1993).

1.2 Research Problem

— Investigating the production of laser from black seed oil and finding new lasers to treat some of the most difficult diseases, and identifying the ingredients of black seed oil and the ability of using it to treat the diseases.

1.3 Literature review

1n 2003 Mohamed et al investigated Nigella sativa oil Crude oils and their fractions were for their radical scavenging activity (RSA) toward the stable galvinoxyl radical by electron spin resonance (ESR) spectrometry and toward 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by spectrophotometric method. Their results the importance of minor components in crude seed oils on their oxidative stability, which will reflect on their food value and shelf life.

Used Nigella sativa samples obtained from the market and irradiated it under 2.5 kGy, 6 kGy, 8 kGy, and 10 kGy doses, respectively. Along with the increase in the dose of irradiation, both the free fatty acid and peroxide values of the samples increased, whereas oil contents, iodine numbers, refraction index and rancimat values decreased. In the composition of fatty acids, while the percentages of unsaturated fatty acids decreased; trans fatty acid levels increased. Microbial count of the samples decreased as the dose of irradiation increased. They observed that total bacterial count as well as total count(Arici and et al; 2007).

In 2013 Ahmed Jasim identified the role of Nigella Sativa oil and olive oil on cholesterol, total protein and triglycerides. His results showed low cholesterol depending on the concentration of Nigella Sativa oil and olive oil, while increases when there are Nigella Sativa oil only. The level of total protein in group treated with Nigella Sativa oil and olive oil while dipping in the treatment group outdoor graduation was drenched Nigella Sativa seeds only. Low triglycerides in treatment depending on the concentration of Nigella Sativa oil and olive oil, while increases when there are Nigella Sativa oil only. He concluded that the impact of LDL, LDH so black bean t. ether doing Nigella Sativa oil on blood depends on the mix concentration of Nigella Sativa oil and olive oil (Mohammad, 2013).

In 2016 Nivetha and Prasanna identified the bioactive constituents present in ethanol extract of Nigella Sativa L. seeds using Gas Chromatography-Mass

Spectroscopic (GC-MS) and Fourier Transform Infrared Spectroscopy (FT-IR). Preliminary phytochemical analysis revealed the presence of anthroquinones, alkaloids, amino acid, tannins, saponnins, flavonoids, protein, steroids, terpenoids and absence of carbohydrates, polyphenols, phlobatannins, phytosterol, cardiac glycosides. GC-MS analysis of plant extract was performed using a Perkin-Elmer GC Clarus 436 system and interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST).The FT-IR spectrum analysis was also carried out and the results confirmed the presence of functional groups such as amines, alkanes, acids, esters, alkyl and alkenes.Result showed using Nigella sativa seeds as herbal alternatives for various diseases.

1.4 Objectives

— To determine the optical properties of Nigella Sativa oil.

— To analyses of oil components.

— To classify of Nigella Sativa oil and its medicinal dependence in treatment.

1.5 Research Methodology

The Nigella Sativa oil will be purchased from market, Saudi companies, Hemani, The cumin oil will be analyzed by GC/MS, and determination of cumin (Nigella Sativa) oil absorbance will carried out using UV-Vis spectrometer, then the appropriate excited source will be selected to record the emission spectrum, and then study the possibility of laser production from it.

1.6 Thesis Layout

This thesis is consist of four chapters, chapter one Introduction and Literature Review ,and chapter two consist Basic Concepts of laser and Oil, and Light interaction with matter, chapter three consist Experimental Part (The materials and device and method), chapter four consist Results and Discussion and Conclusion, Recommendations and finally Reference.

Chapter Two

Theoretical Background

2.1 Fluorescence

Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation. It is a form of luminescence. In most cases, the emitted light has a longer wavelength, and therefore lower energy, than the absorbed radiation. The most striking example of fluorescence occurs when the absorbed radiation is in the ultraviolet region of the spectrum, and thus invisible to the human eye, while the emitted light is in the visible region, which gives the fluorescent substance a distinct color that can only be seen when exposed to UV light. Fluorescent materials cease to glow immediately when the radiation source stops, unlike phosphorescence, where it continues to emit light for some time after.

Fluorescence has many practical applications, including mineralogy, gemology, medicine, chemical sensors (fluorescence spectroscopy), fluorescent labeling, dyes, biological detectors, cosmic-ray detection, and, most commonly, fluorescent lamps. Fluorescence also occurs frequently in nature in some minerals and in various biological states in many branches of the animal kingdom (Coumans et al ,2012).

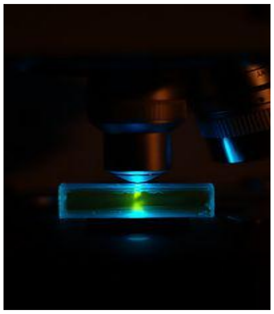

Figure 2.1 A sample of herring sperm stained with SYBR green in cavetti illuminated by blue light in an epifluorescence microscope. The SYBR green in the sample binds to the

herring sperm DNA and, once bound, fluoresces giving off green light when illuminated by blue light.

2.1.1 Fluorescence

Strongly fluorescent pigments often have an unusual appearance, which is often described colloquially as a "neon color" This phenomenon was termed "Farbenglut" by Hermann von Helmholtz and "fluorence" by Ralph M. Evans. It is generally thought to be related to the high brightness of the color relative to what it would be as a component of white. Fluorescence shifts energy in the incident illumination from shorter wavelengths to longer (such as blue to yellow) and thus can make the fluorescent color appear brighter (more saturated) than it could possibly be by reflection alone (Schieber and Frank 2001), "Modeling the Appearance of Fluorescent Colors".

2.1.2 Light Sources

Fluorescence microscopy requires intense, near-monochromatic, illumination which some widespread light sources, like halogen lamps cannot provide. Four main types of light source are used, including xenon arc lamps or mercury vapor lamps with an excitation filter, lasers, super continuum sources, and high-power LEDs. Lasers are most widely used for more complex fluorescence microscopy techniques like confocal microscopy and total internal reflection fluorescence microscopy while xenon lamps, and mercury lamps, and LEDs with a diachronic excitation filter are commonly used for wide field epi-fluorescence microscopes. By placing two micro lens arrays into the illumination path of a wide field epi-fluorescence microscope, (Coumans et al , 2012) highly uniform illumination with a coefficient of variation of 1-2% can be achieved.

Figure 2.2 Fluorescing scorpion (Photograph taken by Jonbeebe, 2007)

2.1.3 Epi-fluorescence microscopy

The majority of fluorescence microscopes, especially those used in the life science is of the epi-fluorescence design shown in the diagram. Light of the excitation wavelength is focused on the specimen through the objective lens. The fluorescence emitted by the specimen is focused to the detector by the same objective that is used for the excitation, which for greater resolution will need objective lens with higher numerical aperture. Since most of the excitation light is transmitted through the specimen, only reflected excitatory light reaches the objective together with the emitted light and the epi-fluorescence method therefore gives a high signal-to-noise ratio. The diachronic beam splitter acts as a wavelength specific filter, transmitting fluoresced light through to the eyepiece or detector, but reflecting any remaining excitation light back towards the source (Coumans, 2012).

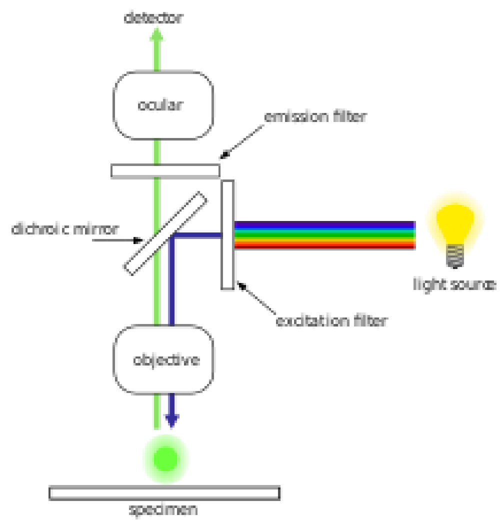

Figure 2.3 diagram of a fluorescence microscope

2.1.4 Fluorescence Microscope

A fluorescence microscope is an optical microscope that uses fluorescence and phosphorescence instead of, or in addition to, reflection and absorption to study properties of organic or inorganic substances (Nikon Microscopy U Retrieved; 2008).The "fluorescence microscope" refers to any microscope that uses fluorescence to generate an image, whether it is a more simple set up like an epi-fluorescence microscope, or a more complicated design such as a confocal microscope, which uses optical sectioning to get better resolution of the fluorescent image (Richard et al,1986).

Figure 2.4 An upright fluorescence microscope (Olympus BX61) with the fluorescent filter cube turret above the objective lenses, coupled with a digital camera.

2.1.5. Laser-Induced Fluorescence (LIF)

Laser-induced fluorescence (LIF) or laser-stimulated fluorescence (LSF) is a spectroscopic method in which an atom or molecule is excited to a higher energy level by the absorption of laser light followed by spontaneous emission of light (Zara and coworkers first reported it in 1968).

Laser-induced fluorescence is used for studying structure of molecules, detection of selective species and flow visualization and measurements. The wavelength is often selected to be the one at which the species has its largest cross section. The excited species will after some time, usually in the order of few nanoseconds to microseconds, de-excite and emit light at a wavelength longer than the excitation wavelength. This fluorescent light is typically recorded with a photomultiplier tube (PMT) or filtered photodiodes (Kinsey, 1977, Laser-Induced Fluorescence).

Laser-induced fluorescence is a resonant technique. Thus, the laser wavelength is chosen to match the transition of a target species, usually from one electronic state to another but sometimes of one rotational vibration level to another. This is illustrated in (**Figure** 2.5) the target species is excited by a laser, the light which is emitted on the basis of the de-excitation process being termed fluorescence. Although the fluorescence produced may be resonant, so that the target emits the same wavelength as that which was absorbed, the rotational and vibration levels in the ground state that are accessible can also differ depending on the structure of the molecules and the selection rules that apply, leading to the wave lengths emitted being slightly different (usually longer) than those of the excitation source as shown in (**Figure** 2.5) This wave length shift makes the detection of the signal technically simpler than for instance for (Rayleigh scattering) where the signal appear at the same wave length as the laser,

Interference or color glass filters can be used to suppress the irradiating laser light. The absorbed and the emitted energies are unique to the target species in question, which can be chosen selectively, identified and information concerning both temperature and concentration extracted from them.

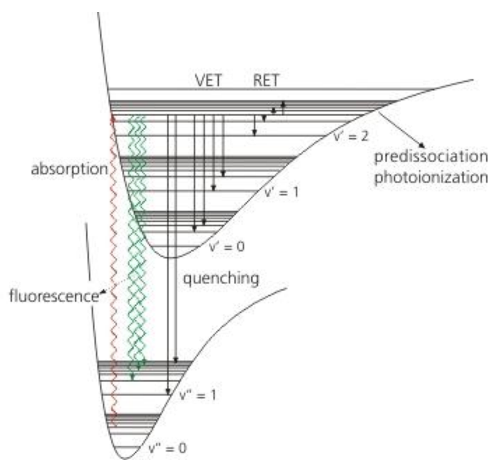

Figure 2.5 Schematic diagram showing the energy transfer processes in a typical radical. v denotes vibrational quantum numbers, VET = vibrational energy transfer and RET = rotational energy transfer

Unfortunately, just as with most diagnostic techniques in this area, LIF has certain inherent difficulties; The long lifetime of the fluorescence (typically on the order of tens of or even hundreds of nanoseconds) makes the signal very sensitive to collisions (typical collision times under atmospheric conditions are ~100 ps), which compete with the signal-generating process. This process termed quenching is what makes quantitative LIF difficult to obtain. Even at the pressure of 1 bar, the quenching rate for most radicals relevant to combustion can be 2-4 times the rate of spontaneous emission of a fluorescence photon. At elevated pressures, the collision times are diminished and can be on the order of only a few picoseconds.

There are a number of different approaches in dealing with quenching, involving either avoiding it entirely or measuring it. In predissociative LIF and ionization LIF, for example, loss terms can be increased to the point of making quenching negligible, but weakening the fluorescence signal dramatically. Saturated LIF, an approach in which a strong excitation laser is employed, is strongly affected by the energy transfer in the ground state, leaving the fluorescence signal largely independent of both quenching and laser irradiance. Use of short excitation pulses, such that the pulse duration is shorter than collision times typically are, is a further option, one that enables quenching-free measurements to be performed, yet the short pulses involved can introduce other problems, such as limited spectral resolution, through the line width of the laser being related to the pulse duration. The shorter the duration is, the broader the spectral profile becomes.

(Kinsey, 1977, Laser-Induced Fluorescence).

2.1.6 Method for laser induced fluorescence of tissue

Laser-induced fluorescence (LIF) is a highly sensitive resonant technique that can readily be employed for studying the distributions of minor species, down to sub-ppm levels, both qualitatively and quantitatively, and for measuring temperature fields and flame front propagation. At present LIF is one of the most widely utilized laser diagnostic techniques in the combustion area, and is by far the most utilized technique at the division.

A method for laser induced fluorescence of tissue in which laser radiation is used to illuminate and induce fluorescence in the tissue under study to determine the chemical composition or pathologic condition of tissue. The laser radiation and the retrieved fluorescing radiation can be conveyed through a catheter using an array of optical fiber. The fluorescence spectrum of the tissue can be displayed and analyzed to obtain information regarding the chemical composition and medical condition of the tissue inside the human body (Kitrell et al; 1996).

2.1.7 The Laser Idea

The word 'laser' is an acronym derived from 'light amplification by the stimulated emission of radiation'. If the light concerned is in the microwave region then the alternative acronym 'maser' is often used. Although the first such device to be constructed was the ammonia maser in 1954 it is the lasers made subsequently which operate in the infrared, visible or ultraviolet regions of the spectrum which have made a greater impact

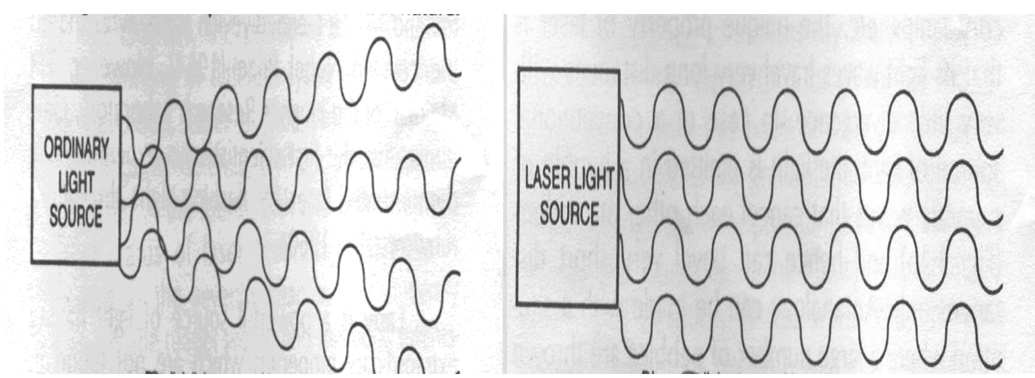

Figure 2.6 Ordinary light source and laser light source

2.1.8 Properties of Laser beams

Light from the laser arises primarily from stimulated emission and the resonator cavity within which the amplifying medium is kept leads to the following special properties:

*Directionality

*Spectral purity

*High power

*Extremely short pulse durations

Table 2.1 Some of the special properties possessed by laser beams from different types of lasers

Directionality	Divergence ~ 10^{-7}rad
Spectral purity	$\Delta\lambda$~10^{-9} µm
High power	P~1018 W/cm^2
Ultra short pulses	Δt~10^{-15} s
High electric fields	E ~ 10^{12} V/m
Small focused areas	~10^{-12} m2

2.1.9 Principle of Laser Action

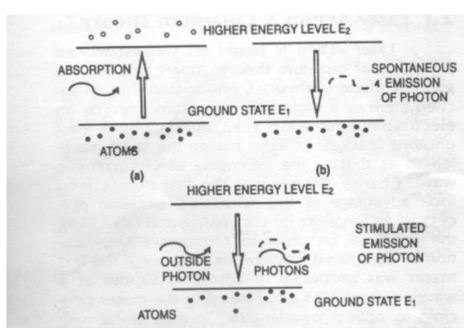

Figure 2.7 principle of laser action

Every atom, according to the quantum theory, can have energies only in certain discrete states or energy levels. Normally, the atoms are in the lowest energy state or ground state.

When light from a powerful source like a flash lamp or a mercury arc falls on a substance, the atoms in the ground state can be excited to go to one of the higher levels. This process is called absorption. After staying in that level for a very short duration (of the order of 10-8 second), the atom returns to its initial ground state, emitting a photon in the process, this process is called spontaneous or an emission.(Svelto and Hanna)

The two processes mainly: absorption and spontaneous emission take place in a conventional light source. In case the atom still in Its urns to excited stat is struck by an outside photon having precisely the energy necessary for spontaneous emission, the outside photon is augmented by the one given up by the excited atom, Moreover, both the photons are released from the same excited state in the same phase, This process called stimulated emission, is fundamental for laser action (Fig, 2.7).Thus, the atom is stimulated or induced to give up its photon earlier than it would have done ordinarily under spontaneous emission the laser is thus analogous to a spring that is wound up and cocked, It needs a key to release it, In this process the key is the photon having exactly the same wavelength as that of the light to be emitted. .(Svelto and Hanna)

2.1.10 Laser Types

The various laser types developed so far display a wide range of physical and operating parameters. Indeed, if lasers are characterized according to the physical state of the active material, we call them solid-state, liquid, or gas lasers. Since the first demonstration of the ruby laser, a variety of other materials has been found to operate as lasers (Orzio Svelt,1998).

2.1.11 Solid-State Lasers

Solid-state lasers include all optically pumped lasers in which the gain medium is a solid at room temperature. Semiconductor lasers do not belong in this category because these lasers are usually electrically pumped and involve different physical processes. In solid-state laser materials the atoms responsible for generating laser light are first excited to higher energy states through the absorption of photons, and the way in which these atoms relax from their excited states determines the quality and quantity of laser light produced (for an overview of this process, see Back to Basics, June 1994, p. 127).

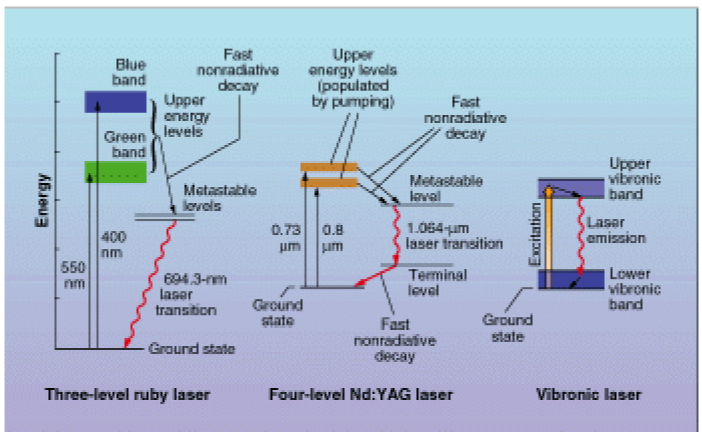

Figure 2.8 Diagram

Figure 2.8 In three-level lasers such as ruby (left), electrons of the active element occupy roughly three energy levels in their transition from excited state to ground state, whereas four-level lasers such as Nd:YAG involve four general energy levels (middle). In tunable vibronic lasers, electron transitions involve broadened energy bands (right) (Higgins, 1995**).**

2.1.12 Gas Lasers

In gas lasers, of course, the gain medium is a gas. The vast majority of these lasers are pumping by an electric discharge, but some designs use RF waves, photons, or even e-beams.

Like most gas lasers, the He-Ne (helium-neon) laser is discharge-pumped, although RF excitation also is possible. The gain medium consists of a narrow glass tube filled with a mixture of helium and neon gases. An anode near one end of the tube and a cathode near the other deliver the dc discharge current down the bore, while mirrors at the tube ends furnish the optical feedback required for stimulated emission. Electrons from the discharge collide with the more numerous helium atoms (which usually outnumber the neon atoms by 10 to 1), exciting these atoms to a higher met stable state. Then, through resonant collisions, the excited helium atoms transfer their energy to the neon atoms, raising them to met stable state nearly identical in energy to the excited helium atoms (Higgins, 1995).

From the met stable state of neon, electrons can return to the ground state via several paths, thus generating different output frequencies of laser light once population inversion is reached. However, the over helming majority of He-Ne lasers are designed to favor 632.8-nm emission (See Back to Basics, June 1994, p. 132, for an energy diagram of the 632.8-nm laser transition) . Because of their notoriously low gain and efficiency (0.01%-0.1%), few He-Ne lasers can exceed 100 mW of CW radiant power at this wavelength, which relegates them to low-power applications such as product-code scanning, alignment, interferometers, and metrology (Higgins, 1995).

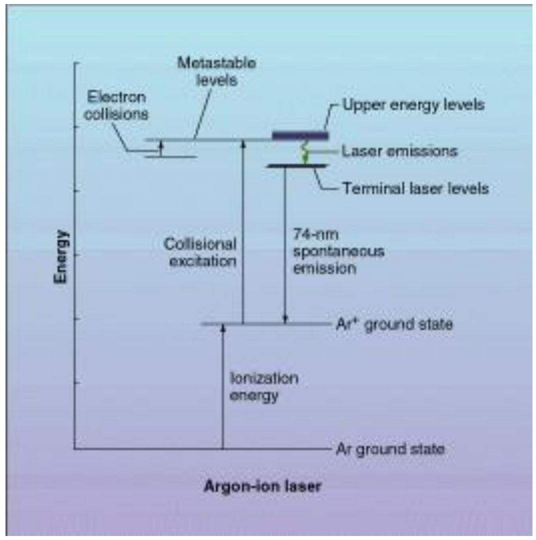

Figure 2.9 In argon-ion lasers, the neutral argon atoms must be ionized before electrons are pumped to higher energy levels from the ground state of the ionized argon.

2.1.13 Liquid Dye Lasers

Liquid lasers are optically pumped lasers in which the gain medium is a liquid at room temperature. In addition, the most successful of all liquid lasers are dye lasers. These lasers generate broad-band laser light from the excited energy states of organic dyes dissolved in liquid solvents. Either output can be pulsed or CW and spans the spectrum from the near-UV to the near-IR, depending on the dye used.

The large organic molecules of the dye are excited to higher energy states by arc lamps, flash-lamps, or other lasers such as frequency-doubled (Nd-YAG), copper-vapor, argon-ion, nitrogen, and even excimer (Higgins, 1995).

The dye solution is usually pumping transversely through the laser cavity and contained by a transparent chamber called a flow cell.

Broadband laser emission originates from interactions between the vibration and electronic states of dye molecules that split the electronic energy levels into broad energy bands similar to those of vibronic lasers. Wavelength-selective cavity optics such as a prism or diffraction grating can be used to tune to a desired frequency.

The efficiency, tenability, and high coherence of dye lasers make them ideal for scientific, medical, and spectroscopic research. In addition, their broadband emission makes them particularly well suited for generating ultra short laser pulses (Higgins, 1995).

2.1.14 Laser Structure

Perhaps the simplest form of a dye laser is the flash lamp-pumped laser, which closely resembles a solid-state laser with a liquid-filled cell instead of a solid crystal rod. Other configurations for a flash lamp-pumped dye laser include a slab configuration in which the dye cell is formed between two slabs of glass that have a different index of refraction than the dye solution between the slabs.

Flash lamp-pumped dye lasers feature large pulse energies and are a useful source for ophthalmological procedures, where they may be used for retinal photocoagulation (Csele, 2004, p336).

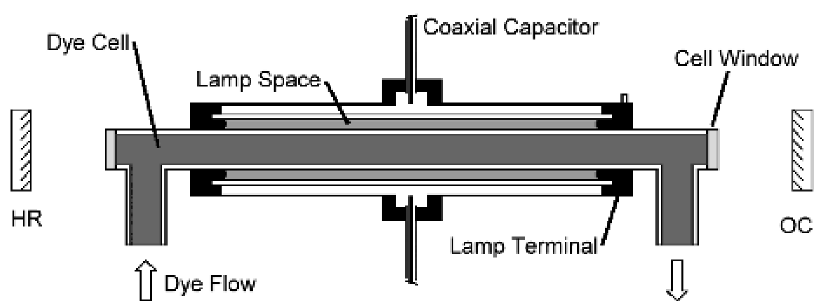

Figure 2.10 Flash lamp-pumped dye laser configuration.

However, pulse rate is severely limited to the rate at which dye can flow through the dye cell to remove heat and suppress thermal gradients that may occur. In addition, electrical discharge paths in a flash lamp-pumped dye laser are critical and must be manufactured with extremely low inductance.

Optics for a laser-pumped dye laser usually consists of an output coupler and a diffraction grating. Although a simple mirror (HR) could be used in the cavity, a grating is invariably used to allow tuning of the laser across the wide gain bandwidth of the laser, the biggest advantage of a dye laser (Csele, 2004 p337).

2.2 Medical Applications

Before we talk about laser applications, we should discuss some of the properties of tissues and interactions that occur within tissues while using laser light

2.2.1 Laser tissue interaction

In general, there exist several methods for the measurement of optical tissue properties. They focus on different quantities such as transmitted, reflected, and scattered intensities. The absorbance itself is difficult to determine, since photons absorbed by the tissue can no longer be used for detection. Therefore, the absorbed intensity is usually obtained when subtracting transmitted, reflected, and scattered intensities from the incident intensity. Depending on the experimental method, either only the total attenuation coefficient or the coefficients of both absorption and scattering can be evaluated. If the angular dependence of the scattered intensity is measured by rotating the corresponding detector, the coefficient of anisotropy can be obtained, as well. However, it is well known that the optical properties of biological tissue are altered during heating which is often associated with exposure to laser radiation. Hence, it is more accurate to measure these properties in the same experimental arrangement and at the same time. One commonly used setup satisfying this requirement is the double-integrating sphere geometry shown in (**Figure** 2.2). It was first applied to the measurement of optical tissue properties by (Derbyshire et al, 1990; Role et al, 1990), and has meanwhile turned into an unsurpassed diagnostic tool.

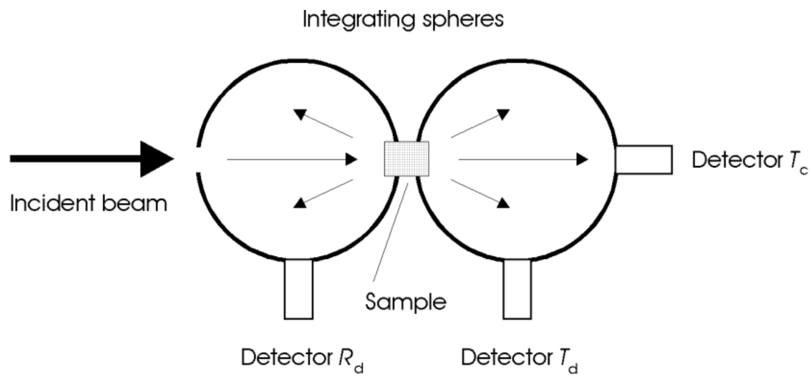

Figure 2.2 Double-integrating sphere geometry for the simultaneous measurement of different optical tissue properties. The detectors measure the transmitted coherent intensity (Tc), the transmitted diffuse intensity (Td), and the reflected diffuse intensity (Rd).

The double-integrating sphere geometry incorporates two more or less identical spheres which are located in front and behind the sample to be investigated. One sphere integrates all radiation which is either reflected or scattered backwards from the sample. Transmitted and forward scattered radiation is absorbed in the second sphere. With three detectors, all required measurements can be performed simultaneously (**Niemz, 2013, p52).**

Before providing a detailed list of optical tissue parameters, it should be noted that absorption and scattering coefficients may change during laser

exposure. However, the occurrence of carbonization is usually avoided during any kind of clinical surgery. Scattering, on the other hand, is already affected at lower temperatures, e.g. when tissue is coagulated7. In Figer. 2.3, the absorption and scattering coefficients are shown for white matter of human brain as calculated by Roggan et al. (1995a) using the Kubelka–Munk theory. Coagulation was achieved by keeping the tissue in a bath of hot water at approximately 75◦C.

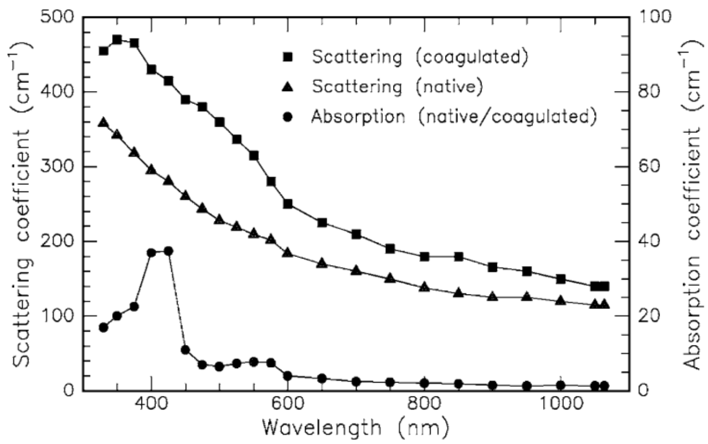

Figure 2.3 Optical properties of white matter of human brain in its native and coagulated state Data according to Roggan et al, 1995a).

Finally, the reader is reminded that biological tissue is something very inhomogeneous and fragile. The in homogeneity makes it difficult to transfer experimental data from one sample to another. Usually, it is taken into account by applying generous error bars (**Niemz, 2013, p54)**.

2.2.2 Interaction Mechanisms

The variety of interaction mechanisms that may occur when applying laser light to biological tissue is manifold. Specific tissue characteristics as well as laser parameters contribute to this diversity. Most important among optical tissue properties are the coefficients of reflection, absorption, and scattering. Together, they determine the total transmission of the tissue at a certain wavelength. Thermal tissue properties such as heat conduction and heat capacity. On the other hand, the following parameters are given by the laser radiation itself: wavelength, exposure time, applied energy, focal spot size, energy density, and power density1. Among these, the exposure time is a very crucial parameter when selecting a certain type of interaction (**Niemz, 2013, p58)**.

During the first decades after the invention of the laser by Maiman (1960), many studies were conducted investigating potential interaction effects by using all types of laser systems and tissue targets. Although the number of possible combinations for the experimental parameters is unlimited, mainly five categories of interaction types are classified today. These are photochemical interactions, thermal interactions, photo-ablation, plasma-induced ablation, and photo disruption. In particular, the physical principles governing these interactions are reviewed. Emphasis is placed on microscopic mechanisms controlling various processes of laser energy conversion. Each type of interaction will be introduced by common macroscopic observations including typical experimental data and/or histology of tissue samples after laser exposure. All these seemingly different interaction types share a single common datum: the characteristic energy density ranges from approximately 1 J/cm^2 to 1000 J/cm^2**(Niemz, 2013, p58)**.

This is surprising, since the power density itself varies over 15 orders of magnitude!

Thus, a single parameter distinguishes and primarily controls these processes: the duration of laser exposure which is mainly identical with the interaction time itself.

A double-logarithmic map with the five basic interaction types is shown in

(Figure 2.4) as found in several experiments. The ordinate expresses the applied power density or irradiance in W/cm2. The abscissa represents the exposure time in seconds. Two diagonals show constant energy fluencies at 1 J/cm2 and 1000 J/cm2, respectively. According to this chart, the time scale can be roughly divided into five sections: continuous wave or exposure times > 1 s for photochemical interactions, 1min down to 1μs for thermal interactions, 1 μs down to 1ns for photo ablation, and < 1ns for plasma-induced ablation and photo-disruption. The difference between the latter two is attributed to different energy densities.

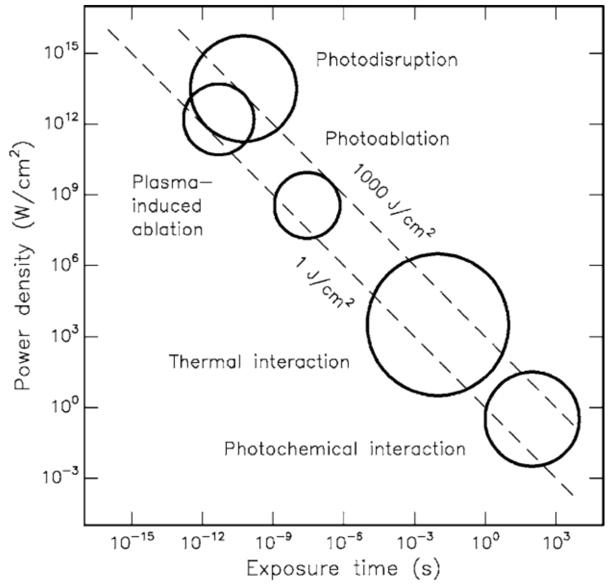

Figure 2.4 Map of laser–tissue interactions. The circles give only a rough estimate of the associated laser parameters. Modified from Boulnois (1986) (Niemz, 2013,p59).

2.2.3 Photochemical Interaction

The group of photochemical interactions stems from empirical observations that light can induce chemical effects and reactions within macromolecules or tissues. One of the most popular examples was created by evolution itself: the energy release due to photosynthesis. In the field of medical laser physics, photochemical interaction mechanisms play a significant role during photodynamic therapy (PDT). Frequently, bio stimulation is also attributed to photochemical interactions, although this is not scientifically ascertained. After a detailed description of the physical background, both of these methods will be discussed in this section.

Photochemical interactions take place at very low power densities (typically 1W/cm^2) and long exposure times ranging from seconds to continuous wave. Careful selection of laser parameters yields a radiation distribution inside the tissue that is determined by scattering. In most cases, wavelengths in the visible range (e.g. Rhodamine dye lasers at 630 nm) are used because of their efficiency and their high optical penetration depths. The latter are of importance if deeper tissue structures are to be reached **(Niemz, 2013,p60).**

Adjacent interaction types cannot always be strictly separated. As shown in the following sections, thermal effects may also play an important role during photochemical interaction. In addition, even ultra-short laser pulses with pulse durations shorter than 100 ps each of them having no thermal effect may add up to a measurable increase in temperature if applied at repetition rates higher than about 10–20 Hz, depending on the laser. However, the basic physics involved in each interaction becomes accessible if enough data are collected to fit unknown parameters (**Niemz, 2013,p60).**

The group of photochemical interactions stems from empirical observations that light can induce chemical effects and reactions within macromolecules or tissues. One of the most popular examples was created by evolution itself: the energy release due to photosynthesis. In the field of medical laser physics, photochemical interaction mechanisms play a significant role during photodynamic therapy (PDT). Frequently, bio-stimulation is also attributed to photochemical interactions, although this is not scientifically ascertained (Niemz, M.H., 2013).

Coagulation, The histological appearance of coagulated tissue is illustrated in **(Figure** 2.5 (a–b)). In one case, a sample of uterine tissue was coagulated using a CW Nd-YAG laser. In a histological section, the coagulated area can be easily detected when staining the tissue with hematoxylin and eosin. Coagulated tissue appears significantly darker than other tissue. In the second photograph, 120 pulses from an Er-YAG laser were applied to an excised cornea.

Again, the tissue were stained with hematoxylin and eosin. During the process of coagulation, temperatures reach at least 60◦C. Vaporization. Another example of an important thermal effect is shown in **(Figure** 2.6(a–b)). A tooth was exposed to 20 pulses from an Er-YAG laser. During the ablation process, complete layers of tooth substance were removed leaving stair-like structures. This observation is attributed to the existence of so called striae of Retzius which are layers with a high content of water molecules. Water strongly absorbs the Er:YAG wavelength at 2.94 μm as shown below, thus leading to vaporization within these layers. The induced increase in pressure water tries to expand in volume as it vaporizes leads to localized micro-explosions with results as demonstrated in the enlargement in Figure. 2.7b. In the literature, vaporization is sometimes also referred to as a thermo mechanical effect due to the pressure build-up involved. The resulting ablation is called thermal decomposition and must be distinguished from photo ablation. Carbonization. In (**Figure** 2.7a), a sample of skin is shown which was exposed to a CW CO2 laser for the purpose of treating metastases. In this case, however, too much energy was applied and carbonization occurred. Thus, the local temperature of the exposed tissue had been drastically increased.

At temperatures above approximately 100◦C, the tissue starts to carbonize, i.e. carbon is released, leading to a blackening in color. A similar effect is seen in (**Figure** 2.7b), where a tooth was exposed to a CW CO2 laser. For medical laser applications, carbonization should be avoided in any case, since tissue already becomes necrotic at lower temperatures. Thus, carbonization only reduces visibility during surgery, melting. Finally, (Figure 2.8a–b) shows the surface of a tooth after exposure to 100 pulses from a Ho-YAG laser. In (Figure. 2.8a), several cracks can be seen leaving the application spot radically. They originate from thermal stress induced by a local temperature gradient across the tooth surface. The edge of the interaction zone is shown in an enlargement in (Figure. 2.8b). Melted and afterwards down-cooled tooth substance as well as gas bubbles are observed similar to solidified lava. The temperature must have reached a few hundred degrees Celsius to melt the tooth substance which mainly consists of hydroxyapatite. Obviously, the pulse duration of a few microseconds is still long enough to enable a sufficient increase in temperature, since the applied repetition rate of 1 Hz is extremely low (**Niemz, 2013**, p72).

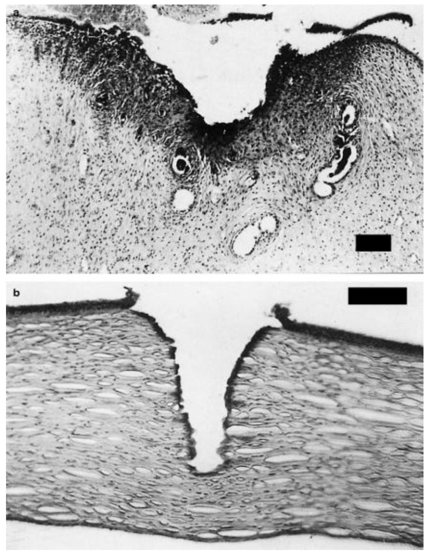

Figure 2.5 (a) Uterine tissue of a wistar rat coagulated with a CW Nd:YAG laser (power: 10W, bar: 80μm). Photograph kindly provided by Dr. Kurek (Heidelberg). (b) Human cornea coagulated with 120 pulses from an Er:YAG laser (pulse duration: 90 μs, pulse energy: 5 mJ, repetition rate: 1Hz, bar: 100 μm (Niemz, 2013,p73)

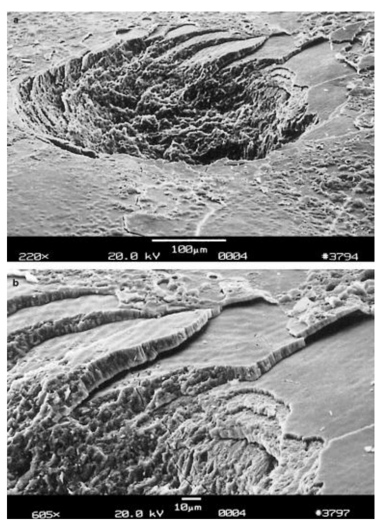

Figure 2.6. (a) Human tooth vaporized with 20 pulses from an Er:YAG laser (pulse duration: 90 μs, pulse energy: 100 mJ, repetition rate: 1Hz). (b) Enlargement showing the edge of ablation (Niemz, M.H., 2013, p74).

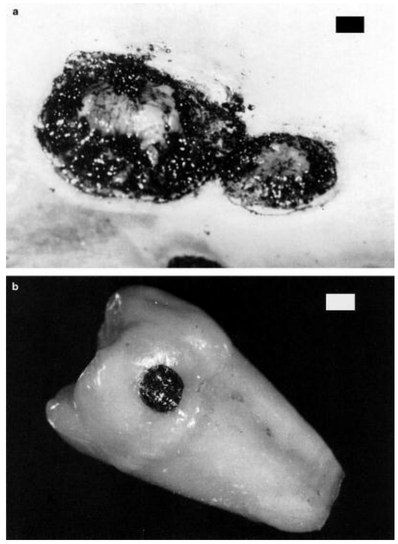

Figure 2.7 (a) Tumor Metastases on human skin carbonized with a CW CO2 laser (power: 40W, bar: 1 mm). Photograph kindly provided by Dr. Kurek (Heidelberg). (b) Human tooth carbonized with a CW CO2 laser (power: 1W, bar: 1mm) (Niemz, M.H., 2013, p74)

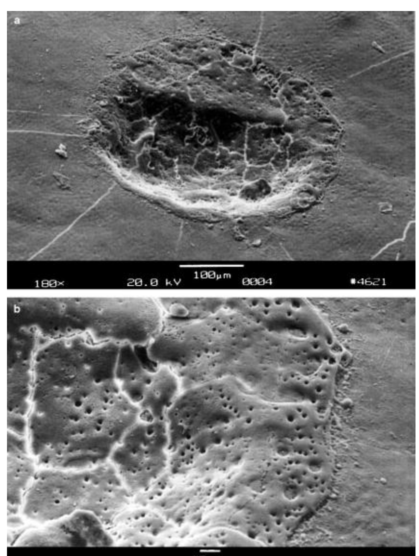

Figure 2.8 (a) Human tooth melted with 100 pulses from a Ho:YAG laser (pulse duration: 3.8 µs, pulse energy: 18mJ, repetition rate: 1 Hz). (b) Enlargement showing

the edge of the melted zone (Niemz, M.H., 2013 p76)

2.2.4 Lasers in Medicine

Lasers are extensively used in medicine and surgery. The first practical application was in eye surgery.

Perhaps the most important use of lasers in the field of medicine is in eye surgery Lasers are also expected to be used extensively in the treatment of cancer (**Thyagarajan and Ghatak, 2011**).

Lasers can also be used for correction of focusing defects of the eye. In the method referred to as LASIK (laser in situ keratomileusis), the cornea of the eye can be crafted to adjust the curvature so that the focusing by the eye lens takes place on the retina (see **Figure**. 2.10). This method can correct for eye defects requiring high lens powers and is a very popular technique.

Extensive use of lasers is anticipated in surgery, dentistry, and dermatology. For further details and other applications of lasers in medicine, (Peng et al, 2008)

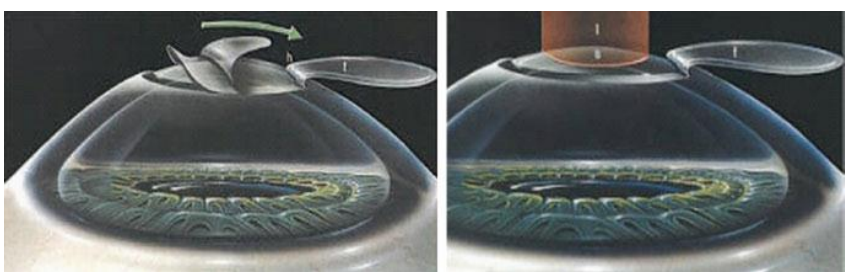

Figure 2.10 Application of lasers in LASIK (Thyagarajan, K and Ghatak, 2011, Lasers in Science. In Lasers (pp. 445-470).

2.2.4.1 Lasers in Ophthalmology

In ophthalmology, various types of lasers are being applied today for either diagnostic or therapeutic purposes. In diagnostics, lasers are advantageous if conventional incoherent light sources fail. One major diagnostic tool is co focal laser microscopy, which allows the detection of early stages of retinal alterations.

Others are, for instance, treatment of glaucoma and cataract (Niemz, 2013,p165).

The targets of all therapeutic laser treatments of the eye can be classified into front and rear segments. The front segments consist of the cornea, sclera, trabeculum, iris, and lens. The vitreous body and retina give the rear segments. A schematic illustration of a human eye is shown in **(Figure** 2.11) in the following paragraphs (Niemz, 2013).

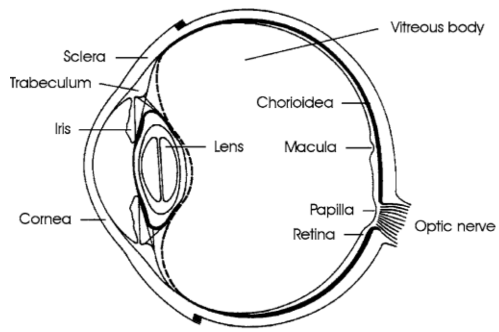

Figure 2.11 Scheme of a human eye (Niemz, M.H., 2013, Laser-tissue interactions: fundamentals and applications, p152).

2.2.4.2 Lasers in Dentistry

Although dentistry was the second medical discipline, Especially in caries therapy the most frequent dental surgery conventional mechanical drills are still superior compared to most types of lasers, particularly CW or long-pulse lasers. Only laser systems capable of providing ultra-short pulses might be an alternative to mechanical drills (Pioch et al,1994). However, many clinical studies and extensive engineering effort remain to be done in order to achieve satisfactory results (Niemz, 2013,p165).

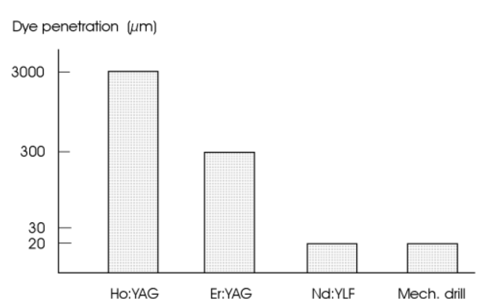

Figure 2.12. Results of dye penetration tests for three different solid-state lasers and the mechanical drill. Listed are the maximum penetration depths inside the enamel of human teeth. Pulse durations: 250 µs (Ho:YAG), 90µs (Er:YAG), and 30 ps (Nd:YLF) (Niemz, 2013, p199)

2.2.4.3 Lasers in Gynecology

Beside ophthalmology, gynecology is one of the most significant disciplines for laser applications. This is mainly due to the high success rate of about 93–97% in treating cervical intraepithelial neoplasia (CIN), i.e. uncommon growth of new cervical tissue, with the CO2 laser. CIN is the most frequent alteration of the cervix and should be treated as soon as possible. Otherwise, cervical cancer is very likely to develop. The cervix represents the connective channel between the vagina and uterus (**Niemz, 2013p214)**.

The CO_2 laser is the standard laser in gynecology. Beside treating CIN, it is applied in vulvar intraepithelial neoplasia (VIN) and vaginal intraepithelial neoplasia (VAIN). Depending on the type of treatment, CO_2 lasers can be operated in three different modes CW radiation, chopped pulse, and super pulse – as shown in (Figure 2.13). Chopped pulses with durations in the millisecond range are obtained from CW lasers when using rotating apertures. Super pulses are achieved by modulation of the high voltage discharge (**Niemz, 2013, p214)**.

Thereby, pulse durations less than 1 ms can be generated. The peak power is inversely related to the pulse duration. The mean powers of CW radiation and chopped pulses are nearly the same, whereas it decreases in the case of super pulses. Shorter pulse durations are associated with a reduction of thermal effects. Hence, by choosing an appropriate mode of the laser, the best surgical result can be obtained. (Niemz, 2013.p214)

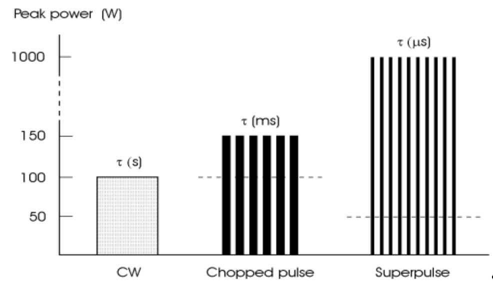

Figure 2.13 CW, chopped pulse, and super pulse modes of a CO2 laser. Dashed lines denote mean powers (Niemz, 2013p215).

2.2.4.4 Lasers in Urology

The workhorse lasers of urology are primarily CO2, argon ion, (Nd-YAG), and dye lasers. CO2 lasers are best in precise cutting of tissue. Argon ion lasers and (Nd-YAG) lasers are used for the coagulation of highly vascularized tumors or malformations (Niemz, 2013. P220)

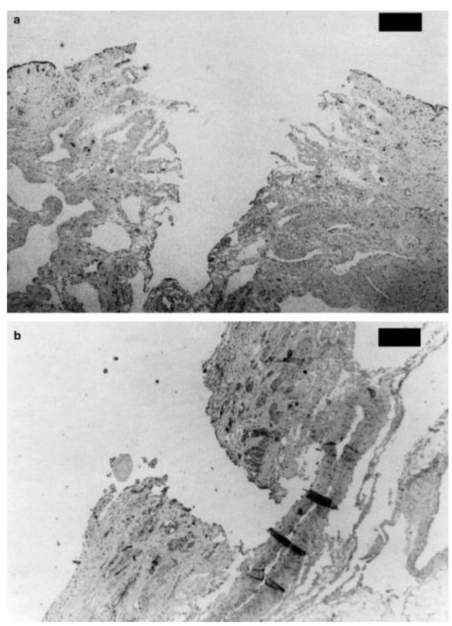

Figure 2.14 (a) Effect of thirty pulses from a Ho:YAG laser (pulse duration: 1ms, pulse energy: 370 mJ, bar: 250 μm) on the urethra. (b) Effect of thirty pulses from a Ho:YAG laser (pulse duration: 1ms, pulse energy: 370 mJ, bar: 250 μm) on the ureter. Photographs kindly provided by Dr. Nicolai (Regensburg)(Niemz, 2013, pp222).

2.2.4.5 Lasers in Dermatology

In dermatology, thermal effects of laser radiation are commonly used, especially coagulation and vaporization. Since the optical parameters of skin, i.e. absorption and scattering, are strongly wavelength-dependent, various kinds of tissue reaction can be evoked by different laser systems. In clinical practice, mainly five types of lasers are currently being used: argon ion lasers, dye lasers, CO2 lasers, Nd:YAG lasers, and ruby lasers (Niemz, 2013p240).

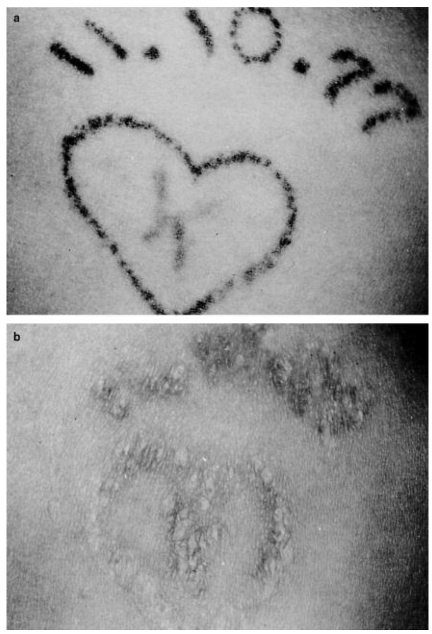

Figure 2.15(a) Preoperative state of a tattoo, (b) Postoperative state of the same tattoo after six complete treatments with an argon ion laser (pulse duration: 0.3 s, power: 3W, focal spot size: 0.5 mm). Photographs kindly provided by Dr. Seipp (Darmstadt) (Niemz, 2013,p244).

2.2.4.6 Lasers in Gastroenterology

Gastrointestinal diseases primarily include ulcers and tumors of the esophagus, stomach, liver, gallbladder, and intestine. The intestine further consists of the jejunum, ileum, colon, and rectum. According to the position of these organs, the gastrointestinal tract is sub divided into an upper and a lower tract. Most intestinal tumors are reported to occur inside the colon or the rectum.

In general, any kind of ulcer or tumor can be treated with lasers if it is Accessible with endoscopic surgery.

Gastroenterology is one of the major domains of the CW Nd:YAG laser.

Only in photodynamic therapy is dye lasers applied. There exist mainly two indications for laser therapy: gastrointestinal hemorrhages and benign, malignant, or nonneoplastic stenoses (Niemz, M.H., 2013,p250).

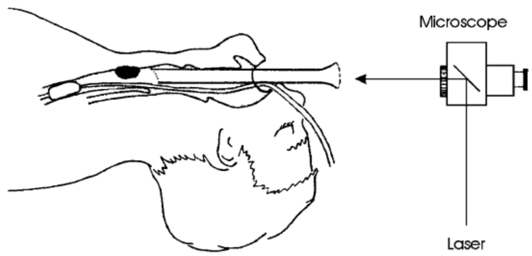

Figure 2.16 Direct laryngoscopy with simultaneous intubation. Reproduced from Boenninghaus (1980) by permission. _c 1980 Springer-Verlag

We have mentioned some medical applications, for example, not limited to, and those who want to use more can refer to the reference (Niemz, 2013, Laser-tissue interaction fundamentals and applications).

2.3 Nigella Sativa Oil

Figure 2.3 (A) Nigella Sativa (B) Nigella Sativa size

2.3.1 Background

Nigella Sativa L. (Family: Ranunculaceae; commonly known as N. Sativa is an annual herb possessing a wide range of medicinal uses **(Chopra,** et al 1982; (Kirtikar, et al 1982) not withstanding its commercial significance as a spice yielding plant (Pruthi, 1976). Black cumin seeds are most revered (Holy herb of the Middle East (Yarnell and Abascal, 2011). Effective utilization of N. sativa for therapeutic purposes as well as for trade will vastly depend upon yield (raw plant product- seeds; bioactive compounds- essential oil) and its quality. Existing germplasms may not substantiate the need for future, if not, at present. Therefore, it is of utmost essentiality to raise desirable plant type(s) in N. sativa through induced genetic variations and efficient breeding Endeavour. Considering nearly all essential aspects of N. sativa, a monograph is conducted with the laid formulation of WHO as well as with other significant parameters which will provide unabridged repository of references for present and future researchers who are looking to eugenize the species as a „potential medicinal herb‟ for human benefits.

2.3.2 Common Names

English: fennel flower, nutmeg flower, Roman coriander, black seed or black caraway, black sesame; India: Assamese - kaljeera or kolajeera, Bengali - kalo jeeray, Kannada – Krishna Jeerige, Tamil - karum jeerakam, Hindi/Urdu - kalaunji/man grail; Russian: Chernushka; Hebrew: Ketzakh; Turkish: çörek out; Arabic: habbat al-barakah; Persian: siyâh Dane; Indonesian: jintan hit am; Bosnian: čurekot (Paarakh, 2010). French: nigelle de Crète, toute épice; Germany: Schwarzkümmel; Portuguese: cominho-negro; Spanish: ajenuz, arañuel; Swedish: svartkummin (Naz, 2011). .

Host to over one hundred different vitamins, minerals, essential fatty acids and other constituents, the precious seeds and oil of black cumin (Nigella sativa) have been used both internally and topically since ancient times. Occasionally referred to as organic N. Sativa oil, the long folk history of organic organic N. Sativa oil caught the attention of the natural health, beauty and personal care industries, and they continue to study and document its wide array of impressive nutritive properties and applications.

2.3.3 The seed oil contains

cholesterol, camp sterol, stigma sterol, β-sit sterol, α-spinasterol, (+)-citronellol, (+)-

limonene, p-cymene, citronellyl acetate, carvone (Djilanim and Dicko, 2011).**,**nigellone, arachidic, linoleniclinolenic, myristic, oleic, palm tic, palmitoleic and stearic acids. Fixed oil: linolenic acid (55.6%), oleic acid (23.4%) and palm tic acid (12.5%). Volatile oil: trans-anethole (38.3%), p-cymene (14.8%), limonene (4.3%), and carvone (4.0%) (Tariq, 2008). , methoxypropyl)-5-methyl-1, 4-benzenediol, thymol and carvacrol (**Nadkarni,** 1976). Root and shoot are reported to contain vanillicacid **(Warrier,** et al 1996).

2.3.4 Organic Nigella Sativa Oil Properties

• Stable Shelf Life

• Golden Yellow to Golden Brown Color

• Moderate Viscosity

• Quick Absorption

• Peppery Aroma

• Anti-oxidant

• Anti-inflammatory

• Anti-bacterial

• Anti-fungal

• Supports Immune System

• Supports Wound Healing

• Vitamin Composition Includes Vitamins A, B1, B2, B6, C, Niacin and Folacin

• Mineral Composition Includes Calcium, Potassium, Iron,
Magnesium, Selenium, Copper, Phosphorus and Zinc

• Linoleic (Omega-6) Essential Fatty Acid Content: 52-67%

• Oleic (Omega-9) Fatty Acid Content: (17-26)%

• Linolenic (Omega-3) Essential Fatty Acid Content: 0.1-1%(Natural sourcing ,LLC.341,Christian Street,Oxford CT06478).

2.3.5 Uses

Organic Black Cumin Seed Oil is anti-inflammatory, anti-bacterial and anti-fungal and it helps support wound healing. Highly regarded for its ability to support the immune system, documented studies confirm that Organic Black Cumin Seed Oil has also been helpful in aiding damaged and problem skin.

Organic Black Cumin Seed Oil absorbs quickly and is highly recommended for inclusion in moisturizers and hair care products where emolliency is desired (Mohammad, A.J., 2013).

2.3.6 Therapeutic Uses

Traditional Uses: In traditional system of medicine black cumin seeds are effective against cough, bronchitis, asthma, chronic headache, migraine, dizziness, chest congestion, dysmenorrheal, obesity, diabetes, paralysis, hemiplegic, back pain, infection, inflammation, rheumatism, hypertension, and gastrointestinal problems such as dyspepsia, flatulence, dysentery, and diarrhea**.** It has also been used as a stimulant, diuretic, emmenagogue, lactagogue, anthelmintic and carminative as well as it is applied to abscesses, nasal ulcers, orchitis, eczema and swollen joints. Seed oil is considered to be local anesthetic. (Salem, 2005; Isik et al, 2005; Raval et al, 2010;Büyüköztürk et al, 2005).

2.3.6.1 Antibacterial Effect

The isolated saponin compounds from N. sativa (seeds) showed significant inhibiting effect on the growth of some bacteria, which include: Staphylococcus aurous, Bacillus subtilis, Salmonella typhi, Klebsiella pneumonia, Proteus vulgaris and Pseudomonas aeruginosa.(Mohammed, 2009).

2.3.6.2 Antifungal Effect

It was found that methanolic extract of black seeds exhibits potent inhibition of fungus growth against Candida Parapsilosis, and Issatchenkia Orientale'swith IC50 Value 4.846 µ g/ml, and 6.795µg/ml, respectively and ethanolic extract also shows significant anti-fungal activity against fungus strain Issatchenkia Orientalwith IC50 value 5.805 µg/ml. (Raval et al, 2010).

2.3.6.3 Ant parasitic Effect

It was revealed that the water extract of N. sativa L. seeds effect against trophozoites isolated from chronic and acute cases of Entamoeba histolyticin Baquba General Hospital, Diyala. (Hassan et al, 2009).

2.3.6.4 Anticancer Effect

N. sativa seed, its oil and extracts and some of its active principles, particularly thymoquinone and alpha-hederin, possess remarkable in vitro and in vivo activities against a large variety of cancers (Randhawaand Alghamdi; 2011).

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2.3.6.5 Anti- hepatotoxicity

The role of N. sativa was investigated in the prevention of carbon tetrachloride (CCl_4) -induced liver toxicity, their results indicated that its' oil decreased significantly the elevated serum levels of liver enzymes and improve the state of oxidative stress induced by CCl_4 (Ahmed, 2010). . Similar study confirm the protective role of vitamin E and flavonoids of N. sativa seed against hepatic dysfunction caused by sodium nitrate manifested by structural and functional change (AL-Oaklyn et al; 2012) . Another study confirmed that the black seeds have protective effect of against $AlCl_3$ induced toxicity in rabbits (Mohammed, 2010).

2.3.6.6 Anti- Diabetic

N. sativa seeds were used as an adjuvant therapy in patients with diabetes mellitus type two added to their anti-diabetic medications. A dose of 2 gm/day of N. sativa might be a beneficial adjuvant to oral hypoglycemic agents (reductions in fasting blood glucose [FBG], blood glucose level two hours postprandial [2hPG], and glcosylated hemoglobin [HbA1]) in type 2 diabetic patients (Bamosa et al, 2010).

2.3.6.7 Hypocholesterolemic and antiatherogeniccardio protective properties

N. sativa produces anti-atherogenic effect by decreasing low-density lipoprotein cholesterol level significantly **(**Bhatti et al, 2009; Hussein, 2012). Serum triglycerides, total and (LDL) cholesterol decreased significantly after treatment with 750 mg of powdered grains of N. sativa enclosed in a capsule twice daily for 28 days, While (HDL) cholesterol increased significantly (Qibi et al, 2006). Similar results revealed that N. sativa oil decreased he levels of total cholesterol , triglycerides, phospholipids ,(LDL) cholesterol and uric acid (AL-Hindi et al, 2006). N. sativa either in powder or oil forms was shown to significantly reduce total cholesterol (TC) and low-density lipoprotein cholesterol (LDL) levels and enhance high-density lipoprotein cholesterol (HDL) levels after treatment for (2, 4, 6) and 8 weeks compared to the positive control group (Al-Naqeep et al, 2011) .

2.3.6.8 Effects on Reproduction

The administration of 1ml/kg/day of N.sativa oil stimulated the secretion of sexual hormones that led to improve protein synthesis of hepatic enzymes, white blood cells count and decrease the serum cholesterol concentration in blood (Jumna et al, 2011)**.**

2.3.6.9 Effect on immunity

Treatment of typhoid-antigen-challenged rat with the volatile oil revealed an immunosuppressant action as evidenced by the significant decreases in the antibody titer and the splenocytes and neutrophils counts (El-Tahir et al, 2006) **.**

2.3.6.10 Impact on the gastrointestinal system

Black cumin seed has been widely used as gastrointestinal disorders. The aqueous extract of the seeds was reported to exhibit anti-ulcer activity by decreasing the volume of acid in gastric juice in acetylsalicylic acid treated rats treated rat (Akhtar et al, 1996). The volatile oil and ethanolic extract inhibited spontaneous movements of the rabbit jejunum as well as agonist induced contractions and the spasmolytic effect involved calcium channel blockade (Al-Hader et al, 1993)**.**

2.3.6.11 Others

Black seeds act as analgesic, anti-inflammatory action, anti-asthmatic, antihistaminic, anti-allergic, antihypertensive, antihypertensive and anti-oxidant **(**Mohammed, M.J.(2009). Biological Effect of Saponins Isolated from Nigella sativa (seeds) on Growth of Some Bacteria. Tikrit Journal of Pure Science (El-Tahir et al, 2006;Randhawa, 2008; Parvardeh et al ,2003;Naz, 2011).

2.3.6.12 Ingredients

Medicinal Ingredients

a. Black Seed Oil (Nigella sativa, seed) 2,000 mg
b. Providing:50% Linoleic Acid and 25% Oleic Acid
c. Vitamin E (mixed Tocopherol Concentrate) 45.5 mg
d. Alpha Tocopherol 25 IU16.75 mg
e. Gamma Tocopherol 20.5 mg
f. Delta Tocopherol 7.65 mg

There are no other ingredients added to this formula.

Directions: Adults: Take a half teaspoon (2.5 ml) once daily with food, diluted in water or juice, or as directed by a health care practitioner

Chapter Three

Experimental Part

3.1 Introduction

This chapter contains the material and apparatus that used in this study with description if the physical and chemical properties of the samples measured with different techniques such as UV spectrometer, GC/MS.

3.2 Materials and Apparatus

3.2.1 Cumin (Nigella Sativa) Oil

Sample of black seed oil were taken from Saudi companies, Hemani black seed oil (100% natural pomegranate seed), *i.e.* natural oils, free of chemicals and preservatives, extracted anew black seed ensure that active substances do not volatilize figure 3.1.

Figure 3.1 Cumin Oil Sample

3.2.2 He-Ne Laser

The (He-Ne) laser is certainly the most important of the noble gas lasers. Laser action is obtained from transitions of the neon atom, while helium is added to the gas mixture to greatly facilitate the pumping process The laser oscillates on many wavelengths; by far the most popular is λ = 633 nm (red). Other wavelengths include green (543 nm) and infrared, at λ = 1.15□□ and λ = 3.39□□. The (He-Ne) laser, oscillating on its λ = 1.15□□ transition, was the first gas laser and the first CW laser to be operated.

In this experiment, we used a helium neon laser with the following specification:

Model: KX= 350-1B

Laser wavelength: 632nm

Laser output power≥6mw

Voltage: AC = (85-26)5v γ= (50-60) Hz

Rated power: 20w

Manufacture NO: Made in China

Figure 3.2 He-Ne Laser

3.2.3 UV-Vis Spectrometer

Is a device used for spectral analysis in the areas of visible and ultraviolet radiation and consist of four main parts:

(Light source-sample cell-uniform wave length-screen), and covers the wave length area of (200-800) nm

In this research, we used UV-Vis spectrometer model (1240) mini from the company Shimadzui-Japan (2002).

The UV mini comes standard with a Spectrum mode that allows for full spectral data acquisition over the wavelength range of 190nm to 1100nm

The UV mini provides flexible print options with the use of either a thermal screen copy printer or a variety of PC printers. The screen copy printer enables instant printing of tabulated data as well as copied information directly from the screen. The PC printers can be utilized for and for finer print resolution of spectral data.

Figure 3.3 UV-Vis mini 1240 spectrophotometer.

3.2.4 Gas Chromatography / Mass Spectrometer

Gas Chromatography Mass Spectrometry (GC-MS) is an instrumental technique, comprising a gas chromatograph (GC) coupled to a mass spectrometer (MS), by which complex mixtures of chemicals may be separated, identified and quantified. This makes it ideal for the analysis of the hundreds of relatively low molecular weight compounds found in environmental materials. In order for a compound to be analyzed by GC/MS it must be sufficiently volatile and thermally stable.

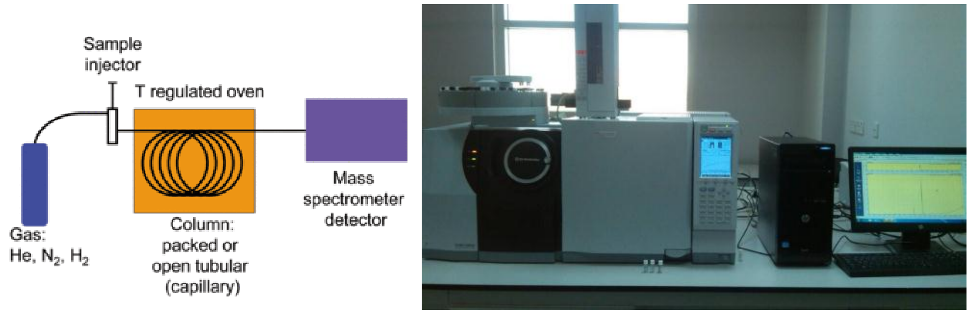

Figure 3.4 Gas Chromatography /MS

3.2.5 USB Detector

The Detector used in this work was USB2000 Spectrometer. The Ocean Optics USB2000 Spectrometer include an advanced detector and powerful high-speed electronics to provide both an unusually high spectral response and high optical resolution in a single package the result is a compact, flexible system, with no moving parts, that's easily integrated as an OEM component.

The detector used in the USB2000 spectrometer is a high-sensitivity 2048-element CCD array from Sony, product number ILX511.

The USB2000 has a 10 pin external interface to easily integrate with Ocean Optics other modular components to for an entire system

Figure 3.5 USB2000 Spectrometer.

Ocean Optics permanently secures all components in the USB2000 at the time of manufacture. Figure 3.5 shows The USB2000 Spectrometer Component.

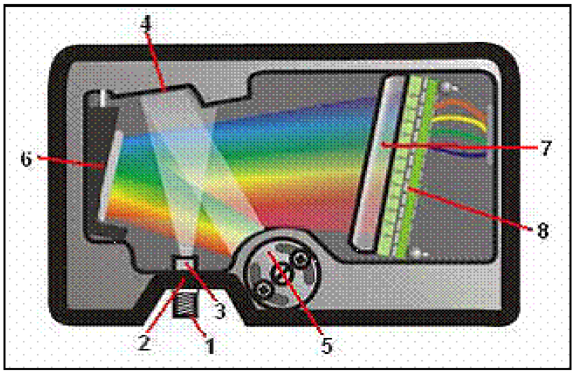

Figure 3.6 USB4000 Spectrometer Components.

1- **SMA Connector:** the SMA Connector secures the input fiber to the spectrometer. Light from the input fiber enters the optical bench through this connector.

2- **Slit:** The Slit is a dark piece of material containing a rectangular aperture, which is mounted directly behind the SMA Connector. The size of the aperture regulates the amount of light that enters the optical bench and controls spectral resolution.

3- **Filter:** The Filter is a device that restricts optical radiation to pre-determined wave length regions. Light passes through the Filter before entering the optical bench.

4- **Collimating Mirror:** The Collimating Mirror focuses light entering the optical bench towards the Grating of the spectrometer.

5- **Grating:** The Grating diffracts light from the Collimating Mirror and directs the diffracted light onto the Focusing Mirror. Gratings are available in different groove densities, allowing you to specify wavelength coverage and resolution in the spectrometer.

6- **Focusing Mirror:** The Focusing Mirror receives light reflected from the Grating and focuses the light on to the CCD Detector or L2 Detector Collection Lens (depending on the spectrometer configuration).

7- L2 Detector Collection Lens: The L2 Detector Collection Lens (optional) attaches to the CCD Detector. It focuses light from a tall slit onto the shorter CCD Detector elements.

8- CCD Detector (UV or VIS): The CCD Detector collects the light received from the Focusing Mirror or L2Detector Collection Lens and converts the optical signal to a digital signal.

3.3 Method

3.3.1 Optical Absorbance Measurement

In this study that some different physical parameters such as wavelength, absorption and then the study of any wavelengths is absorbed N.Sativa oil.

The N.Sativa oil was placed in a transparent plastic tube, then placed inside the UV-Vis spectrometer device and the oil was then calculated, and then taken for reading.

3.3.2 Photoluminescence Process

In this method, the sample was placed in a transparent plastic between two sensors to amplify the emitted light and then excited it with (He-Ne) laser beam. The emissions were the received using a USB detector and recording the results.

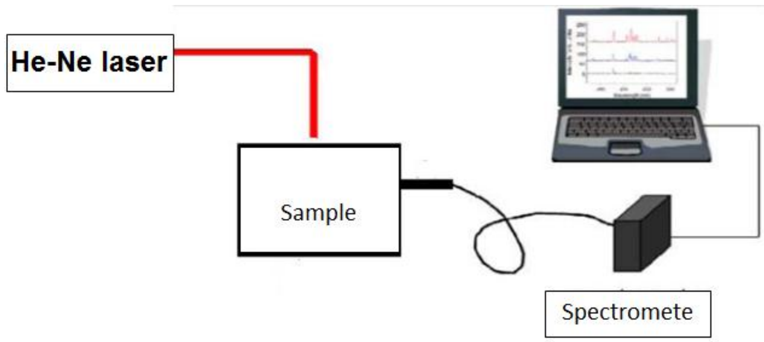

Figure 3.7 Photoluminescence Setup

Chapter Four

Results and Discussion

4.1 Introduction

This chapter contains the results (figures and tables) of sample survey using GC/MS, oil absorption using by UV-Vis spectrometer and emission induced by helium neon laser (632.8 nm). In addition to results the discussion, conclusion and the list ofreferences.

4.2 Results and Discussion

4.2.1 Optical Absorbance

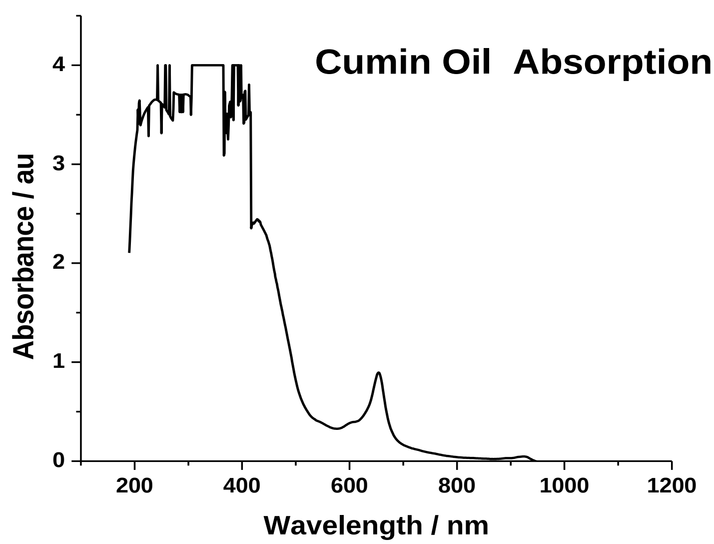

Figure 4.1 N. Sativa Oil Absorption

Figure 4.1 shows absorption of N. Sativa oil used by UV-Vis spectrometer, it was found that the absorption spectrum ranged from 600 to 680 nm.

4.2.2 Photoluminescence

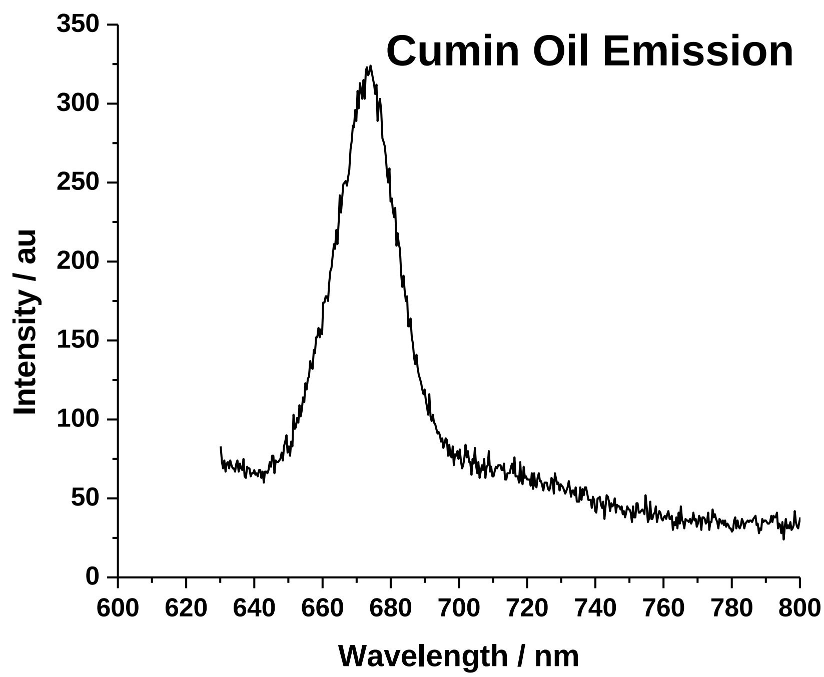

Figure 4.2 N. Sativa Oil Emission excited by He-Ne Laser (632.8 nm)

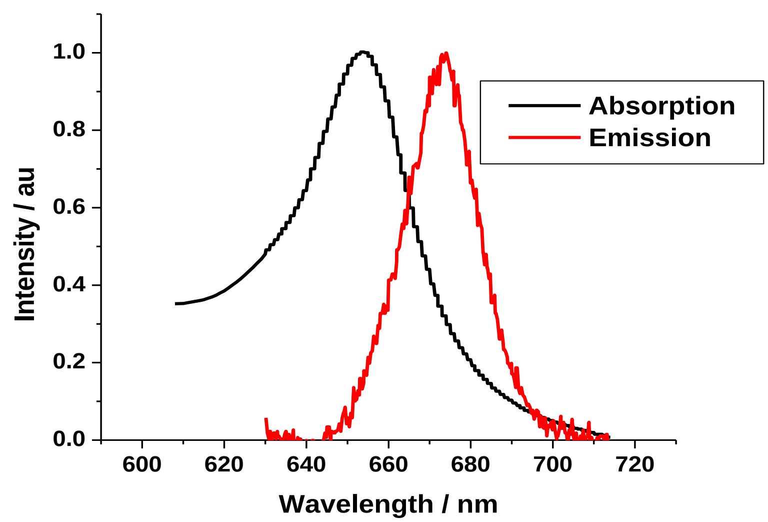

Figure 4.3 N. Sativa Oil Absorption and Emission

Figure 4.3 shows the absorption and emission of cumin oil, it was found that the absorption spectrum ranged from 600 nm to 680 nm, while the emission spectrum ranged from 650 nm to 700 nm.

The fluorescence quantum yield of a fluorophore is the ratio of fluorescence photons emitted to photons absorbed.

The fluorescence quantum yield is an intrinsic property of a fluorophore and is important for the characterization of novel fluorescent probes. The fluorescence quantum yield is the ratio of photons absorbed to photons emitted through fluorescence. The quantum yield Q can also be described by the relative rates of the radiative kr and non-radiative knr relaxation pathways, which deactivate the excited state.

The fluorescence quantum yield= Number of photons emitted / Number of photons absorbed

=7858.7/1230.5

= 6.38659.

The difference between positions of the band maxima of absorption and fluorescence of the same electronic transition is known as Stokes shift. Stokes shift is important for practical applications of fluorescence because it allows separating strong excitation light from weak emitted fluorescence using appropriate optics.

The importance of the Stokes shift is not only a practical one, but it can also give you insight on what happens to the fluorescing system in the excited state. Small stokes shift means small geometrical relaxation and small solvation effects once the chromophore is excited. Large stokes shift means large geometrical relaxation and/or solvation effects.

The stokes shifts = the spectrum emission maxima – the spectrum absorption maxima

= 674.54 – 653.99

= 20.55 nm.

The range 650 nm to 700 nm includes the laser wavelengths that used to treat some diseases such as 66o nm and 670 nm which used treat to wound healing and wrinkles and 690 nm used in PDT (Mang,2004)(Desmet et al, 2006)(Nam et al, 2017).

4.2.3 Chemical Analysis

Table 4.2 GC/MS analysis of N. Sativa oil

S. No.	RT	Name of the compound	Molecular Formula	Area %	Molecular Weight
1	8.127	Butylated Hydroxytoluene(phenol)	$C_{15}H_{24}O$	0.77	220
2	10.856	Tetradecanoic acid(Myristic acid),Isopropyl ester	$C_{17}H_{34}O_2$	0.52	270
3	11.877	Hexadecanoic acid(palmitic acid),methyl ester	$C_{17}H_{34}O_2$	8.15	270
4	13.587	9-12Octadecadienoic(Linoleic acid),methyl ester	$C_{19}H_{34}O_2$	39.24	294
5	13.633	9-Octadecenoic acid(Oleic acid),methyl ester	$C_{19}H_{36}O_2$	48.16	296
6	13.857	Methyl stearate(Stearic acid),methyl ester	$C_{19}H_{38}O_2$	1.89	298
7	15.479	Oxacycloheptadec-8-en-2-one,(8Z),Musk ambrette	$C_{16}H_{28}O_2$	1.28	252

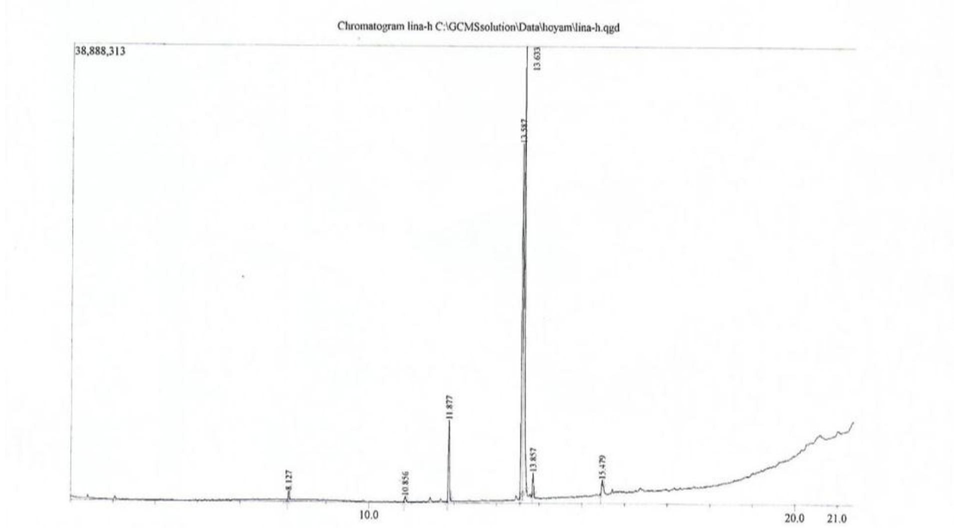

Figure 4.4 GC/MS analysis compounds of N. Sativa oil

Table 4.3 GC/MS analysis of N. Sativa oil

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Nar
1	8.127	8.100	8.158	1182646	0.77	752318	0.95	1.57	MI	
2	10.856	10.825	10.892	799092	0.52	443958	0.56	1.80	MI	
3	11.877	11.842	11.925	12521284	8.15	6898187	8.75	1.82	MI	
4	13.587	13.550	13.608	60316905	39.24	30051448	38.13	2.01	MI	
5	13.633	13.608	13.708	74020404	48.16	37875190	48.06	1.95	MI	
6	13.857	13.833	13.883	2903608	1.89	1860273	2.36	1.56	MI	
7	15.479	15.442	15.517	1960940	1.28	923218	1.17	2.12	MI	
				153704879	100.00	78804592	100.00			

The compounds were identified through mass spectrometry attached with GC with respect to their peak area and retention time. Totally 7 compounds were identified namely butylated hydroxytoluene (phenol) (0.77%),tetradecanoic acid (Myristic acid) (0.52%),Isopropyl ester,hexadecanoic acid (palmitic acid) (8.15%),methyl ester,9-octadecenoic acid (Elaidic acid) (48.16%), 9-12Octadecadienoic, methyl ester (39.24%), methyl stearate (Stearic acid) (1.89%), methyl ester,oxacycloheptadec-8-en-2-one,(8Z), musk ambrette (1.28%), (these compounds are very important in medical application as we mentioned earlier), besides there is a new compound not previously reported: (oxacycloheptadec-8-en-2-one, (8Z)), musk ambrette.

Among the seven compounds, major peak values were obtained for the compounds such as 9-octadecenoic acid (Elaidic acid) (48.16%), 9-12octadecadienoic, methyl ester (39.24%), hexadecanoic acid (palmitic acid) (8.15%).

Table 4.4: GC-MS analysis of N. Sativa oil based on Dr. Duke's ethnobotanical and phytochemistry database

S. No	RT	Name of the Compound	Compound Nature	Activity
1	10.856	Tetradecanoic acid	Myristic acid	Antioxidant, Lubricant, Hypercholesterolemic, Cancer-Preventive, Cosmetic
2	13.587	9,12-Octadecadienoic acid,(z,z)	Linoleic acid	Antiinflammatory, Nematicide, Insectifuge, Hypocholesterolemic,Cancer preventive,Hepatoprotective, Antihistaminic, Antiacne, Antiarthritic, Antieczemic
3	11.877	Hexadecanoic acid methyl ester	Fatty acid ester	Antioxidant ,Hypocholesterolemic,Nematicide, pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5- Alpha reductase inhibitor
4	13.633	Oleic Acid	Palmitic acid	Monoacylglycerol, Antioxidant, antiatheroselerotic and protein glycation inhibitory activities

Conclusion

From this study, it could be concluded that Cumin oil can give fluorescents by excited with He-Ne laser in the range of 650 nm to 700 nm and this could be used in a liquid laser system to treat some diseases. Cumin oil also contains various bioactive compounds such as antioxidant, anti-inflammatory, pesticide, cancer preventive, insectifuge, hypocholesterolemic, antiarthritic, anti-inflammatory, Cosmetic, or protein glycation inhibitory activities. The presence of these bioactive compounds justifies the use of the oil for various ailments by traditional practitioners.

Future studies

Based on the results obtained in this study, its suggest that there will be a good medical applications of **N. Sativa** oil such as antioxidant, anti-inflammatory, pesticide, cancer preventive, insectifuge, hypocholesterolemic, antiarthritic, anti-inflammatory, Cosmetic, or protein glycation inhibitory activities.

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