PHYSICAL EFFECTS ON THE INTERACTIONS OF CO_2 LASER ON THE TEETH

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KESAN FIZIKAL INTERAKSI LASER CO_2 KE ATAS GIGI

ABSTRAK

Dewasa ini, kajian telah membuktikan bahawa laser CO_2 berketumpatan tenaga rendah berjaya digunakan untuk meleburkan enamel, dentin, dan apatit. Dalam kajian ini, empat puluh sembilan sampel dari jenis gigi manusia yang berbeza yang telah dicabut telah disinari dengan laser CO_2 gelombang selanjar berkuasa rendah. Jisim, ketumpatan, dan ketebalan setiap jenis gigi telah direkodkan sebelum dan selepas penyinaran. Ketumpatan dan ketebalan sampel itu masing-masing telah diukur menggunakan piknometer gas dan spektroskopi gama dengan sumber Americium-241. Sebatian-sebatian kimia gigi ditentukan menggunakan Spektroskopi inframerah jelmaan Fourier (FTIR), garis pusat penembusan dengan mikroskop biologi, dan kedalaman penembusan telah dikira dengan pembezaan ketebalan sebelum dan selepas penyinaran. Keputusan menunjukkan garis pusat penembusan itu bertambah dengan kuasa laser kerana penyatuan dan peleburan hablur-hablur enamel selepas penyinaran, menjejaskan permukaan enamel. Apabila kuasa laser dinaikkan, kedalaman penembusan laser itu meningkat beransur-ansur tetapi tak seragam. Bila kuasa laser melebihi 9 W digunakan, abu terbentuk di atas permukaan gigi dan ini mengurangkan penembusan laser selanjutnya. Kuasa laser melebihi 18 W, bagaimanapun, akan merosakkan gigi. Jisim gigi selepas penyinaran adalah kurang daripada sebelum penyinaran, berdasarkan fakta bahawa pembakaran berlaku apabila gigi telah disinari dengan laser CO_2 dan menyebabkan sebatian berhablur menjadi cair pada suhu tinggi. Bagaimanapun, keputusan

yang diperolehi menunjukkan ketumpatan sesetengah gigi meningkat selepas penyinaran. Keadaan ini berlaku disebabkan oleh faktor-faktor lain seperti suhu kerana ketumpatan bergantung terhadap suhu dan isipadu satu bahan boleh berubah dengan perubahan suhu dan ianya juga disebabkan oleh kehadiran air. Sebatian-sebatian kimia utama gigi yang ditentukan selepas penyinaran menunjukkan kuasa laser yang tinggi mempunyai peratus kepantulan lebih tinggi kerana kebergantungan suhu terhadap pemalar kadar, dan oleh itu, kadar tindak balas kimia, boleh dijelaskan menggunakan persamaan Arrhenius.

PHYSICAL EFFECTS ON THE INTERACTIONS OF CO_2 LASER ON THE TEETH

ABSTRACT

Recent studies have shown that CO_2 lasers can successfully be used at low-power densities to fuse enamel, dentin, and apatite. In this study, forty nine samples of different types of extracted human teeth were irradiated under low power continuous wave CO_2 laser. The mass, density, and thickness of each type of teeth were recorded before and after irradiation. Density and thickness of the sample was measured using gas pycnometer and gamma spectroscopy with Americium-241 source, respectively. Chemical compounds of teeth were determined using Fourier Transform Infrared Spectroscopy (FTIR), diameter of penetration with biological microscope, and the penetration depth was calculated by differentiation of thickness before and after irradiation. Results show that the diameter of penetration increased with laser power because fusing and melting of enamel crystals after the irradiation, affected the enamel surface. As the laser power is increased, the depth of laser penetration gradually increased but nonuniform. When laser power above 9 W is used, ash was formed on the surface of the teeth and this further reduced the laser penetration. Laser power above 18 W, however, will damage the teeth. The mass of teeth after irradiation is less than before irradiation due to the fact that combustion occurred when the teeth were irradiated with CO_2 laser and caused the crystalline compounds melting at high temperature. The results obtained, however, indicate that the densities of some teeth increased after irradiation. This situation occurs due to other factors such as temperature since density

depends on temperature and the volume of a substance can change with temperature and also because the present of water. The main chemical compounds of teeth identified after irradiation showed that higher laser power has a higher percentage of reflectance because the temperature dependence of the rate constant, and therefore, rate of chemical reaction, which can be explained using Arrhenius equation.

CHAPTER 1

INTRODUCTION

1.1 Background

Teeth are hard tissue and were embedded inside the bone of the jaws of vertebrates. It performed the primary function of mastication. Humans and most other mammals have temporary set of teeth, the primary teeth. They usually erupt between the 6th and 24th months of age for humans. It comprises of two central incisors, two lateral incisors, two canines, and four premolars in each jaw. The preliminary teeth begin to be shed as the permanent set replaces them about six years of age. The last of the permanent teeth or wisdom teeth may not appear until the 25th year, and in some persons do not erupt at all. The permanent teeth generally have a 32 in all. They included four incisors, two canines, four incisors, and four (or six, if wisdom teeth develop) molars in each jaw. Canines are the smallest teeth.

The teeth have a lot of problems such as tooth decay or cavities, dental plaque, stained or discoloured and teeth sensitivity. Tooth decay or cavities is one of the most common human diseases. Dental caries formed because loss of minerals from the tooth enamel due to the action of acids produced by dental plaque. In pits on the chewing surfaces of the back teeth, in between teeth, and near the gumline or at the unprotected root are most likely to develop cavities if it is exposed by gum recession. Tooth decay can destroy the tooth through the enamel to the dentin and down to the pulp of the tooth if they did not get early treatment. Factors as nutrition habits, quality of oral hygiene, dry mouth problems, and presence of fluoride in water or toothpaste and heredity could be played a significant role to cause tooth decay.

Dental plaque is a sticky, soft and colourless film of bacteria that constantly builds up on the surfaces of teeth and gums. Tooth plaque bacteria from the mouth, can lead to tooth decay and dental cavities, or periodontal problems (such as gingivitis and periodontitis). Meanwhile, stained or discoloured teeth make a lot of people to feel uncomfortable in talking or smiling, due to the colour of their teeth and it is a very common dental problem. This problem occurs because of the acquisition of colored substances onto the tooth pellicle and this situation caused a yellow teeth lost their natural white color. Human teeth naturally vary in color. Slightly yellow teeth are normal for most people. Tooth enamel is the underlying dentin's color that actually determines the tooth color because it is more or less translucent. Normal variations in dentin structure can affect to the color of the reflected light, resulting in not perfectly white teeth. This problem requires teeth whitening treatment. Teeth sensitivity problem or known as dentinal hypersensitivity occur in condition when a cold or hot, sweet or sour sting stimulus in the mouth or even regular brushing causes an intense pain to some teeth.

Different types of laser have been used in dentistry to solve teeth problems such as tooth decay, gum disease, biopsy or caries removal and teeth whitening. Lasers have become more popular in dentistry for soft-tissue surgery in recent years. It was approved by the Food and Drug Administration for limited hard-tissue procedures in 1997 and one potential application of dental lasers is as a preventive laser treatment of dental hard tissues to increase their resistance to caries.

Laser produced the energy in the form of coherence light. The laser acts as a cutting instrument or a vaporizer of tissue that it comes in contact with it used for surgical procedures. When it was used for "curing" a filling the laser helps to strengthen the bond between the filling and the tooth. For a teeth whitening procedures, the laser acts as a heat source and enhances the effect of tooth beaching bleaching agents. Laser help to minimize bleeding, pain and swelling during soft tissue treatments and also preserve healthier tooth during cavity removal. Recent studies have shown that CO_2 lasers can successfully be used at low-power densities to fuse enamel, dentin, and apatite. An obvious possible utilization of lasers in dentistry is for the fusion of enamel for cavity preparation.

CO_2 laser classified as class IV laser and emitted in an infrared region. When the CO_2 laser operates, it will produce a very large amount of heat which must be dissipated because of wavelength around 9,400 nm to 10,600 nm. The infrared beam is a heat beam and most materials appear opaque to them and will absorb energy and heat up. If the energy absorbed is more then the material can dissipate, the material will melt and burn.

CO_2 laser is absorbed strongly by water that formed more than 80% of soft tissues. CO_2 laser with the wavelength 9,300 nm and 9,600 nm are highly absorbed by the apatite mineral and wavelength of 10,300 nm and 10,600 nm to an order of magnitude smaller level. CO_2 laser also can be use for ablation because the wavelength in this range is strongly absorbed by the tissue. Apart from that, it is also was used for caries prevention because it has a wavelength that will alter the mineral to make it less soluble.

In this research, CO_2 laser was used to investigate the interaction of the extracted human teeth. Three different types of teeth were used in this research. They are molars, premolars and incisors. A various parameters such as distance between laser aperture and teeth, exposure time, and power were used to irradiate the teeth. Then, the teeth was analysed using a few methods to be analysed from a few aspects such as differentiation of density, mass, the depth of hole produced, the diameter of penetration and the composition of the teeth. The equipments involved in this research such as CO_2 laser, power meter, biological microscope, gamma spectroscopy, Fourier Transform Infrared (FTIR) and gas pycnometer.

1.2 Problem Statement

CO_2 laser are very important in dentistry nowadays because CO_2 laser is absorbed strongly by water that formed more than 80% of soft tissues. Besides, CO_2 laser is effective for a dental hard tissue since it strongly absorbs light in certain regions of the infrared spectrum because of the phosphate, carbonate and hydroxyl groups in the crystal structure. It was used for caries detection, ablation, $CO_{2,}$ and caries prevention. Apart from that, previous studied show that, CO_2 laser caused no damaging thermal effect of the pulp and induced only very subtle defence responses if pulpodentine complex when used with the specific energy settings, pulse duration within thermal relaxation time, emitting radiations at a wavelength of 9,600 nm and with provision for water cooling.

In this research, low laser power was used because recent studies have shown that CO_2 lasers can successfully be used at low-power densities to fuse enamel, dentin, and apatite since CO_2 laser is the highest-power continuous wave laser and it was classified as class IV laser and this will damage the teeth if using a high laser power. Furthermore, CO_2 laser just can penetrate the enamel surface and could not penetrate through the teeth.

1.3 Research Objectives

Nd-YAG lasers are widely used in dentistry. Recent studies proved that pulsed CO_2 lasers successfully be used at low-energy densities and find useful applications in dentistry. However, a lot of research has to be carried out to improve the technology since the pulsed CO_2 laser is less superior as compare to the Nd-YAG laser in its dental applications and addressing teeth problems.

The main objective of this research is to investigate the suitability of a continuous low power continuous wave CO_2 in dental applications and to study the laser-teeth interactions at low laser power. Another objective is to find out the best parameters for the continuous CO_2 laser beam interactions with different types of teeth. These parameters are very important for optimum absorption of the beam since higher power or longer beam irradiation will damage the teeth.

1.4 Scope of Research

In this research, 10,600 nm continuous wave CO_2 laser will be employed to irradiate different types of extracted human teeth samples. The teeth samples will be acquired from the Health Centre of the Universiti Sains Malaysia, Penang, Malaysia. Teeth which are heavily damaged will be excluded from the selected samples. The low power range for this laser will be limited to 3 W to 18 W.

1.5 Organization of Thesis

This thesis contains six chapters and the chapters are organized according to their scope. Chapter one focused on the introduction, followed with the literature review as a background and nurturing the idea and understanding of this research. This chapter also outlined the objectives and scope of this research.

Chapter two describes the theory of teeth and some literature reviews. Materials and equipments as well as functions of each equipment used will be discussed in chapter three. Chapter four will explain about the methodology of this research, including sample preparation, experiment set-up, laser operation and acquisition of data. Chapter five will focus on the experimental results and discussions pertaining to the interactions of low power continuous wave CO_2 laser beam with human teeth. Finally, chapter six will conclude the research done. Some suggestions for further work will also be included in this chapter.

CHAPTER 2

THEORY

Possible applications of lasers in dentistry have been suggested and studied for more than 30 years. Nowadays research on laser increases to gear up and develop the laser technology. One of the most important applications of laser is in medical laser especially in dentistry. Laser dentistry is one of the most exciting advances in dental technology. Lasers have been proven to reduce the pain, swelling, and bacterial formation that can occur during dental procedures.

2.1 Human Teeth

The human teeth anatomy has 2 primary components, the crown and root as shown in Figure 2.1. The part of the teeth that is visible, which is not covered by the gum (the pink, fleshy part), is called the crown. It is covered by a hard shell of enamel known as the anatomical crown. It is made up of the enamel, dentine and pulp. The appearance of teeth varies in shape and size. The enamel is the white hard covering over the crown of the tooth. It is hard enough to endure the strain of a lifetime of biting, chewing and grinding. It does not have a blood and nerve supply. It also protects the teeth from bacteria and changes in temperature when eating hot or cold foods. Nonetheless, it is brittle and may crack or chip. The enamel is translucent, and most of the color of the teeth actually is produced by the dentin below the enamel (Roberto D. S., Ambrosio L. & Nicolais L., 2002).

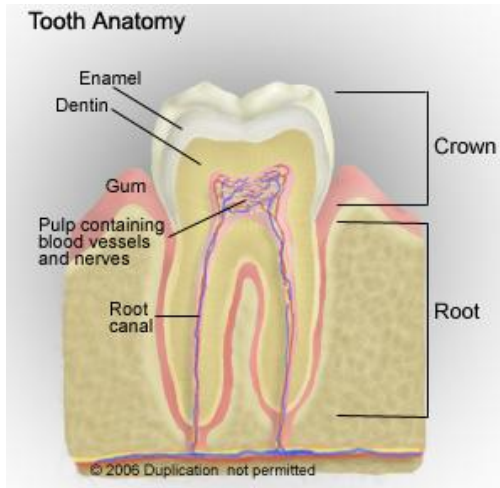

Figure 2.1: Teeth anatomy (Download from : http://yourtotalhealth.ivillage.com/oral-dental-anatomy.html?pageNum=3 accessed December 15, 2008)

Dentin makes up the largest part of the teeth. It is covered by enamel on the crown, and by cementum on the roots. Dentine is not mineralized as enamel; therefore it is softer than enamel but harder than bone. It is composed of collagen and hydroxyapatite and is more compressible and elastic than enamel. This substance resembles bone and makes up most of a teeth's structure and is responsible for the teeth's colour. It typically has a whitish to yellowish hue. The dentine surrounds and protects the nerves and blood vessels (pulp) in the crown and roots (The Anatomy of Teeth and Jaws, 2008).

The pulp occupies the root canals, and the pulp chamber in the crown of the teeth. It is where each tooth's nerve endings and blood supply are found. It is composed of blood vessels, nerves and connective tissue which the blood provides the nutrients that keep the teeth alive and healthy. The pulp goes down into the root of the teeth, which is under the gum.

The root is the part of the teeth that embedded in the tooth socket in the jaw bone, the upper (maxilla) or lower (mandible) jawbone, and the structure of the root is different for all teeth. The front incisors and canines each have a single root, premolars have one or two roots, and the molars can have two or three roots. Each root has a tiny opening at the bottom called the apical foramen. Blood vessels and nerves enter the root through this space (Niemz M. H., 2007). Roots are covered by cementum and held in place by the periodontal ligament.

Cementum makes up the root of the teeth and attaches to fibers that fasten the root to the jawbone. It meets the enamel at the neck of the tooth and it is a hard protective layer of the teeth but it has no nerve supply. Cementum is produced by cementoblasts that form two types of cementum, cellular and acellular. Cellular cementum is less hard than enamel or dentine. Cellular also does not resorb and reform, rather, it grows by apposition, layer by layer. Since cementum surrounds the tooth root, fiber of the periodontal ligament passes from the alveolar bone to attach to the cementum in helping to support the tooth in its alveolus. The size and strength of a tooth as well as protecting the underlying dentine is added by cementum (Kenneth C. & D.D.S, 2006).

2.2 Types of Teeth

Human beings (homo-sapiens), have two sets of teeth in their life-time. This set is the primary dentitions and secondary dentitions. The first set of 20 primary teeth (Figure 2.2) usually begins to erupt between 6 months and 1 year old. Most children have all their primary teeth by age 3. Normal teeth eruption at about six months is known as teething and can be painful and a second set of 32 permanent teeth begin to erupt.

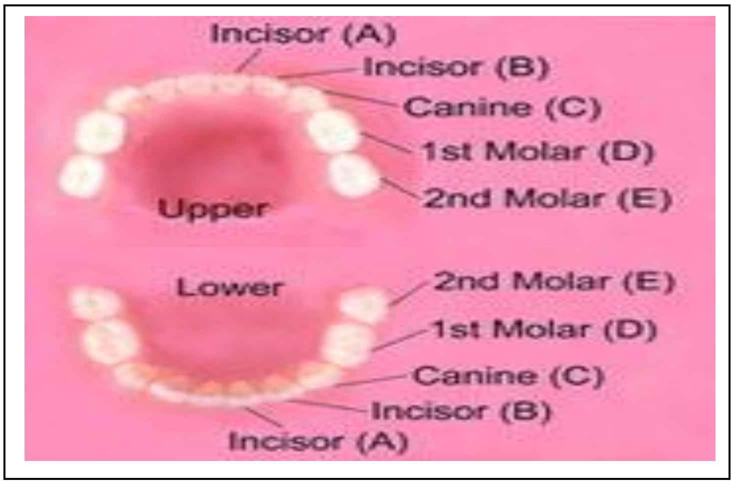

Figure 2.2: Primary teeth (Download from : http://www.simplyteeth.com/ category/sections /Adult/AboutTeeth/Anatomy. accessed December 9, 2008)

There are several types of permanent teeth or called as secondary teeth (Figure 2.3), including incisors, canines, premolars and molars. The square teeth called incisors are located in the front of the mouth, with four on the bottom and four on top. Incisors are shaped like tiny chisels, with flat ends that are somewhat sharp. The pair of teeth at the center of mouth, top and bottom, are called the central incisors and the teeth on each side of the central incisors are the lateral incisors. They have a single root. They are good for cutting or snipping off pieces of food (Types of Teeth, 2008).

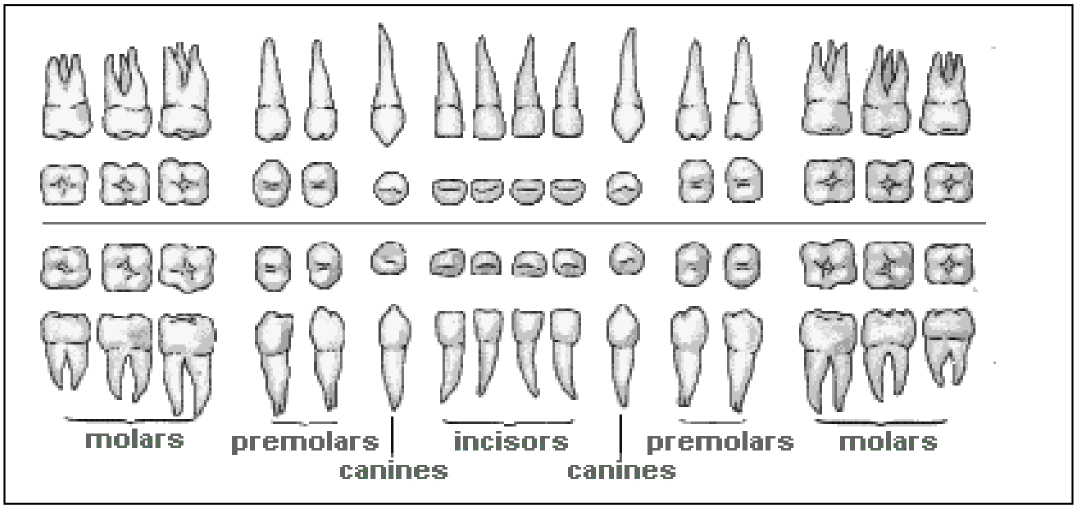

Figure 2.3: Secondary teeth

Sharp teeth called canines are located on either side of the incisors. Sometimes called eyeteeth or cuspids, canines are the longest and most stable teeth in the mouth. There are four of them, two on top and two on bottom. They are thick and come to a single sharp point and have a long single root because these teeth are pointy and also sharp thus, they are used for ripping and tearing at foods that might be tough, such as meat, and for piercing and holding.

Behind the canines are the premolars which are also called bicuspid teeth. Premolars are a cross between canines and molars. Eight premolars are located in each jaw four on top and four on the bottom. Premolars are bigger, stronger, and have ridges. Their shape is completely different from both incisors and canines because they have sharp points for piercing and ripping, but they also have a broader surface for chewing and grinding. The first upper premolars (directly next to the canines) have two roots, and the second upper premolars have one root. All the lower premolars only have one root. The incisors and canines are known as the anterior teeth; premolars and molars are the posterior teeth (Your Teeth, 2008).

The last three upper and lower teeth on both sides are the molars that have a total of twelve. They are numbered first, second or third molars depending on their location. The first molars, also called 6-year molars, are those closest to the front of the mouth, directly next to the second premolars. The third molars are also called the wisdom teeth and they are the last teeth, farthest back in the mouth on all sides. In between are the second molars, also called 12-year molars. A molar is a large tooth with broad surfaces. It was used for crushing, grinding and chewing food. On the upper jaw, the molars have three well-separated roots. On the lower jaw, the molars have two roots (Types of Teeth, 2008).

2.3 Teeth Compounds

The tooth comprised many compounds and it depends on the food taken. A few compounds that were important to the teeth are hydroxy, carboxylic acid, amine, amides, phenols, ketones, ester, hydrocarbon, nitrogen, carbonate and carbonyl. Hydroxy is a molecule that consists of an oxygen atom and hydrogen atom connected by a covalent bond that is also known as single bond. The neutral form is a hydroxy radical and the hydroxy anion called a hydroxide.

Carboxylic acid is most found in the form of salts or acid derivatives in living systems. The comparatively high boiling points for carboxylic acids are due to intermolecular attractions resulting from hydrogen bonding. Like mineral acids such as HCI, carboxylic acid and phenols, ionize in water to produce hydronium ions and anions. Carboxylic acids are only slightly ionized in water since it was a generally weak acid. Under biological conditions, most carboxylic acids form anions. It turns, living cells respond to changes in ionic charge (Morris H, Leo R. B, Scott P, & Susan A, 2005).

The amines are major classes of nitrogen-containing compounds. An amine is a substituted ammonia molecule with basic properties. Simple aromatic amines are liquids or solids. When freshly prepared, they are colourless or almost colourless, but when exposed to air and light it become dark brown or red. Living cells use amine linkage to create proteins. In proteins, small reactants (amino acids) are connected via amide bonds. Amines, substituted amines, and amide occur in every living cell and they have important physiological effects.

Amides also the major classes of nitrogen-containing compound as amine. It is a nitrogen derivative of carboxylic acids. Carboxylic acids react with ammonia to form ammonium salts. Since ammonium salts of carboxylic acids are ionic substances, when heated it lose a molecule of water and are converted to amides. Amides are neutral (nonbasic) molecular substances. They are existing as molecules (not ions) both in the crystalline form and when dissolve in water. Many amides are odorless and colourless. Low-molar-mass amides are soluble in water, but it solubility decreases quickly as molar mass increases. The amide functional group is polar, and nitrogen is capable of hydrogen bonding. This polarity and hydrogen bonding between molecules affect to the solubility of these molecules and their exceptionally high melting point and boiling points.

Phenol is a colourless crystalline solid with a melting point of about 41 ^{o}C and a characteristic odor in the pure state. Phenol is highly poisonous and this characteristic caused the ingestion of even small amounts of it may causes nausea, vomiting, circulatory collapse, and death from respiratory failure. Phenol is classified as a weak acid. It is more acidic than alcohols and water but less acidic than acetic and carbonic acids (Morris H, Leo R. B, Scott P, & Susan A, 2005).

The structure of ketone contains the carbonyl group, a carbon-oxygen double bond. It has at least alkyl or aryl (aromatic, denoted Ar) groups bonded to the carbonyl group. Ketone cannot hydrogen-bond to themselves like alcohols because no hydrogen atom is attached to the oxygen atom of the carbonyl group. However, it has lower boiling points than alcohols of comparable molar mass.

An ester is an organic compound that formed by the reaction of an acid with an alcohol or phenol and water is the product in this reaction. Esters are alcohol derivatives of carboxylic acids and they are found throughout nature. Simple ester derived from monocarboxylic acids and monohydroxy alcohols that are colourless, generally nonpolar liquids or solids. The low polarity of ester molecules are affected by both of their water solubility and boiling points that are lower than those of either acids or alcohols of similar molar masses.The most important reaction of ester is hydrolysis. This reaction is the splitting of molecules through the addition of water. The majority of organic and biochemical substances react only very slowly, if at all, with water (Morris H, Leo R. B, Scott P, & Susan A, 2005).

Hydrocarbon is the organic compound consisting entirely of carbon and hydrogen atoms bonded to each other by covalent bonds. It was classified into two major categories that is aliphatic and aromatic. Aromatic is the compounds that contain benzene rings and all the hydrocarbons that not included in aromatic are often known as aliphatic.

Nitrogen is a chemical element with atomic number 7 and atomic mass 14.00674 u. it is colourless, odorless, tasteless and mostly inert diaotomc gas at standard conditions. Nitrogen is a nonmetal element with an electronegatively of 3.04. The triple bond in molecular nitrogen (N_2) makes it strongest in nature. This bond makes it difficulty of converting N_2 into other compounds. At atmospheric pressure molecules nitrogen condenses at 77 K and freezes at 63 K. Nitrogen is generally unreactive at standard temperature and pressure. N_2 reacts spontaneously with few reagents, being resilient to acids and bases as well as oxidants and most reductants. Nitrogen is present in all living organisms, usually in proteins, nucleic acids and other molecules. It typically around 3% of the weight of the human body. When nitrates burn or explode, the formation of the powerful triple bond in the N_2 produces most of the energy of the reaction (Morris H, Leo R. B, Scott P, & Susan A, 2005).

Carbonate is a salt or ester of carbonic acid that is characterized by the presence of the carbonate ion, CO_3^{2-} or a carbonate functional group. Carbonate mineral is extremely varied and ubiquitous. Carbonate, bicarbonate, carbon dioxide, and carbonic acid exist together in aqueous solution in a form of dynamic equilibrium. The bicarbonate ion is prevalent and CO_3^{2-} is a moderately strong base (Morris H, Leo R. B, Scott P, & Susan A, 2005).

Carbonyl group is a functional group composed of a carbon atom double-bonded to an oxygen atom. Carbonyl groups characterize the aldehyde, ketone, carboxylic acid, ester, amide, enone, acyl halide and acid anhydride.

2.4 Carbon Dioxide Laser

Laser is device which uses a quantum mechanical effect, stimulated emission to emit intense beam of light which is monochromatic, coherent, and highly collimated. Laser light is usually spatially coherent, which means that the light either is emitted in a narrow, low-divergence beam, or can be converted into one with the help of optical components such as lenses. Light from a laser typically has very low divergence (Thyagarajan K. & Ghatak A. K, 1981).

CO_2 laser is the highest-power continuous wave laser and it was classified as class IV laser (Synrad, 2004). The ratio of output power to pump power can be as large as 20% and this make it quite efficient. It produced a beam of infrared light. When the CO_2 laser operates, it will produce a very large amount of heat which must be dissipated because infrared beam are heat beam. Most materials appear opaque to them and will absorb energy and heat up. If the energy absorbed is more than the material can dissipate, the material will melt and burn (Synrad, 2004). CO_2 laser produced wavelength around 9,400 nm and 10,600 nm. The laser levels involved consist of molecular energy levels not as ruby laser or helium laser that consist electronic energy levels of an atom or an ion. The constituent atoms can vibrate in relation to each other and the molecules as a whole can also rotate. Hence, the molecules have rotational as well as vibrational energy levels in addition to the electronic energy levels. The energy difference between various electron levels correspond to the visible and the ultraviolet transitions where as the energy difference among the various vibrational levels involve infrared region and the transition corresponding to the rotational levels lie in the microwave region or in the far infrared (Joseph D. B, 2000, Siegman & Anthony E., 1986).

The CO_2 molecule consisting of one carbon atom and two oxygen atoms and it is a triatomic molecule and also a linear molecule. The number of vibrational or vibrational frequencies in a molecule depends upon the number of atoms by which it is formed. CO_2 molecule is a vibrational mode because it has four vibrations possible. Out of the four vibrations possible modes in CO_2 two of them are degenerate that means they have a same energy. Thus, only three vibrational modes in CO_2 exist. They are symmetric stretch, asymmetric stretch and bending mode (Bass & M., 1972).

Three gases CO_2, N_2 and He are mixed and fed into one end of a discharge tube. The gas flows down the end of the tube in about one second and is pumped out the far end with a mechanical forepump. At 10,600 nm and at 9,400 nm, the population inversion is achieved through a gas discharge and the lasing action produces outputs. Electron will impacts excite the molecules to higher electronic and vibrational rotational levels when a discharge is passed in a tube containing CO_2. The resonant transfer of energy from N_2 added to the gas increases the pumping efficiency. N_2 does not possess a permanent dipole moment. Thus, the N_2 molecules are excited to the upper vibrational ground level and it is seen that the energy difference between this level and the vibrational ground level of the N_2 molecule matches very well with the energy required to excite the CO_2 molecule to the upper energy level. This situation shows that there has efficient transfer energy between N_2 and CO_2 molecules and this give the excitation of the CO_2 molecule and the efficiency of CO_2 laser also increase. With a fully reflecting mirror on the left and a partially transmitting mirror on the right (Figure 2.4), the device becomes a laser which radiates in the far infrared at 10 600 nm. Typically, the mirrors are made of coated silicon, molybdenum, or gold. Windows and lenses are made of either germanium or zinc selenide. The levels for lasing action are in molecules and not depend on electron excitation but on the vibrational and rotational movements of the molecule (Siegman & Anthony E., 1986, Hawkes J. & Latimer I., 1995).

In CO_2 laser, the levels taking part in laser transitions are the vibrational rotational levels of the lowest electronic level. A large portion of the input energy is converted into the output laser energy because these levels are very close to the ground level and hence resulting in very high efficiencies. This situation explains that the CO_2 lasers are much more efficient than other gas lasers. CO_2 laser produce output powers from several Watt to several thousand Watt because it is a high-power continuous wave laser. CO_2 laser is absorbed strongly by water that formed more than 80% of soft tissues. It widely used by surgeons as a scapel and is the most commonly used medical laser (Siegman & Anthony E., 1986).

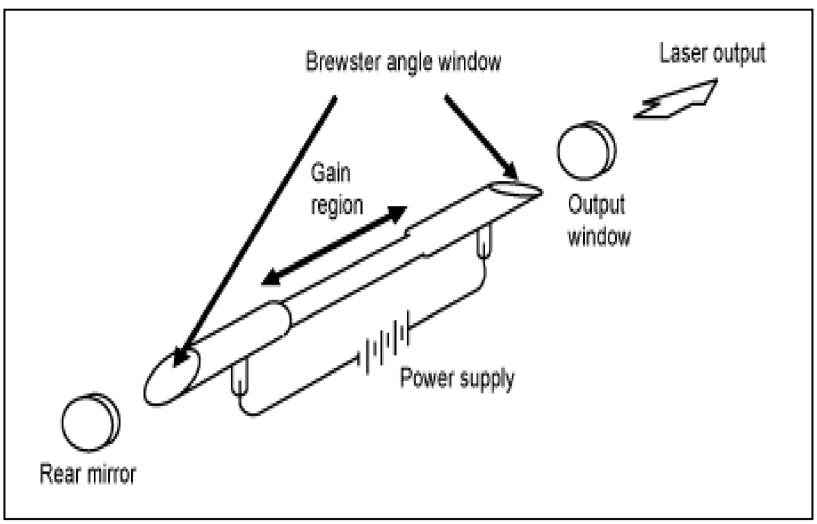

Figure 2.4: Sealed tube CO_2 laser scheme (Download from : http://www.twi.co.uk/ content/kspah002.html accessed September 18, 2008)

2.4.1 Laser ablation

In laser ablation , matter from a target surface such that the stoichiometry of the material is preserved in the interaction was evaporated by a high-power laser pulses. As a result, a supersonic jet of particles (plume) which similar to the rocket exhaust, expands away from the target with a strong forward-directed velocity distribution of the different particles is ejected normal to the target surface (Laser-Ablation Technique, 2010).

The ablated species condense on the placed opposite to the target. The laser-target interactions will be sensitively dependent both on the nature and condition of the target material, and on the laser pulse parameters (wavelength, intensity, fluence, and pulse duration). Besides, laser-plume interactions also depend on the properties of the laser radiation, while the evolution and propagation of the plume sensitive to collisions and thus to the quality of the vacuum under which the ablation is conducted and/or the presence of any background gas (Laser Ablation, 2010).

2.5 Principle of Biological Microscope

A main principle of microscope is a simple lens system for magnifying small objects. The objective is a first lens and has a short focal length with a few mm. it creates an image of the object in the intermediate image plane. This image then, in turn can be looked at with another lens called eye-piece. Further magnification was provided by this lens. The resolution of the image is limited by diffraction.

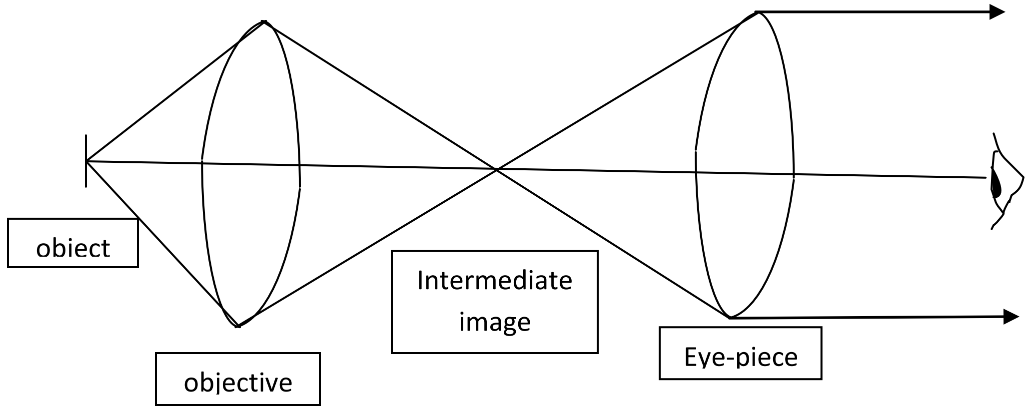

Figure 2.5: Principle scheme of an microscope. The objective lens has a much shorter focal length than the eye-piece, in order to magnify the intermediate image

This resolution can be explain by the Abbe-Rayleigh criterion. This criterion state that, for a wavelength λ, the smallest distance $d_{\min}$ resolvable between two point sources as deduced from diffraction theory is:

$$d_{\min} = 1.22 \text{ x } \frac{\lambda}{2NA} \qquad (2.1)$$

where NA = n x sin α is called numerical aperture of the objective lens.

n is the index of refraction in the object space,

α half the maximum angle under which the objective lens collects light from the object.

The numerical aperture must be as large as possible because the spatial resolution improves for larger *N*A and the collection efficiency, i.e. the brightness of the image increase faster with *N*A, quadratically for small apertures. The numerical aperture is inversely propotional to the refraction index. This feature make it can collect light through glass or through oil when possible. Special water-immersion objectives are used for biological samples to make the index matched metween sample and objective glass and this situation give benefit from high index. The other benefit of immersion is the higher efficiency of fluorescence collection (an air gap leads to total internal reflection losses).

Image a planer onto a plane field (aplanetism) without distortion and without change in image with wavelength (achromatism) is the features for the idea microscope objective. To achieve these features, correction of aberration was necessary. The corresponding aberrations are:

i) chromatic aberrations from the dispersion of glass (the refractive index is larger in the blue than in the red)

ii) geometrical aberrations: spherical (change of focal point with distance from axis), coma, due to changes of the image point with ray direction, field curvature (image on a sphere), field distortion (pincushion or barrel images), etc.

Simple spherical lenses made out of ordinary dispersive glass suffer from all these aberrations. This condition cannot fulfill the requirements of the ideal lens. Designation for objectives and other multilens system require codes calculation imaging with rays far from paraxial for arbitrary systems of lenses. As much as 10 lenses which have to be spaced with specifications as narrow as microns for some of them is a feature for a good objective. Anti-reflection coated have to be provided to the air-glasses interfaces in order to reduce reflection losses and bridged with high-index medium, usually UV-polymerizable glue, after the respective position of the lenses has been adjusted at small gaps between the lenses.

The field at the focus of a linearly polarized laser wave is due to a vector quantity for the electric field of a laser wave. The polarization of the spot is same as of the incident beam and by symmetry it also the same at the focal point itself for low N.A. Meanwhile, part of the PSF away from the focus, interface of the incoming rays leads to deviations from this polarization at high N.A.

The resolution of microscopic images can be improve and the selected volume in single-molecule studied can be reduce by reduced the spot size below the Abbe diffraction limit. Optics at ranges smaller than the wavelength is known as near-field optics. Interaction with microscopic objects is necessary to produce and analyze optical fields with variations less than λ. Thus, to enhance or constrain the optical field, near-field optics has to use small objects, usually tips.

2.6 Principle of Gamma Spectroscopy

Gamma spectroscopy involves the spectroscopy of radionuclides which provides identification and measurement of activity of radionuclides which emit gamma radiation or X-rays. The energies and the photon yields are characteristic for specific nuclides. It used Geiger counter to determine the count rate. Detectors such as semiconductors, scintillators or proportional counters are sensitive to the deposited gamma energy because gamma spectrometer also determines the energy of gamma rays emitted by radioactive substances while a Geiger counter determines the count rate. By using pulse height analysis gamma spectra, it also can be acquired. Usually, most of radioactive sources produce gamma rays of various energies and intensities. These emissions were collected and analyzed with a gamma spectroscopy system and at the same time a gamma energy spectrum can be produced. This spectrum was used to determine the identity and quantity of gamma emitters present in the source by a detailed analysis. The gamma spectrum is characteristic of the gamma-emitting nuclides contained in the source, just as in optical spectroscopy and the optical spectrum is characteristic of the atoms and molecules contained in the probe.

A complete gamma spectroscopy system consists of a detector, electronics to collect and process the signals produced by the detector, and a computer with processing software to generate, display, and store the spectrum. Other components that provide such as rate meters and peak position stabilizers may also be included. The detectors wait for a gamma interaction to occur in the detector volume. This situation make the detectors are passive materials. The photoelectric effect, the Compton effect, and pair production is the most important interaction mechanisms involved in this instrument. Because it absorbs all of the energy of the incident gamma ray so, the photoelectric effect is most preferred than other mechanisms. When a series of these interaction mechanisms take place within the detector volume, full energy absorption is also possible. The background rate in the spectrum will increased by one count when a portion of the energy escapes from the detector volume without being absorbed and a gamma ray undergoes a Compton interaction or pair production. This count will appear in a channel below the channel that corresponds to the full energy of the gamma ray. This effect can be reducing by using larger detector volumes.

2.7 Principle of Density Measurement

Gas pycnometer was used for measuring the density or more accurately the volume of solids. The materials can be regularly shaped, porous or non-porous, monolithic, powdered, granular or even comminuted. This device was applied some method of gas displacement and the volume: pressure relationship called as Boyle's Law. Because it was used helium gas it is also sometimes referred to as a helium pycnometer.

Density was calculated from the ratio of mass to volume. So, before use this device they should know the mass first by weighing. Because of this situation, while pycnometer is known as density measuring devices they are actually devices for measuring volume only. The volume measured in a gas pycnometer considering the finest scale of surface roughness depend on the atomic or molecular size of the gas because the volume measured is the amount of three-dimensional space which is inaccessible to the gas used, i.e. that volume within the sample chamber from which the gas is excluded. Helium gas was used to operate this device because is most often prescribed as the measurement gas. Besides, helium also is a small in size, inert and the most ideal gas.

2.8 Literature Review

Interaction of CO_2 laser radiation with enamel and dentine was studied by Lobene et al. (1968). The aim of their studies is to determine the feasibility of fusing enamel using high power density, CW radiation at 10,600 nm. Extracted human teeth with intact enamel surfaces were exposed to CW CO_2 laser radiation with constant output power at 20 Watts. Adjusting the excitation voltage and gas mixture was made by measured with a thermal equilibrium thermopile to make sure that laser output is always constant. The desired spot size was achieved by reflective focusing of the beam or by use of a lens. Finding from this study shows that the region of exposure in enamel appeared fused, chalky white, and opaque and it was hard and brittle and fractured easily. It also shows that both the region of irradiation and the surrounding region of the crown were hot to touch that means the temperature of teeth increases after lasing.

The study about the ultrastructural observations of pulsed CO_2 laser effects on irradiated enamel has been done by Ralph H. S., Johanna V. and Reidar F. S. (1972). The purpose of his study is to determine about the macroscopic enamel surface changes can be induced with a pulsed CO_2 laser, and about the extent the structure and appearance of the enamel surface can be affected by an energy density range from 1.3 to 50 J/cm^2. Another purpose of his study is to investigate the correlations between SEM and microradiographic examinations after in vitro subsurface demineralization for lased enamel. Freshly extracted noncarious human third molars were used in this study. This study found that the enamel showed some gross enamel surface alteration immediately after laser exposure when exposed to 50 J/cm^2 and the surface was crazed and mottled, and exhibited white surface opacities and light brown discoloration. For enamel that exposed at 25 J/cm^2, the gross enamel surface alterations were reduced significantly, and only slight crazing and surface opacity were visible on dried enamel. But at the energy density of 13 J/cm^2 there has no gross enamel changes appear after laser exposure.

Human teeth with and without caries studied by laser scattering, fluorescence, and absorption spectroscopy has been by Robert R. Alfano, W. Lam, Hassan J. Zarrabi, Michele A. Alfano, Julius Cordero, Darayash B. Tata, and Charles E. Swenberg (1984). In their study,