SYNTHESIS OF SILVER NANOPARTICLES VIA PULSE LASER ABLATION IN LIQUID FOR ANTI-BACTERIA APPLICATIONS

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by

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To my sweetest, who left soon….to my mom.

Your spirit with me all the way …

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Even with a single word, to all whom

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
AFM	Atom Force Microscopy
ALD	Atomic Layer Deposition
ATP	Adenosine Triphosphate
CVD	Chemical Vapor Deposition
DDDW	Double Deionized Distilled Water
DNA	Deoxyribonucleic Acid
E. Coli	Escherichia Coli
EM	Electromagnetic
IR	Infra-Red
IZ	Inhibition Zone
KDP	Potassium, Deuterium, Phosphate
KTP	Potassium, Titanium, Phosphate
LP-PLA	Liquid Produced Plasma by Laser Ablation
LSP	Localized Surface Plasmon
MBC	Minimal Bactericidal Concentration
MBC	Minimum Bacteria Concentration
MIC	Minimal Inhibitory Concentration
Nd:YAG	Neodymium-doped Yttrium Aluminum Garnet; Nd:$Y_3Al_5O_{12}$
NPs	Nanoparticles
PLAL	Pulsed Laser Ablation in Liquid
PVD	Physical Vapour Deposition

ROS	Reactive Oxygen Species
SDS	Sodium Dissolve Sulphate
SERS	Surface Enhanced Raman Scattering
SP	Surface Plasmon
SPA	Surface Plasmon Absorption
SPR	Surface Plasmon Resonance
TEM	Transmission Electron Microscope
USM	Universiti Sains Malaysia
UV-Vis	Ultraviolet- Visible
WWTP	Wastewater Treatment Plants

LIST OF SYMBOLS

σ_{abs}	Absorption cross section
α	Absorption Coefficient
k_B	Boltzmann Constant
ρ	Density
E	Dielectric Constant
d	Diameter
E	Electron Charge
σ_{ext}	Extinction cross section
m_e	Electron Effective Mass
J	Joule
I_o	Laser Intensity
ω	Photon Frequency
ω_P	plasma frequency
θ_s	Probability
R	Radius
R	Radius
n_m	Real Refraction Index
n	Refraction Index
σ_{sca}	Scattering cross section
C_p	Specific Heat
T	Temperature
K	Thermal Conductivity
I_t	Transmitted Intensity
ε_o	Vacuum Dielectric Permittivity

V	Volume
T_v	Vapor Temperature
Λ	Wavelength
K	Wavevector

SINTISIS NANO PARTIKEL PERAK MELALUI ABLASI LASER DENYUT DALAM CECAIR UNTUK APLIKASI ANTIBAKTERIA

ABSTRAK

Pelbagai jenis bakteria boleh menjejaskan kesihatan manusia dan menyebabkan penyakit yang berbeza seperti jangkitan kulit, saluran hidung, keracunan makanan dan sebagainya. Bakteria ini memperoleh imuniti dan membangun kendiri terhadap beberapa antibiotik yang biasa. Oleh itu, permintaan yang besar muncul pada nanopartikel logam yang mempunyai keupayaan untuk menghapuskan jenis bakteria tersebut. Laser denyut Nd:YAG suis-Q 10 ns digunakan untuk menghasilkan larutan terampai partikel nano perak menggunakan laser ablasi. Kaedah ini menyediakan proses kawalan saiz, taburan saiz dan bentuk partikel nano. Kesan terhadap beberapa parameter laser di periksa ke atas kecekapan ablasi seperti panjang gelombang, ketumpatan tenaga, dan bilangan denyut laser. Nanopartikel perak yang dihasilkan dalam karya ini dicirikan menggunakan penyiasatan morfologi (HRTEM dan AFM) dan kaedah spektroskopi (penyerapan UV-Vis). Data eksperimen itu dipasang dengan model teoretis Mie-Gans untuk menganggarkan saiz nanopartikel dan bentuknya. Nanopartikel perak yang telah disediakan diuji pada beberapa jenis bakteria yang terkenal di dalam tubuh manusia seperti Staphylococcus, Streptococcus, Proteus dan E. coli. Didapati bahawa panjang gelombang asas laser 1064 nm, boleh menghasilkan ketumpatan partikel nano perak yang tinggi dengan saiz terbesar 100nm dan taburan saiz yang lebih luas. Manakala saiz partikel nano yang lebih kecil dengan diameter purata (70nm) disintisis melalui generasi harmonik kedua dengan panjang gelombang 532nm. Pencirian partikel nano yang dihasilkan dalam kajian ini dilakukan

mengunakan penyiasat morfologi (HRTEM dan AFM) dan kaedah spektroskopi (UV-Vis penyerapan) ini sesuai dengan model teoritis Mie-Gans untuk menentukan saiz dan bentuk partikel nano. Keputusan yang diperolehi mendedahkan bahawa bentuk yang terkawal dihasilkan melalui parameter laser yang bersesuaian. Sifat partikel nano yang diperolehi sangat berkesan ke atas aktiviti antibakteria bagi melawan bakteria. Partikel nano perak dengan diameter purata (75nm) diperolehi mengunakan laser dengan tenaga 600 mJ, manakala taburan saiz nano partikel yang seragam dengan purata minimum (20nm) diperolehi apabila ketumpatan tenaga laser mencapai 20 J/cm^2. Didapati bahawa aktiviti antibakteria partikel nano perak bergantung kepada saiz dan bentuk partikel nano tersebut. Seterusnya, aktiviti antibakteria partikel nano perak dibandingkan dengan antibodi yang diketahui umum (*Amoxicillin, Streptomycin, dan Penicillin*). Pemerhatian utama menunjukkan bahawa partikel nano perak aktivitinya lemah (tidak mencapai 15mm diameter zon perencatan walaupun kepekatan partikel nano perak yang tinggi) berbanding dengan antibiotik kerana kesan kumpalan. Namun begitu aktiviti antibakteria meningkat dengan mendadak apabila partikel nano perak ditambah kepada antibiotik menjadikan zon perencatan maksimum meningkat dari (20mm) kepada (38mm) untuk Staphylococcus terhadap penicilin berbanding penicilin dengan partikel nano perak. Didapati juga bahawa, bakteria menunjukkan sensitiviti yang berlainan terhadap partikel nano dan bakteria paling sensitif ialah Steptococous dengan kepekatan perencatan minimum nano partikel perak (0.468 μg /ml). Oleh itu, nanopartikel perak dengan ciri-ciri yang betul boleh digunakan untuk meningkatkan kesan antibiotik sesetengah bakteria yang hidup di dalam tubuh manusia dan terutama pada kulit.

SYNTHESIS OF SILVER NANO-PARTICLES VIA PULSE LASER ABLATION IN LIQUID FOR ANTI-BACTERIA APPLICATIONS

ABSTRACT

Various types of bacteria affected the human health and cause different diseases like skin infection nasal passage, food poisoning, etc. These bacteria acquired immunity and develop self-resistance against some familiar antibiotics. Therefore, considerable demand is emerged on nobel metal nanoparticles which have ability to eliminate those types of bacteria. Q – Switched Nd:YAG laser pulse of 10 ns was employed to produce colloidal suspension of silver nanoparticles by laser ablation in distilled water. This technique provides controllable process for size, size distribution and shape of nanoparticles. Effects of various laser parameters were examined on the ablation efficiency such as laser wavelength, laser energy density and number of pulses. Silver nanoparticles produced in this work were characterized using morphological investigation (HRTEM and AFM) and spectroscopic method (UV-Vis absorption). The experimental data were fitted with Mie-Gans theoretical model to estimate the nanoparticles size and their shape. The prepared silver nanoparticles were tested on some famous types of bacteria live in human body such as Staphylococcus, Streptococcus, Proteus and E. coli. It is found that the fundamental laser wavelength of 1064 nm could produce high density of silver nanoparticles with larger size of 100 nm and wider size distribution. While smaller nanoparticles of average diameter of 70 nm were synthesized by the second harmonic generation of 532 nm wavelength. Our results reveal formation of controllable shapes via using proper laser parameters. The silver nanoparticles features were found very effective on the antibacterial activity

against the bacteria. Silver nanoparticles of mean diameter 75 nm was obtained using laser energy of 600 mJ, while uniform silver nanoparticles size distribution can be obtained with minimum mean diameter of 20 nm was achieved by laser energy density of 20 J/cm^2. It is found that the antibacterial activity of silver nanoparticles depends on the size and shape of nanoparticles. Moreover, the antibacterial activity of silver nanoparticles on these bacteria was compared with those of familiar antibiotics (Amoxicillin, Streptomycin and Penicillin). The main observations indicate that silver nanoparticles have weak activity (not exceed 15 mm diameter of inhibition zone even at high silver nanoparticles concentration) compared with that of antibiotics due to the aggregation effect, but the antibacterial activity could increase drastically when silver nanoparticles added to antibiotics and maximum inhibition zone was increased from 20 mm to 38 mm for Staphylococcus for Penicillin and Penicillin with silver nanoparticles, respectively. Furthermore, it is also found that the bacteria show different sensitivity to silver nanoparticles and the most sensitive bacteria was Streptococcus at minimum inhibition concentration of 0.468 μg/ml for silver nanoparticles. Thereby, silver nanoparticles of proper features could be used to enhance the effect of antibiotics of some bacteria live in human body and especially on the skin.

CHAPTER ONE

INTRODUCTION

1.1 Introduction

This chapter includes overview of nanotechnology, laser ablation process in liquid and historical review of literatures involve applications of metals (gold & silver) nanoparticles in bacteria treatments such as antibacterial activity of some types of bacteria live in human body.

Nanotechnology can be defined as design, synthesis and application of nanomaterials and devices whose size and shape have been engineered at the nanoscale. These materials exploit unique physical, chemical, mechanical features that emerged when matter is structured at the nanoscale (C .Buzea et al., 2007). Recently, nanotechnology researches and applications are emerge as cutting edge technology interdisciplinary with physics, chemistry, biology, material science, medicine and device engineering (N. Drogat, et al., 2010). Therefore, nanotechnology is considered a revolution not evolution in science and technology.

Nanotechnology has been expanded rapidly last decade impinging on diverse area like economy and environment. Thereby, large numbers of commercial products comprising nanomaterials, household devices, medical devices and drug delivery systems were emerged.

There have been impressive developments in the fields of nanotechnology recently with numerous methodologies to control the synthesis, size and shape of nanomaterials depending on specific requirements (B. Ashe, 2011).

Metals nanoparticles are very attractive for biophysical, biochemical and biotechnological applications due to their unusual physical properties according to their sharp Plasmon absorption peak at the visible region (S. Eusita and M. El-Sayed, 2006).

The resonance frequencies are strongly depending on particle shape, size as well as on the optical properties of the material within the near-field of the particle (J. Prekulis et al., 2004), and (A. Templeton et al., 2000). Silver nanoparticles have gained considerable attention in recent years for potential applications in nanomedicine due to their interesting size dependent chemical, electronical and optical properties. Moreover, silver nanoparticles show promise in enhancing the effectiveness of various targeted tumor treatments such as radiotherapy and photothermal therapy (N. Dodgostar, 2008).

The main features of metal nanoparticles are (D. Mirela, 2005)

1. Large surface – volume ratio compared with those of bulk
2. Large surface energy
3. Plasmon excitation
4. Short range ordering
5. Quantum confinement

Gold and silver nanoparticles (AuNP and AgNP) are playing a protagonist role. The reason for AuNP and AgNP success lies in a favorable combination of physical – chemical properties and advances in chemical synthesis. The main characteristic of AuNP and AgNP is the Surface Plasmon Absorption (SPA), which has $10^5 – 10^6$ μm^2 larger extinction cross section (the total attenuation including the scattering and absorption from the incident light) than that for ordinary molecular chromophores and is also more intense than that of other metals particles, due to the weak coupling to interband transitions (V. Kattumuri, 2006) and (K. Vernon et al., 2011). Moreover, the frequency of gold and silver SPA can also be tuned from visible to near infrared acting on shape, size or nanoparticles assembly. Furthermore AuNP and AgNP have high chemical stability and photostability and especially AuNP are nontoxic for living organisms (X. Xu et al., 2004) and (A. R. Bijanzadeh et al., 2012).

Silver is a nontoxic, safe inorganic antibacterial agent used for centuries and is capable of killing about 650 type of diseases causing microorganisms. Silver has been described as being ‘oligo dynamic’ because of its ability to exert a bactericidal effect at minute concentrations. It has a significant potential for a wide range of biological applications such as antifungal agent, antibacterial agents for antibiotic resistant bacteria, preventing infections, healing wounds and anti-inflammatory (M. Raffi et al., 2008). Silver ions (Ag^+) and its compounds are highly toxic to microorganisms exhibiting strong biocidal effects on many species of bacteria but have a low toxicity towards animal cells. Therefore, silver ions, being antibacterial component, are employed in formulation of dental resin composites, bone cement, ion exchange fibers and coatings for medical devices. Bactericidal behavior of nanoparticles is attributed to the presence of electronic effects that are brought about as a result of changes in local electronic structures of the surfaces due to smaller sizes. These effects are considered to be contributing towards enhancement of reactivity of silver nanoparticles surfaces. Ionic silver strongly interacts with thiol groups of vital enzymes and inactivates them. It has been suggested that Deoxyribonucleic Acid (DNA) loses its replication ability once the bacterium is treated with silver ions. Two dimensional electrophoresis and proteins identification analysis of antibacterial action of silver nanoparticles have disclosed accumulation of envelope proteins precursors. Silver nanoparticles destabilize plasma membrane potential and depletion of levels of intracellular Adenosine Triphosphate (ATP) by targeting bacterial membrane resulting in bacterial cell death (O. Choi, K. Deng and N. Kim 2008).

One of the most active application of silver nanoparticles is usage in health care and microbial treatments due to their high antibacterial activity. There are very broad band of bacteria which commonly affected the human health and many recent studies and publications were conducted on various types of bacteria. Those bacteria are classified into two groups Gram-positive and Gram-negative bacteria and some of famous types are Staphylococcus, Escherichia coli and Streptococcus.

Staphylococcus is a facultative anaerobic Gram-positive coccal bacterium, it is frequently found as part of the normal skin flora on the skin and nasal passages. It is estimated that 20% of the human population are long-term carriers of Staphylococcus bacteria**.** This type of bacteria is the most common species of staphylococcus to cause Staph infections and food poisoning.

Staphylococcus can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, carbuncles and abscesses, to life-threatening diseases such as pneumonia, meningitis, toxic shock syndrome and sepsis. Its incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections. Each year, about 500,000 patients in American hospitals suffer a staphylococcal infection (K. Matthews et al., 1997).

Moreover**,** another Gram-positive bacteria of spherical shape called Streptococcus. This kind of bacteria is a genus and belonging to the lactic acid bacteria group. Streptococcus species are responsible for many cases of meningitis, bacterial pneumonia, endocarditis, erysipelas and necrotizing fasciitis. However, many streptococcal species are nonpathogenic, and form part of the commensal human microbiome of the mouth, skin, intestine, and upper respiratory tract. Furthermore, streptococci are a necessary ingredient in producing emmentaler cheese (V. Ilic et al., 2010).

Escherichia coli commonly abbreviated E. coli is a Gram-negative. These bacteria have a rod-shaped and commonly found in the lower intestine of warm-blooded organisms. Most E. coli are harmless and pathogenic strains which cause disease, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls due to food contamination. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2 and by preventing the establishment of pathogenic bacteria within the intestine. E. coli cells are able to survive outside the body for a limited amount of time, which makes them ideal indicator organisms to test environmental samples for fecal contamination (R. Vogt and L. Dippold, 2005).

1.2 The Problem Statement

Many bacteria have developed multi-resistance properties against various types of antibiotics. Therefore there was a need to overcome such a problem throughout improving the antibacterial activity using more toxic, and inexpensive material and find out new antibacterial substances that inhibit the bacteria. Silver nanoparticles are good candidate as an antibacterial agent due to his superior physical- and chemical properties, stable and nontoxic. Thereby, this problem can be solved by silver nanoparticles which have unique features to kill a wide range of spectrum of bacteria. Laser offers high possibility to synthesize silver nanoparticles with controllable features by pulse laser ablation in liquid. This process is costly effective, swiftly adapted, updated, and adjusted.

1.3 Objective of the Study

Aim of this work can be summarized as following

- To prepare silver nanoparticles in easy, fast and controllable process via pulse laser ablation in liquid,
- To optimize and improve the formation rate of silver nanoparticles by studying the effects of laser parameters and control the size, and distribution of Ag NPs.
- To examine the antibacterial activity of the prepared silver nanoparticles on some types of bacteria living in the human body.
- To study the antibacterial activity using silver NPs with, and without antibiotics.

1.4 Thesis Layout

This thesis is divided into five chapters. The First Chapter involves introduction of laser ablation in nanotechnology to fabricate metal nanoparticles, the problem statement and aim of the work. Chapter Two include the literature review and explains in detail a theoretical background approach to characterize the properties of nanoparticles and their interaction with light. Chapter Three describes the experimental procedures and methodologies adopted in synthesis and measurements of silver nanoparticles. Chapter Four presents the experimental results of this study and their discussions. Chapter Five gives the summary the conclusions for the current work and future pathways.

CHAPTER TWO

LITRARURE REVIEW

2.1 Researches Review

The aim of this review is to summarize earlier and recent published researches in the field of interest concerning preparation of silver NPs using Pulsed Laser Ablation in Liquid PLAL (PLAL) method and its applications as antibacterial material.

In 1990s, various works were published on preparation of metals nanoparticles using pulse laser ablation in liquids. Henglein et al., (1993) was used pulse laser ablation colloidal to prepare silver nanoparticle suspension in various solvents such as water, propanol. This work encourages preparation of metal nanoparticles by t op-down approach. While J. Neddersen et al. in (1993) presented the production of Ag, Au, Pb, Pt and Cu nanoparticles in distilled water and organic solvents by laser ablation. They examined the stability of properties of those nanoparticles in the colloidal suspension using Surface Enhanced Raman Scattering (SERS) measurements. They employed pulsed Nd:YAG laser of 1064 nm, 10 nanosecond and 55mJ/ pulse at 10Hz repetition to prepare metals nanoparticles. Moreover, M. Prochazka et al., (1997) has reported production of silver (Ag) colloids as active surfaces for Surface-Enhanced Raman Scattering (SERS) spectroscopy by laser ablation of an Ag foil in pure water, in the presence of chlorine ions. They found that silver nanoparticles have strong Raman scattering due to the large surface to volume ratio.

Then after, Mafune et al., (2000) produced silver nanoparticles by laser ablation of a silver plate in water and Sodium Dissolve Sulfate SDS. High number of laser pulses (50000) was used. They found that the size distribution of silver nanoparticles shifts toward smaller sizes when (SDS) concentration was increased. Moreover, Tsuji et al., (2000) have studied effects of various parameters of Nd:YAG laser on the ablation efficiency. Influence of different preparation parameters such as the laser wavelength, focusing conditions on the ablation efficiency of silver and copper nanoparticles in water were examined. They found that the ablation efficiency at shorter wavelengths was higher, while the ablation efficiency at longer wavelengths increased when high laser flounce was employed. Similar research was conducted by Tsuji et al. (2001) have studied the influence of the laser wavelength and focusing conditions on the ablation efficiency of silver and copper nanoparticles in water. It was found that the ablation efficiency at shorter wavelengths and low flounce was higher, while higher ablation efficiency was obtained at longer wavelengths and high flounce.

Dolgaev et al., (2002) have reported the formation of some metals nanoparticles (Ag and Au) by pulsed laser ablation metal target in various liquid environments (H_2O, C_2H_5OH, $C_2H_4Cl_2$) using cooper vapor laser. They compare the ablation efficiency when a laser wavelength of 510 nm was used. They found that this method provides high formation rate of nanoparticles. Furthermore, Tsuji et al., (2002) were studied influences of laser wavelengths of 1064, 532, and 355 nm on synthesis of silver nanoparticles in water. It is found that the ablation efficiency decreases with decrease the laser wavelength. It is found that smaller laser wavelength produces smaller silver nanoparticles.

Moreover, another research group (Kabashin et al. 2003) have reported two different mechanisms of material ablation in the liquid environment to produce gold nanoparticles. The first, associated with femtosecond laser ablation. This mechanism occurs at laser flounces around 400 J/cm^2 and leads to produce small nanoparticles in the range 3–10 nm. The second method of ablation in liquid is correlated to the plasmainduced heating. While Tsuji et al., (2003) have studied the preparation of silver nanoparticles by laser ablation in water by femtosecond and nanosecond pulses for laser wavelength of 800 nm. The found that the formation efficiency when femtosecond laser pulse was significantly lower than longer pulses of (nanoseconds). Furthermore, laser ablation of a gold target in aqueous solutions was also studied in (2004) by Sylvestre et al., using femtosecond laser pulses to produce nanoparticles with controlled surface chemistry. A detailed chemical analysis revealed that gold nanoparticles were partially oxidized by the oxygen presented in the solution.

Various groups in 2005 were also published their work on laser ablation of silver in various media. Pyatenko et al., (2005) have prepared silver nanoparticles using 532 nm Nd:YAG laser, with low laser flounce of about 0.2 J/cm^2 in acetone at different wavelengths 532, 266, 400 and 800 nm. While Tarasenko et al., (2005) have studied the surface modification for silver nanoparticles. Their experimental conditions lead to form spherical size-controlled silver nanoparticles.

Very extensive researches were conducted in 2006 to prepare different metals nanoparticles. Kawasaki et al., (2006) have found that laser-induced fragmentation of thin Au and Ag flakes in acetone by 1064nm nanosecond laser with low flounce of 2 J/cm^2 offers highly productive pathway to produce stable metal nanoparticles. While Zhao et al., (2006) were studied the spectroscopic properties of silver nanoparticles prepared by pulse laser ablation. Also, V. Amendola et al., (2006) have studied preparation of gold nanoparticles in organic media. Tarasenko et al., (2006) have reported the formation of alloyed nanoparticles Au–Ag and, Ag–Cu is reported under laser exposure of a mixture of individual nanoparticles. Moreover, Yamada et al., (2006) have prepared gold nanoparticles with average diameter of 8 nm by a tightly focused pulse laser at 355 nm in an aqueous solution of (SDS). While Mafune et al.,

(2006) have studied the concentration dependence of the abundant liquid. It is found that the stability of the nanoparticles in the solutions is related to the charge state on the nanoparticle surface.

Zheng et al., (2007) have reported a new method for the tunable production of monodisperse silver nanoparticles has been presented using different laser wavelengths to irradiate initial solutions of seed crystals. It is found that the size and shape of silver nanoparticles can be controlled in this method.

Jimenez and his group in (2008) have explored a novel technique concerning laser ablation of a solid target immersed in a water solution of a metal salt. Silicon was chosen as a target to synthesize silver and gold nanoparticles from a water solution of metal salt (either $AgNO_3$ or $HAuCl_4$). Moreover, a novel Au-core–Ag-shell nanoparticles was also prepared by irradiating mixture of Au–Ag nanoparticles using pulsed laser ablation of metallic targets in liquid medium.

Pyatenko et al. (2009) were presented mechanisms for synthesis silver and gold particle size reduction by laser ablation. The results presented in this work are interesting for researchers who using lasers in particle size controlling, resizing, and reshaping. While Petersen et al., (2009) were employed a high repetition rate laser (5 KHz) and 800 nm wavelength to prepare gold NPs using a femtosecond laser system delivering 120 fs laser pulses.

The influences of temperature on the hydrodynamic diameter has been studied by Manjon et al., (2010) have studied the preparation of gold nanoparticles using Infra-Red laser beam. The target is ablated by femtosecond laser in water at different stabilized liquid temperatures in the range 283-353 K. It is found that the maximum diameter was observed at 330 K. In the same year, Karimzadehet al., (2010) have synthesized silver nanoparticles by nanosecond pulsed laser ablation for silver plate immersed distilled water. The results revealed that narrow size distribution of the nanoparticles with radius centered at about 9 nm with a standard deviation of 3 nm can be obtained.

In (2011), Zamiri et al., were fabricated silver nanoparticles by Q-switched Nd:YAG laser ablation of silver plate immersed in a vegetable oil (castor oil). The results showed that silver nanoparticles of 5 nm diameter were very stable for long time. The same research group (Zamiri et al., 2012) have reported a comparative study on the efficiency of stabilizing silver nanoparticles prepared by laser ablation using natural polymers. They found that natural polymers can improve the formation efficiency and stability of silver nanoparticles when dissolved in starch polymer.

Dorranian et al., (2013) have studied effects of the laser flounce on the characteristics of silver nanoparticles. They found that silver nanoparticles exhibit two peaks in the photoluminescence emission due to the inter band transition and the electron-electron recombination. They also found that increasing the laser flounce produces smaller nanoparticles due to the secondary interaction of the laser pulse and the ablated nanoparticles.

Moreover, a literature survey on application of silver nanoparticles on bacterial activity was investigated. Many work have been devoted on antibacterial activity of silver nanoparticles against different types of bacteria. V. Ilic et al., (2010) have used radio frequency to enhance the binding efficiency of the colloidal silver nanoparticles and studied the stability of antibacterial effect on S. aureus. While Kim and his research group (2007) have also studied the antibacterial activity of silver nanoparticles on E. coli and S. aureus. Moreover, Li et al., (2010) have examined the Minimum Bacteria Concentration (MBC) of silver nanoparticles and found that it was

20 µg/ml. They also found that longer time of interaction with silver nanoparticles to 12 hours leads to breakdown the bacteria cell wall.

Many groups have investigated the antibacterial activity of silver nanoparticles against gram-negative bacteria such as E. coli bacteria. Georgious et al., (2010) have compared the antibacterial activity of silver nanoparticles and the released silver ions. While Raffi et al., (2008) have prepared the preparation of silver nanoparticles by inert gas condensation method. They have attributed the antibacterial activity of silver nanoparticles to the large surface area of silver nanoparticles. Furthermore, Pal et al. (2007) have studied effect of silver nanoparticles shape and they found that the antibacterial activity against E. coli is shape-dependent interaction.

While another research group emphasizes that silver ions are released through the oxidation dissolution due to the surround O_2 (L. Benjamin and S. Francesco, 2016).

In 2016, one group state that the effect of silver nanoparticles can be summarized by the subsequent steps; adhesion of silver nanoparticles to microbial cell, penetration inside the cell, generation of Reactive Oxygen Species (ROS) and modulation of microbial signal transduction **(**T. Dakal et al., 2016). Moreover, the fluorescence study demonstrates that the outer cell membrane disruption is an important toxicity mechanism **(**J. Chen et al, 2016).

Recent research in 2017 reveal that silver nanoparticles have great influence on multi-resistant bacteria and can be mixed with antibiotics to enhance the antibacterial activity **(**R. Salomoni et al., 2017). Moreover, some recent work on silver nanoparticles activity indicates that silver nanoparticles and silver ions have different physic-chemical properties and therefore, different interaction with bacteria cell and subsequently, different toxicity on the bacteria (Anna Kedziora, 2017).

2.2 Nanotechnology and Nanoparticles

Nanotechnology is the science and technology which deal with materials of nanometer sizes. This field has attracted great attention last two decades due to the unique properties of nanomaterials and control the structure and composition of nanomaterials would control those properties. Reducing dimensionality leads to major consequences of the nanomaterials properties compared with those of bulk. This is attributed to the quantum confinement effect. The quantum confinement could take different structures (U. Kreibig and M. Vollmer, 1995)

1. Quantum wells, which confine electrons or holes in one dimension and allow free propagation in two dimensions.
2. Quantum wires, which confine electrons or holes in two spatial dimensions and allow free propagation in the third.
3. Quantum dots, which confine electrons in all three spatial dimensions.

The previous mentioned quantum confinement structure can be represented by schematic diagram in Figure 2.1. Larger quantum dots have more energy levels which are more closely spaced and that means the density of states increased when the size decrease.

Nanoparticles are of two types: non-engineered and engineered NPs. Non-engineered NPs present in the environment are derived from natural events such as terrestrial dust storms, erosion, volcanic eruption, and forest fires. While engineered NPs are manmade using many different materials, such as metals (including Au, Ag, Zn, Ni, Fe, and Cu) (Fedlheim and Foss 2001), metal oxides (TiO2, Fe 2O4, SiO2, CeO2, and Al2O3) (Fernández-García and Rodriguez 2011), nonmetals (silica and quantum dots) (Ehrman et al., 1999), carbon (graphene and fullerene) (Endo et al. 2013), polymers (Paques et al., 2014) (Rao and Geckeler, 2011), and lipids (soybean lecithin and stearic acid) (Ekambaram et al. 2012), where metal nanoparticles are subject matter type of nanoparticles for our study.

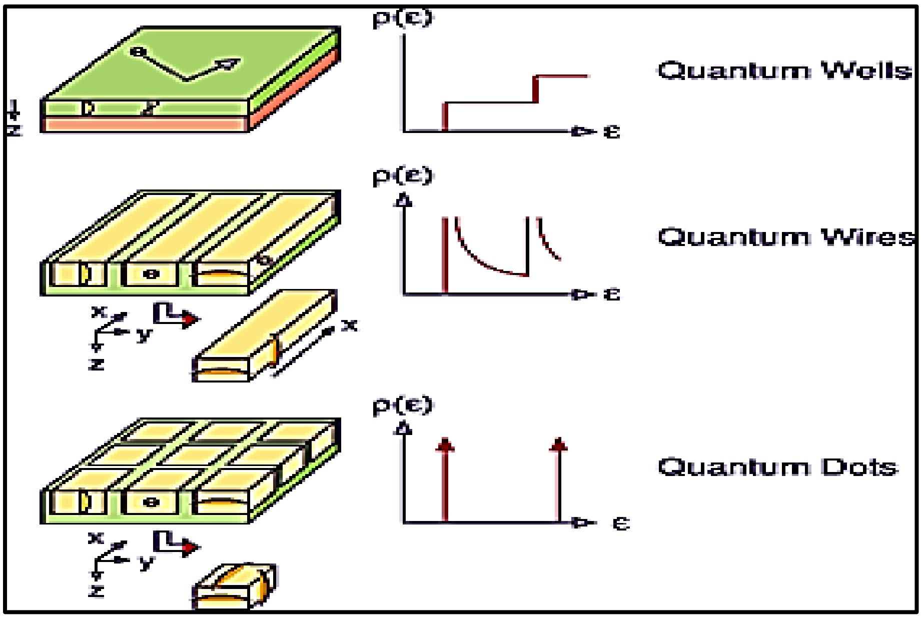

Figure 2.1: Quantum Confinement Structures and their corresponding density of states (P. J. Thomas, 2003).

2.3 Metal Nanoparticles

In the area of nanotechnology, metal nanoparticles NPs have played an important role in the development of new sensors especially biosensors to fulfill the demand for more specific and highly sensitive biomolecular diagnostics. The unique physicochemical properties of such noble metals at the nanoscale have led to the development of a wide variety of Nanobiosensors **(**G. Doria et al., 2012).

Noble metal nanoparticles such as; Ag, and Au NPs, have been a source of great interest due to their novel electrical, optical, physical, chemical and magnetic properties (R. Tilaki et al., 2006) and (M. Ullmann et al., 2002). They have different shapes and can be composed of one or more inorganic compounds, such as noble metals, heavy metals, iron, *etc.* Most of noble metals exhibit size-related properties that differ significantly from those observed in microparticles or bulk materials.

Depending on their size and composition, tremendous properties such as quantum confinement in semiconductor nanocrystals, surface Plasmon in some metal NPs can be distinguished. Noble metal nanoparticles in particular gold and silver NPs, are among the most extensively studied nanomaterials and have led to the development of innumerous techniques and methods for many targeting applications. Most of their unique physicochemical properties at the nanoscale range, such as Localized Surface Plasmon (LSP), have been explored for the development of new type of biosensors. Another important advantage of Ag and Au nanoparticles prepared by Pulsed Laser Ablation in Liquid (PLAL) process were chemically stable for a period of months. Additionally, gold and silver nanoparticles are exhibit Surface Enhanced Raman Scattering (SERS) in the visible range, where they may cause a tremendous increase in various optical cross-sections. These resonance frequencies strongly depend on nanoparticle shape and size as well as on the optical properties of the material within the near-field of the particle (U. Kreibig and P. Zacharias, 1970).

2.4 Synthesis of Metals Nanoparticles

There are two approaches to synthesize nanomaterials;

1. Top – down Approach

Many techniques can be used to reduce the material size to the nanometer scale. The main challenge of the top – down approach is creation of increasingly small size with good accuracy. The Top down method typically starting from bulk, involves laser ablation (R. Ganeev et al., 2005), and arc discharge (S. Hosseynizadeh et al., 2012). The nucleation takes place starting from the plume and continues till a solid substrate comes in its way. Control of particle size is achieved by tuning the flounce, wavelength irradiation time, etc. (B. Rasheed, 2013).

2. Bottom – up Approach

This approach has a better chance to produce nanomaterials of less defects, homogeneous chemical composition and better short and long range ordering. That is attributed to the reduction of Gibbs free energy which leads to form nanomaterial close to the thermal equilibrium (A. Siekkinen et al., 2006).

The bottom up method starting from atoms, include chemical (J. Liu et al., 2010), electrochemical (R. Khaydarov et al., 2009, sol-gel method (S. Eustis, 2006), etc., have been used to generate nanoparticles. Bottom up synthesis techniques usually employ an agent to stop growth of the particle at the nanoscale.

Synthesis of metal nanoparticles can be classified into two methods; physical and chemical method:

2.4.1 Chemical Methods

This method includes the following techniques:

1. Chemical reduction of metal salts
2. Microemulsions
3. Electrochemical synthesis
4. Chemical vapor deposition

Chemical Reduction of Metal Salts is simplest and most commonly used to prepare silver and gold nanoparticles in aqueous reduction. Nanoparticles silver may be prepared by citrate reduction, such as silver nanoparticles from $AgNO_3$ (M. Muzamil et al., 2014). While gold nanoparticles can be prepared from $HAuCl_4$ by sodium citrate at boiling point and other reducing agents, the size control of the nanoparticles can be done by adding a stabilizing agent. The drawback of the chemically produced colloidal gold nanoparticles is their contamination with reaction by products such as an ions and reducing agents (Y. Lee et al., 2013).

Moreover, the micro emulsionis a technique for synthesis of nanoparticles in which two immiscible fluids are mixed together with the help of surfactant. For example, water in oil or oil in water become a thermodynamically stable dispersion with the aid of a surfactant. A typical emulsion is a single phase of three components, water, oil and a surfactant (X. Liu et al., 2010). Normally oil and water are immiscible but with the addition of a surfactant, the oil and water become miscible because the surfactant is able to bridge the interfacial tension between both fluids (Y. Lee et al., 2013).

The micro emulsion consists of surfactant aggregates in the ranges less than 100 nm. The geometry of aggregate is strongly affected by the location of water, oil and surfactant phases. The product of oil in water and surfactant (O/W) is called micelles, which is an aggregate formed to reduce free energy. Hydrophobic surfactants in nanoscale oil and micelles point toward the center of aggregate, whereas the hydrophobic head groups toward water, the bulk solvent. The water in oil microemulsion carries oil or organic solvent as bulk. The system is thermodynamically stable and called reverse micelles (X. Liu et al., 2010).

Furthermore, electrochemical method can also be used to synthesize method for the production of metal nanoparticles. Electrochemical synthesis can be conducted by passing an electric current between two electrodes separated by an electrolyte such as hydrofluoric acid. The synthesis takes place at the electrode electrolyte interface (A. Umer et al., 2012).

Many advantages of electrochemical techniques among other methods and these can be represented by; avoidance of vacuum systems as used in physical techniques, low costs, simple operation, high flexibility, easy availability of equipment and instruments, less contamination (pure product) and environment-friendly process (A. Umer et al., 2012).

Chemical Vapor deposition (CVD) is a widely used technology for fabrication of metal nanomaterials. The majority of its applications involve applying solid thin-film coatings to surfaces, but it is also used to produce high-purity bulk materials and powders, as well as fabricating composite materials. CVD has been used to deposit a very wide range of materials (H. Rashid et al., 2015).

Typically, CVD process can be used to prepare metal nanoparticles involves complex flow dynamics since gases are flowing into the reactor, reacting, and then by-products are exhausted out of the reactor. The sequence of events during a CVD reaction as follows and shown in Figure 2.2:

1) Precursor gases input into the chamber by pressurized gas lines.
2) Mass transport of precursors from the main flow region to the substrate through the boundary layer (2a);
3) Adsorption of precursors on the substrate (normally heated) (2b).
4) Chemical reaction on the surface (2c)
5) Atoms diffuse on the surface to growth sites.
6) Desorption of by-products of the reactions (2d).

Mass transport of by-products to the main flow region (2e) (H. O. Pierson, 1992).

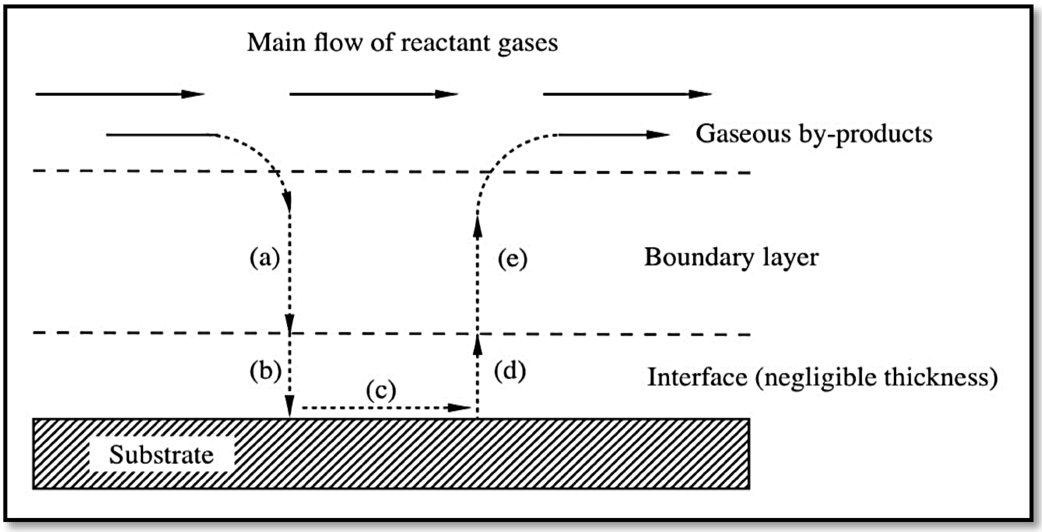

Figure 2.2**:** Sequence of events during CVD: (a) diffusion of reactants through boundary layer, (b) adsorption of reactants on substrate, (c) chemical reaction takes place, (d) desorption of adsorbed species, and (e) diffusion out of by-products through boundary layer (H. O. Pierson, 1992).

2.4.2 Physical Methods

Many interesting Physical methods can also be used to synthesize nanomaterials. Those methods provide nanomaterials and metal clusters of various features (U. Kreibig and M. Volmer, 1995).

The physical methods include:-

1. Thermal Evaporation
2. Exploding Wire Technique
3. Sputtering Deposition
4. Laser Ablation

Thermal evaporation is used to prepare nanofilms and also many other applications like formation of optical interference coatings using high and low index of refraction materials, mirror coatings, decorative coatings, permeation barrier films on flexible packaging materials, electrically conducting films and corrosion protective coatings. When depositing metals, thermal evaporation is sometimes called vacuum metallization (W. Prusseit, 1999).

This process Physical Vapor Deposition (PVD) process where material from a thermal vaporization source reaches the substrate without collision with gas molecules in the space between the source and substrate. This technique can be used to prepare a variety of metal compounds nanoparticles. Typically, thermal evaporation takes place in low gas pressure range of 10^{-5} to 10^{-9} mbar, depending on the level of contamination that can be tolerated in the deposited film. And vaporization sources are resistively heated stranded wires, boats or crucibles (for vaporization temperatures below 1500 C^{o}, while high-energy electron beams that are focused over the surface is used to evaporate materials of higher melting pints.

Moreover, the explosion is a familiar technique to produce nanoparticles when very high current density is applied to a thin metal wire, causing the wire to explode to very small fragments. This process involves wire heating and melting followed by wire evaporation followed by vapor/plasma expansion and shock waves. Metals nanoparticles are prepared using a simple apparatus consisting of a vacuum chamber, a powder collection filter and a discharging circuit (W. Jiang and K. Yatsui, 1998).

Synthesis of nanoparticles can be performed using the exploding wire technique. This technique can be conducted via either pulsed discharge to supply a high power pulsed current or by continuous discharge system (DC discharge). The pulse discharge is useful for hard materials like titanium and tantalum, while continues discharge could be used for materials of low hardness such as silver, gold, lead. The metal who need to prepare as nanoparticles should use as an anode in thin wire of few millimeter diameter, while the cathode represented by graphite or carbon rod in the wire explosion system. In this technique, metal vapor then cooled by an ambient gas to form nanoparticles. Preparations of metal, oxide and nitride nanoparticles by wire explosion can also be performed (G. Kawamura et al., 2015). This method has some advantages and limitations. It has potentially a high production rate. While in the same time, some other limitations have to be considered. This process is not used conventionally for common industrial purposes because it is not only very expensive but also impossible to use explicitly for different metals. It is mainly useful for metals of high electrical conductivity that are easily available in the thin wire form (C. Cho et al., 2010).

Sputtering is a method of vaporizing materials from a solid surface by bombardment with high-velocity ions or electrons in inert gas, causing an ejection of atoms and clusters. Vacuum systems, below 10^{-3}mbar are normally used in this technique. While Sputtering sources such as an ion gun or a hollow-cathode plasma sputter source and sometimes, electrons from electron gun instead of ions can be also used. This technique is one of the physical methods that can be employed to prepare a wide variety of metals nanoparticles.

Laser Ablation of solids has been extensively explored the last years for formation of metals nanoparticles. This process can be employed either in a gas-solid or liquid-solid interface. Many parameters such as target materials, background gases and the laser wavelength, flounce, and pulse duration are possibly used to produce a wide variety of compounds nanoparticles (S. Barcikowski et al., 2009). Moreover, Pulse Laser Ablation in Liquid (PLAL) can be seen as the extension of this concept. Therefore, the process of laser interaction with the target is similar for both laser ablations in a vacuum and ablation at the solid-liquid interface. Both produce plasmas and create a strong confinement of the emission species, resulting in an efficient electron-ion recombination. The difference occurs when the plasma begins to expand, which occurs freely in vacuum, while is confined by any liquid layer. Generation of various NPs by PLAL is an alternative technique to Chemical Vapor Deposition (CVD) method due to its relative simplicity and the low cost of the experimental setup. Moreover, nanoparticles produced by laser ablation of solid targets in a liquid environment are free of any counter-ions or surface-active substances.

2.5 Laser Ablation in Liquid

The initial process of laser ablation is interaction of light with solids surfaces and this causes vaporization of the target with small amount of the surrounding liquid. Due to the high energy, these ablated species may interact with the liquid molecules. Laser ablation in liquids has a similar concept with that of laser ablation in gases. Both processes produce plasmas and create strong confinement of the emission species but the main difference occurs when the plasma expands freely in the gas while it is confined in the liquid for the other.

Laser ablation in liquids, has many advantages and can be summarized as follow;

1. Chemically clean and simple process.
2. Nanoparticles of metals, semiconductors and insulator can be formed.
3. Single and compound metallic nanomaterials can be obtained by this process.
4. Feasibility and Low cost experimental set up.
5. The nanomaterials produced by this process is easy controlled (H. Kobayashi et al., 2013).

2.6 The mechanism of PLAL

Pulse laser ablation in liquid is a significant process which occurs by four subsequent stages; laser-induced melting, laser-induced boiling, explosive evaporation & plasma formation and plasma expanding in liquid & condensation.

2.6.1 Laser-Induced Heating and Melting

The absorption of laser light by metal nanoparticles gives rise to energy transformation processes. These involve the successive excitation and interaction with the lattice. Afterwards, several thermal processes like heating, melting or evaporation can be activated. In the case of nanosecond-pulsed laser, the heat diffusion from the metal particle to the support takes place on a time scale much shorter than the pulse width. This process ends with thermodynamic equilibrium (K. Zimmer, 2009).

The temperature distribution in this process is governed by the heat conduction equation (C. Liu, 2005):

$$\rho C_p \frac{\partial T}{\partial t} = \nabla \cdot (K \nabla T) + (1 - R) I_0 \, \alpha \, e^{-\alpha z} \tag{2.1}$$

Where (ρ, C_p, K and T) represent density, specific heat, thermal conductivity and temperature, respectively. The second term on the right hand side of the equationis related to the laser source which absorbed by the material at a depth z from the surface, where R is the surface reflectivity, and I_0 is the laser intensity and α is the absorption coefficient. The reflectivity was found to be strongly dependent on the laser pulse duration (W. Chan et al., 2008).

2.6.2 Explosive Boiling

When a very high-energy laser flounce is directed to the target surface, a dramatic change is occurred. When the surface region is heated beyond the limit of its thermodynamic stability during short-pulse laser irradiation, the surface is supposed to undergo a rapid transition from a superheated liquid to a mixture of vapor and liquid droplets. The experimental results reveal that the transition from normal vaporization to phase explosion is happened (Q. Xia and S. Y. Chou 2010).

2.6.3 Evaporation

When the temperature increased rapidly, some of the metal mass evaporated and forms a vapor plume which moves away from the surface with a high temperature and pressure. The evaporation rate is given by (C. Liu, 2005):

$$R = \left(\frac{k_B T_l}{2\pi m_a}\right)^{\frac{1}{2}} \exp\left(-\frac{h_{lv}}{k_b T_l}\right) - \theta_s n_v \left(\frac{k_B T_v}{2\pi m_a}\right)^{\frac{1}{2}} \qquad (2.2)$$

Where the subscripts *l* and v represent liquid and vapor, respectively, and are the latent heat of vaporization h_{lv} and T_v vapor temperature, m_a is the atomic mass of the metal and k_B is the Boltzmann constant. The first term in this equation represents the evaporization rate from a liquid surface temperature while the second term represents the condensation rate of molecules back to the liquid surface. The coefficient θ_s represents the probability that a vapor atom returning to the liquid surface is adsorbed as in (L. Zhigilei et al., 2009) and (K. Gouriet et al., 2009).