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Preparing and Studying Nano Silver Particles and Their Anti Bacterial Effect

A Thesis

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بسم الله الرحمن الرحيم

وَمَا تَوْفِيقِي إِلَّا بِاللَّهِ ۚ عَلَيْهِ تَوَكَّلْتُ
وَإِلَيْهِ أُنِيبُ

صدق الله العلي العظيم

سورة هود: آية ٨٨

DEDICATION

To my dear mother, who was the basis of my encouragement and success in all areas of my life and to all my family where they shared with me the most accurate details of my life.

ACKNOWLEDGMENT

Praise is to **“ALLAH JALA JALALAH”** for being the best and the best for me before everything and peace be upon his messenger **“Mohammad”.**

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Abstract

The target of this research is to prepare nano silver particles by laser ablation in both deionized and magnetized water. The properties of nano silver particles and their bacterial effects with different parameters' were studied. The magnetic water was laboratory preparing by square magnets with magnetic flux of 200 mT applied on the deionized water which pass through it 10ns Q.swiched Nd-YAG laser of wave length 1064nm with different energies and different laser pulses number were used with and without applied electric field. Optical properties for the nano silver colloidal by UV-Vis technique was investigate. The absorption for the silver spectrum was found lied at 420-430nm, i.e. involved visible region. The absorption peaks have different height due to different both the shape and concentration of nano silver in the colloidal.TEM technique was used for investigating both size and shape of nano silver particles. The nano silver particles have different shapes such spherical, spindles, etc…. depending upon two parameters is laser energy and laser pulses number. The effect of nano silver particles on bacteria growth of three types of bacteria (staph, ps., E.coli) were studied in details under effect of applied electric field of 3000V/m. There is no inhibition zone was obtained when used different silver colloidal except the colloidal prepared under 600mJ and 1000laser pulses, where this colloidal gives an effected property and ability to determine the bacteria growth type ps. In the range of inhibition of 25nm.The results of the TEM show that the shape of nanoparticles affecting the growth of bacteria was spherical and its size ranges between32-50nm.

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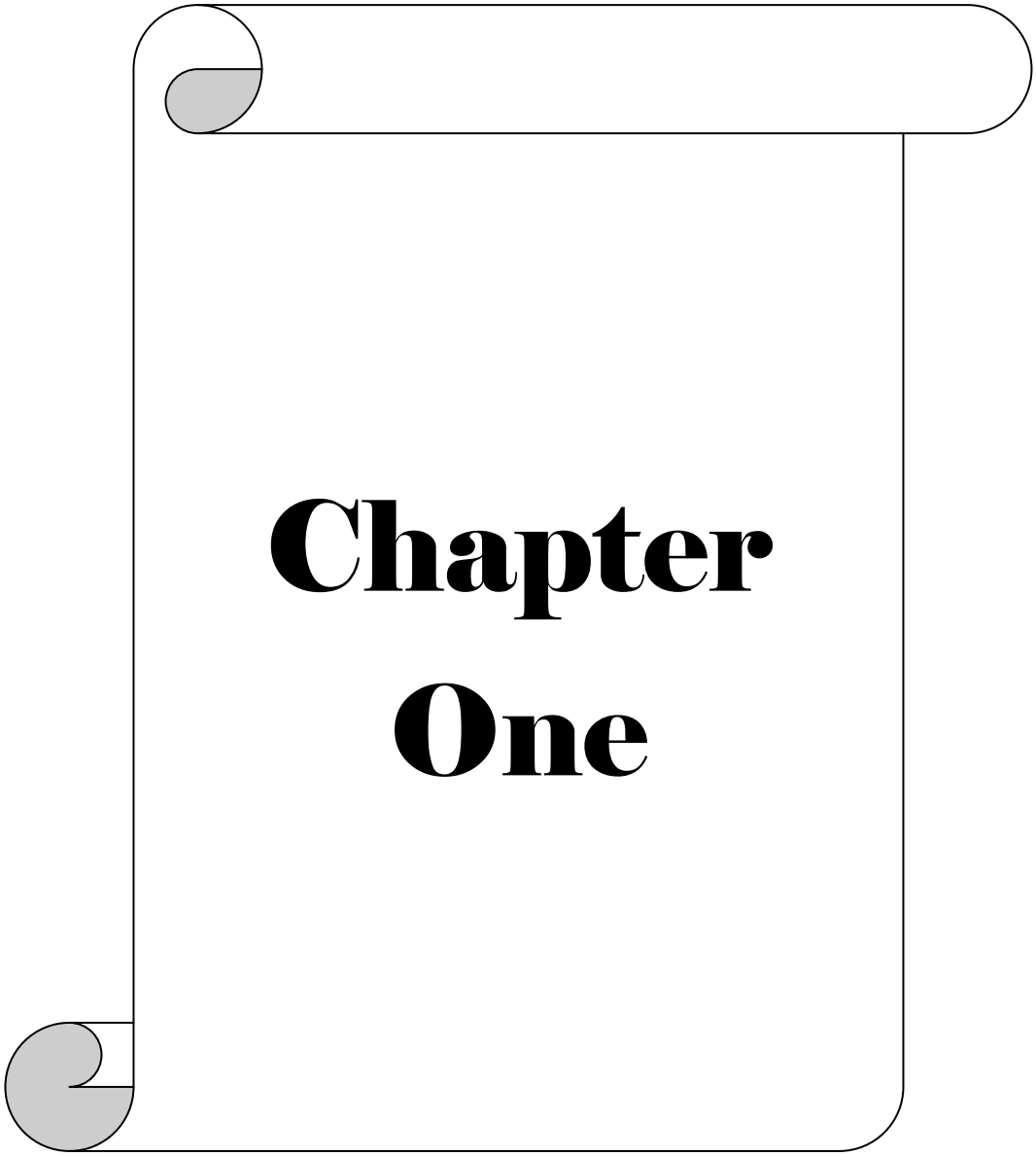
List of Abbreviations

AgNPs	Silver nano particles
Nd:YAG	Neodymium YAG laser
TEM	Transmission electron Microscopy
SEM	Scanning electron microscopy
E.coli	Escherichia coli
Staph.	Staphylococcus aurous
PS.	Pseudomonas
XRD	X-ray diffraction
UV-Vis	Ultraviolet –visible spectroscopy
BC	Bacteria cellulose
PLA	Pulsed laser ablation
DDW	Double distilled water
AuNPs	Gold nano particles
SPR	Surface Plasmon resonance
EDS	Energy Dispersive X-ray
µS	Micro Siemens
Mag(water)	Magnetized water
Dei(water)	Deionized water


Chapter
One

Chapter One

Concept nano science and Literature Review

1.1 Introduction

Today the nano size material and technology have induced major scientific advancement in the field of science, medicine, and industry.The fabrication of small size object which can be used throughout all fields such as physics, biology, medicine and engineering nanotechnology. Nano size particles are a core material which carries out as a whole unit in terms of property and transportation. The term nanomaterial pointed to the nano size in which means a billionth of unit Because of ultra small size of material it occupies a location and importance in several fields of nano science and nanotechnology the range of its size already taken from 1-100nm [NAI10].

1.2 History of nanomaterial

The particles which have nano size are quite unique- in nature due to the nano size increase surface area to the weight or volume ratio as well as their physical biological and chemical properties is different to the relative of bulk material. So the purpose of study the field of nano material and introducing small size is to trigger and excite chemical activity with distinct crystallography that tend to increases the surface area. Thus in 10th latest years much researches and scientific literatures are going to metallic nano size particle and its properties such sensing to optics antibacterial activity information steerage capacity [NAL10, SHA09].Nanotechnology is a vast growing area in the field of science and technology that give increase the idea of investing and regulating at cell level among synthetic material and biological system. [DU07, SIN09].Nanoparticles of metal for instance silver copper gold zinc…etc which is formation via plants has been stated

in several articles. Colloidal contains silver nano size had exhibited distinct properties such as anti bacterial activity, good conductivity and chemical satiability[SHA09].Silver nano sizes have several purposes in the field of bio labeling detector optics antibacterial electronic and other medical application such drug and diagnosis [JON08].

Nano size of particles is utilized in industries in the field of medical, military and physical activities. The unique properties of these nonmaterials are highly dependent upon their size and shape. Nanometer scale sized particles tend to exhibit huge different properties than their bulk scaled of the same material. For example the small size of the silver Nanoparticles is mentioned in several researches reported to improve the antibacterial properties of silver in addition to improving chemical durability [TOS99]. The antibacterial properties of silver nanoparticles have led to several new products such as nanoparticles embedded clothes dryers sheets to reduce clothing (socks) odors and antibacterial soap and toothpaste. The enhanced properties are due to the large surface area to volume or weight ratio. This larger ratio significantly may be increases the antibacterial effects by allowing easier interaction with other particles [LEE03].

Silver nanoparticles can be synthesized and prepared experimentally using several different techniques such as chemical reduction laser ablation photochemical sol-gel and electrochemical methods [SIL06]. Nano particles, of silver metal (AgNPs) are widely increasingly used in several fields involving health care medical and industrial chemical and biological properties. These targets include different application for instance optics, magnetic, thermal, electrical conductivity, and biological application [LIW10, MUK01]. Nanosilver is much more efficient as antimicrobial than

bulk silver [JON10]. The nanosilver appears to be more efficient than bulk silver at generating silver ions [WIJ09].

1.3 Literatures Review

I. **Tsuji, T. *et.al.* [TSU02]**: They prepared silver nanoparticles via laser ablation using Nd: YAG with different wavelength (1064, 533,355nm) an electron microscope joined with energy dispersive X-ray was used to investigate the characteristic of silver nanoparticles. They conclude that the diameter of particles become smaller in between 29-12nm with decrease in laser wavelength the particles size can be controlled by changing the number of laser pulses and the wavelength of laser beam from 1064 to 355nm.

II. **Abid, J.P. *et.al.* [ABI02]:** They used silver salt and by laser irradiation to prepare the silver nanoparticles in solution as a colloidal. This technique considered an alternative method for preparation metallic salt solution with using Nd: YAG laser system as they conclude one can get the metallic nanoparticles with narrow size distribution as well as the shape of the prepared particles are also exclusively spherical owing to the continuous irradiation of the solution.

III. **Ganeev, R.A. *et.al.* [GAN 04]:** They studied the characterization of optical and nonlinear optical properties of silver nanoparticle prepared by laser ablation with Nd: YAG Laser (wave length =532nm). They used the silver plate of purity (99.99%) placed inside 10mm thickness with liquid of different viscosity. The solution was prepared cauterized (TEM) and spectral measurement have shown temporal dynamic of Ag nanoparticles size distribution.

IV. **Sondi, I. *et.al.* [SON04]**: They are showing the ability of nano silver as antimicrobial agent and study its activity on E.coli as a model against gram-negative bacteria by using Luria-Bertani culture. They noticed when silver nanoparticles were present on LB agar plate they could completely inhibition bacterial growth as well as the inhibition depend on the concentration of the silver Nanoparticles they concluded that silver nanoparticles have an excellent antibacterial activity against E.coli.

V. **Jose, R.M. *et.al.* [JOS05]:** They used the nanotechnology for synthesized silver nanoparticles. They obtained the powder of silver nano size solution of carbon matrix. They found the silver Nanoparticles is suspended in water to make colloidal in order to perform the interaction of the silver nano particles with the bacteria. The analysis of silver nanoparticles was achieved by TEM where found the nano silver may be act primarily in three main methods against gram-negative bacteria:

1- Nano particles have the sizes in the range of 1-100nm close to the surface of the cell membrane and drastically disturb its proper function.

2- Nano particles can going and penetrate inside the bacteria cells and; cause further damage by possibly interaction with some compound such as DNA.

3- Nano sizes of silver ions will have an additional contribution to the bacterial affective the silver nano particles.

VI. **Kim, J.S. *et.al.* [KIM07]:** They studied the effect of Ag nanoparticles against Yeast Escherichia Coli and Staphylococci aurous. They used Muller Hinton agar plates and used the Ag nonoparticles of various concentrations. To inhibited the Yeast and E.coli they found the Ag nanoparticles can be used to effective the growth inhibitors in various microorganisms and discuss the ability of Ag ions and Ag based

compounds effect with the antimicrobial. From test found the AgNPs were toxic both the p.arruginosa. E.coli and their antibacterial efficiency when depend on the size and shape also noticed the spherical shape of Ag NPs against the bacteria is less efficient in bactericidal action than Triangle shaped.

VII. **SHR, S. *et.al.* [SHR07]:** They could synthesize silver Nanoparticles in range of size 10-15nm and found that with increased stability is enhanced anti-bacterial activity. The conclusion of this study found the silver Nanoparticles have stronger antibacterial potency and the effect was found dose dependent and was more pronounced against negative organisms than gram-positive ones.

VIII. **Martinez, G.M. *et.al.* [MAR08]:** Silver nano particles with different size were prepared by virtue chemical technique named Gallic acid in an aqueous reduction method .They used several analysis techniques such XRD, TEM, UV-Vis, for characterized silver Nanoparticles .They found the antibacterial activity of silver nanarticles can be modified with the size of silver nano particles where it' decrease with an increase of the particles size.

IX. **Song, Ki. *et.al.* [SON09]:** The Colloidal of silver nanoparticles was obtained by chemical reduction of silver nitrate in water mixed with sodium borohydride (NaBH4) in the presence of sodium dodecyl sulfate (SDS) work as a stabilizer. The prepared nanoparticles were characterized; by the UV-Vis absorption spectra and transmission electron micrograph (TEM) images. The UV-VIs absorption spectra showed that NaBH4 served not only as a reducing agent but also as a stabilizer which protects against

aggregation of silver nanoparticles. The TEM images showed that the particles were dispersed better with increasing the NaBH4 concentration**.**

X. **Sadeghi, B. *et.al.* [SAD10]:** Chemical reductions technique was used to prepare the solution of Ag (0.074%) and produced the nano size particles agglomerated with uniform size distribution of silver nano size. They found the concentration of silver nanoparticles is 0.076 to 100mg/ml. The solution was used versus both types of bacterial gram positive gram negative via virtue known disk diffusion. They showed the stabilized the AgNPs and high antibacterial activity. This study showed the AgNPs is strong versus antibacterial activity.

XI. **Maria, L.C.S. *et.al.* [MAR10]**: They prepared and studied silver Nanoparticles and their impregnated in bacterial cellulose by used a simple method for pregnancy a much amount of silver nanoparticles into- bacterial cellulose (BC). Nonporous structure as an effective nano reactor the colloidal. Protector particularly gelatin and this very important in keeping-silver particles from conglomerate and regulating the size of the particles anchored on the BC membrane as they said. They stated that the strong of bacterial activity of the Ag composite based on bacterial cellulose is attributed to the action of silver nanoparticles obtained when combination of hydroxylamine colloid was employed.

XII. **Dass, R. *et.al.* [DAS11]:** Preparation silver nanopartocles and studying their antibacterial activity is the goal of this project .Chemical reduction of silver ion by ethanol in presence of sodium linoleate was used .TEM technique was used to show a uniform distribution growth inhibitors against staphylococcus Basillus, Staphylococcus Aureus, and Pseudomonas

Aureginosa .They conclude that nanoparticles can be used as an effective growth inhibition in various microorganisms.

XIII. **Hwan, K.S. *et.al.* [HWA11]:** They used the silver Nanoparticles against Gram-positive and Gram negative bacteria. They stated the observation taken from the field emission scanning microscope (FE.SEM).And by using 5% silver brown as a silver soluble with Muller-Hinton agar as a culture media. They noticed that AgNPs could be used as an effective antibacterial media.

XIV. **Umadevi, M. *et.al.* [UMA11]**: They obtained silver nanoparticles by the technique of ultrasonic field via chemical reduction method with solution of sodium borohydride. They studied the activity of some types of bacteria such staphylococcus aurous Bacillus subtitles streptococcus mutants Escherichia coli and pseudomonad aerations. It was found the B.subtitles and E.coli were more sensitive's to the AgNPs and they conclude that the size of AgNPs is seemed to be very sensitive on the activity of these bacteria.

XV. **Mahmood, M.A. [MAH12]:** The AgNPs were prepared with (PLA) and silver plate immersed in double distilled water (DDW) and used them Nd: YAG Laser of 1064nm. She studied the activity of silver nano particles on the bacterial activity of different four common antibiotics versus gram positive and gram negative isolates use the disk diffusion mechanism for determine the antibacterial effect, noticed when use the AgNPs with amoxicillin and penicillin Gram positive isolates is the synergistic effect and between the AgNPs with both cefriaxone and chloramphenicol for Gram Negative isolates.

XVI. Jumma, T. *et.al.* [JUM14]: They studied the effective of electric field on the antibacterial activity by Au nanoparticles .They prepared nano gold particles by (PLA) ((Nd: YAG) of gold plate (with purity 99.99%) immersed in deionized water. The UV-Visible spectral analysis of gold nanoparticles colloidal (TEM). And showed the morphology and size distribution; of gold nanoparticles. When applied the electric field on the both gold nanoparticles colloidal and standard agar for different time they showed the positive effects of AuNPs on the activity of bacterial growth inhibition and both the concentration of gold nanoparticles and the applied electric field are effective parameter on the bacteria growth.

XVII. Hamza, L.F. *and* Ibrahim, I.M [HAM14]: Silver nonoparaticles have been prepared by using pulsed laser ablation (Q -switched Nd: YAG) 1064nm pulse duration and (E= 100mJ to 400mJ) of pure Ag metal plate immersed in distilled water and deionized water. The synthesized nanoparticles are characterized using transmittance electron microscopy; (TEM) and UV-VIS spectrophotometer. The effect of the pulses laser energies and pulses number; denoted the; activity of AgNPs against bacteria; growth. The silver nanoparticles exhibited surface Plasmon resonance effect with wave length (λspr =400nm).They could production of pure and spherical Ag with average size of (1-15nm) .All the size measurements have been confirmed by TEM.

XVIII. Raúl, B.S. [RAU14]: The solution of silver nanoparticles was obtained by laser ablation in order to analyze the affectivity of this method. The laser used was Nd: YAG with 1064 nm of wavelength. Characterization of these particles was done using a transmission electron microscope (TEM) and a UV-Vis spectrophotometer of double beam in order to measure both the size and the absorption spectrum of these Nanoparticles respectively. The

average diameter of the produced nanoparticles increases from; 9 to 22 nm as the laser pulse energy increases from 9 to 13 mJ. These particles obtained in solution present a strong absorption due to Plasmon resonance around 400 nm. It is observed that the peak absorbance of each sample is directly related with the concentration and the size of silver nanoparticles. The position and the maximum value of the peak absorbance varieties when ablation time energy density or laser energy is changed. Ablation efficiency is reduced as time progresses during the process due to the absorption and dispersion of laser light by the nanoparticle solution**.**

XIX. **Purushotham, E.** and **Krishna, N.G. [PUR14]**: They used the mechanisms of ball milling for fabricate silver nano particles. They used XRD technique to investigate many parameters such as particle size lattice strain and root mean square. They conclude from the correlation between the strain and Deby-Waller factors for zero strain have been given and calculated for Ag .The relation among the vacancy formation and lattice strain had been investigated.

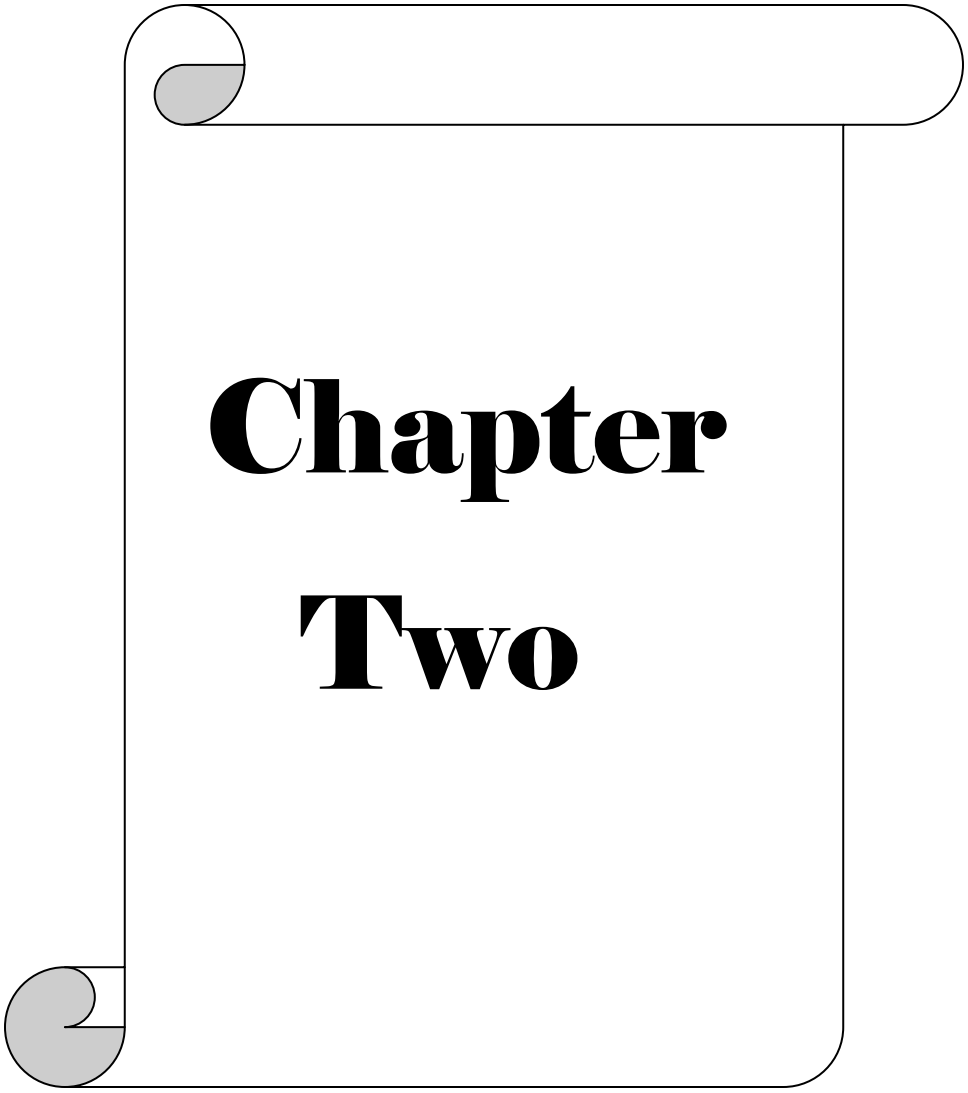
XX. **Raza, M.A. *et.al.* [RAZ16]:** The Ag nanoparticles of different shape and size of silver nanoarticles had been introduced via solution upon a reduction method routs technique. He used the silver nitrate was added to a precursor and (TSC) Trisodium citrate and sodium borohydride as reducing agents. (PVP) used as a stabilizing agent the morphology shape size and structure properties of obtained Nanoparticles were characterized by SEM, UV-vis and X-ray techniques respectively. noticed the spherical shape of AgNPs and found the diameter of particles in the range of 15-90 nm while the particles length is 150nm, also noticed the absorption peaks of different

AgNPs at the wavelength 397nm to 504nm it was found the smallest spherical size of AgNPs have a good efficient versus the bacterial strains compared with the triangular as well as with larger spherical shaped of AgNPs.

1.4 Aim of the work

The aim of this project can be summarized in the following remarks:

1. Preparation nano silver particles by laser ablation method and use the energy and the number of different pulses and see the effect of each energy and intensity on the silver nano particles.
2. Preparing both deionized and magnetized Water.
3. Magnetized water production in a new method.
4. Visually discriminate through the difference between deionized water and magnetized water.
5. The choice of silver to work because of physical and chemical properties enjoyed by silver.
6. Studying the effects of nano silver particles anti positive and negative gram of bacterial.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Chapter Two

Theoretical Considerations and concepts

2.1 Introduction

Fabrication of nano size particles via laser ablation technique of metals either in noble gas or in vacuum condition has been explored early the last two decades. A modern; methodology based on laser ablation in; liquids such water has received much attention as a novel nanoparticles-production technique. Laser ablation technique represents dramatic laser-material-interaction phenomenon. The amount of mass removed from such material was found depending on the laser parameters such as: pulse duration, pulse energy, Laser wavelength, target properties and the surrounding environment[LIU05, XIA10].The synthesis of silver nanoparticles from top-down method the effect of nanosilver against the bacterial. Silver is a nontoxic material safe inorganic antibacterial agent used for centuries and is capable; of killing more than 650 types of diseases causing micro organisms. It's found the ability of silver nano sizes to exert a bactericidal effect at same minute. It has a significant energy versus wide range of biological purpose for instance antifungal at agent infections and anti-inflammatory [RAF08].

2.2 Nanoparticles

The term "nanoparticles" is use to explain a particle with size in the range of 1nm-100nm, at least in one dimension. In this limit of sizes will be exchange in respect basic methods relative to the properties of both individual atoms/molecules- and of corresponding raw material.

Nanoparticles -can be made of bulk- materials of have different chemical nature, the most common being metals, metal oxides, silicates, nonoxide-ceramics, polymers, organics, carbon and bimolecular.

Nanoparticles exist in different shapes for examples spheres, cylinders, tubes, and other different shapes. Because of their nano size feature over available chemical imaging drug and drug agent, inorganic particles have been attempted as a potential tools for purposes the medical imaging [PIN06]. Nanoparticles have a big surface area to volume ratio together with size activity (give quantum confinement) which gives nano particles characteristically variations property for instance, optical, electronic, chemical, etc…from those of natural material [BAR04]. Silver Nanoparticles can be synthesized by some several techniques such as chemical reduction, laser ablation, photochemical and electrochemical methods [SIL06]. Silver nanoparticles are of importance because of the characteristic properties (e.g., size and shape depending optical, electrical, and magnetic properties) which can be involved into antimicrobial treatment, biosensor materials , composite fibers, cryogenic superconducting materials and electronic components. Several physical and chemical methods have been used in recent years for synthesize and stabilize silver nanoparticles [KLA99, SEN05]. Pure silver as known has the better electrical and thermal conductivity than most metals and alloys [RAM12]. Due to uses variety of nanosilver permits exposure throughout several routes of entry into the cells body. Silver in any form have not a toxic to many tissues such nervous, cardiovascular, etc…in the same time it has not carcinogenic effect[FUR78].Therefore one can conclude that the

silver is relatively non-toxic in general [CHE08]. Silver is yet request in many new recently uses especially in industry medicine [RAM12].

2.3 Synthesis of AgNPs

In general nano size particles were fabricated *via* chemical and physical methods. Evaporation condensation mechanism by using a tube furnace under ambient pressure is used commonly to prepare nano silver particles. [GUR94, SCH88] Spark discharging, laser ablation and paralysis were used for fabricated of AgNPs [TIE08, POW93].These technique characteristic to the physical methods. The main feature of physical methods is speed process and pure material resultants by reducing agents and no hazardous but the downsides are low yield and high energy consumption solvent contamination and lack of uniform distribution [ELS15, SHA10, TSU05, and ABO10]. In Chemical methods solvent such as organic solution or water are communally used to prepare nano silver or nano gold particles [TAO06, WIL05]. Three main important parameters are effecting in chemical methods reducing agents metal precursors and stabilizing agents. In case of reduction of silver performed by two stages first nucleation and second subsequent growth. It is known two main techniques used for synthesis the nano silver particles [DEE11]. The top-down technique in which is required grinding and refining process of bulk material as shown in Figure (2.1)[AMU98, MAL04]. The down-top technique as shown in Figure (2.2) involved chemical reduction and electrochemical methods. The future of chemical methods is high yield and low energy consumption but in the same time are expensive. In addition the material which used in chemical methods is more toxic and hazardous such material is glycerol and citrate [MAL04]. One of disadvantage of chemical

methods is not of expected purity as their surfaces were found to be sediment with chemicals. It is also very difficult to prepare AgNPs with a well-defined size requiring a further step for the prevention of particle aggregation [MAL02]. In addition one can be expected, many toxic and hazards by material product during synthesis of nanoparticles [SER99], laser ablation [MAF00], lithography [HUL99], electrochemical reduction [ZHU01], laser irradiation [ABI02], Decomposition [TAL10], thermal decomposition [MOS14] and chemical- reduction [ZHA11]. The advantage of the chemical synthesis of nanoparticles is the ease of production low cost and high yield however the use of chemical reducing agents are harmful to living organisms [GUR15].

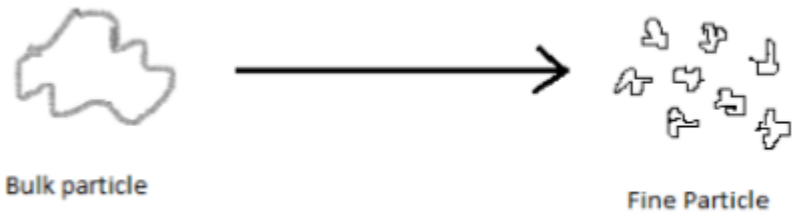


Figure (2-1): Top –down techniques [SON13].

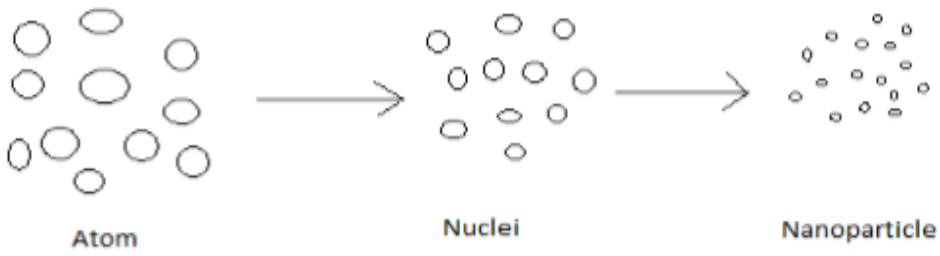


Figure (2-2): Down- Top technique [SON13].

2.4 Properties of AgNPs

The properties of silver nano particles, including surface activity shape of particles, size distribution, particle composition, agglomeration and particle reactivity are critical factors for classification of nano silver particles [CAR08, ZHA11, and GLI14]. It is expected that silver nano size can be fabricated in different shapes and sizes, spherical, hexagonal and rod are a common shapes appeared during synthesis. Figure (2.3) presents different shape and size of synthesis particles. More studies stated the assertions that smaller size particles may be more toxicity than larger particles due to their surface area [SRI12].

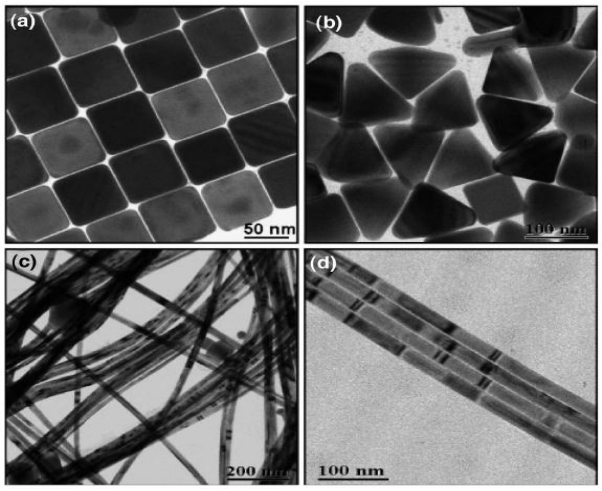


Figure (2.3): various shapes of AgNPs (a) cubes ;(b) triangles;(c)wires;(d) an alignment of wires [SHA09]

2.5 Biological applications of silver nano particles

Silver nano particles have wide been used in several fields in industry and medicine. It is found that it is used in food storage, health care industry biomedical application and environmental applications. Scientists and

researchers have been published several books and literatures concerning the application of silver nano particles .Now advanced researchers are interesting in emphasizing the application of silver nano particles especially in the field of biological application such antibacterial as shown in Figure (2.4)[SON04].

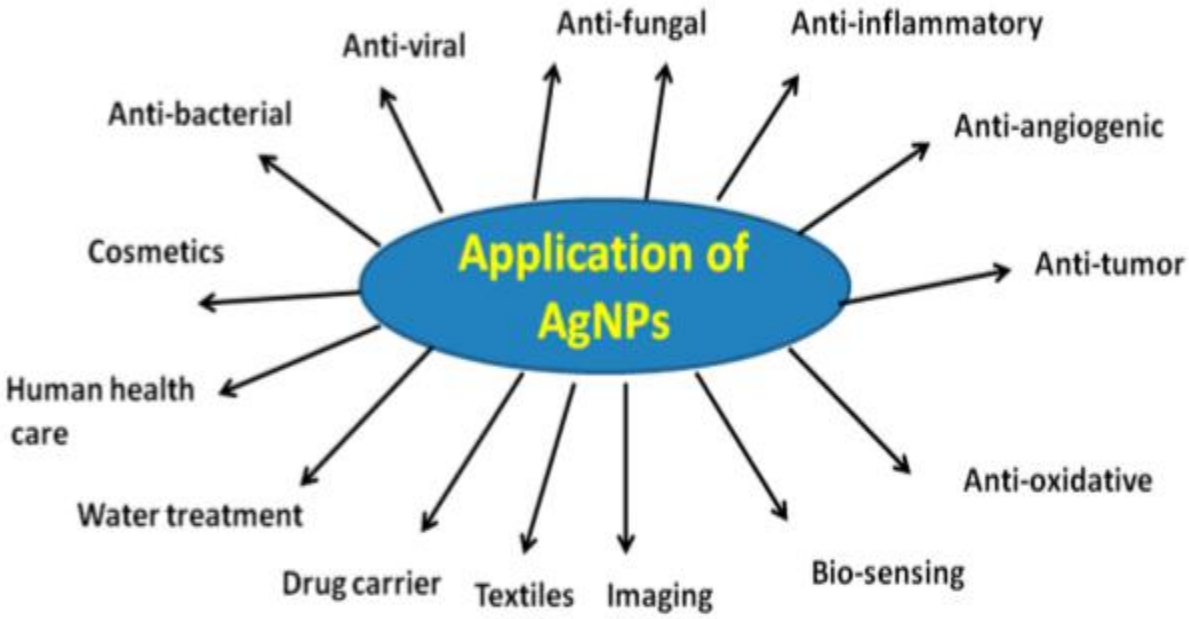


Figure (2.4): Several Applications of AgNPs.

2.6 Antibacterial activity of silver nano particles

In recent times the silver nano particles considered to be alternative solution versus antibacterial agents to antibiotics. It is found the AgNPs have capability to overcome the antibacterial versus antibiotic agents. [SON04].They found the AgNPs has a promise metal which be considered

have a potential antibacterial against antibiotic because of their unique physical property. They illustrated in their researches the activity of AgNPs against E-coli bacteria. In addition the research found the ability of AgNPs against bacteria resistivity is due of the size and shape of particles. Other research showing the ability of AgNPs against gram-positive and gram-negative bacteria compared with other antibiotic agents [BAK05]. Furthermore, more studied were achieved versus other types of bacteria and investigated the ability of AgNPs for determine the activity of bacteria growth [MOR05]. AgNPs were synthesized by four different types of saccharine with an average size of 25 nm showing high antimicrobial and bactericidal activity against gram-positive and gram-negative bacteria including highly multi resistant strains such as methicillin resistant Staphylococcus aurous. As mentioned before the size and shape of nanoparticles are important for determining the efficiency because AgNPs undergo a shape dependent interaction with the gram-negative organism E. coli [PAL07]. Furthermore a detailed study was to investigate the efficiency of the antimicrobial effects of AgNPs against yeast E. coli and Staphylococcus aurous. The efficiencies of various antibiotics such as penicillin amoxicillin, erythromycin, clindamycin, and vancomycin against Staphylococcus aurous and E. coli were increased in the presence of AgNPs **[**SHA07]**.**

2.7 Importance of silver nanoparticles

Silver nano particles have several physical and chemical properties make it an important metal used in many applications as the summarized points in following:

1- Silver nano particles use as a filter purification of air and water in many fields of industry, medicine and environment.

2- Nano silver particles can also fabricated *via* biological methods. Nano particles resultant has several applications in the field of solar energy detector receptors and antibacterial due to their heavy reaction.

3- Silver nano particles have enormous application in the field of bimolecular detection due to their sensitivity.For this characteristic feature its can be used in diagnostic and therapeutic, although the silver nano particles have some human toxicity.

4- In the field of industry application, the nano silver particles play a significant role in air condition, refrigerators and household [SHR07].

2.8 Characterization of nano particles

The nonomaterial's can be characterized by various physical or chemical techniques which provide feature for the understanding of different physiochemical features. The most extensively used techniques can be summarized into the following:

a) Scanning electron microscopy (SEM)

b) Transmission electron microscopy (TEM)

c) Ultraviolet visible (UV-Vis) spectroscopy

2.8.1 Scanning electron microscopy

The technique of electron microscopy (SEM) considered a verification method for specification of nanomaterial.The SEM technique characterized by high resolution with a few nanometer and high magnification above one million. This inspection technique is easily adjusted and operated. The SEM technique gives information about topography, chemical composition of material near surface. This information gives up through interaction between the electron beam and the specimen surface [NEW86].

2.8.2 Transmission electron microscopy (TEM)

Nano structure and composition analysis can be achieved in high precision by use a typical technique which is named transmission electron microscopy due to high resolution imaging .This technique involves three main components:

i) Subjected a very thin film to the electron beam of high energy .These electrons suffered diffracted by lattices of the material subjected to test.
ii) Imaging and angular distribution analysis of the forwarded scattered electrons.
iii) Energy analysis of the emitted x-ray.

Both structural characterization and identification of various phases of nano material can be inspected using TEM technique. TEM has many other uses in nano materials and nanotechnology inspection [WAN00].

2.8.3 UV-Vis spectroscopy

Electronic transitions between atomic or molecules level deals with the spectroscopy of UV-Vis. The absorption peaks characteristic with the metallic nano particles and exhibit different colors according to the size and shape of nano particles. Mie was study and explain the origin of established color theoretically earlier of 20^Th century. He was starting to solve the Maxwell's equations for the absorption and scattering of electromagnet waves by metallic particles. The nanoparticle contains colloidal will be absorb the electromagnetic waves, and the electrons of the valence band will be coherently oscillation by the interaction with the electromagnetic field. The resultant resonance is called a surface Plasmon, in which happen just associated with nanoparticles. Therefore the ultra violet-visible spectroscopy technique is considered useful for determining the optical properties of nano particles [LIN99]. The concentration of the substance in the solution can be measured by determining the absorption of light within this specific wavelength if the color formation is related to the concentration of the substance in the solution If a light (monochromatic) passes through the solution, there is a quantitative relationship and a law (the law of Beer), a relationship between the amount of material and the intensity of the passing light as illustrated below equation:

$$\mathbf{I=I_{\circ}\ exp\ -\alpha\ t}\quad \mathbf{[PAR15]}$$

$I_{\circ}$: The intensity of light passing through the pure solution.

I: The intensity of light when adding colored compound.

α: Absorption coefficient

t: Thickness

2.9 Nano particle formation by laser ablation

Since a photon of laser reaches to the surface of selective metallic plate such silver. The photon energy will be divided into two main parts, some reflected by the metallic surface and the other will be absorbed by the material surface. It is know principle the reflectivity of electromagnetic waves depends on the laser wavelength and type of material [ZHI09]. The metallic specimen will be absorbs laser energy. This energy is suffered transferred from laser photon to the specimen electrons and followed transferred to the crystal lattice. Hence, the energy will be diffuses into the whole specimen [LIU05]. Many reaction may be happened between the atoms or molecules and the absorb photon. If the energy of laser is high enough, then the laser pulses may cause the photochemical effect which releases the atoms and molecules from the surface of specimen. The temperature of metallic surface is will be rise by the laser pulses and reach to the sufficient quantity close to the critical temperature then the vaporization state was arrived .The plasma which consists of ionized vaporized atoms and electrons as a result of vaporization process. The plasma cloud contains of ions and electrons may be absorb some of laser energy and then allow the laser energy to reach the plasma shielding. The absorption photons make a corona of plasma around the laser attract then the corona will be heated and extended. When the vapor is cooling then the particles start to agglomerate. Depending on the sufficient laser energy, some of this energy will be diffuse into material *via* heating process. The other energy going to the specimen surface then became melted as form a liquid state. The liquid state may be released from the molten state as form of droplet as a result of higher laser ablation pulses(number of laser pulses

and energy of laser photon) in which are found the main affected variables which be influence on the concentration of ejected particles (atoms or molecular) [LIU05, LVZ09].

2.10 Antibacterial activity

Antibacterial activity *via* NPs is one of the most important applications in health field. Generally antibacterial agents are capable to locally destroy bacteria without causing side effect of several toxicity to the surrounding tissue [VON06].However with the several usages of antibiotics bacterial resistance is on the rise particularly due to genetic mutations these antibiotic resistant strains are responsible for the production of new fatal diseases. The drug resistant strains that cause these diseases bring about the need for high dosage treatment methods which produce adverse side effects and increased cost certain classes of NPs have antimicrobial activity and the ability to be a carrier for antibiotic delivery in vitro and in animal models. The mechanisms of antibacterial activity of the NPs depend on the bacterial species as well as the intrinsic properties of NPs and there surface modification and composition [HAJ12]. The energy of the NPs increases due to the increase in ratio of surface to volume which reduces the NPs stability and in turn enhances the antimicrobial properties in comparison with the bulk form. A distinct advantage of NPs lies in the avoidance of nonspecific interaction between the drug and unaffected tissue [SUN02].Ag NPs eristic toxic to multidrug resistant bacteria which in turn indicate the potential for use in biomedical applications [JAI09]. The mechanism of the antimicrobial effect of Ag lies in it being released into solution and higher ratios of NPs to bacteria resulting in quicker bactericidal activity (Figure 2.5). However these results are variable and

the NPs do not function with the same level of success on other bacterial species [PIS10]. It is unexpected some of microbes has enough resistance versus nano silver likely they do versus antibiotic. That the nano silver particles will be aggression the organism targets which means the microbes have to develop a host of mutations instantaneous to protect themselves [PAL07]. Table 1 provides a summary of the advantages and disadvantages that antimicrobial NPs have over free antimicrobial agents.

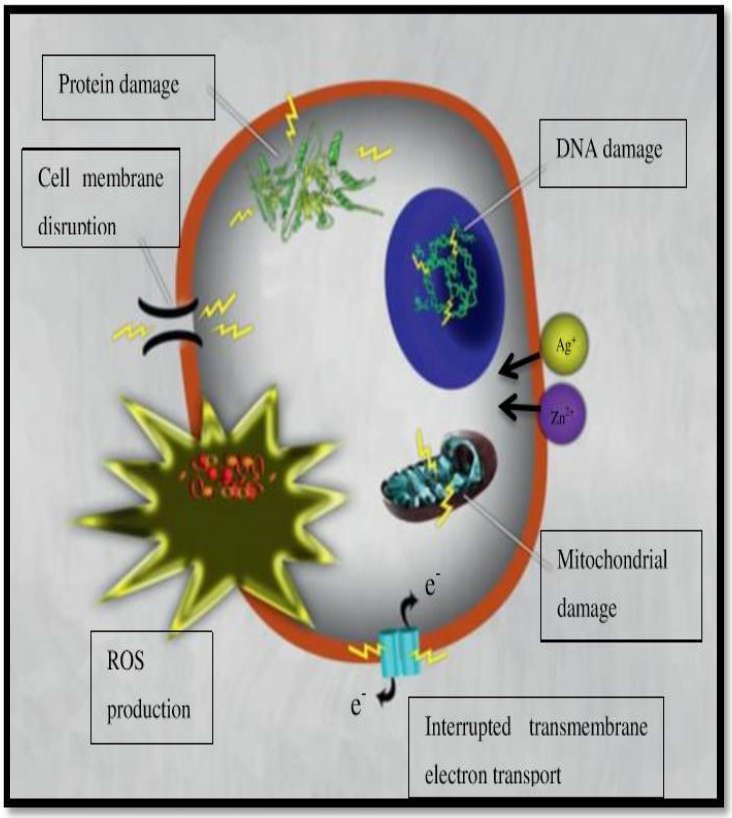


Figure (٢.5): Mechanisms of toxicity of Ag NPs on bacterial cell (HAJ12).

Table (2-1): Comparison of the advantages and disadvantages of antimicrobial NPs versus free antimicrobial agents [HUH11].

Antimicrobial NPs	**Free antimicrobial agents**
Advantage	**Advantage**
Targeted drug delivery via specific accumulation Lowered side effects of chemical antimicrobials Low antimicrobial resistance Extended therapeutic lifetime due to slow element Controlled drug release Broad therapeutic index Improved solubility Low immunosuppressant Low cost	Absence of nanomaterial in the whole body Low systemic exposure to locally administrated drugs Absence of nano toxicity Well-established characterization techniques
Disadvantage	**Disadvantage**
Accumulation of intravenously injected nanomaterial in tissue and organs High systemic exposure to locally administrated drugs Nano toxicity (lung, kidney, liver, brain, germ cell, metabolic, etc.) Lack of characterization techniques that are not affected by NPs properties	No specific accumulation High side effects of chemical antimicrobials High antimicrobial resistance Short half-life due to fast elimination Usual pharmacokinetics of free drugs Narrow therapeutic index Sometimes poor solubility Immunosuppressant High cost

2.11 Mie theory

The situation solution of the interaction mechanism of an individual homogeneous sphere of radius R and of any material with an incident electromagnetic field was first explained given by Mie in 1908[MIE08]. Mie was introduced a solution to Maxwell's equations that describes the extinction spectra of spherical particles of different shape and size embedded in a homogeneous solution. Mie's theory has remained important for so long is that it is presented exact solution to Maxwell's equations that is relevant to particles. As well as it has worth mentioning that in his calculation he introduces the dielectric function at the angular frequency to conduct the material problem which can incorporate all the

size effects. The spherical symmetry suggests the use of a multiple extension of the fields giving Mie's calculations a series of multiple oscillations such dipole and quadruple for the absorption and the scattering cross section of the particles as a function of the particle radius. The extinction spectrum is then composed of the sum of absorption and scattering modes each of which has a contribution that depends on the particle size. Higher-order modes become more dominant with increasing particle size. Physically if the particle has a larger size then the light cannot polarize for two reasons:

i. Due to homogeneous nano particle

ii. Retardation effect lead to the excitation of higher- order modes.

Mie's theory is agreement with and experimental spectra until for bulk metals the vertical incidence absorption no longer shows a Plasmon resonance. Also Mie's theory did not explain the meaning of collective oscillation of the conduction band electrons[JAI07, GIE08, LEE98].The term; Plasmon refers to a plasma oscillation excited by electromagnetic waves and a surface polarization is the origin of the plasma oscillation [XIA10]. Therefore the surface Plasmon resonance SPR in such metal nano particles is an oscillation Plasmon absorbs or scatters light resonantly of certain wavelength. For electromagnetic waves at a certain wavelength incident on spherical NPs much smaller in radius than the incident wavelength which induce the resonant coherent oscillation of metal free electrons across the nanoparticles as shown in Fig (2-6). The penetration depth of electromagnetic waves in such metal element is more than 30nm and because the diameter of particle is few nanometers thus the incident wavelength able to propagate through metals NPs. The propagate electric

field inside the particle drives the conduction free electrons collectively with respect to the fixed positive lattice ions. As a result of this interaction a net charge difference appears on the surface at one side of nanoparticle. The attraction conduction electrons lattice ions leads to a restoring force. This force depends on the separation of the surface charges.

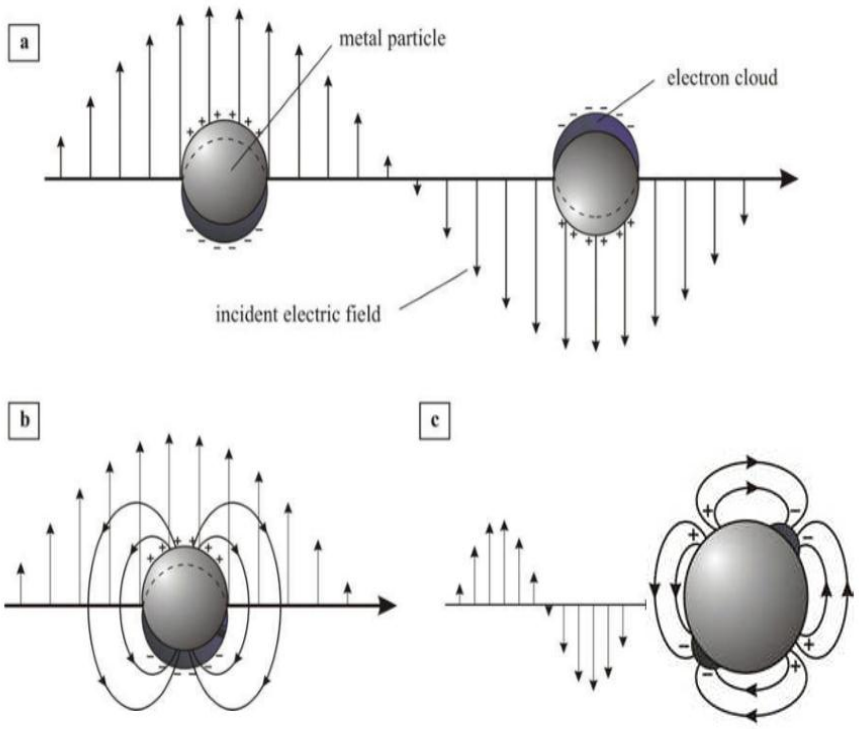


Figure (2.6): interface of a small metal nanoparticle with light (λ>>R) (a), particle dipolar radiation (b) and quadruple radiation of larger particles (c) [SAN07].

2.12 Effect of reaction modes with bacteria

There are some effective reaction parameters of silver nano particles with the microorganism as described in following:

i. Silver nano particles may be destructed the micro-organism cells

ii. The monovalent nano silver can substitute hydrogen ions and hence prevent the unwinding of DNA in viruses [RLD97].

iii. It is confirmed that silver nano particles can be absorb and accumulate in bacteria cells leading to make shrinkage of the cytoplasm.

According the DNA molecules losses their capability to replicate. Also the silver nano particles may be make a shield block tends to inactivate proteins *via* interact with their bonds [PET08]. When the silver nano particles absorb oxygen, follow the oxygen diffuses into silver particles more easily due to repulsion of silver atoms. The absorb oxygen is converted to nascent oxygen by silver elements. Hence, the oxygen plays an important parameter causes antibacterial properties of silver NPs [RLD97].When the concentration of silver NPs is in between 0.01-0.1mg/l, then the metabolism of bacteria suffered destructive. Therefore some less soluble silver compounds also act against bacterial [BRU09].

Chapter Three

Chapter Three

Experimental Work and procedures

3.1 Introduction

The practical chapter involves the material to be used, deionized and magnetized water preparation system set up inspection techniques and procedure and finally antibacterial activity by nano silver. As well as the decontamination process along each step of practical section in which are stated in details in the following sections. The experimental work describe in Figure(3-1).

3.2 Preparation of typical water

Two types of typical water were prepared deionized and magnetized water was used as a medium solution for the ability to suspend the nano silver particles in which obtain able from the laser ablation process.

3.2.1 Deionized water

This type of water could obtain originally from the distilled water and by using the conducting system (100GPD Deionized Ultra pure water equipment model number xx-RO-20L). The features of deionized water are nearly empty from cations and anion units and therefore do not get the polar ion.

3.2.2 Magnetized water

Magnetized water was prepared at the University of AL Nahrain /College of Science/ Department of Physics by exposing a magnetic field

by parallel square magnets where we measured the magnetic flux of square magnets by Gauss meter device with 200m T as shown in Figure (3-2).

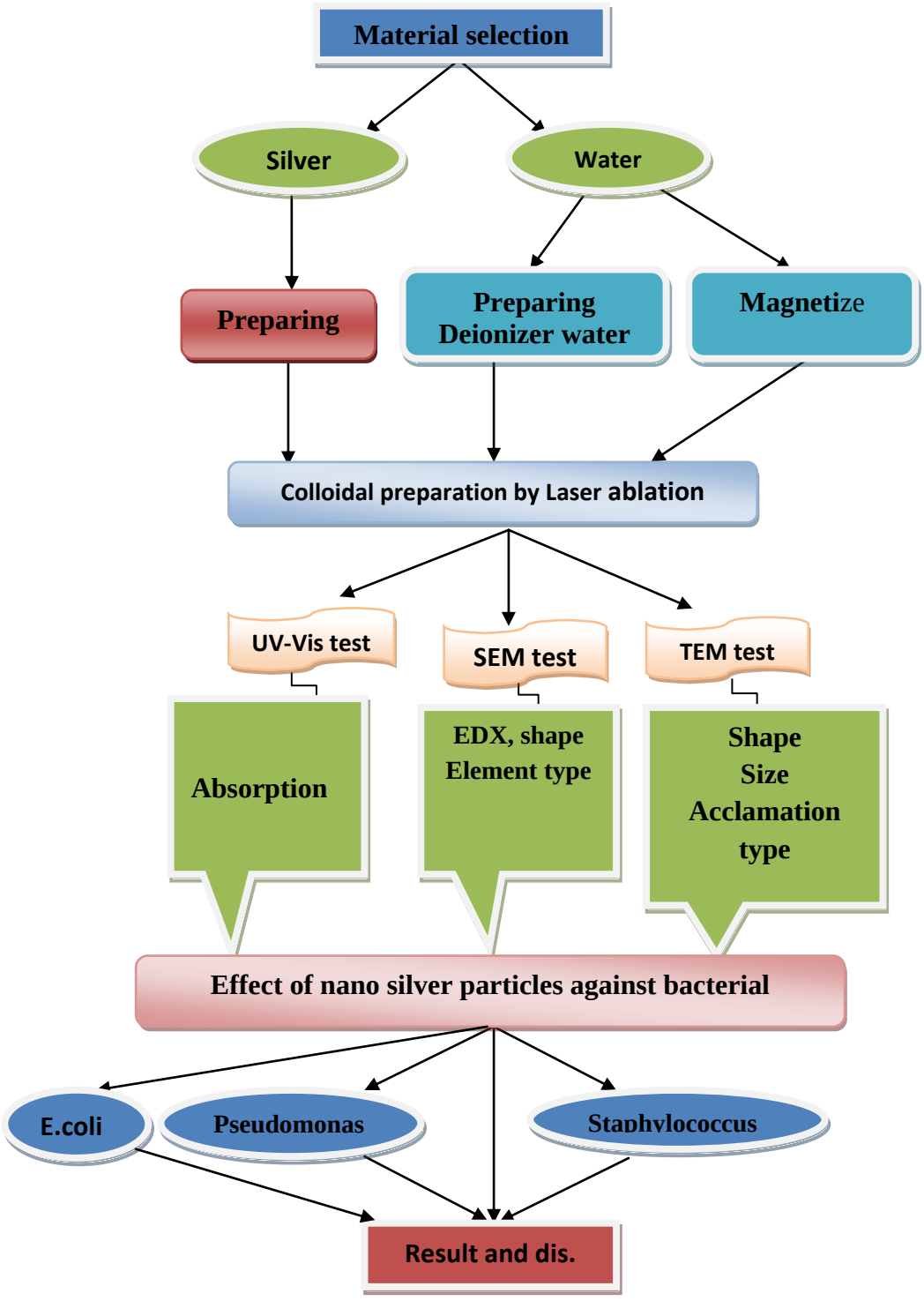


Fig (3-1): diagram of the Experimental work and procedures.

The dimension of each square magnet is (30x30x6) mm² and the face surface area is 900mm² .The purpose of our use of this measurement is to find out the best magnetic over flow for square magnets. The square magnets were imported previously from China, the greats magnetic can be obtained when put 8 square magnets.

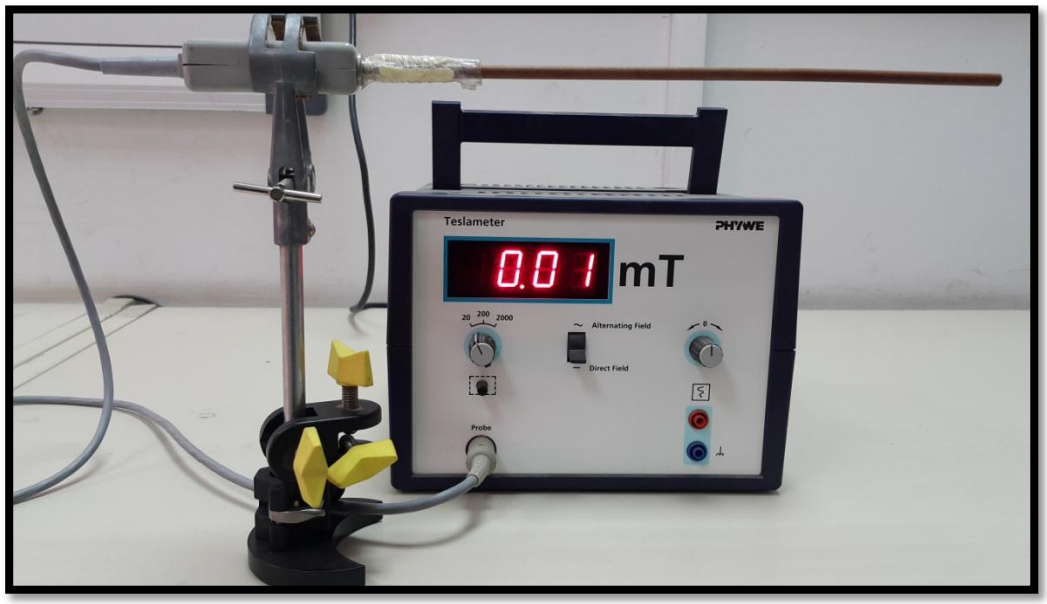


Fig (3-2): Gauss meter device.

3.3 Preparation of magnetized water

The system was equipped to prepare the magnetized water through the passing of distilled water among the magnets and placing a funnel tube into the beaker as shown in Figure (3-3). 16 square pieces of magnets were used by placing 8 parallel pieces on each side of the tubing and the distance between two sides was 1 cm. The magnetized water was prepared through Pour the water into the funnel and pass through the elastic tubular connection downwards with the effect of gravity, only magnets will affect the water vertically and this means that the gravity of the earth is

responsible for the decline of water from the top to down, but industrial magnets are responsible for the manufacture of magnetized water.

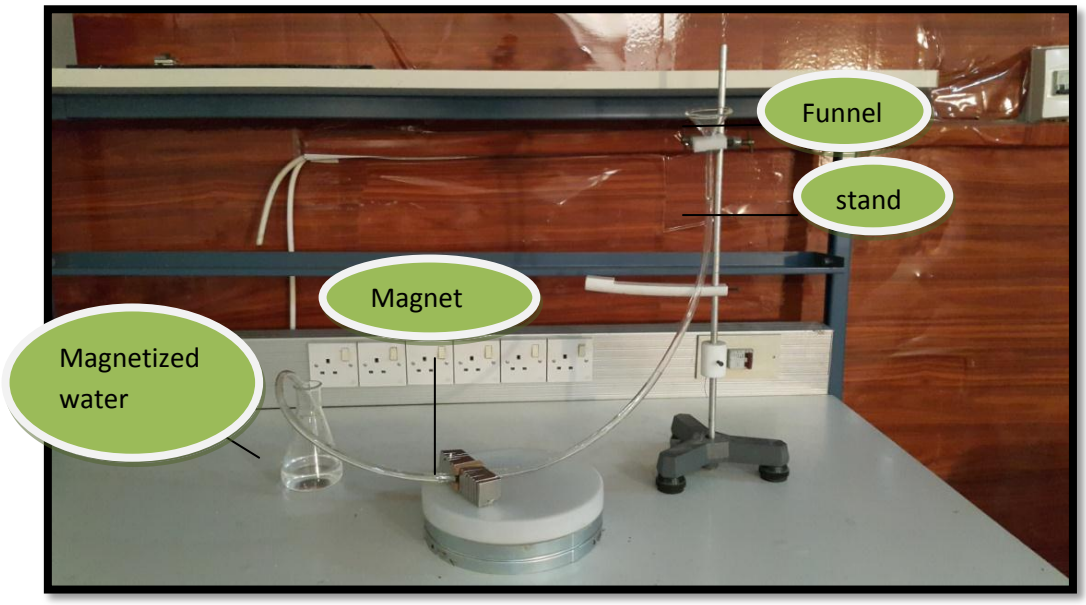
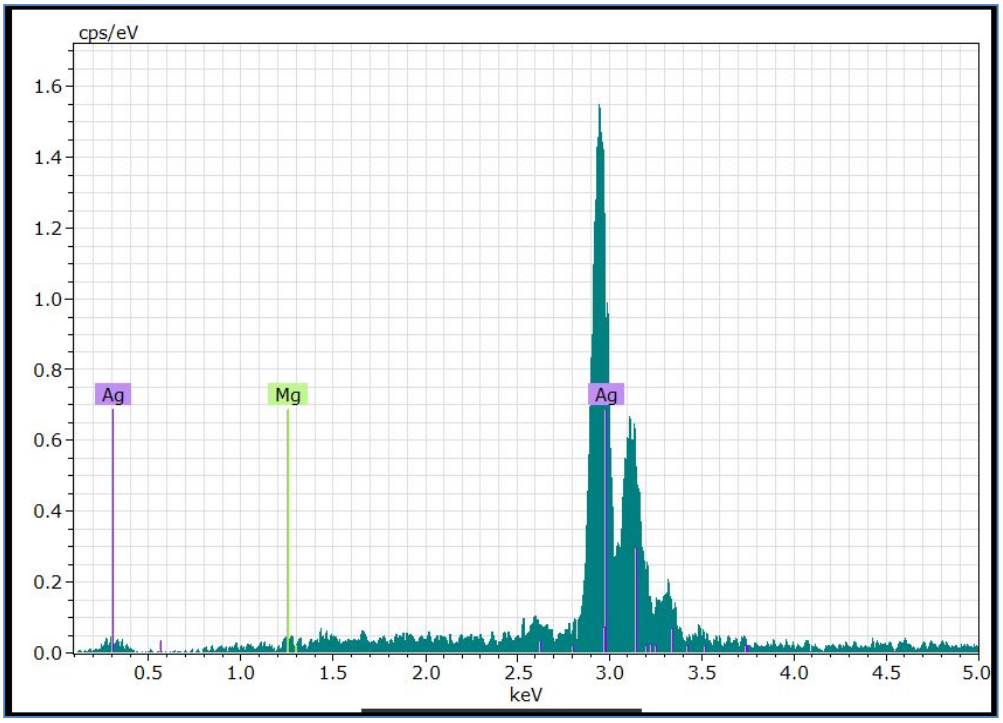


Figure (3-3): Magnetized water preparation system.

3.4 Silver plate assertion

High purity of silver plate was of dimension (18.1x16.6x05) mm^3 available in Iraq market used as a target was tested by energy dispersive x-ray (EDS) technique joined with the scanning electron microscopic (SEM) device. This device was available at University of AL-Nahrain/College of Science/Department of Physics .The purity of silver plate was found more than 97% as shown in figure (3-4). The pure silver is necessary condition to prepare the nano silver as suspension particles in the both magnetized and deionized water tend to approach preparation of colloidal involved nanosilver particles.



Bruker Nano GmbH, Germany 11/2/2016

Quantax

Results Objects 18490
Date: 11/2/2016

Element	AN	series	[wt.%]	[norm. wt.%]	[norm. at.%]	Error in wt.% (1 Sigma)
Silver	47	L-series	82.15249	99.30903	97.00455	2.937207
Magnesium	12	K-series	0.571602	0.690974	2.995451	0.164164
		Sum:	82.72409	100	100	

Fig (3-4): Energy dispersive spectrum for pure silver.

3.5 Magnetic water testing

Water quality has been tested through two methods:

3.5.1 Method of solubility

Where the sugar and salt were used to test its solubility by magnetized water and distilled water as a solvent, 5 g of sugar and salt individually were used. The solubility of both sugar and salt was found taken more time if used the magnetized water than any type other water was taken where dissolved in 10 ml of magnetized water and distilled water. A difference was observed in the solubility method in both species.

3.5.2 UV-Vis device method

The magnetized water and deionized water were subjected to the UV-Vis spectroscopy for comparing among these two types of water as shown in Figure (3-5) it is observed that the magnetized water has more absorbility than deionized water. This is an efficient technique was used for improve the existence of magnetization was after treated by magnets as previously mentioned.

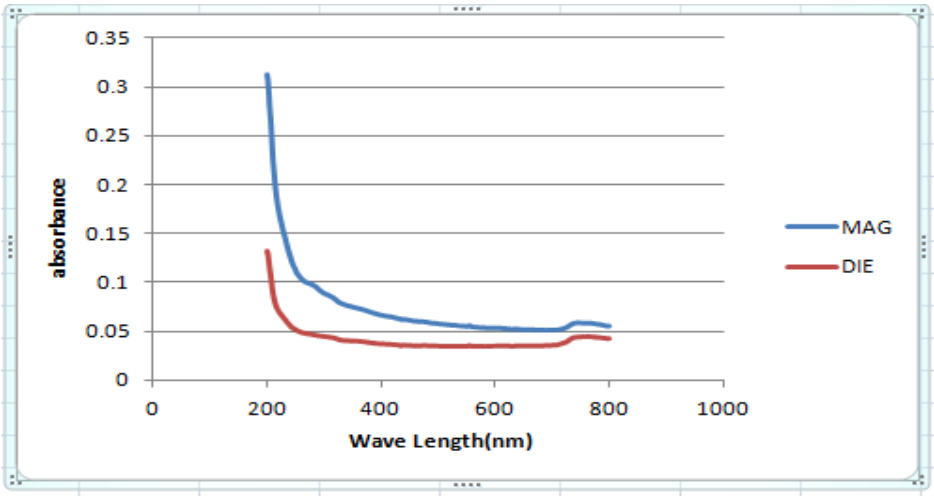


Fig (3-5): UV-Vis spectrum of magnetized and deionized water.

3.6 Preparing nano silver particles

Pulsed laser ablation technique was used to prepare nano silver particles. Nd: YAG laser apparatus of wavelength 1064nm, made in China, was used as a main source of laser power in this investigation. The procedure starting by placing a pure silver sheet of dimension (10x10x0.3) mm^3 in a glass vessel containing 3ml Deionized water at the first step and magnetized water in the second step. Several laser energies and pulses were selected The depth of water above the silver plate in the vessel was 10mm as shown in Figure (3-6). Repeated other experiments by placing the magnets around the vessel in the first try and placing the vessel in the electric field in the second try for enhancement the colloidal containing nano silver particles.

Fig (3-6): Nd: YAG laser device.

3.7 Measurement of colloidal density

The colloidal density was measured firstly with a limited volume by using a 4-digit balance (stanton462AL) as shown in Figure (3-7). A certain volume of colloidal was taken at each time. Then the density was measured according to the laser strikes (150,250,350and 500 pulses).

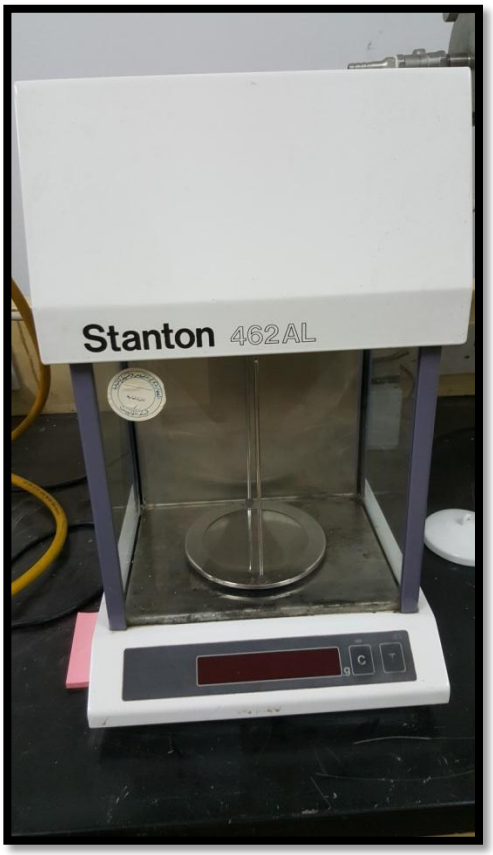


Fig (3-7): Sensitive balance.

3.8 Measurement of PH and conductivity

The conductivity and (PH) of pure water and the nano-silver solution were measured by a PH-Meter as shows in Figure (3-8):

Fig (3-8): PH-Meter.

3.9 Well diffusion method

This method is used to achieve antibacterial effect through nanoparticle. In this method, the bacteria were planted and wiped completely on the pallet media. Then we put the solution containing nano silver particles in the plate of the media and this work steps was achieved all inside the fume hood, where it is important in removing odors, smoke, fumes, heat and air filtration through the discharge and filtration. The incubator was set at a 310 ºK for 18-24 hours. The growth of bacteria is well tested and we see

how Nanoparticles affect the growth of bacteria by observing the inhibition zone, which means growth stops and is measured by the ruler.

3.10 Antibacterial activity of silver nanoparticles

Three types of bacteria staph, Sidom and E.coli as a source of infection were used. Hinton media was prepared according to the specific standardization. It is the minimum nutrient necessary for bacteria. The bacteria are then neutralized by normal saline, containing high salts. This is done by adding 5 ml diluted to 100 µl of bacteria. The bacteria were then wiped out by cotton soap on the pallet and plant each type of bacteria and then put the solution containing the particles of silver nanoparticles inside the prepared placed well for each type of bacteria where 150 µl was added to each type of bacteria inside well as shown in Figure (3-9). A part of which we control only the colloidal and another part, we control the solution by applied DC electric field for 30 minutes by applied voltage/distance is 3000 V/m as shown in Figure (3-10) and then put the assembly inside the incubator at 310^{o} K and for a period of 18-24 hours .

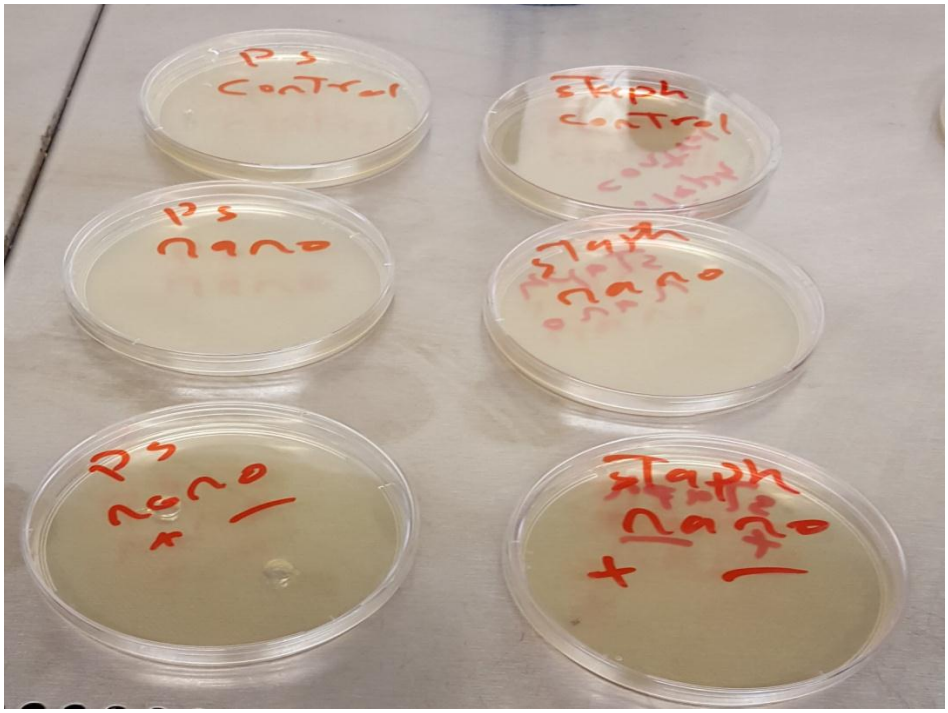


Fig (3-9): Agar plate with well.

3.11 Measuring devices

In this work, several devices were used for investigating the nano silver particles and their anti bacteria effects:

3.11.1 Scanning electron microscopy (SEM)

A detector and amplifier device, which is more than a million times larger, gives the shape of the model with the element type. The advantages of the surface and the coupling with this device are the EDX detector, where the analysis of samples and components in the component gives the model of the device is(Inspect S50) and the manufacturer is (FEI) as in Figure (3-11).The test of the SEM carried out in the college of Science Department of Physics at the University of AL-Nahrain.

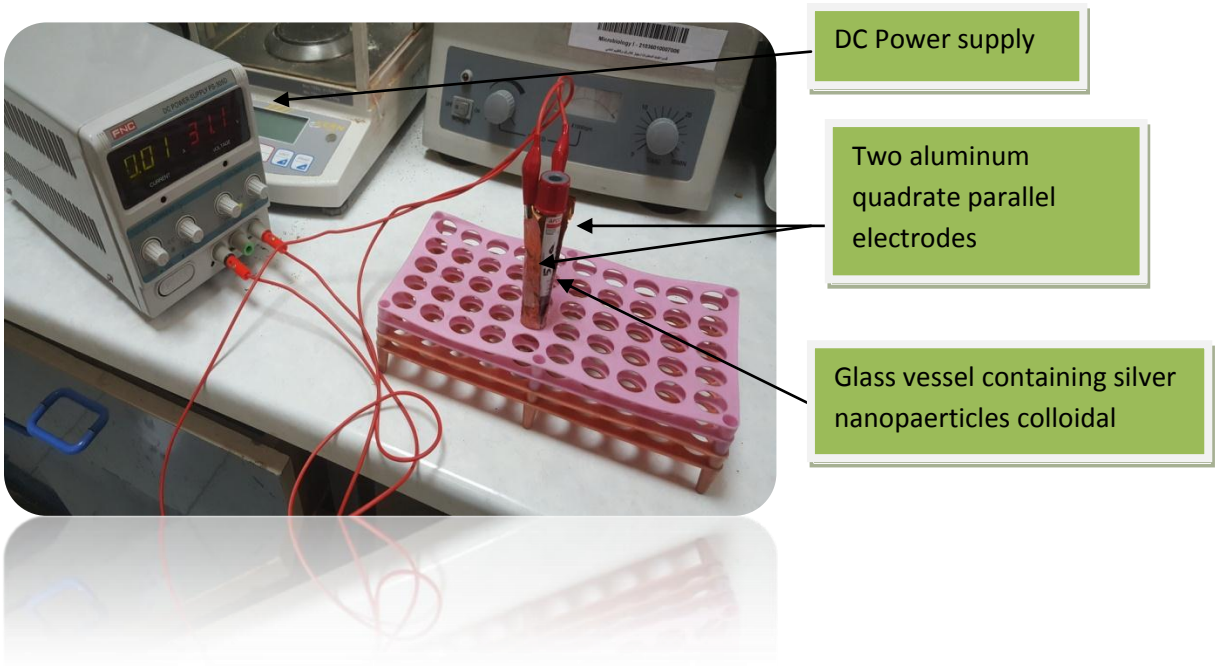


Fig (3-10): The process of applying DC electric field on the colloidal.

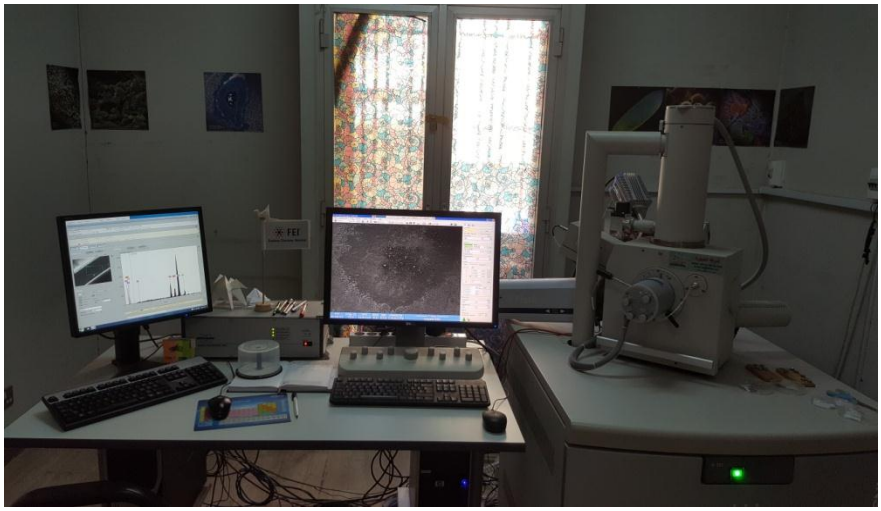


Fig (3-11): SEM device.

3.11.2 UV-Visible device

This device gives a beam of wavelength extending from visible wavelength to the wavelengths specified for the ultraviolet. The elliptical device consists of two parts, spectrometer for the production of light at any wavelength and the other part is photometer to measure the intensity of light. The amount of light passing through the tube is measured through the photometer, where it's sends a voltage signal to the projector and the signal changes with the amount of light absorbed through the liquid. Magnetized and distilled water were tested without containing nanoparticles as a references solution. The nanoparticles solution was then tested by directing a beam several wavelengths on the silver test tube solution where attenuation will occur (reducing the energy and intensity of the radiation). This attenuation varies from solution to solution depending on its density (silver density in solution). So when the tested of the device is to know the viability of the materials to attenuation of the falling rays and was tested by this device (UV-1601PC UV-Visible spectrophotometer SHIMADZU) at Al-Nahrain University / College of Sciences/ Department of Physics as shown in Figure (3-12).

3.11.3 Transmission electron Microscopy(TEM)

Machine mechanics is the production of X-Ray with rectified electrons, where the test was performed by the TEM system, showing the shape of the assemblies, size, shape and type of assembly. Is it cluster or spherical or random (unspecified) tested the TEM system, where we have a solution containing the minutes of silver nanoparticles randomly where penetrates the depth of the material and see through the parameters of the internal

medium of the material and gives an enlargement of the image more than a million times and the clarity of the image depends on the type of material and how to prepare. This device type (Philips CM 10) in the College of Medicine, University of Nahrain. This devices work with a voltage of 60 kV as shown in the Figure (3-13).

Fig (3-12): UV-Vis device.

Fig (3-13): Transmission electron microscopic (TEM).

Chapter Four

Chapter Four

Results and Discussion

4-1 Introduction

This chapter concerning the practical results and the test analysis of the experimental measurement, which involve the structure, optical and antibacterial analyzing of nanosilver are discussed in some details.

4-2 Characterization of silver colloidal

Some of the physical and chemical properties are changed when the silver material become nano particles when the nano silver particles are dissolved into either magnetized or deionized water. Then the nano silver particles become suspended approach to synthesized colloidal silver. The color of colloidal silver was found tend to yellow color due to scattering phenomena. It is found the colloidal color was transformed from the Yellow to more darken as the density of nano silver increases as shown in Figure (4-1).

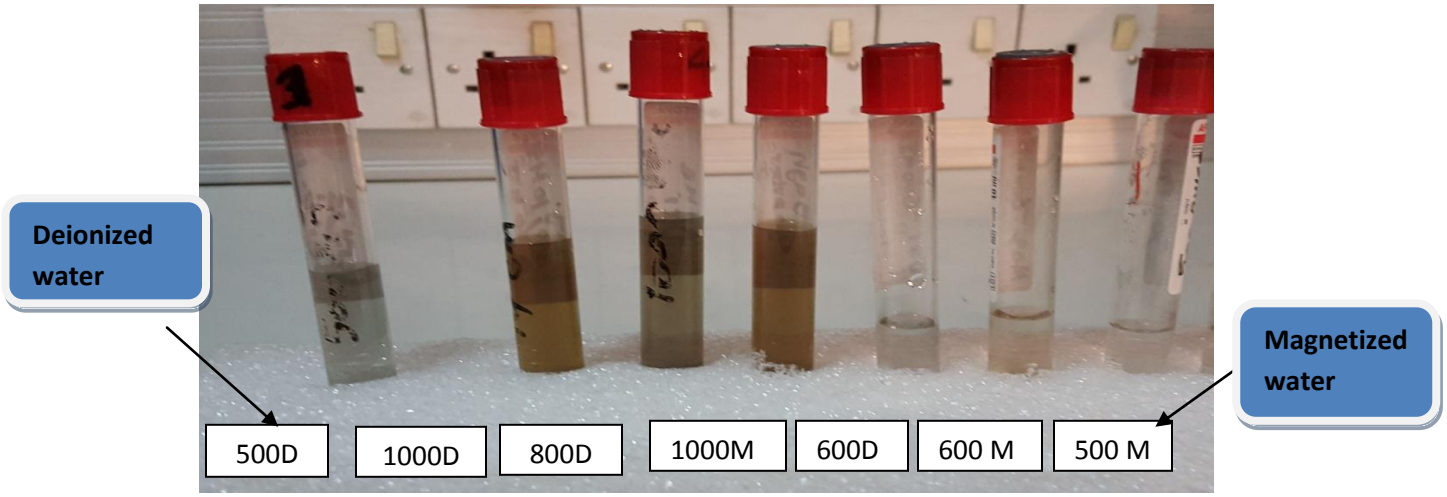


Figure (4-1): The color of the solution changes from laser pulses (500,600,800and1000pulses).

That is meaning the change in colloidal color depending on increase the concentration of silver nano particles in the solution. This fact was studied experimentally throughout the correlation between the absorption peaks and laser pulses as shown in Figure (4-2).

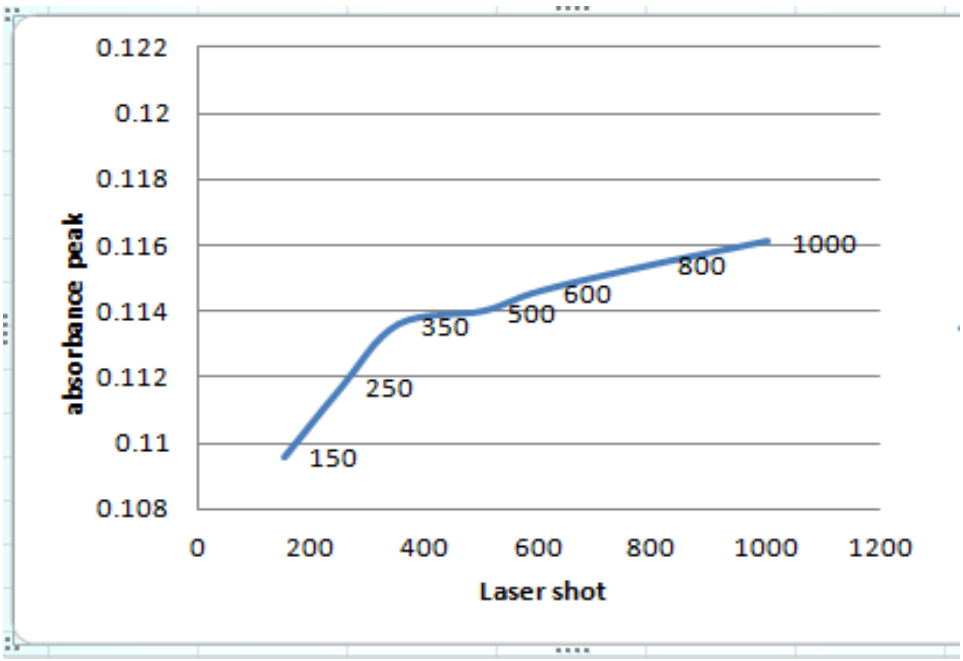


Fig (4-2): The increase absorbance when the laser shot increase.

From this Figure one can be noticed the direct proportional and non linear of absorption as a function of laser pulses number. The absorption peaks increases sharply when the laser shots number increase until 350 pulses and follow the absorption ability to decreasing slightly until the laser shots number increases to 1000 pulses. That the conductivity of colloid was studied through the concentration of nano silver particles in water. It is expected the conductivity of colloidal is increasing as the nano silver particles is increasing too, due to the electrical property of silver. It is found practically that the colloidal conductivity associated to the density of nano silver as demonstrated in Table (4-1).

Table (4-1): Represent the conductivity of colloidal.

Colloidal state	Conductivity(μS)
Deionized water (pure)	Zero
Mgnitized water (pure)	zero
Deionized water (800m J)	20μS
Magnitized water(800m J)	20μS
Magnitized water (600 m J)	50μS

Level of acidity (pH) of silver colloidal was measured experimentally. The pH of different type of water and different colloidal were measured and the result stated in table (4-2).

Table (4-2): Measurement of pH.

Type of water	pH
Distilled water(pure)	7
Deionized water(pure)	6.9
Deionized water (800mJ)	6.3
Mgnitized water (800mJ)	6.5
Mgnitized water (600m J)	6.5

The pH of pure water was approximately (7), while the pH of both deionized and magnetized water contains nano silver particles was found reduces slightly to the range between (6.3-6.5) i.e. as the nano silver particles are exist then the acidity of colloidal will be tend to reduced.

It is well known, the pH value of any solution depends on the concentration of positive hydrogen ion and negative hydroxide ion.

4-3 Optical properties of nano silver

The absorption phenomena by the nano silver particles can explain according to the Gustavo Mie theory. The colloid colure is found depend on the particle size and shape of nano particles. The color of colloidal contained different amount of nano silver changed from bright yellow to the darken yellow and to the light green relative to the concentration of nano silver and its shape as illustrated in Figure (4-1).In addition, it is found that the absorption peaks defined surface Plasmon band in the region (420-430) nm which is indicated of nano silver and the altitude of absorption peaks are found variation between about 12.5%-46% as shown in Figure (4-3).

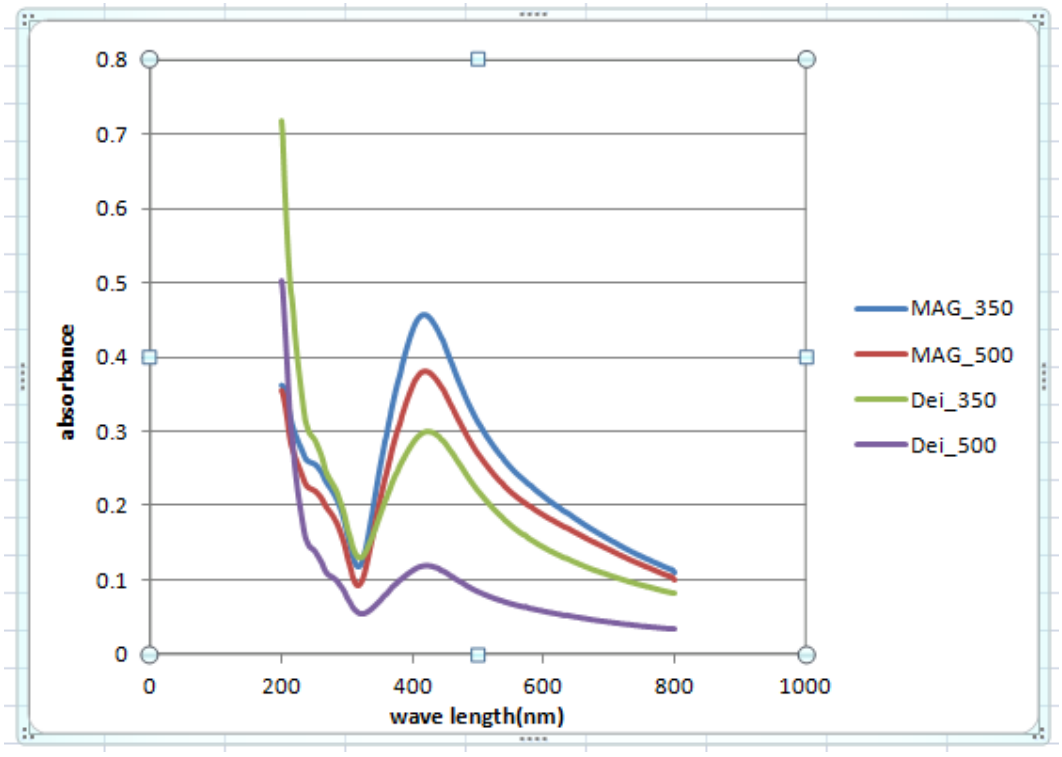


Fig (4-3): UV-Visible absorption spectra of nano silver colloidal (1000mJ, 350and 500pulses) for Magnetized and Deionized.

The variation in absorption regions is due to the nano silver, while the variation of altitude peaks is due to the concentration of nano silver in colloidal. The absorption spectra result due to the interactions of free electron confined to nano silver objects with incident radiation.

4-4 Identification of magnetic water

The deionized water was prepared laboratory and subjected to UV-Vis test. As well as the deionized water was to prepare the magnetized water. Several square magnets were placed parallel closely together as mentioned in section (3.2.2). The flux density as a function of number of magnets on opposite sides and a function of distance between the magnets poles is listed in the Table (4-3).

Table (4-3): Measurement of magnetic flux of magnet.

No. of magnet	Distance from the center of square surface of magnet(cm)	Flux density(m T)
One magnet	1	30
Two magnet	1 2 3	60 20 9
Three magnet	1 2 3	70 25 12
Eight magnet	1 2 3	100 40 18

From the Table one can noticed the flux density increases when the number of parallel magnets poles increases.

The deionized water was passing through the magnets poles at a distance (1cm), then one can predicated became magnetized water. For improving this fact, both deionized and magnetized water subjected to UV-Vis spectroscopy as mentioned in Fig (3-5).One can see the difference absorbility of each type of water. This is the improvement that the deionized water was become magnetized water.

4-5 Influence of laser pulses on colloidal

The colloidal contains nano silver particles which produced *via* laser ablation by using Nd: YAG solid state laser of wavelength (1064nm). Several laser pulses were used (150,250,350,500,600,800and1000) pulses per limited laser energy. Although the color of colloidal are changed as previously discussed, also the density of colloidal will be changed too. The colloidal density found increases proportionally to increases of laser pulses. Weighing method was used to verify this fact. Where taken a limited volume from each type of concentration colloidal, and then the colloidal density was found changed according to the laser pulses regarding to the laser energy. As listed in Table (4-4). It is found the density of colloidal increase as the laser pulses increase too.

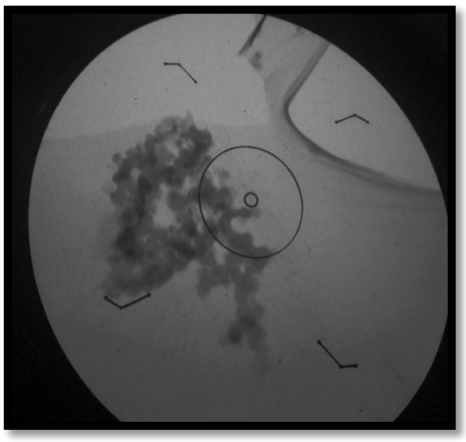
Table (4-4): Shown the density of colloidal of nano silver.

Type of water	Energy of Laser (mJ)	No. of pulses	Density(g/ml)
Deionized	1000	150 250 350 500	0.978.5 0.97975 0.9861 1.0216
Deionized	800	1000	1.0664
Magnetized	600	1000	1.0268
Magnetized	800	1000	1.00665

Figure (4-2) is ensured and enhanced this fact. However, it is not predicated that the density increase linearly with laser pulses number, where it is found the relation was exponentially, due to the effect of accumulated nano silver particles. These phenomena appear to make attenuation of laser intensity. The degree of the reduction of colloidal density may be also due to the surface condition, laser incident angle and free electrons in colloidal.

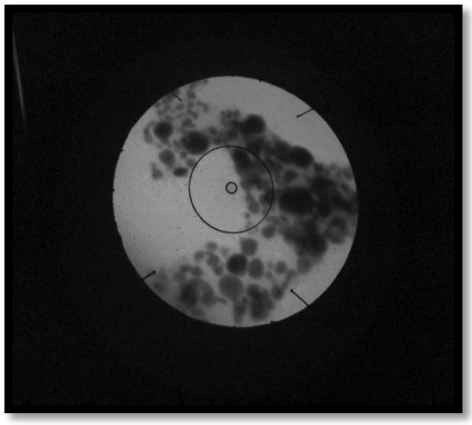
4-6 Structure of silver nano particles by Transmission electron microscopic

The accumulation nano silver state are clearly revealed on the TEM image for both deionized and magnetized water at different laser pulses as shown in Figure(4-4).

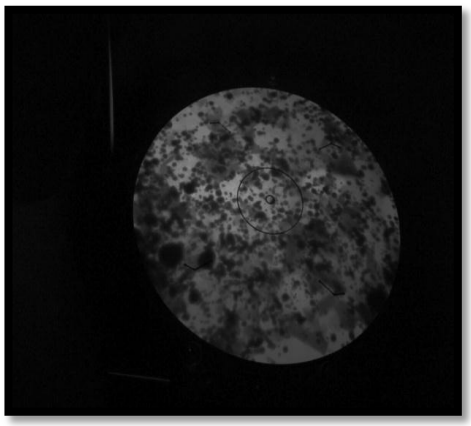


Mag water, 350 pulses,
average size (25-30) nm

Mag water, 500 pulses,
average size (33-53) nm



Die water, 350 pulses,
and average size (12-25) nm



Die water, 500 pulses, and
average size (29-60) nm

Fig (4-4): TEM image for silver nano particles (Die and Mag water) (350and 500 pulses).

4-7 Influence of laser energy on silver particles

Independently the effect of laser pulses on the laser ablation. It is found another effective parameter which influence on the laser ablation and both the nano size and shape of silver particles. Several laser energies were taken (500,600,800and1000) m J / pulse. A sensible deviation of absorption

peaks were observed on the UV-Vis spectra as in Figure (4-3). The nano size of silver particles is found changed according to the laser energies as noticed in Table (4-5).

One can see that the laser energy reduced, the nano size particles suffered reduction depending upon the sufficient photon energy which is could ablated the silver crystal from the metal. The laser energy should have related to the critical threshold of crystal ablated.

In addition, the type of water can be affected on the nano size particles as seen in Table (4-5).The relation between the nano sizes silver absorbility against laser energy was plotted as shown in Figure (4-5).

Table (4-5): The average size as a function of energy of laser pulses.

Energy of laser shots (mJ)	Average size of spherical silver nano particles (Deionized water)	Average size of spherical nano particles (Magnetized water)
350	12-25 nm	25-30 nm
500	29-60 nm	33-53 nm

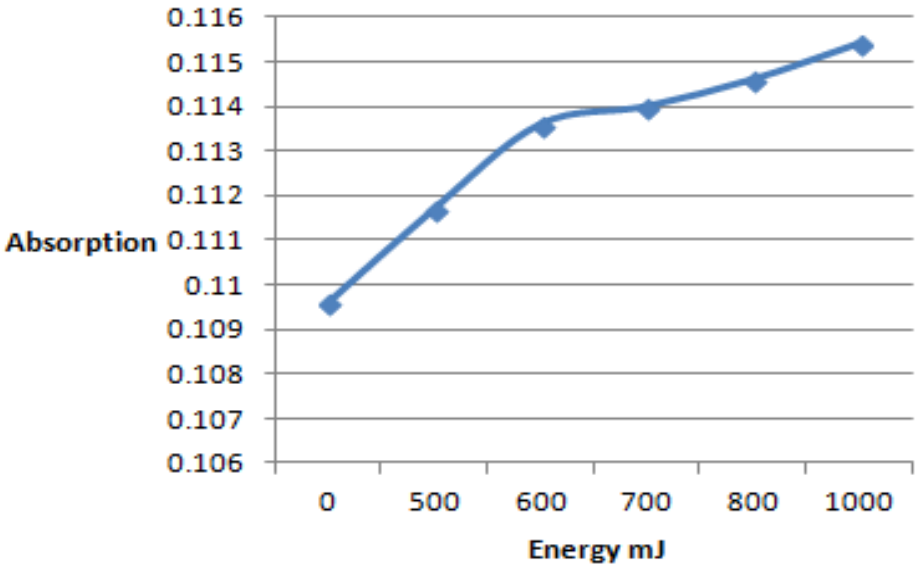


Fig (4-5): The relation between the absorption and Energy

Almost linear relation is found between the above two variables .Since with higher laser energies both the concentration and shape of nano size particles are changed where the particles become large size compared with others produced with low energy. In addition, the shape of silver particles has different configuration as illustrated in the TEM and SEM images.

Figs (4-6, 4-7 and 4-8) present several regular and irregular shapes, spherical, rhombic and spindles. These particles shapes will be affected on the absorption phenomena and it can be expected affected on the antibacterial and that is a good agreement with reference [LEN14].

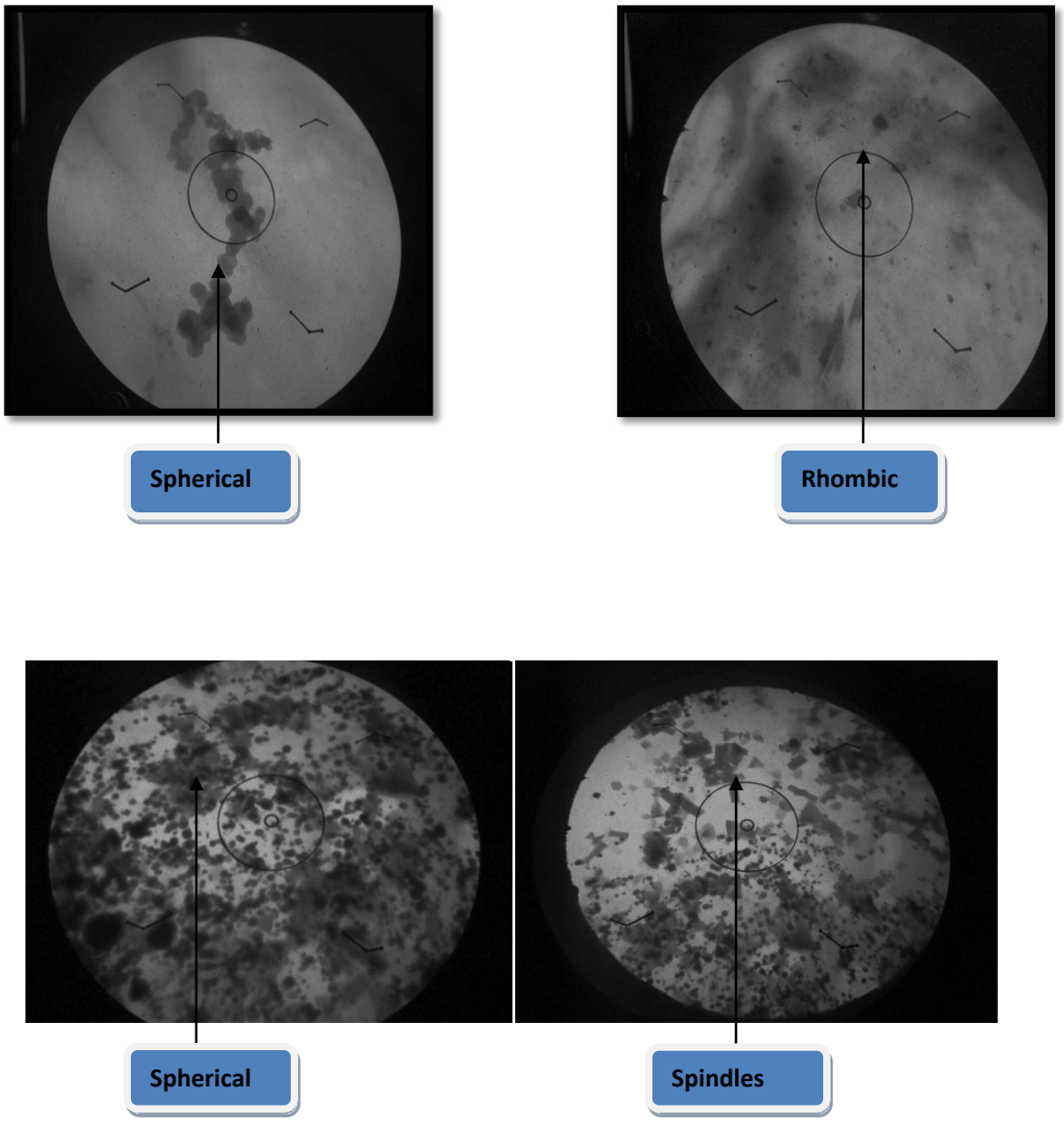
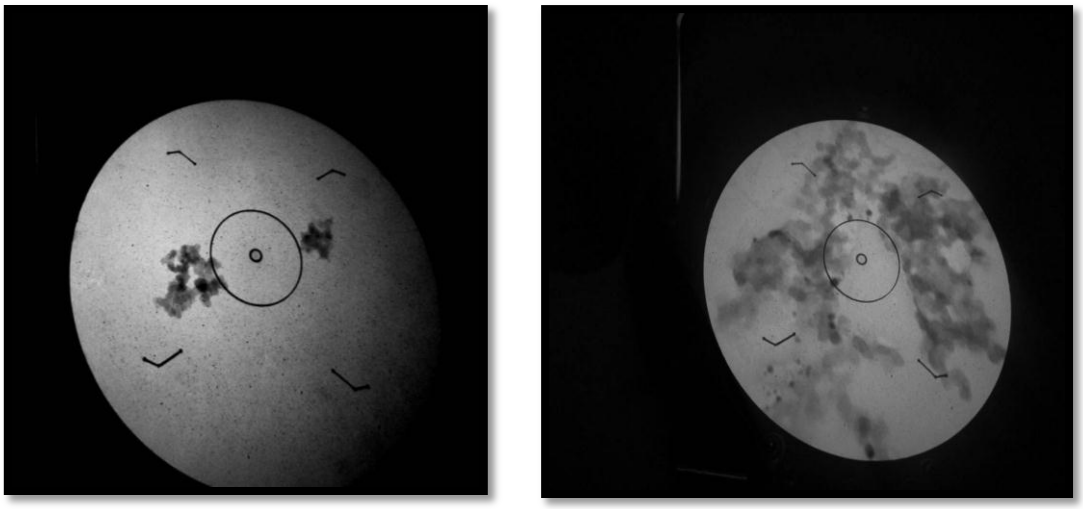


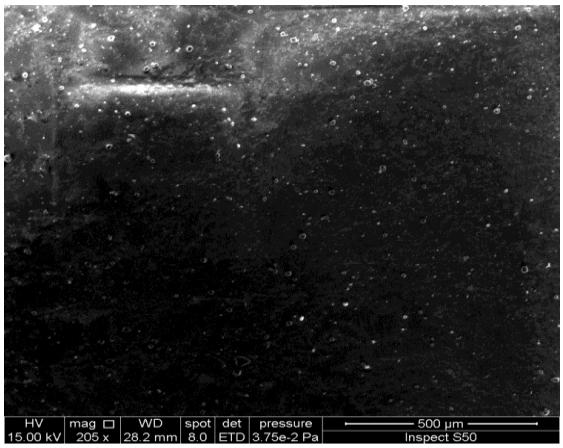
Fig (4-6): TEM image show the size and shape of silver nanoparticles with (scale bar=100nm).

The silver particles solution exposed to the D.Celectric field is (600 V/m) using the TEM teqnique and the colloidal with the energy of 600 and 500 mJ, which was examined for the purpose of knowing the shapes and sizes of nanoparticles for the effect of bacteria as shown in the Figure (4-7). It is noticeable that the shapes of nanoparticles are spherical.

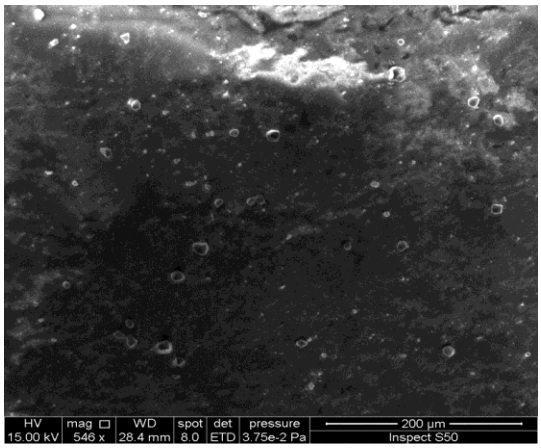


500mJ,92k,53nm **600mJ,130k,32nm**

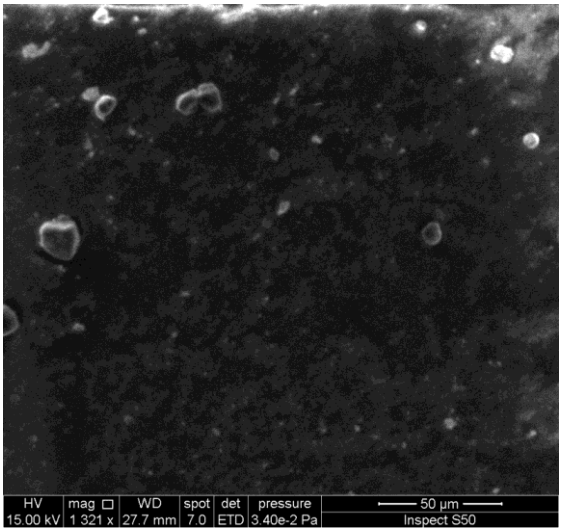
Fig(4-7): TEM image show the size and shape of silver nanoparticles(500 and 600 mJ) with (scale bar=100nm).



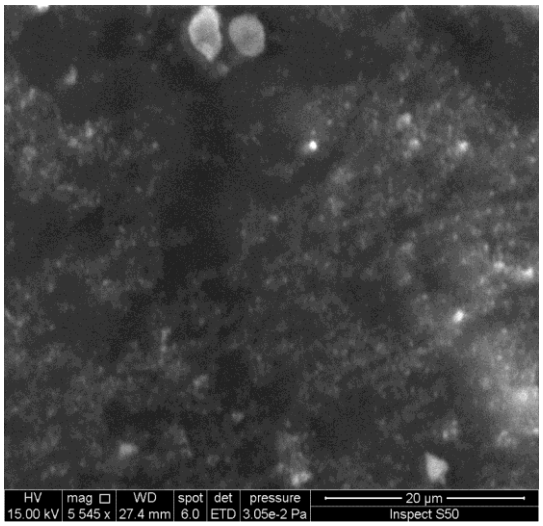
(a)



(b)



(c)



(d)

Fig (4-8): shape and type of assembly of the silver particles
(a) 500µm (b)200µm (c) 50µm (d) 20µm

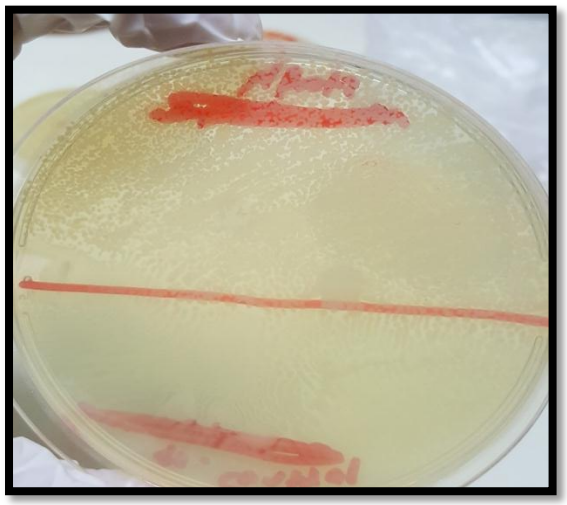
4-8 Antibacterial activity of nano size silver

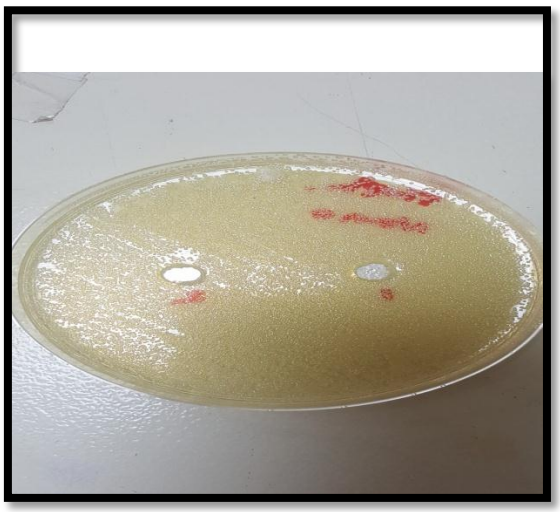
The effect of the nano size silver solution against the negative gram and positive gram bacteria was studied in detail. The issue of antibacterial activity of nano size silver colloidal is corresponding to discharging energy of nano silver particles into bacteria or such micro bacterium for stopping their growing.

Several types of colloids concentration contains nano silver which prepared *via* laser ablation association with different laser energies and laser pulses as mentioned before, were undergo for determining or stopping of bacteria growth. Two mechanisms of conducted bacteria with and without applied D.C electric field were applied as explained earlier in the Fig(3-11).We have made several bacteria by using different agents, laser energies and different pulses count as shown in the Table(4-6).we did not notice any inhibition zones were obtained in bacteria growth as shown in Figure (4-9).

Table (4-6): shown the attempts to inhibit bacteria

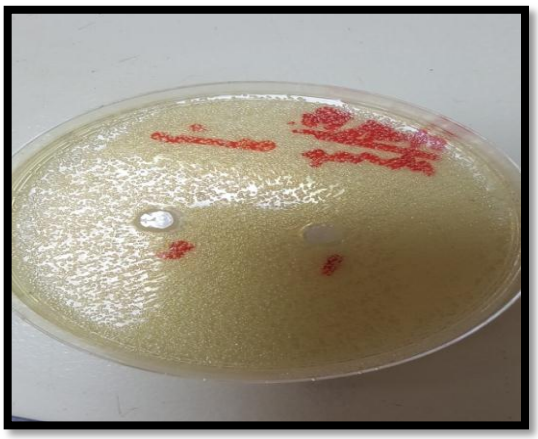
No. of attempts	Laser energy(m J)	No. of pulses	Electric field time(minute)	Type of bacteria	The resultant
1	1000	500	30	Staph ,Sidom	Nil
2	1000	1000	30	Staph, Sidom	Nil
3	600	1000	30	Staph, Sidom	Nil
4	800	1000	60	Staph, Sidom	Nil
5	800	1000	60	Staph, Sidom	Nil





(a) Staphylococcus aurous and Pseudomonas silver nano particles

(b) Staphylococcus under effect control



(c) Pseudomonas under effect silver nanoparticle

(d) staphylococcus aurous under effect of electric field and nano particle

Fig (4-9): Shows did not inhibition Zone was obtained in bacterial growth.

Another step was the preparation of the silver nano particles solution by the Nd: YAG Laser with the energy (500 and 600m J), 1000 pulses, frequency 6 Hertz and the electric field was given to the solution during the preparation with a voltage of 600 V/m. The electric field was then applied to the colloidal by 3000V/m and for half an hour each. The electric field was also applied to the nano gold solution to test the bacteria and for comparison between the silver solution and the nano gold solution. The colloidal of (500 and 600 mJ) was then applied to the E. coli bacteria. The nano-gold solution was placed on the Staphylococcus and pseudomonas bacteria and placed in the incubator for 24 hours. The result was that the growth of the bacteria was no stopped when the nano-solution of 500 mJ was applied to the E. coli bacteria as shown in Fig (4-10).while the growth of bacteria is stopped when effect of colloidal(nano silver) with the energy (600m J). The zone size was (25 mm) as shown in Fig (4-11). While the gold nano solution was observed to stop bacterial growth when shedding on the bacteria pseudomonas and staphylococcus and the zone scale (20 mm) as shown in Fig (4-12), also the Fig (4-13): shows the relation between the laser energy used to prepare the nanoparticles and the inhibition zone, observed zone when the laser energy is at 600 m J.

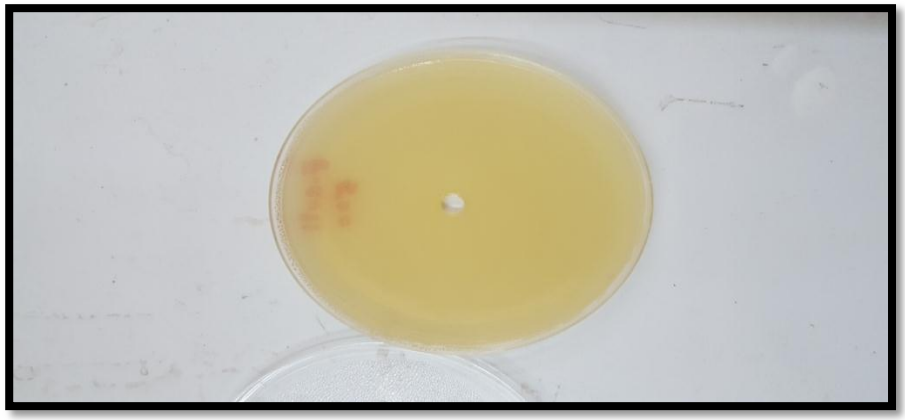


Fig (4-10): Shows no inhibition zone of AgNPs on the growth of E.coli.

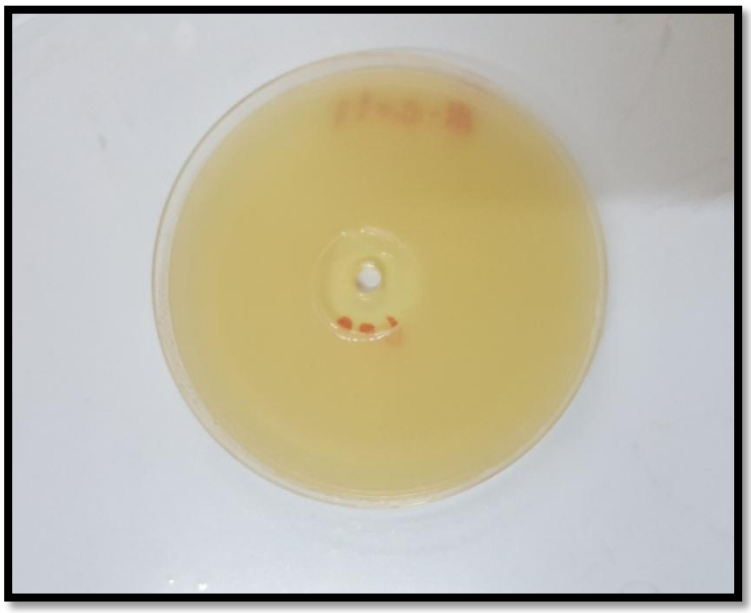


Fig (4-11): Effect of colloidal (AgNPs) on E.coli shows inhibition zone is (25mm).

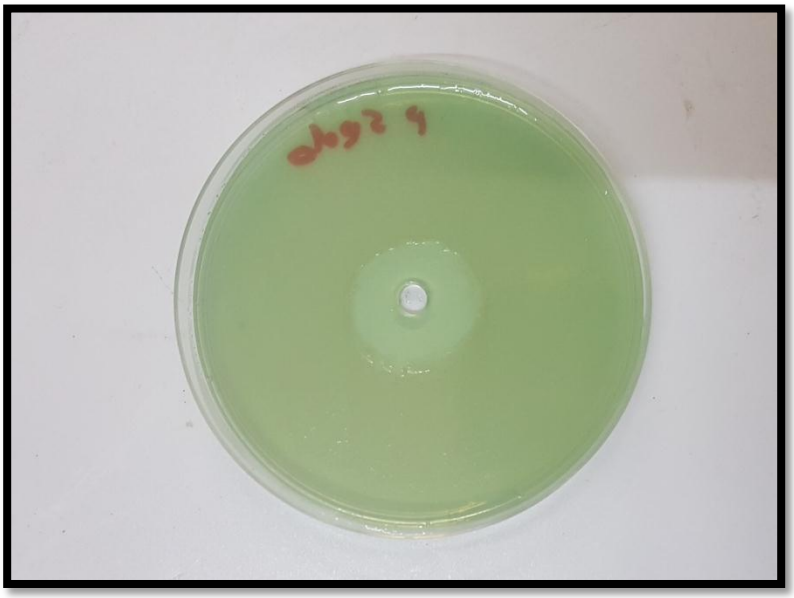


Fig (4-12): Effect of colloidal (AuNPs) on pseudomonas, the inhibition zone (20mm).

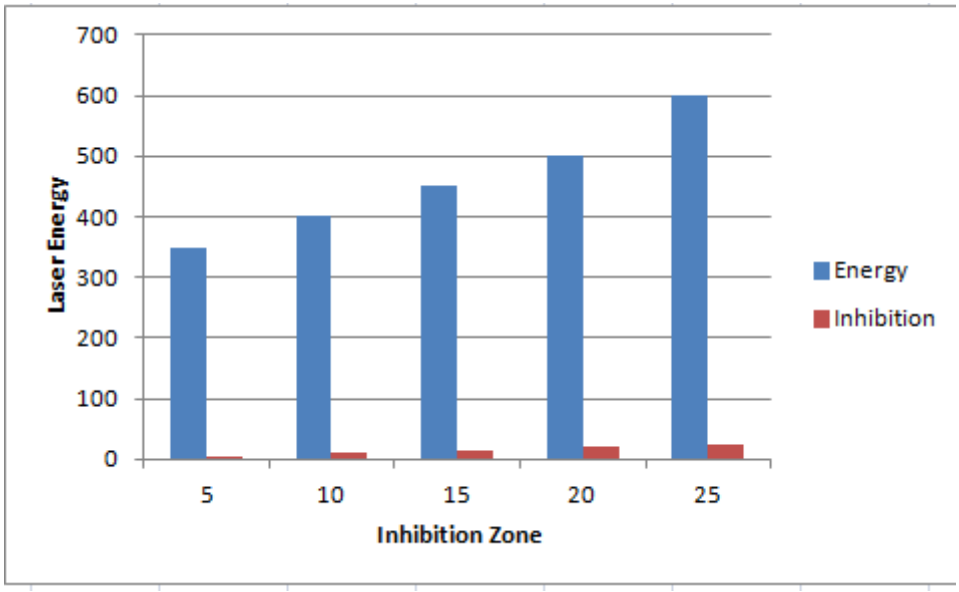


Fig (4-13): The relation between the laser energy and inhibition zone.

Also, Fig (4-14): shows the relation between the number of pulses and inhibition zone, noticed the inhibition zone when the number of pulses is 1000 pulses.

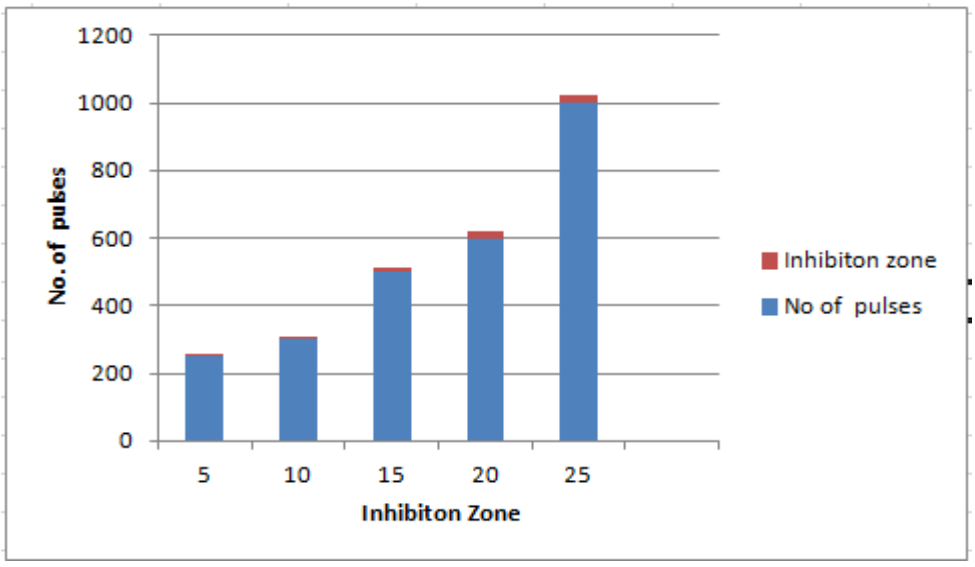


Fig (4-14): The relation between number of pulses and inhibition zone.

When we raised the colloidal with the energy of 500 m J and did not see a clear effect of bacterial growth, this may be because energy is not enough for nanoparticles where it did not affect bacterial growth while the silver solution with the energy of 600 m J, it's found the size of the inhibition zone was 25 mm. This indicates that the energy was sufficient to effect the bacterial growth as well as for gold testing on bacteria. Where the size of the zone was observed by 20 mm and this indicates that the nanoparticles of gold have stood the growth of bacteria. In the bacterial test it was observed that bacterial growth or cell death depended on the concentration of nanoparticles in the solution as well as on the size of the nanoparticles and the type of bacteria. At the small concentration of nanoparticles it was observed that there was no obvious effect while it was observed that the particles of silver at the energy of 600 m J. The number of silver particles increased in solution, which affected the bacterial growth and the result is clear as shown in the previous pictures.

The last step we made was to use the nano-gold solution on staph bacteria and under the same conditions, but we did not saw a clear effect on bacterial growth as shown in Figure (4-15).

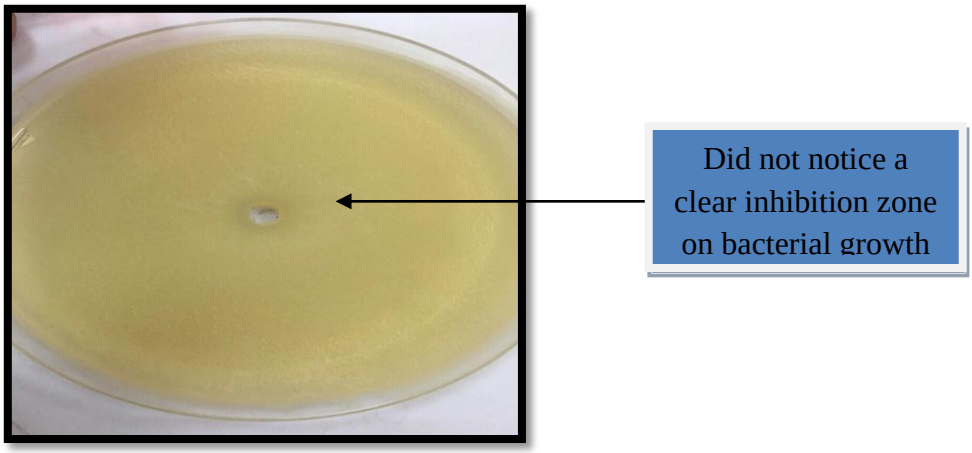


Fig (4-15): Shows no inhibition zone of AuNPs on the growth of (staph).

Also performed a test on the bacteria type (pseudomonas**)** by the use of anti-Biotic type (MITROX) and see the extent of its effect on bacterial growth and using the same conditions previous obtained inhibition zone by (46 mm), as shown in Figure(4-16):

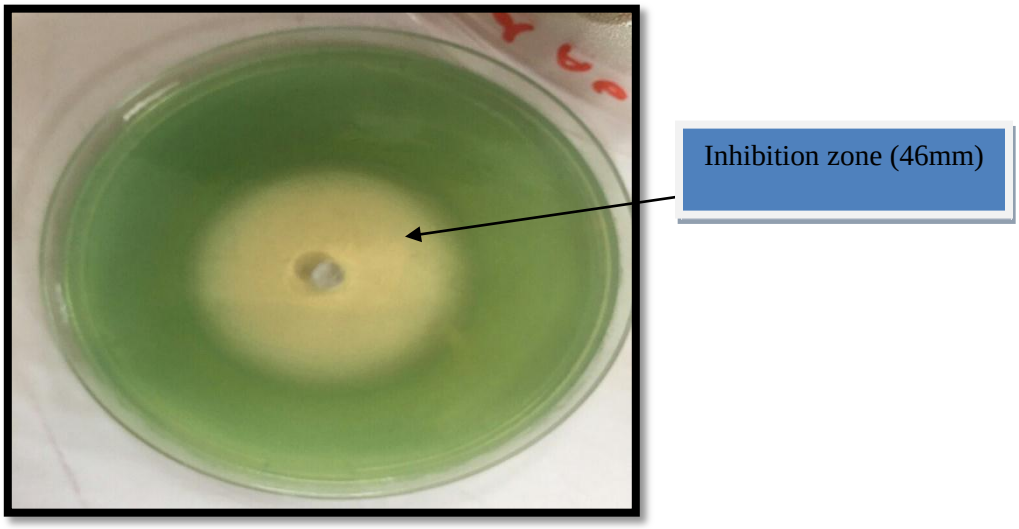


Fig (4-16): Effect of antibiotic (MITROX) on bacteria (ps.).

Also, a combination of nano silver solution was used by 15 µL with ante Biotic with 15µL. The effect of the mixture on the bacteria (PS.), and we obtained inhibition zone (43 mm) as shown in Figure (4-17).

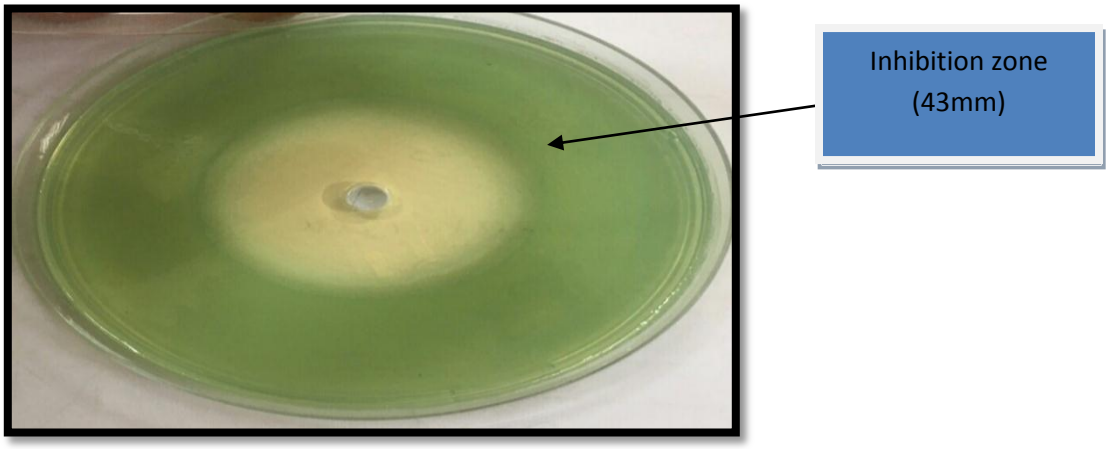
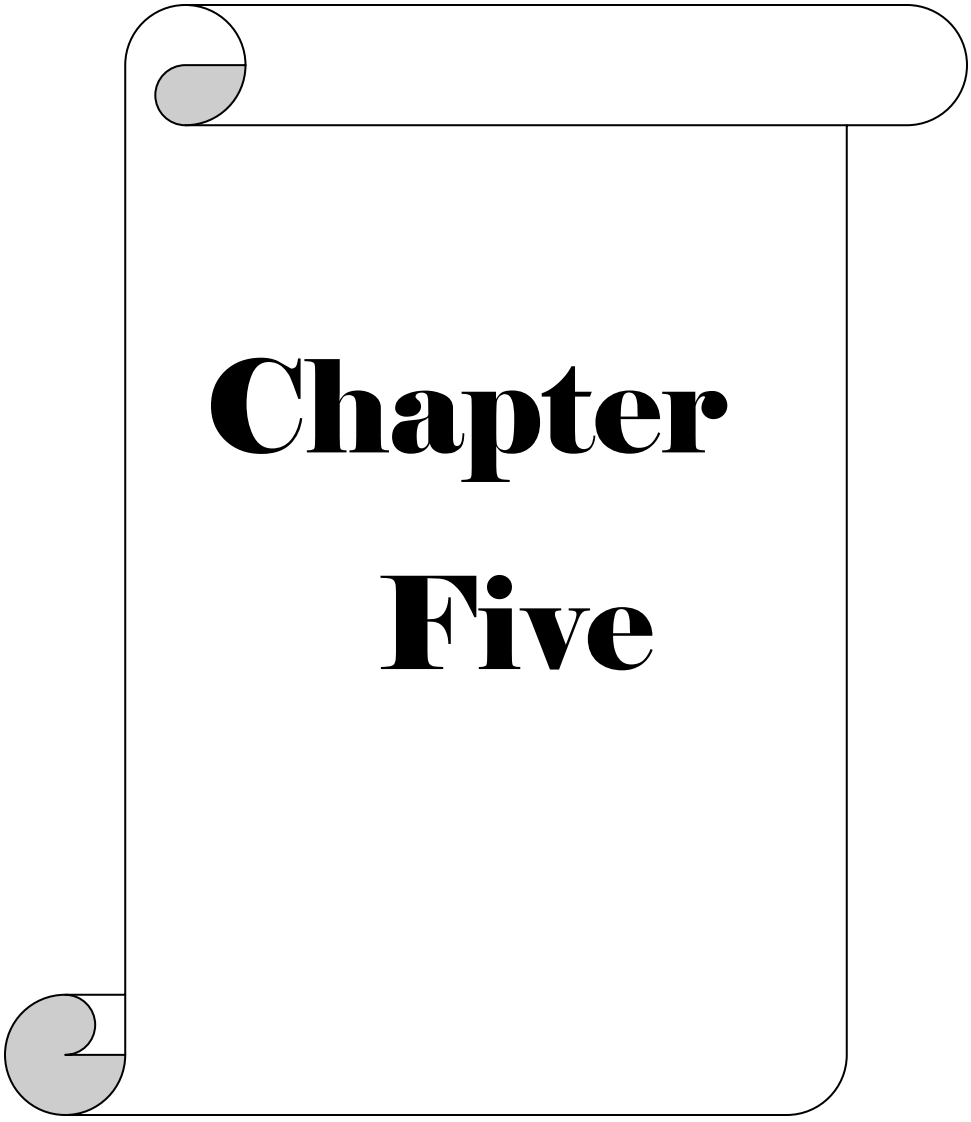


Fig (4-17): effect (AgNPs + antibiotic (MITROX)) on bacteria (ps.).

Through the results shown above, we obtained zone in the work steps and did not get in the other steps and this is due to several reasons perhaps working conditions in terms of lasers used on silver or perhaps the nature of resistance to bacteria used solutions as well as the conditions of preparation and time and the amount of electric field used are all conditions leading to the results as mentioned earlier.


Chapter
Five

Chapter Five

Conclusions and future work

5.1 Conclusion

1. The color of nano size silver is changed depending on the size of particles due to the absorption and scattering phenomenon. Also noticed that the colors of the solution change from transparent to yellow and to deeper color.

2. The nano size silver and it is shape is affected on the absorption spectra either on region or height of absorption peaks.

3. Preparation of silver nanoparticles by laser technology with wave length of 1064 nm and different energy where the energy can be controlled as well as particle size through the laser energy , wavelength , number of pulses and frequency of the laser.

4. An electric field was exposed to the nanoparticle to provide a card. This energy is discharged by the bacteria as the silver absorbs the electromagnetic wave controlled by it. This means that it is good absorption of energy and has an effect on the bacteria**.**

5. The first steps of the work showed that we did not have clear results for bacterial growth. When the electric field was subjected to a voltage of 600 V/m during the preparation of the nanoparticles by the laser device, it had a significant effect, unlike the solid steps through. The results it expect the surface area of the nanoparticles to be more effective as the size of the particles so that the storage of energy with nanoparticles more as small size

and then we can rewarded energy with nanoparticles on the energy needed by the bacteria to kill.

5.2 Suggestions for Future work

1. Preparing the silver nano particles by ultrasonic field and studying the activity of their antimicrobial.
2. Studying and preparing the silver nano size by laser ablation with different wavelength.
3. Synthesis of nano size silver by a reduction method and studying their anti bacterial activity.
4. Optical properties of nano size and shape of silver particles, then studying the effects against bacteria.

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الملخص

الغاية من هذا البحث هو تحضير جسيمات الفضة النانوية بطريقة الأستئصال الليزري في وسط مائي لا ايوني تارة وممغنط تارة اخرى تمت دراسة خواص جسيمات الفضة النانوية وتأثيرها على البكتيريا بأستخدام معلمات مختلفة.

حضر الوسط المائي الممغنط مختبريا بأستخدام مجموعة مغانط مربعة الشكل بفيض مغناطيسي مقداره 200 ملي تسلا للتأثير على الماء اللاايوني المار خلالها. أستخدمت منظومة الليزر نوع نديميوم ياك بطول موجي 1064 نانومتر بأمد نبضي مقداره 10 نانو ثانية وبطاقات مختلفة وبعدد نبضات مختلفة تحت تأثير مجال كهربائي تاره وبعدمه تارة اخرى.

اجريت فحصوصات بصرية على المحلول الغروي الفضي بوساطة تقنية تسليط أشعة فوق البنفسجية واشعة مرئية واظهرت النتائج ان طيف امتصاص الفضة يقع عند 420-430 نانومتر اي ضمن المنطقة المرئية وبينت النتائج ان ارتفاع وانخفاض قمم الأمتصاص تعتمد على تركيز جسيمات الفضة النانوية في المحلول الغروي وكذلك على شكل تلك الجسيمات .أستخدمت منظومة المجهر الألكتروني النافذ للتعرف على ابعاد واشكال جسيمات الفضة النانوية واظهرت النتائج ان ابعاد جسيمات الفضة تراوحت بين 20-60 نانومتر وبأشكال مختلفة منها الكروية والمغزلية وغيرها ووجد ان كل من طاقة الليزر وعدد النبضات عاملان رئيسان في تحديد كل من الشكل والحجم. تأثير جسيمات الفضة النانوية على انواع البكتيريا (ستاف وسيدو واي كولي) قد درست تحت تأثير مجال كهربائي مقداره 3000 فولط /متر وبينت النتائج انه لم يلاحظ تأثير لمحلول الفضة النانوي على ايقاف نمو البكتيريا عدا المحلول الغروي المنتج بطاقة 600 ملي جول و 1000 نبضة حيث كان مدى تأثيره على ايقاف نمو البكتيريا نوع سيدو بمدى 25 ملي مترومن خلال نتائج المجهر الألكتروني النافذ تبين ان شكل جسيمات الفضة النانوية المؤثرة على نمو البكتيريا كان كرويا وحجمها الدقائقي يتراوح بين (32-50) نانومتر.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم

تحضير ودراسة جسيمات الفضة النانوية وتأثيرها المضاد للبكتيريا

رسالة
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كأستكمال جزئي
من متطلبات نيل درجة الماجستير في علوم الفيزياء

من قبل

سماح صباح عبدالرزاق
بكالوريوس علوم فيزياء جامعة النهرين (٢٠١٤)

بأشراف

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