The Study of Silver and Gold Nanoparticles Effect Against Local Bacteria

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ABSTRACT

Gold and silver nanoparticles colloids were produced by irradiating a metallic target plates with a thickness of 1mm immersed in distilled water with a pulsed laser beam. The size and size distributions of the metals nanoparticles were examined by the transmission electron microscope TEM analysis. The nanoparticles concentrations were also characterized by atomic absorption spectroscopy AAS measurement.

Antibacterial properties of silver and gold nanoparticles are attributed to their total surface area, as a larger surface to volume ratio of nanoparticles provides more efficient means for enhanced antibacterial activity.

دراسة تأثير دقائق الفضة والذهب النانوية على أنواع من البكتريا المحلية .

الخلاصة

دقائق الذهب والفضة النانوية المكونة لمحلول العالقة في الماء والمحضرة بواسطة بقصف نبضات من شاع من الليزر لقطعة معدنية من الذهب والتي هي بسمك 1ملم مغمورة في ماء مقطر تم فحص حجم والتوزيع الحجمي للدقائق النانوية والخصائص البصرية المتكونة باستعمال تحليلها بالمجهر الالكتروني الماسح TEM وتوزيع تركيز محلول الذهب والفضة النانوية تم قياسه باستخدام تقنية طيف الامتصاص الذري ASS.

تعزى الخصائص المضادة للبكتريا لدقائق الذهب والفضة النانوية لمجمل المساحة السطحية كلما كانت المساحة لدقائق النانوية اكبر كلما أكثر كفاءة لتعزيز النشاط المضاد للبكتريا .

INTRODUCTION

Laser ablation of bulk target immersed in liquid environment which is simple method, recently has attracted much attention for nanoparticles formation[1-4] . Nanomaterials display unique, superior and indispensable properties and have attracted much attention for their distinct characteristics that are unavailable in conventional macroscopic materials. Their uniqueness arises specifically from higher surface to volume ratio and increased percentage of atoms at the grain boundaries. They represent an important class of materials in the development of novel devices that can be used in various physical, biological, biomedical and pharmaceutical applications[5-8].

Synthesis of nano sized drug particles with tailored physical and chemical properties is of great interest in the development of new pharmaceutical products[9]. Emergence of new resistant bacterial strains to current antibiotics has become a serious public health issue, which raised the need to develop new bactericidal materials. However, the phenomenon of enhanced biological activity and certain material changes resulting from nanoparticles is not yet understood fairly. Investigations have shown encouraging results about the activity of different drugs and antimicrobial formulation in the form of nanoparticles[10].

Silver is a nontoxic, safe inorganic antibacterial agent used for centuries and is capable of killing about 650 type of diseases causing microorganisms[9]. Silver has been described as being 'oligodynamic' 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[11]. 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[12,13].

The implication of microbial infection as a causative agent in arthritis was the stimulus for the investigation of the antimicrobial properties of gold complexes. The early work by Robert Koch demonstrated that gold compounds were active against the tubercle bacillus. Subsequent extensive work in the 1930's and 1940's
demonstrated that a variety of gold compounds were active against a broad spectrum of microorganisms. Activity in invitro test systems was demonstrated against both Gram negative and Gram positive bacteria, a number of strains of mycoplasma, and the protozoan Leishmania. Gold complexes were also able to modify the course of a number of in vivo infections in a variety of animal hosts. Many of these early studies were flawed, however, and the lack of evidence for the role of an infectious agent in rheumatoid arthritis meant that this work was not pursued. Since then there has been little novel work on the antimicrobial activity of gold complexes. There are indications that the antiarthritic gold complexes may suppress Helico bacteria infections in the gastric mucosa , a causative agent for peptic ulcers, and that gold phosphine complexes in vitro are cytocidal towards Pseudomonas putida (14,15).

The present study was conducted to synthesize gold and silver nanoparticles by laser ablation of bulk target immersed in liquid environment which is simple method, and estimation of mean size and distribution of their nanoparticals by TEM techniques and U.V-Vis absorption spectroscopy. The ultimate objective was to study the interaction between bacteria and nanoparticles of gold and silver.

Materials and methods

Nanoparticles Preparation

Gold and silver nanoparticles colloids were produced by irradiating a metallic target plates with a thickness of 1mm immersed in distilled water with a pulsed laser beam. The ablation was performed with the (1064 nm) of a Nd:YAG laser (HUAFEI) operating at

10 Hz repetition rate, with a pulse width of 10 ns. The beam was focused on the surface of the target through a lens with 11cm of focal length. The spot size was about 1.5mm in diameter(16). The size and size distributions of the metals nanoparticles were examined by the transmission electron microscope TEM analysis, using a CM10 pw6020, Philips-Germany.

UV–vis absorption spectroscopy measurements were carried out on a double beam, CECIL C. 7200 (France) spectrophotometer. The nanoparticle concentrations were also characterized by Atomic absorption spectroscopy AAS measurement (model GBS 933, Australia), was carried out for the prepared samples.

Antibacterial susceptibility test

The concentrations of silver (11,14,17,20)PPM and gold(11,14,15)PPM nanoparticles were prepared by sterile deionized water by autoclave. The agar diffusion method was used to notice the effect of the concentrations of both silver and gold against bacteria (Pseudoronas aeruginosa No.1 isolated from urinary tract infactions (UTI) ,P.aeruginosa No.2 ,Streptococcus sp No.1 isolated from Nosal swao,Strepto coccus sp No.2 isoleted from sputum, Echrichia coli isolated from UTI and Staphylococcu sp) according to Mcltosh,R.M.(17,18).The evaluation depended on measuring the diameter of inhibition zone of bacterial growth by millimeter.

Results and Discussion:-

This research addresses on preparation of pure noble metals of Au and Ag nanoparticles and investigation of the effects on antibactiral activity.

Fig. 1(A and B), shows the extinction spectra of colloidal solutions of Ag and Au samples, respectively. The Nd-YAG laser of 1064 nm was utilized as an ablation source. The pulse energy at the target surface was varied in the range (300-600 mJ) and the beam was focused to have a diameter near the outer edges of the target of 1.27 and 0.85 mm for Ag and Au, respectively. The metal plate was fixed in a glass vessel filled with 1 ml DDDW thus the smokelike colloids above the metal plate was observed. The plate was located at 8 and 7 mm from the liquid surface for Ag and Au, respectively. Laser ablation listed for 15 pulses and the solution gradually turned to colored with the increase of the number of laser pulses. Fig. 1(A) shows the Absorbance peaks that occurred at around 400 nm is the characteristic SPE signature of Ag nanoparticles[16]. Fig 1-B shows broad band with the Absorbance peak around 526 nm with the peak position remaining practically constant, that indicates the production of gold nanoparticles[19]. We observed a faint pink coloration of the solution after several pulses of the experiment. In the absorption spectra of the solutions, the surface plasmon related peak could be clearly distinguished. This peak was around 520–530 nm, which was consistent with the presence of small 3–30 nm particles in the solution[20], which also confirmed by TEM.
Figure 2(A and B) shows the TEM images and corresponding size distributions of silver and gold nanoparticles, produced by laser ablation of silver plate immersed in pure water. The nanoparticles thus produced were calculated to have the average diameters of 14 nm. It is observed that the average diameter and size distribution was increased with the increase of the laser energy. The origin of the surface morphology of the irregularly

shaped particles sizes and the size distribution broadens can be explained by absorption by defects and thermally induced pressure pulses which cause cracking[21].
Figures(3) and (4) showed the zone inhibition on nutrient agar plates as a function of concentrations of silver(11,14,17,20)ppm and gold (11,14,15)ppm nanoparticles .The zone inhibition increased significantly with increasing the concentration of silver and gold nanoparticels .

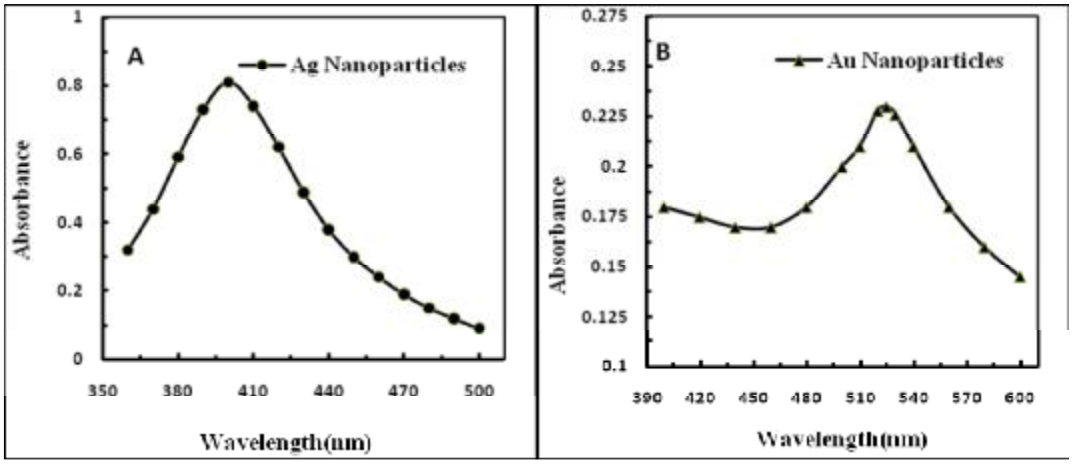
Bactericidal behavior of nanoparticles is attributed to the presence of electronic effects that are brought about as a result of changes in local electronic structures of the surfaces due to smaller sizes. These effects are considered to be contributing towards enhancement of reactivity of silver nanoparticles surfaces. Ionic silver and gold strongly interacts with thiol groups of vital enzymes and inactivates them. It has been suggested that DNA loses its replication ability once the bacterium are treated with silver ions[14,22,23]. 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[24]. Compounds of silver such as silver nitrate and silver sulfadiazine are used to prevent bacterial growth in drinking water, sterilization and burn care. It is economical to consolidate silver in polymers, composites, fabrics and catheters for antibacterial functionality[25,26].

Bacterium have different cell well structures on the basis of which these are classified as Gram negative or Gram positive. The structural difference lies in the organization of peptidoglycan, which is the key component of cell wall structure. Gram-negative bacterium exhibit a thin layer of peptidoglycan (about 2–3 nm) between the cytoplasmic membrane and the outer cell wall. Outer membrane of *E. coli* cells is predominantly constructed from tightly packed lipopolysaccharide (LPS) molecules, which provides an effective permeability barrier[27]. The overall charge of bacterial cells at biological pH values is negative because of excess number of carboxylic groups, which upon dissociation makes the cell surface negative. The opposite charges of bacteria and nanoparticles are attributed to their adhesion and bioactivity due to electrostatic forces. It is logical to state that binding of nanoparticles to the bacteria depends on the surface area available for interaction. Nanoparticles have larger surface area available for interactions, which enhances bactericidal effect than the large sized particles; hence they impart cytotoxicity to the microorganisms[28]. The mechanism by which the nanoparticles are able to penetrate the bacteria is not understood completely, but studies suggest that when *E. coli* was treated with silver or gold , changes took place in its membrane morphology that produced a significant increase in its permeability affecting proper transport through the plasma membrane, leaving the bacterial cells incapable of properly regulating transport through the plasma membrane, resulting into cell death[29]. It is observed that nanoparticles have penetrated inside the bacteria and have caused damage by interacting with phosphorus and sulfur containing compounds such as DNA and proteins. Silver tends to have a high affinity to react with such compounds [26,30].

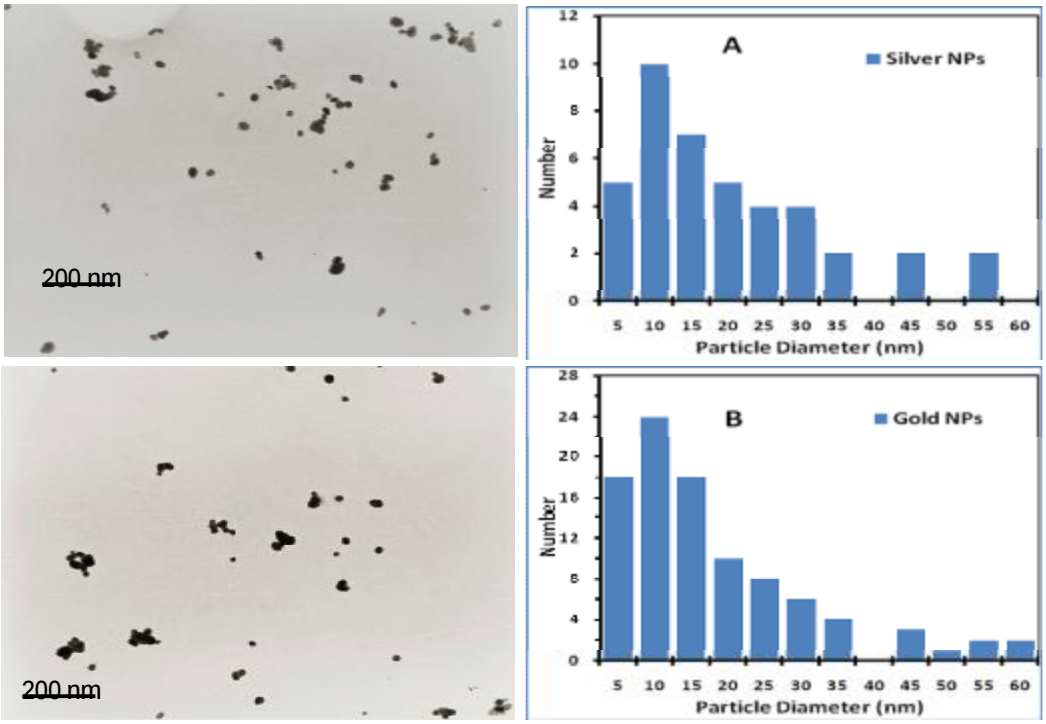
In our study, it is may be that DNA may have lost its replication ability and cellular proteins become inactive after treatment with silver or gold nanoparticles. Another reason

would be the release of silver or gold ions from nanoparticles, which will have an additional contribution to the bactericidal efficacy of nanoparticles.
Heavy metales are toxic and react with proteins,therefore they bind protein molecules[8], heavy metales strongly interacts with thiol groups of vital enzymes and inactivates them [31].In addition,it is believed that Ag and Au bind to functional groups of proteins,resulting in protein deactivation and denaturation [30,32]. as a result cellular metabolism is inhibited causing death of microorganism[4]. High activity of silver nanoparticles is attributed to species difference as they dissolve to release Ag^0,Au^0, Ag^+ Au^+ clusters, whereas other silver and gold sources such as silver nitrate and silver sulfadiazine and [Au(SCN)(PMe)3] release Ag^+ and Au^+ only. It is believed that silver and gold nanoparticles after penetration into the bacteria have inactivated their enzymes, generating hydrogen peroxide and caused bacterial cell death[14,30].

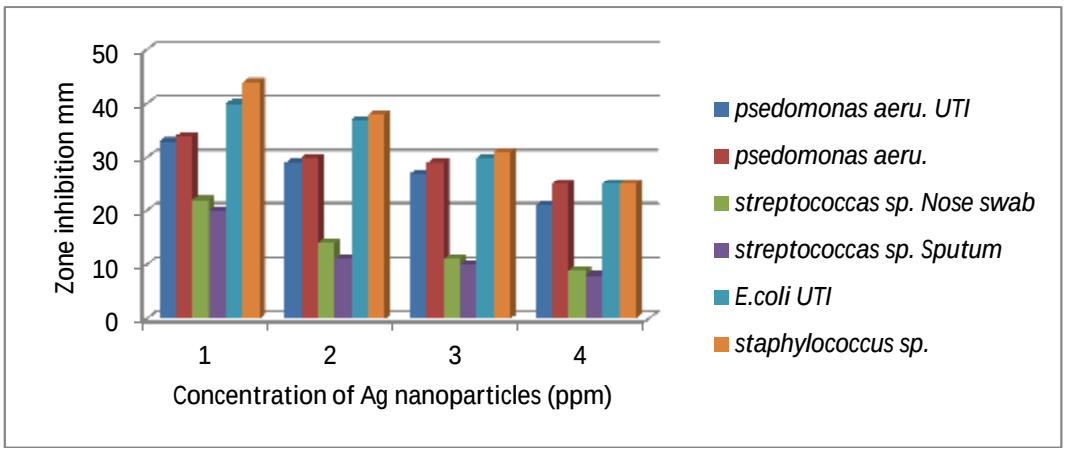
Experimental observations of previous study have explained significantly the antibacterial behavior of silver and gold nanoparticles. When *E.coli* was treated with highly reactive metal oxide nanoparticles, an inhibitory effect took place[14,30]. Silver nanoparticles after adherence to the surface of the cell membrane disturbed its respiration as Ag+ interact with enzymes of the respiratory chains of bacteria[33,34]. It is observed in our study that a degradation of the membrane structure of *E. coli* took place. Metal depletion causes formation of irregular-shaped pits in the outer membrane of bacteria which is caused by progressive release of LPS molecules and membrane proteins. In addition, it is believed that silver binds to functional groups of proteins, resulting in protein denaturation[14,30]. Complete bacterial inhibition depends upon the concentrations of silver nanoparticles and on the number of bacterial cells. It reflects that silver and gold nanoparticles have an excellent biocidal effect and potential in reducing bacterial growth for practical applications.



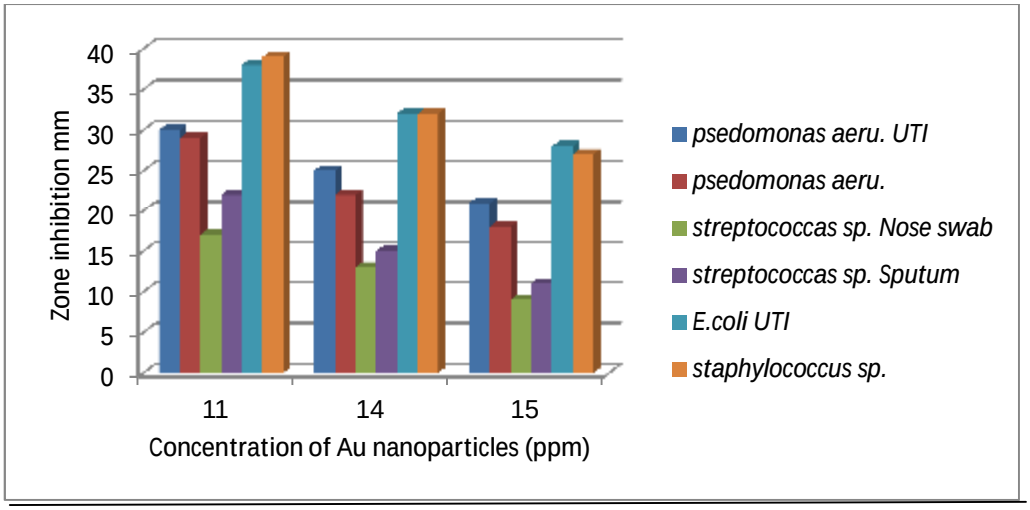
Figure(1): Absorbance spectra of silver nanoparticles (A), and gold nanoparticles (B), obtained by laser ablation of metal plates immersed in DDDW with laser energy of 600 mJ, laser shots of 15 pulses and wave length is 1064 nm of Nd-YAG.



Figure(2): TEM images and size distributions of silver (A), and gold nanoparticles (B), produced by laser ablation of metal plats immersed in pure water, (λ=1064 nm and laser shots of 15 pulses).



Figure(3) Antibacterial characterization by zone inhibition as a function of silver nanoparticles concentration on nutrient agar plates after 24 h incubation time.



Figure(4).Antibacterial characterization by zone inhibition as a function of gold nanoparticles concentration on nutrient agar plates after 24 h incubation time.

REFERENCES

[1] S. Barcikowski, A. Menendez-Manjon, and B. Chichkov (Generation of nanoparticle colloids by picosecond and femtosecond laser ablations in liquid flow) APPLIED PHYSICS LETTERS 91, 083113 _2007_

[2] S. BESNER, A.V. KABASHIN, M. MEUNIER_ (Two-step femtosecond laser ablation-based method for the synthesis of stable and ultra-pure gold nanoparticles in water) Appl. Phys. A 88, 269–272 (2007)

[3] A. Pyatenko, M. Yamaguchi, and M. Suzuki (Mechanisms of Size Reduction of Colloidal Silver and Gold Nanoparticles Irradiated by
Nd:YAG Laser) J. Phys. Chem. C 2009, 113, 9078–9085

[4] Y. Fong, J. R. Gascooke, B. R. Visser, G. F. Metha, and M. A. Buntine (Laser-Based Formation and Properties of Gold Nanoparticles in Aqueous Solution: Formation Kinetics and Surfactant-Modified Particle Size Distributions) J. Phys. Chem. C 2010, 114, 15931–15940.

[5] Prashant K. Jain ,Ivan H. El-Sayed, Mostafa A. El-Sayad; Au Nanoparticles target Cancer; Nanotoday, 2007,2,1, 18-29

[6] Nam Jm,Thaxton CS,Mirkin CA: Nanoparticle-based bio-bar codes for the ultrasensitive detection of proteins,Science 2003,301:184-16.

[7] Tkachenko AG,Xie H,Coleman D,Glomm W,Ryan J,Anderson MF,Franzen S,Feldheim DL : Multifunctional Gold Nanoparticle- Peptide Complexes for Nuclear Targeting. J Am Chem Soc 2003,125:4700-4701.

[8] Hirsch LR,Stafford RJ,Bankson JA,Sershen SR,Rivera B,Price RE,Hazle JD,Halas NJ,West JL:Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance PNAS 2003,100:13549-13554.

[9].Brigger,I.,C.Dubernet and P.Couvreur.2002,Nanoparticles in cancer therapy and diagnosis Adv.Drug Delivery Rev.54:631-651.
[10].Mulvaney,P.,1996.Surface plasmon spectroscopy of nanosized metal particles.Langmuir 12:788-800
[11].S.Pal,Y. K.Tak and J.M.Song, Does the Antibacterial activity of Silver Nanoparticles depend on the shape of the nanoparticle?A study of the Gram-Negative Bacterium Escherichia coli. Applied and Environmental Microbiology,2007; vol.73 ,no.6,1712-1720.
[12].Oka,M.,T.Tomioka,K.Tomita,A.Nishino and S.Ueda.1994Inactivation of enveloped viruses by a silver-thiosulfate complex.Metal-Based Drugs 1;511.
[13].Oloffs,A.,C.,Cross-Siestrup,S.Bisson,M.Rinck,Rudolvh,and U.Gross.1994.Biocompatibility of silver-coated polyurethane catheters and silver-coated Dacron material biomaterials 15;753-758.
[14].Fricker S.P.; Medical Uses of Gold Compounds:Past,Present ,Future,Gold Bulletin ,1996,29(2)53-64.
[15].G.L.Buryging.B.N.Khlebtsov.A.N.Shantrokha,L.A.Dykman.V.A.Bogatyrev.N.G.Khlebtsov, On the Enhanced Antibacterial Activity of Antibiotics Mixed with Gold Nanoparticles.;Nanoscale Res Lett(2009),4;794-801.
[16] X.P. Zhu, T. Suzuki, T. Nakayama, H. Suematsu, W. Jiang, K. Niihara (Underwater laser ablation approach to fabricating monodisperse metallic nanoparticles) Chemical Physics Letters 427 (2006) 127–131
[17].Mclntosh,R.M.(1996); Laboratory Manual Experimental Microbiology.1st edition,Mosby-Year bok,Inc.
[18].Mahmoud,M.J.;Jawad,A.J.;Hussain,A.M.;Al-Omeri,M.;and Al-Naib,A.; Invitro Antimicrobial activity of Sasolia rosmarinus and Adiantum capillusveneris.Int .,J.Crude. Drug. Res. 27:14-16(1989).
[19]. N.V. Tarasenko, A.V. Butsen, E.A. Nevar, N.A. Savastenko (Synthesis of nanosized particles during laser ablation of gold in water) Applied Surface Science 252 (2006) 4439–4444.
[20]. F. Mafune, J. Kohno, Y. Takeda, T. Kondow (Full Physical Preparation of Size-Selected Gold Nanoparticles in Solution: Laser Ablation and Laser-Induced Size Control) American Chemical Society 106 (2002) 7575-7578.
[21]. O.R. Musaev, A.E. Midgley, J.M. Wrobel, M.B. Kruger (Laser ablation of alumina in water) Chemical Physics Letters 487 (2010) 81–83.
[22].Shrivastave S.,Bera T.,Roy A.Singh G.,Ramachandrarao P. Dash D.,Characterization of enhanced antibacterial effects of novel silver nanoparticles,Nanotechnology,2007,18,225103.
[23].Feng,Q.L.,J.Wu,G.Q.Chen,F,Z.Cui,T.M.Kim and J.O.Kim.2002,Amechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus, J, Biomed, Mater. Res.52:662-668.
[24].Yamanaka,M.,K.Hara,and J.Kudo.2005.Bactericidal actions of silver ion solution on Escherichia coli ,studied by energy-fltering transmission electron microscopy and proteomic analysis.Appl.Environ.Microbiol.71:7589-7593.

[25].Vertelov G.K.,Krutyakov,Y.A.,Efremenkova,O.V.Olenin,A.Y.,Lisichkin,G.V.A versatile synthesis of highly bactericidal Myramistin stabilized silver nanoparticles,Nanotechnology 2008,19,355707.
[26].Morones J.R.,Elecheguerra J.L.,Camacho A.,Holt K.,Kouri J.B.Ramirez J,T.Yacaman M.J.,The bactericidal effect of silver nanoparticles,Nanotechnology 2005,16,2346-2353.
[27].Lee D.:Cohen R.E.,Rubner,M.F.Antibacterial properties of magnetically directed antibacterial microparticles Langmuir 2005,21,9651-9659.
[28].Bhupendra Chudasama,AnJana K. Vala,Nidhi Andhariya,R.V. Upadhyay,and R.V.Mehta,Enhanced Antibacterial activity of biofunctional Fe3O4-Ag Core-Shell nanostructures ,Nano Res (2009),2:955-965.
[29].Sondi I. and B. Salopek,-Sondi. 2004,Silver nanoparticles as antimicrobial agent,acase study on E.coli as a model for Gram- negative bacteria.J.Colloid Interface Sci275:177-182.
[30]. M.Raffi.F.Hussain,T.M.Bhatti,J.I.Akter,A.Hameed ,M.M.Hasan; Antibacterial Characterization of Silver Nanoparticles against E.Coli ATCC 15224.,J.Mater.Sci.Technol.,24,2,2008,192-196.
[31] J.L.Elechiguerra,J.L.Burt,J.R.Morones ; Interaction of silver nanoparticles with HIV-I,Journal of Nanobiotechnology;2005,(3),6,1-10
[32] Bhupendra Chudasama. Anjana K.Vala., Nidhi Andhariya.R.V.Mehta. ,R.V.Updhyay,;Highly bacterial resistant silver nanoparticles:synthesis and antibacterial activities, J.Nanopat Res.10,January, 2010.
[33].McDonnell,G. and A.D.Russell 1999.Antisptics and disinfectants activity action and resistance .Clin.Microbiol.Rev.12:147-179.
[34].Bragg.P.D. and J.Rainnie 1974.Theeffect of silver ions on the respiratory chains of E.Coli,Can.J.Microbiol.20:883-889.