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EFFECTS OF AG NANOPARTICLES PREPARED BY ND-YAG LASER AND STUDY THEIR ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY

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Abstract

This study describes one of the most important parameters that effect on the shape and of size Ag NP, It has contain chemical, physical, biological and photovoltaic properties for this reason has applications in many scientific fields. Silver NPs were successfully prepared via – laser (*Nd-YAG)* of (1064 nm wavelength) with and (energy 600 mJ per pulse), The purpose is to get smaller size of (Ag-NPs). studied by different analysis such as measuring absorbance of color, UV-Visible absorption spectrum, X-Ray diffraction, and Transmission electron microscopy. This study used the recurrence rate of 1, 3 and 10 kHz to radiate the Ag target in liquid. Then we saw the effect of laser pulse recurrence rate, on NPs size,structure and morphology, it was also the aim are the preparation of Ag-NPs via antioxidant and inhibition activity against *Pseudomonas aeruginosa*.

Key words: Ag NPs; Laser pulse recurrence rate; antioxidant; antibacterial activity.

Introduction

A nanomaterial is via mean of definition a particle which each its three dimensions are nanometer in size. They are known to be in various forms eg. triangular, spherical, cubical, ellipsoidal and so forth (Park, J.H. *et al.,* 2004) nanoparticles have an very wide range of possibility applications in biological, physical and chemical fields. nanoparticle is widely range utilize by for in diagnose certain diseases, photography, biological labeling, catalysis, biosensors, and in physical ystem such as photonics and optoelectronics (Huang, X. *et al.,* 2010). The nanoparticles is have special property of is caused by their large surface area-to-volume ratios and Nanoparticles have many potential applications, such as, communication, ultrafast data, optical data storage (Schmid, G. G. 1994), solar energy conversion (Graetzel, M. *et al*., 1992) and catalysts (Králik, M. *et al.,* 2001). Compos of many material, for such as, semiconductors, metals (Trindade, T. *et al.,* 2001). Organic polymers (Xu X. J. *et al.,* 2002) and core-shell composite architectures (Spalt M.Z. 2007) in small nanoparticles used the Idiom "Cluster" that have a surface structure of atoms or molecules associated with an bound by forces of ionic, hydrogen and covalent. As for the Idiom "Colloid" is the nanometers range from to several hundreds of micrometers (Xu, X.J. *et al.,* 2002). Silver nanoparticles possess chemical and physical characteristic due to its large effect of volume to surface, as the ratio of surface to volume rises millions of times, which alters the optical and structural characteristic due to its effect on the bulk size.The current study aims to test the effectiveness against microbes of some types of bacteria by laser removal in the deionized water of the nanoscale silver metal, and compare it with a group of antibiotics. (Raffi, M. *et al.,* 2008).

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Materials and Methods

Silver metal were prepared by laser ablation with purity 99.81%, were used as targets, a pellet with 1.5cm in diameter, 3mm in thickness. Cleaned with distilled water and ethanol to remove impurities. Laser {Nd: YAG} laser (1064 nm) with (energy 600mJ per pulse), the liquid in this work was de-ionized water, put at the undermost of a glass container include (8ml) of sterile liquid, rising of liquid (4mm) on the Ag target, pulse repetition frequency 1 Hz, 150ns (pulse width) of utilized for remove of target. The various recurrence average of laser at 1, 3 and 10 kHz for pattern 1-3 respectively as show Fig. 1.

Study of properties of silver –NPs:

UV-Visible Spectrophotometer:

UV-Vis absorption spectra is use for the optical test of Ag NPs with the spectral range (350-650 nm) for Ag nanoparticles, solution were measured

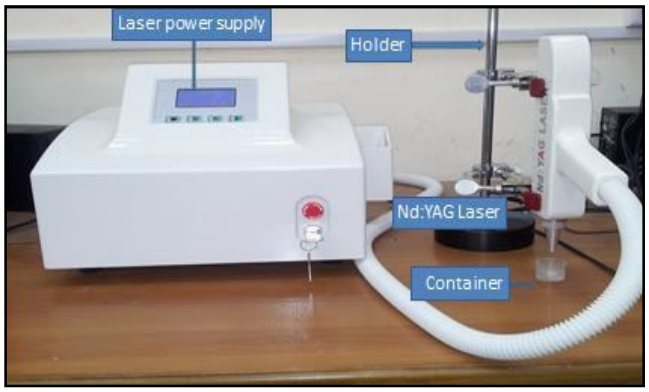

Fig. 1: Laser ablation (Nd: YAG).

by UV-Vis double beam ectrophotometersSP-3000 plus (CE OPTMA TOKYO), the device was utilized in Departments of medical engineering, University of technology, Iraq.

XRD (X-Ray diffraction):

XRD Pattern in order to research the structural characteristic, and the crystal expansion of silver colloidal nanoparticles, the XRD test were made using (XRD, 6000 SHIMADZU, Japan). The exporter of X-ray irradiation was CuKá radiation with (0.15405 A0) wavelength and scan corner was 2θ = (20-60) grade. utilize the sherrer technigue (Swarnkar R.K. *et al.*, 2011):

$$D = 0.94\ \lambda/\beta \cos\theta \quad (1)$$

Transmission electron microscopy (TEM):

Metal oxides nanoparticles suspension in order to get the morphology properties. TEM (kind CM10 pw6020, (Philips/Germany). TEM images of the nanoparticles were obtained at operating voltage of 200 KeV, with 0.3 nm resolution. The analysis Pattern for TEM were prepared via spreading a drop of nanoparticles sol onto standard silver (200 meshes), the instrument was used in electronic microscope center - College of Medicine/ Al-Nahrien University, Iraq.

Antibacterial activities of AgNPs:

Determination of minimum Inhibition concentrations (MICs) of Silver nanoparticales against bacteria. The Inhibition activity from the synthesized AgNPs is estimated through the fixing from the minimum inhibitory concentration (MIC) via the micro dilution technigue in the culture broth{8}. Further dilutions were prepared to concentrations ranging from (10000-50) mg/ml. Briefly, 100 µl of sterile Muller Hinton liquid was placed into the wells of the first column and others columns of the 96-well micro plate, Subsequently, 100 µl of nanoparticles of highest concentration (10000mg. ml^{-}) was added to the first place of the microtiter plate and mix with the medium; this results in a Ag NPs conc. of 5000 mg/ml; serially, 100µl were transport to the following wells reject 100 µl of the mixture in the last column the total size for any well was 100 µl. All wells were inoculated with 5 µl of an overnight culture (1.5 × 108 CFU/ml) of pathogenic bacteria isolates. Microtiter plates were covered and save at 37°C for 24h. The MIC was determined at a concentration which no visible growth could be observed after sub culturing on Nutrient agar (Gudiña, E. J. *et al.*, 2010).

Study the ability of Ag NPs to scavenge Free radicals using (DPPH):

The Antioxidant activity was measured using DPPH assay with minor adjustments according to Tailor and Goyal method. each model compounds (10 µL) were mixed with ethanol at concentration 100% (490 µL) and then add the amount to 1 mL via complet of DPPH liquid (Corina, C. *et al.*, 2013). Thence subsquent together keep at room temperatures at fifteen 15 minute. And was calculated conferring to the equation:-

Scavenging % = Absor of control-Abosrof sample\Absor of control × 100%

Statistical Analysis:

All of the tests were conducted in triplicate. Data were reported as means ± standard deviation.

Results and Discussion

Fig. 2A. shows the prepared samples of Silver colloidal NPs, Where we can see that the color of Ag NPs *Suspension* in deionized water at different pulse number (50, 75 and 100) pulse. The color of colloidal NPs are varied of yellow to brown as the removal pulse number growth, the colors changes depends size of the nanoparticles and strongly concentration.

Fig. 2B. We see the absorption spectra of nanoparticles solutions. The absorption peaks around 420nm..The growth in the production of nanoparticles concentrations is due to the growth in the number of pulses as well increased laser pulse repetition rates through the movement of the wavelength lead to a decrease in the wavelength. count on to the Mie theory (Binh, N.T., *et al.*, 2008). increasing of laser pulse recurrence lead to particles size reduction That is, the higher the repetition the laser, the greater the interactions of the particles produced with the laser light.

Fig. 3. The TEM image of Ag NPs prepared at 600 mJ for 100 pulses presents the histograms of size distributions of synthesized nanoparticles. The size distribution Ag NPs having a diameter in the range 5-40 nm were obtained and spherical shape nanoparticles were dominated. While the higher peak corresponds to

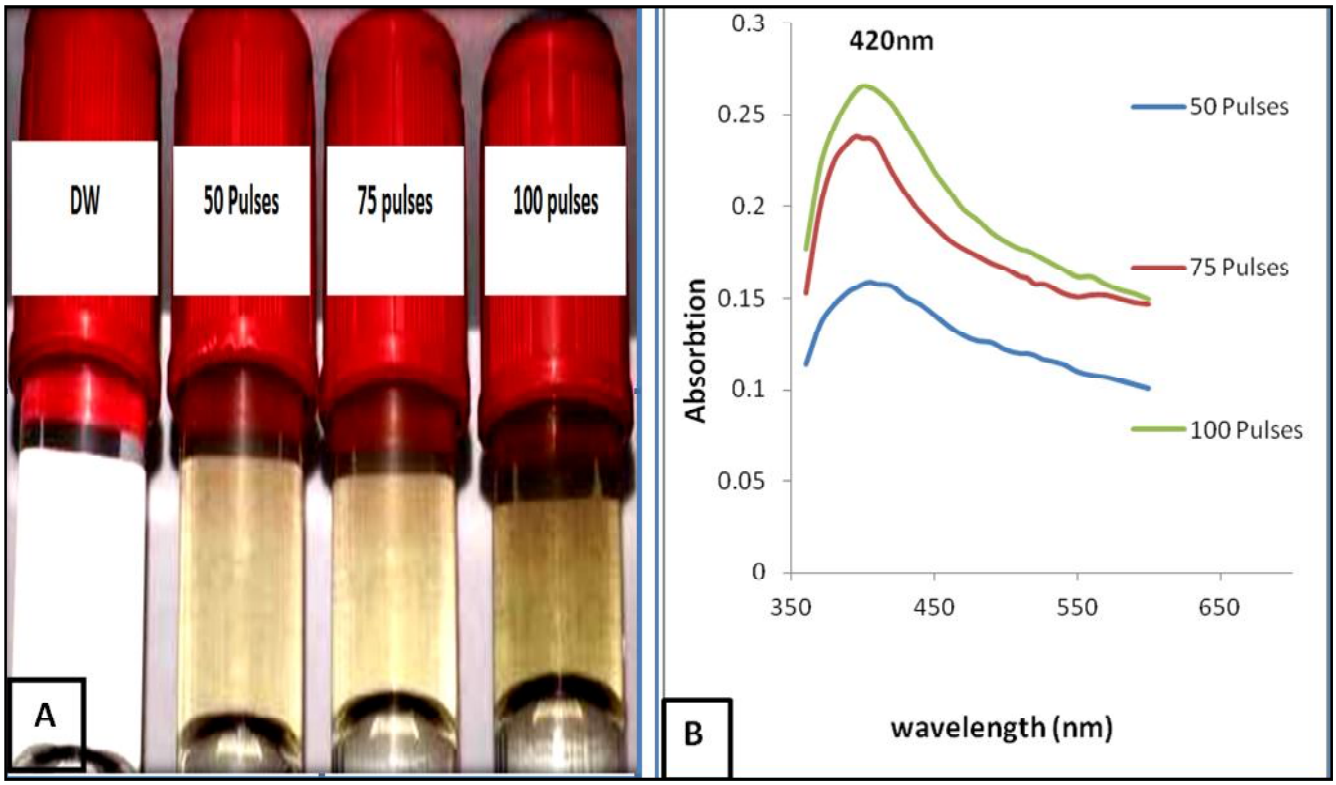

Fig. 2: (A) Color colloidal and (B) UV-vis of AgNPs prepared by laser ablation in DIW and different pulses number 50, 75 and 100pulses.

nanoparticles with smaller sizes (centered around 20 nm), and the number of particles used in these distributions was 122 particles. TEM results suggest a critical dependence of the laser energy and ablation process duration on the size.

Fig. 4. The crystalline nature of the Silver NPs, strong XRD peaks were appeared equal (311), (220), (200),(111) level at 2θ angles 77.64°, 64.54°, 44.38° and 38.28°. was this perfect compatibility with the unit cell of the face centered cubic structure (JCPDSileNo.04-783). The volume of Silver NPs approved to the XRD was about 5.2nm. This outcome was symmetrical with the TEM study.

Antibacterial activity:

Determination of MICs of silver nanoparticles against bacteria. Standard Broth Dilution Method has been used

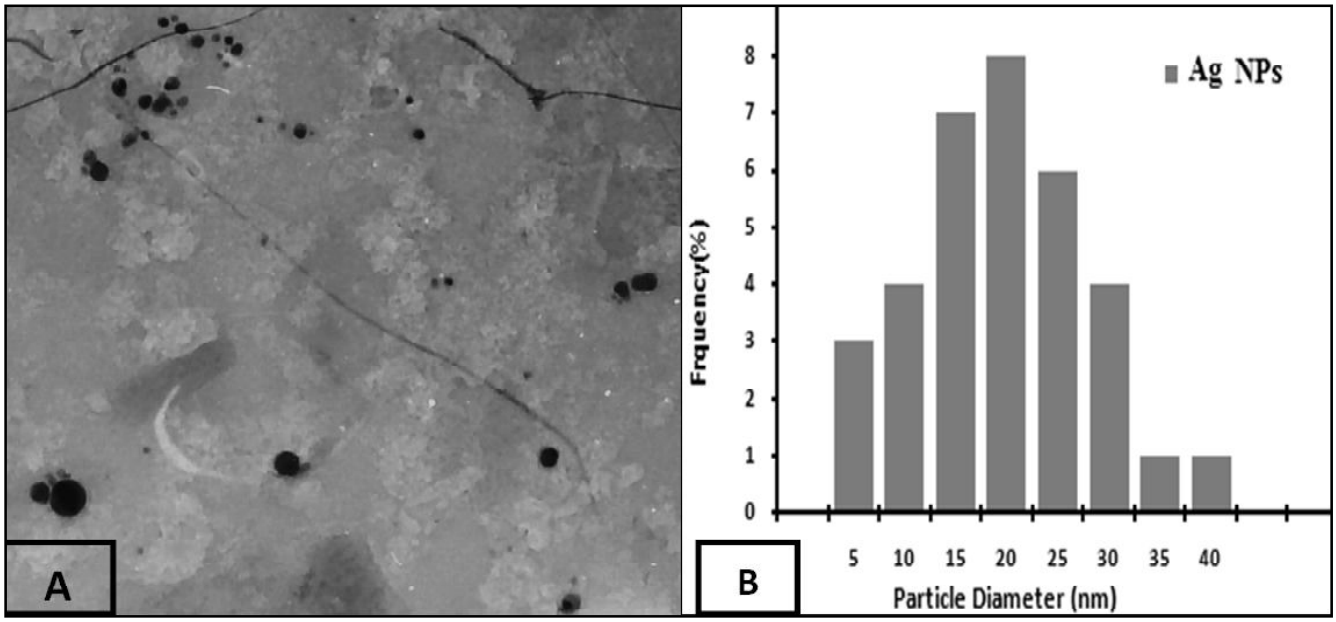

Fig. 3: (A) TEM image of AgNPs prepared via laser remove in DIW with 600 mJ and for 100pulse (B) size distribution of Ag NPs.

to detect the inhibition activity of Ag NPs, where serial dilutions of Ag NPs (10000, 5000, 2500, 1250, 625, 312) µg/ml were prepared and the antibacterial activity was determined depending on minimum inhibitory concentration (MIC), and table 1 shown these results.

The MICs of Ag NPs were determined against nine clinical isolates of *Pseudomonas aeruginosa*. P. (1, 2 and 3). (1000µg/ml) was for seven isolates p. (1, 2, 3, 4, 5, 6 and 7) 4000 mg/ml for one isolate (p.8) and 2000 for one isolate (p.9). According to the results mentioned it was finished that the high active of Silver NPs with concentration (5000µg/ml) that inhibit growth of all the seven isolates out of the nine bacterial isolates. Previous research about the biogenic Ag NPs detected a variable pattern of inhibition activity of Ag NPs against p. aeroginosa the MIC values were 125mg/ml (Ahmadi, M., *et al*., 2017). Another study reported by parveen *et al*., (2018) that found the Minimum inhibition concentrations of silver nanoparticales were 10mg/ml for *proteus vulgaris* as example for gram negative bacteria. The mechanisms that the silver nanoparticles effected on bacteria described by many studies, as it may be due to interaction with the cell wall of bacteria that drive to the conformation of pore in these walls, the effect of NPs on the proteins in the cytoplasm of the cells which drive to damage the regulation in cell function, also the NPs can effect on the DNA replication which will disrupt the replication mechanism (Singh, K. *et al.,* 2014). The other suggestion of the mechanism of silver nanoparticles recorded in previous studies, that silver nanoparticle revealed the antibacterial effect in two ways either by their changing the membrane possibility or decrease ATP {adenosine triphosphate synthase} activities, so decrease the metabolism process; they reject the subunit of the ribosome for tRNA binding, so inhibition its biological action (Fidel Martinez-Gutierrez *et al.,* 2010).

Antioxidant Activity:

The outcome appear that the color of Silver NPs - DPPH incubation solution was slightly changed following the incubation period. This is an indication of antioxidant property of Ag NPs. The Ag NPs nanoparticles were noted to have so antioxidant which detect their ability of

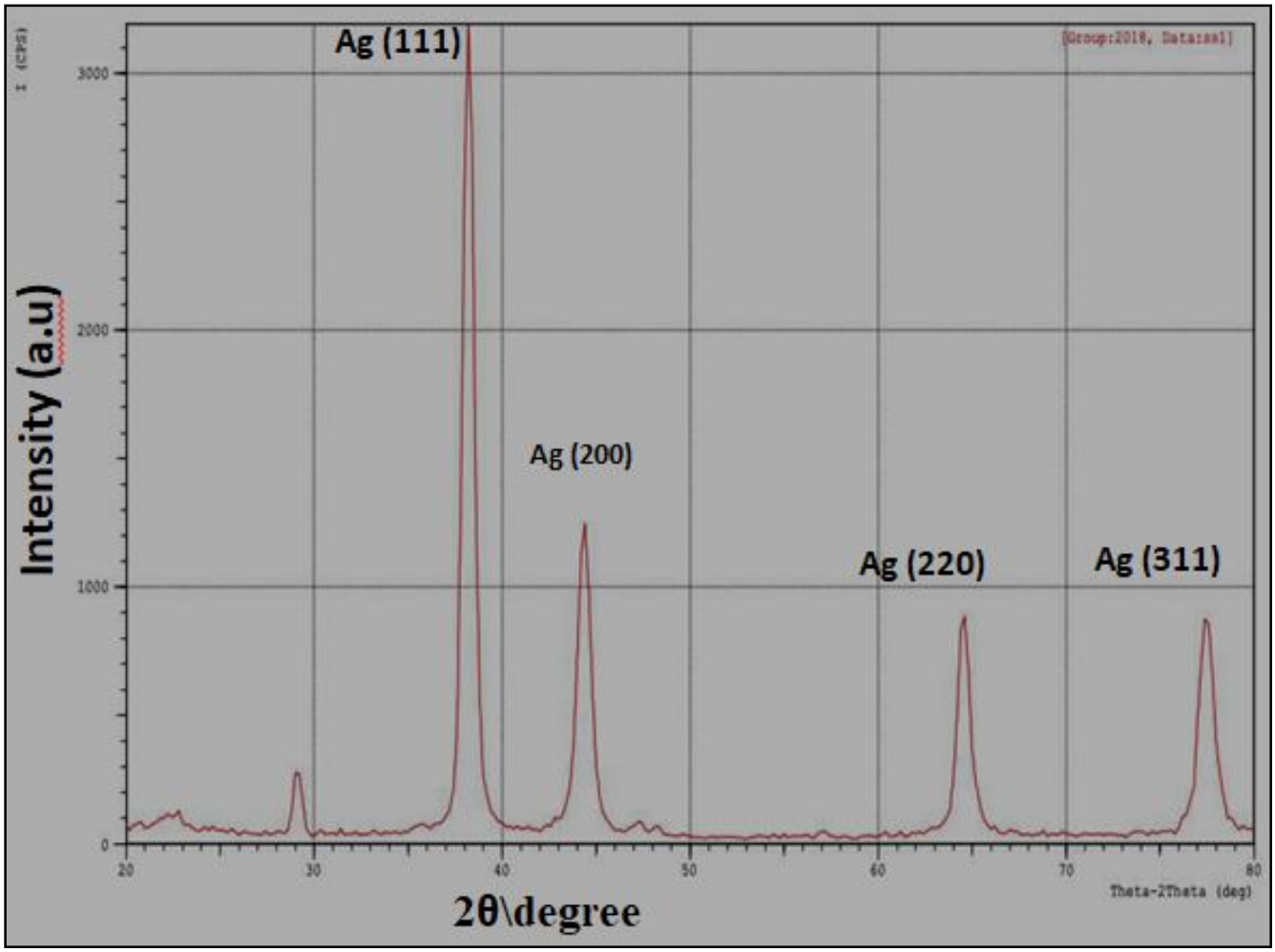

Fig. 4: X-ray diffraction pattern from Ag NPs prepared via laser ablation in DIW with 600 mJ and for 100pulse.

Table 1: Inhibition action of Ag NPs against clinical isolates of *Pseudomonas aeroginosa*.

Bacterial Isolates	Silver nanoparticles concentrations					
	10000 µg/ml	5000 µg/ml	2500 µg/ml	1 250 µg/ml	625 µg/ml	312 µg/ml
P1	+	+	+	+	-	-
P2	+	+	+	+	-	-
P3	+	+	+	+	-	-
P4	+	+	+	+	-	-
P5	+	+	+	+	+	-
P6	+	+	+	+	-	-
P7	+	+	+	+	-	-
P8	+	+	+	+	-	-
P9	+	+	+	+	+	-

*The *G-ve* (+) means inhibition of bacterial growth,*The *G-ve* (-) means bacterial growth.

give electrons and hence interact with free radicals more converting their to form more stable products. antioxidant nature increased with the increase in concentration of This suggests that Ag NPs nanoparticles can act against disease causing free radicals. The Ag NPs concentrations of 25, 50, 75 and 100 µg/ml are given to free radicals at 65.6, 70.0, 79.3 and 88 respectively and compared with positive control. The present results also showed that Ag NPs keep a up plane of antioxidant activity as discuss to ascorbic acid, with the up activity to the NPs synthesized as shown in Fig. 6. Such conclusions is in conformity with the results of other studies (Tsuang S. *et al.*, 2014).

Conclusion

Through the current study shows that Silver nanoparticles possess an anti bacterial, Antioxidant Activity, and it can be used in the pharmaceutical field as a treatment of wounds and infections.

Table 2: Antioxidant activity of Ag NPs by DPPH assay.

NO.	Concentrations µg/m of Ag NPs				Control positive
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	
1	66	69	79	88	95
2	67	71	78	87	95
3	64	70	81	89	95
Total of scavenging	65.6	70	79.3	88	95

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