

Preparation and Characterization of Lead Oxide Nanoparticles by Laser Ablation as Antibacterial Agent

Halah H. Rashed^{1*}

Fattin A. Fadhil²

Iman H. Hadi³

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Abstract

In this work, lead oxide nanoparticles were prepared by laser ablation of lead target immersed in deionized water by using pulsed Nd:YAG laser with laser energy 400 mJ/pulse and different laser pulses. The chemical bonding of lead oxide nps was investigated by Fourier Transform Infrared (FTIR); surface morphology and optical properties were investigated by Scanning Electron Microscope (SEM) and UV-Visible spectroscopy respectively, and the size effect of lead oxide nanoparticles was studied on its antibacterial action against two types of bacteria Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) by diffusion method. The antibacterial property results show that the antibacterial activity of the Lead oxide NPs was inversely proportional to the size of the nanoparticles in both Gram-negative and Gram-positive, and also it has been found that Gram-positive bacteria possess have greater sensitivity and less resistance to the lead oxide nanoparticles compared with Gram-negative bacteria.

Keywords: Antibacterial property, Lead oxide NPs, Laser ablation DIW.

Introduction:

Nanotechnology offers opportunities to re-explore the biological properties of already known antimicrobial compounds by manipulating their size to alter the effect [1]. Because of bacteria, it may possess resistance or immunity to many types of organic antibiotics. One of the most important reasons is that some types of bacteria produce enzymes that cause damage in antibiotic. A change in development may occur in the composition of their cell or secrete of proteins as new membrane prevent entering the antibiotics[2]; therefore, the scientists think about finding an alternative antibiotics in the use of nanoparticles as anti bacterial in different medical fields. Antibacterial activity of nanoparticles is related to compounds that locally kill bacteria or slow down their growth [3]. Antibacterial properties of nanoparticles come from, (1) the diameter of the nanoparticles is small compared with the diameter of bacterial cells which has a few micrometers. Thus, the particles are able to penetrate the bacterial cell [4],(2) the large ratio of surface area to volume which leads to freemetal ion toxicity arising from dissolution of the metals from surface of the nanoparticles[5],

(3)Resulting in the occurrence of the electrostatic interaction between bacteria and particle surface, along with hydrophobic interactions and polymer bridging that may be responsible for the phenomenon of bacterial adhesion onto the particles[6], (4) as well as reactive oxygen species (ROS) that can be generated on the surface of nanoparticles, and lead to ion release, membrane dysfunction; and nanoparticles internalization into cell and cause damage of Deoxyribonucleic acid (DNA) by exerting oxidative stress[7].Actually, there are plenty of factors that could influence the antibacterial printability of material NPs, such as size, concentration, shape, and physicochemical characteristics[8].Lead oxide (PbO), is an important semiconductor industrial material which has both forms α -PbO and β -PbO with band gap energies of 1.92eV and 2.7eV respectively[9], due to its unique electronic, mechanical and optical properties [10]. It has a wide range of industrial applications as the basic material of electrode active mass in leading acid batteries [11], the glass industry and pigments [12]. Among these applications, PbO NPs have a high antibacterial activity whereas T. Theivasanthi studies the antibacterial of spherical Pb nanoparticles prepared by electrolytic process that is capable to kill the E-coli bacteria with high efficiency[13]. For preparation of lead oxide NPs,

^{1, 2, 3} Department of Applied Science, University of Technology, Iraq, Baghdad

*Corresponding author: hala_hassan8140@yahoo.com.

there are several methods, for example, wet synthesis[9], chemical precipitation technique [14], sol-gel method[15], chemical bath deposition technique[16] spray paralysis technique[17], among these methods is pulsed laser ablation in liquid. This technique is easy, uncontaminated and has the ability to control the shape, size and concentration of particles by changing preparation conditions [18].

In this article, the antimicrobial activity of lead oxide nanoparticles and effect of their size on the diameter of the inhibition zone for two types of bacteria Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus*) strains by diffusion method.

Experimental work:

Synthesis and characterization of lead oxide NPs

The procedure of pulsed laser ablation was done by employing of lead plate that has purity 99.99 % as target which is provided from China company. Lead plate was cleaned with ethanol and distilled water before every synthesis and fixed in the bottom of the container which contains 10 ml deionized water (DIW). A pulsed Nd-YAG laser system type (Astanza) provides pulses with 1064 nm wavelength used for ablation while the laser energy, pulse duration is 400 mJ/pulse and 9 ns respectively.

The laser beam is focused by convex lens with 10 cm; effective beam diameter is 0.5 mm, and 50 and 200 laser pulses. There are many of mechanism reactions taking place in this process. The incident laser beam have energy that is larger than the threshold energy of the target. This energy is capable to form of plasma at solid –liquid interface due to the vaporization of the target surface in the effective beam diameter, excited species that react to the liquid (DIW), so that the reaction mechanism can be explained in reaction equations. After laser ablation, PbO suspension was dried on clean glass substrate and silicon at 40°C in an oven via drop

casting technique. The chemical bonds and surface morphology of the samples were investigated by (FTIR) and (SEM) respectively. Finally the optical absorption spectrum was recorded at room temperature using double beam UV-Visible spectrophotometer from (shimadzu).

Antibacterial test

Antibacterial activity of lead oxide NPs was analyzed against two different types of bacteria; Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus*) strains by diffusion method.

Gram –positive and Gram –negative, Bacteria farms can be prepared in nanotechnology center laboratory by diffusion method. It has many necessary steps that must be carried out: Firstly, The bacterial farms has been developed at the nutrient agar with PH 7.3 and placed in an incubator for 24 hours at 37° temperature in order to have good growth, after that the bacteria is diluted by normal saline in order to obtain the 10^5 to 10^7 Colony-Forming Units per ml (CFU./ml) cell comparable with the tube of McFarland Standard number 0.5, then the positive and negative bacteria are distributed on the surface of the separated petri dish, which contain the nutrient agar.

To determine the inhibitory effect, Filter papers were prepared as discs with 5mm diameter and immersed in the lead oxide suspension, then after the confirmation the discs were saturated, and put on the bacteria that's covers the surface of agar, the petri dishes are placed in an incubator for 24 hour at 37° temperature, then measure the diameter about the discs, which represents the inhibition zones. The Diameter of inhibition zone reflects magnitude of susceptibility of microbes.

Results and Discussion:

Characterizations of lead oxide NPs

FTIR spectrum for the lead oxide nanoparticles is shown in Figure (1).

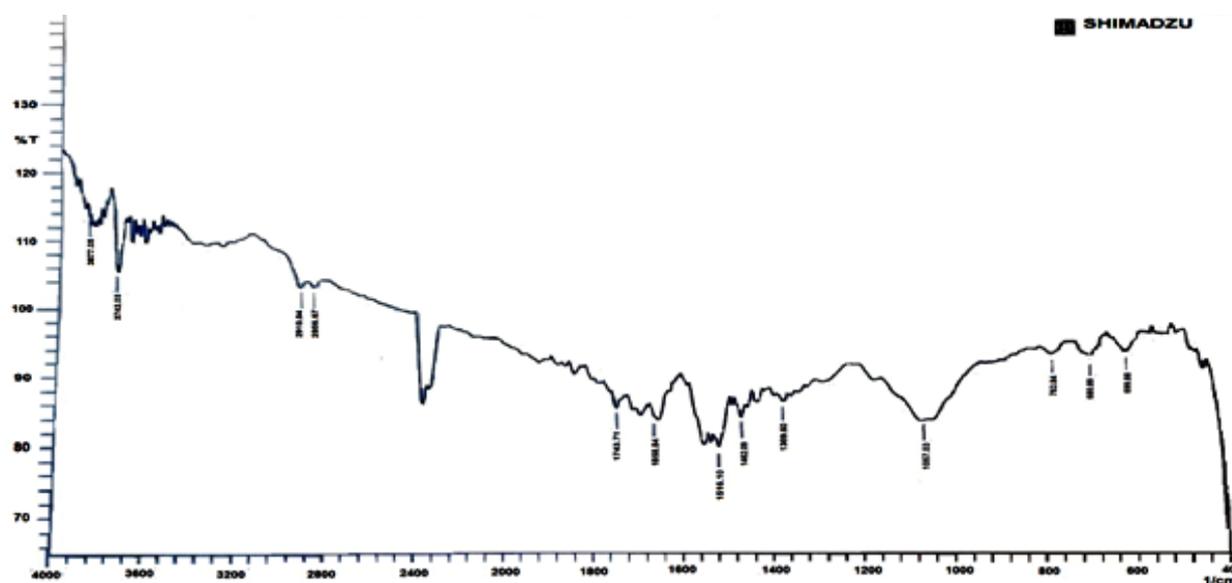
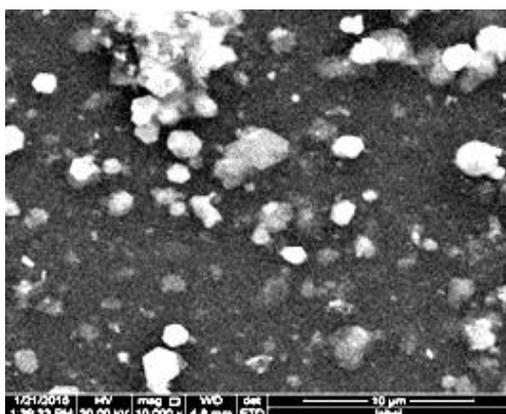


Figure 1. FTIR spectrum of lead oxide nanoparticles synthesis at 1.06 μm wavelength, 400 mJ energy and 200 laser pulses.

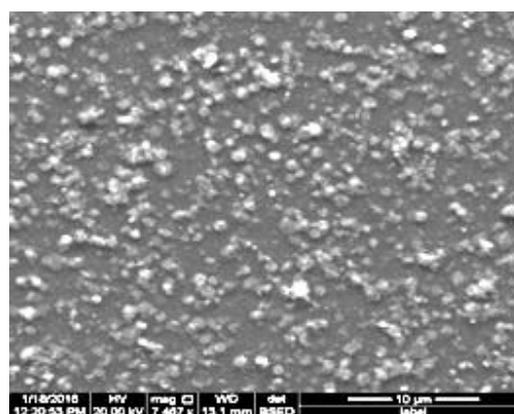
This spectrum has different transmission peaks. The transmission peak at 460 cm^{-1} indicated the presence of (Pb-O) stretching vibration mode [15], the peaks located at $(763.84, 1057.03)\text{ cm}^{-1}$ and the peaks at $(3877.05, 3742.03)$ are corresponding to the bonding of (O-H) and harmonics of H-OH stretching bonding modes of water respectively [19]. While the infrared peaks located at $(2918.67,$

$1743.1)$ and 1658.84 cm^{-1} are related to the (C=O) stretching vibration modes that refer to little contribution of CO_2 dissolution from air contain [20].

The surface morphology of the obtained particles synthesized at 50 and 200 laser pulses was observed by scanning electron microscope (SEM) as shown in Figure 2 (a, b).



(a)



(b)

Figure 2. SEM image of lead oxide NPs prepared at (a) 50 laser pulses and (b) 200 laser pulses.

Fig. 2(a) demonstrates that the obtained products have typical diameter $\sim \pm 300\text{ nm}$, with spherical and hexagonal in shape for sample prepared at 50 pulses while the typical diameter of nanoparticles were approximately $\sim \pm 75\text{ nm}$ and spherical shape only for sample prepared at 200 laser pulses respectively. Its smaller particle size was obtained by employing larger laser pulses. The reason belongs to the decreasing of the particle size when there was an increasing in the number of incident laser pulses upon the target, the quantity of the excised particles is increased. The excised particles will gather at the

surface of target and near the surface of the solution which represented as an obstacle in the path of the incident laser beam. These particles absorbed the laser energy. This energy is working for splitting and the fragmentizing of these particles and produced particles that are smaller in size. This leads to reduce the beam intensity that reached the target which caused no further increase in the number of ablated material [21].

The Ultraviolet -Visible spectrophotometer was carried out to investigate the optical properties of lead oxide colloidal prepared by laser ablation in

deionized water. It is very important to give information about the absorption edge, optical band gap between the electronic transitions, absorption

coefficient, etc... Fig (3) illuminated the transmittance and absorbance curves as a function of incident wavelength of samples.

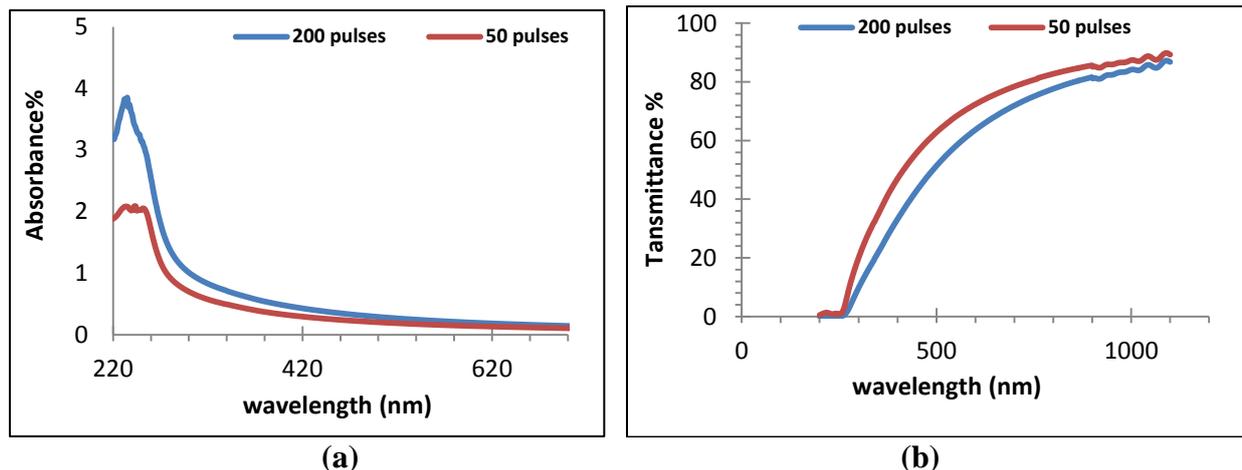


Figure 3. (a)Absorbance curves as function of incident wavelength,(b)transmittance curves as function of incident wavelength of lead oxide nanocolloids prepared at 50 and 200 laser pulses

Fig. 3(a) shows a change in the absorption spectrum as a function of light incident wavelength and a maximum absorption located at shorter wavelength (ultraviolet region) and significantly less with increase of the wavelength until it reached lower absorbance at visible and near infrared region, so that this material can be used as a window in solar cell application. It is noticeable that the absorbance increase with increasing the number of laser pulses compared with least number of laser pulses because of increasing the amount of ablated material and then increase the concentration of lead oxide [22], in addition to a slightly blue shift that could be recognized in the absorbance of lead oxide colloidal as a result to the smaller particle size (larger energy gap). Table (1) shows the effect of number of laser pulses on UV-Visible absorption peak of PbO.

The transmittance spectrum is behaving opposite absorbance as shown in Figure 3(b) of lead oxide colloids. This figure investigates that transmittance increases with the increase of the wavelength and the highest value was at (89 %) and (86%) for the samples prepared at 50 and 200 laser pulses

respectively. The optical band gap can be calculated by using the experimental relationship between the absorption coefficient and band gap energy (Tauc model)[23].

$$(\alpha h\nu) = A(h\nu - E_g)^n \dots\dots\dots(1)$$

Where α is the absorption coefficient, $h\nu$ is the photon energy and E_g is the energy of the band gap.

For direct band gap $n= 1/2$, which can be obtained by a plotting between $(\alpha h\nu)^2$ versus photon energy $(h\nu)$.By taking the best line aligns the straight part of the curve to cut off the axis photon energy at point where the photon energy is equal to the direct band gap.

Table 1. Effect of number of laser pulses on UV-Visible absorption peak of PbO ($\lambda=1064$ nm, $E=400$ mJ/pulse)

Sampl e	Number of laser pulses	Absorption peak (nm)	Absorption peak intensity
1	50	246	2.018
2	200	235	3.853

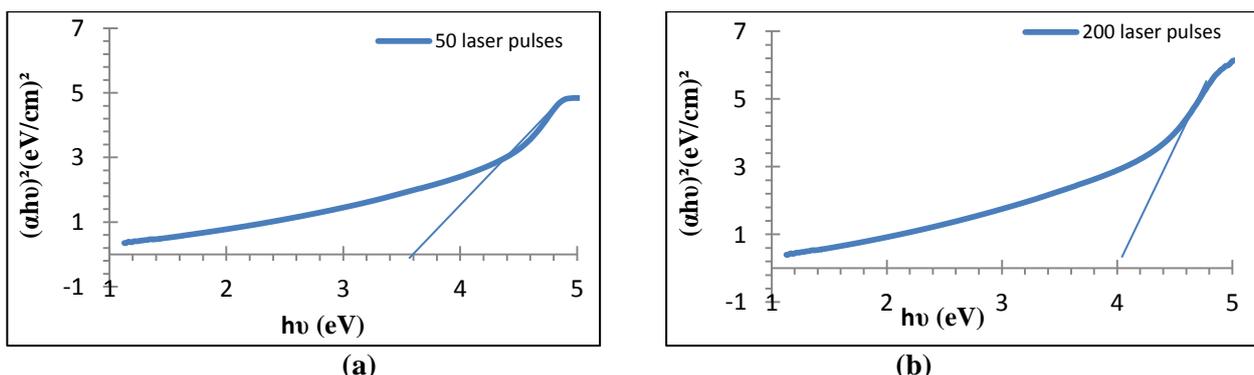


Figure 4. Direct band gap energy for lead oxide nps prepared at (a) 50 laser pulses, (b) 200 laser pulses.

Table (2) indicates the band gaps energy of lead oxide prepared at different laser pulses; the sample was prepared at 200 laser pulses that have higher value of band gap compared to the same material prepared at 50 laser pulses due to quantum confinement effect[14].

Table 2. Effect of number of laser pulses on band gap energy PbO ($\lambda=1064$ nm, $E=400$ mJ/pulse)

Sample	Number of laser pulses	Band gap energy (eV)
1	50	3.6
2	200	4

Antibacterial results

Fig. (4),(5) illustrate the inhibition zones of lead oxide nanoparticles prepared by laser ablation in water at 50 and 200 laser pulses against two types of bacteria, Gram –negative (*Escherichia coli*) and Gram –positive (*Staphylococcus aureus*) respectively, and the antibacterial affectability may be measured concerning illustration zone of restraint over mm in breadth.



Figure 4. Zone of inhibition for lead oxide Nps synthesis at 50 laser pulses at (a) Escherichia coli and (b) Staphylococcus aureus

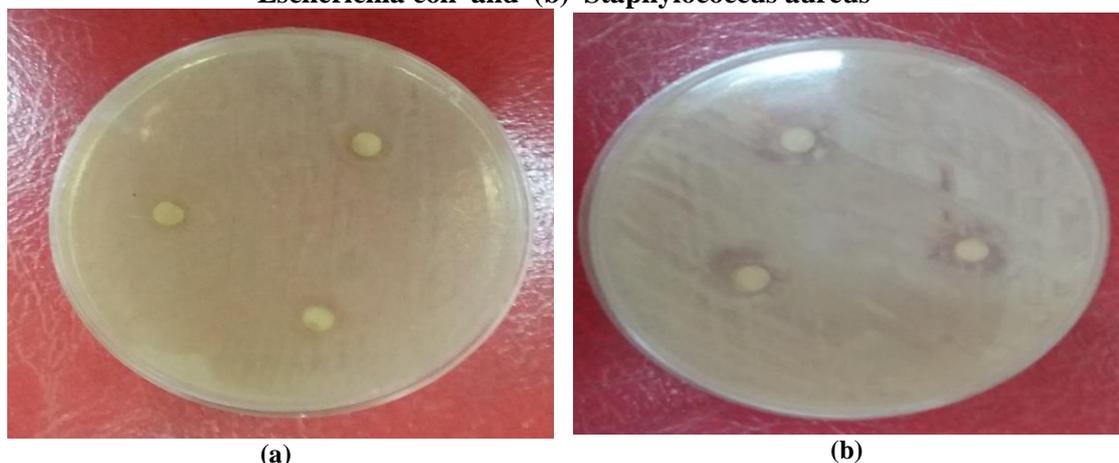


Figure 5. Zone of inhibition for lead oxide NPs synthesis at 200 laser pulses at (a) Escherichia coli and (b) Staphylococcus aureus

Table (3) shows the diameters of inhibition zone around each filter paper for each sample and each type of bacteria.

Table 3. Diameters of inhabitation zone of lead oxide NPs

sample	G-negative (E-coli)	G-positive (Staph)
1	5mm	13mm
	5mm	12mm
	6mm	9mm
	13mm	13mm
2	13mm	12mm
	12mm	12mm

The inhabitation zonediameters of *Staphylococcus aureus* are larger than the inhabitation zonediameters of *Escherichia coli*. This means that *Staph-aureus* bacteria had the highest sensitivity toward PbONPs while *E- coli* aurous bacterial showed the least sensitivity and higher negative resistance/tolerance against lead oxide NPs. Our finding is in agreement with Premanathan et al, who reported that the metal oxide nanoparticle effect is more pronounced against Gram-positive bacterial strains than Gram-negative bacterial strains[24]. These figures indicated the antimicrobial action of

the lead oxide nanoparticles in deniable. It can be a perfect type for damage or inhibit bacterial growth especially in high laser pulses, as a result to the increase the concentration, in addition to decrease in the particle size. The effect of particle size can be explained on antibacterial efficiency by the surface-area-to-volume ratio (SA:V) or Specific Surface Area (SSA), its substantial factor illustrates the effectiveness of the nanoparticles[25]. The particles have large SA: V (very small diameter), this means that the surface of these particles contains a large number of reactive oxygen species (ROS) such as (H_2O_2 , $\cdot OH$ and $O_2^{\cdot -}$) with high mobility and ready to interact, which is responsible of damage of the proteins and DNA in bacteria at a quick rate with the bacteria cell compared with larger particles. Therefore, it's leading to the laceration of cell wall and thereafter cell death and enhanced antibacterial activity[26].

Conclusions:

This work aimed to the studying of the size influence of lead oxide nanoparticles on its antibacterial properties. Lead oxide NPs was prepared by one step pulsed laser ablation of lead target immersed in DIW method. This technique was carried at constant laser energy and different laser pulses. The surface morphology characteristics indicated that the particle size of the nanoparticles decreased with increasing the number of laser pulses, the reason was due to excised particles absorbed the incident laser energy; thus, it was divided into smaller particles. The optical properties characteristics showed that the absorbance increased with the increase of laser pulses due to an increase in the concentration while the energy gap decreases with increasing the number of laser pulses. Finally, lead oxide NPs possess antibacterial properties upon both types of positive and negative bacteria, it was noted that the smaller particles size has larger ability to kill bacteria.

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تحضير وتشخيص جسيمات اوكسيد الرصاص النانوية بواسطة الاستئصال بالليزر كمضاد بكتيري

ايمان حسن هادي³

فاتن عبد الاميرفاضل²

هاله حسن رشيد¹

^{1,2,3} قسم العلوم التطبيقية، الجامعة التكنولوجية، بغداد، العراق.

الخلاصة:

في هذا العمل، تم تحضير جسيمات اوكسيد الرصاص النانوية باستخدام الاستئصال بالليزر باستعمال ليزر النديوم-ياك النبضي لهدف من الرصاص مغمور في الماء المقطر اللايوني بطاقة الليزر 400 مللي جول / نبضه ونبضات ليزر مختلفة، تم تشخيص الاواصر الكيميائية لجسيمات اوكسيد الرصاص بواسطة طيف نفاذيه الاشعه تحت الحمراء ، و دراسة طوبوغرافيه السطح والخصائص البصرية بواسطة المجهر الإلكتروني الماسح ومطياف الاشعه فوق البنفسجية-المرئية ، ودراسة تأثير حجم جسيمات اوكسيد الرصاص النانوية على فاعليتها المضاده للبكتريا ضد نوعين من البكتريا ،بكتريا سالبه الغرام (*Escherichia coli*) والبكتريا ايجابية الغرام (*Staphylococcus aureus*) بطريقه الانتشار. أظهرت النتائج الخاصيه مضادة للجراثيم أن النشاط المضاد للبكتيريا لجسيمات اوكسيد الرصاص النانوية يتناسب تناسباً عكسياً مع حجم الجسيمات النانوية في كلا نوعين من البكتريا سالبة الجرام وموجبة الجرام، وكذلك تبين ان البكتريا ايجابية الغرام تمتلك حساسيه اكبر و مقاومه اقل لجسيمات اوكسيد الرصاص النانوية بمقارنه مع البكتريا سالبه الغرام .

الكلمات المفتاحية: الخاصيه المضاده للبكتريا، جسيمات اوكسيد الرصاص النانوية، الاستئصال بالليزر في الماء المقطر اللايوني.