



THE EFFECT OF ROD AND SPHERICAL GOLD NANOPARTICLES IN INHIBITING SOME TYPES OF MICROORGANISMS

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ABSTRACT

In this study, GNPs and GNRs were prepared using the chemical method. The characterization technique used scanning electron microscopy (SEM), UV-visible spectroscopy, and zeta potential XRD (X-ray diffraction). The GNP results were (16.75-26.5) nm, (525) nm, (26.91 -) mV respectively, while the GNR results were (23.24 - 51.96) nm, (528 and 775) nm, and (39.96) mV respectively. The XRD results showed the spectrum at the peaks (38.03°, 45.09°, and 65.62°). The biological activity was also studied against the group of gram-positive and negative bacteria: (*E. coli* ,*Pseudomonas aeruginosa*, *S. typhmuri*um, *Klebsiella pneumoniae* , *Staphylococcus aureus* , *Streptococcus pneumonia* , *Enterococcus faecalis*), for concentrations (150, 100, 50) mg/ml, GNP showed the highest zone inhibition against *Streptococcus* bacteria, which was (12, 18, 24) mm, respectively. The lowest zone inhibition was for *Klebsiella pneumoniae*, where it reached (9, 11, 16) mm, while the inhibitory effect of GNR reached the highest zone inhibition against bacteria *Klebsiella pneumoniae*, where it was (28, 22, 18) mm. The lowest zone of inhibition was against *Staphylococcus aureus* bacteria, amounting to (17.9.8) mm.

Key words: Biological activity, pathogenic bacteria, Gold Nanosphericalm Gold Nano rod.

تأثير جسيمات الذهب النانوية العنصرية والكروية على بعض انواع الاحياء المجهرية

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الخلاصة

في هذه الدراسة تم تحضير جسيمات الذهب النانوية بشكلها الكروي (GNP) والعنصري (GNR) باستخدام الطريقة الكيميائية وأجريت عملية التوصيف باستخدام عدد من التقنيات شملت Scanning electron microscopy (SEM)، UV-visible spectrometer (UV)، zeta potential، X-Ray XRD Diffraction () اذا كانت نتائج GNP (16.75-26.5) nm، (525 nm)، (26.91 -) mV على التوالي في حين أشارت نتائج XRD إلى القمم المميزة عند القمم ° 38.09 و ° 44.49 و ° 64.42 و ° 77.89 اما GNR كانت (23.24 - 51.96) nm، (528 و 775) nm، (39.96) mV على التوالي، ونتائج XRD يظهر الطيف عند القمم (° 38.03 و ° 45.09 و ° 65.62). كما تم دراسة الفعالية البيولوجية اتجاه مجموعة من البكتريا الموجبة والسالبة لصبغة كرام: *Staphylococcus aureus*، *Pseudomonas aeruginosa*، *S. typhmuri*um، *Klebsiella pneumoniae*، *E. coli*، *Streptococcus pneumonia*، *Enterococcus faecalis* للتركيز (150,100,50) mg/ml، أظهر GNP أعلى منطقة تثبيط ضد بكتريا *Streptococcus pneumoniae* حيث كان (12 ، 18 ، 24) ملم على التوالي. أما أقل منطقة تثبيط فكانت للبكتريا *Klebsiella pneumoniae* إذ بلغت (9 ، 11 ، 1) ملم، بينما التأثير التثبيطي



GNR بلغ أعلى منطقة تثبيط ضد بكتريا *Klebsiella pneumoniae* حيث كان (18, 22, 28) ملم, أما أقل منطقة تثبيط فكانت ضد بكتريا *Staphylococcus aureus* إذ بلغت (8, 9, 17) ملم .

الكلمات المفتاحية: النشاط البيولوجي، البكتيريا المسببة للأمراض، الذهب النانوية الكروي، الذهب النانوية العصوي.

INTRODUCTION

Nanoparticles are a general challenge for the present and future of science, as nanoparticles (NP) mostly cover all types of science and manufacturing technologies, and the properties of these particles rise above barriers. Scientific Journal has gone beyond the limits of conventional science which is the reason behind the evaluation of nanoparticles for use in many fields, a long list of scientific fields has been included in the confrontation list. Shortly, we will study the possibility of transforming the traditional roles of science to explore the available fields to use nanoparticles (**Rafique et al, 2020**). Nanomaterials have a very long history, however, there have been significant developments in nanoscience.

Over the past two decades, the idea of nanotechnology has been highlighted for the first time by award-winning scientists. Nobel Prize Richard Feynman in his famous lecture at the California Institute Technology on December 29, 1959. In 1970, Norio Taniguchi first identified the term nanotechnology as “the technology which essentially consists of manipulating, separating, deforming and uniting materials by a single atom or by a single molecule” (**Cai et al, 2018**). Metal nanoparticles, especially silver, and gold, have contributed to many vital activities (**Barath ManiKanth et al., 2010**), as their antimicrobial properties have led to their widespread use in various technologies such as water treatment, toothpaste, shampoo, rinsers, and infant bottle nipples, fabrics, deodorants, filters, kitchen utensils, toys, and moisturizers).

NPs have been used as powerful sterilizers to disinfect and sterilize equipment necessary for the food industry and containers against bacteria that cause foodborne diseases (**Ahmed et al, 2010**). They can act as antibacterial and antifungal agents in foodstuffs, due to their ability to interact with microorganisms as antibacterial through the fusion of these substances on the cell surface cause changes in the structure of the cells and disruption of their vital functions, such as permeability and disruption of the activity of respiratory chain enzymes, leading to cell death. Some studies have shown the antibacterial effect of niacin loaded onto NP against germs. The results showed that niacin loaded onto NP was 95-90% effective compared to the effect of niacin alone (**Maryam et al., 2010**). During the first years of the beginning of this century, some companies conducted a number of scientific research on NP of silver metal to determine the extent of its possibility of employing it in the field of infection resistance and killing different types of harmful bacteria and viruses. The results indicated that NP of silver metal has tremendous effects on killing multiple types of harmful bacteria. Viruses and germs, this is due to the reduction of atoms to diameters of less than 5 nanometers. Decreasing the diameters of atoms and increasing the surface area leads to a significant increase in their chemical activity, as well as an increase in their interaction with atmospheric oxygen. As a result, toxic silver ions are formed that are responsible for killing germs and viruses. One of the Korean companies specializing in the manufacture of electrical and electronic devices has monopolized the manufacture of home refrigerators covered from



the inside with a thin layer of silver metal, with the aim of killing bacteria and germs that may exist in order to protect preserved foods from contamination bacterial. (Selvaraj & Alagar, 2017). The aim of our study of this research paper is to inhibit some microorganisms by using rod and spherical gold nanoparticles and compare them with each other.

MATERIALS AND METHODS

-Preparation of GNP and GNR by chemical method:

Preparation and testing of gold nanoparticles GNP, GNR was carried out at the Technological University / Nano Research Center (But SEM examination in Iran).

-Prepare GNP:

Gold nanoparticles were prepared from the reduction of HAuCl_4 (1 mM) by sodium citrate (1%). 10 mL of HAuCl_4 was heated to a boil, and then 1 mL of sodium citrate solution was added. We continue stirring until the color changes from yellow to cherry red, then we cool it to room temperature (Shamkhi & Odeh, 2022).

-Prepare GNR:

GNR was prepared with some modifications (Wei et al, 2021):

Mixture A :(seed solution): Prepared by mixing 5 ml of 0.5 mM HAuCl_4 with 5 ml of 0.008 M CTAB solution in a 20 ml beaker. Then 0.6 ml of 0.01 M NaBH_4 diluted in 1 ml with ion-free distilled water was added and mixed. Mix the mixture well with a magnetic stirrer (1200 rpm) until the solution changes color to yellow-brown. Stirring is stopped, then the mixture is stored at room temperature.

Mixture B: (growth solution): - It consists of mixing 7.0 g (0.037 M) of CTAB with 1.234 g of NaOL dissolved in 250 ml of warm water ($\sim 50^\circ\text{C}$) in a 1-liter beaker and leaving the mixture until it reaches temperature. Room temperature, then add 18 ml of AgNO_3 solution (4 mM), keep the mixture at room temperature for 15 minutes, then mix with it 250 ml of HAuCl_4 solution (mM1), after stirring (700 rpm) for 90 minutes until the solution becomes Colorless. Then adjust the pH to 1.65 by adding HCl after another 15 minutes of slow stirring (400 rpm). Then mix 0.4 ml of mixture A with mixture B for 30 seconds and leave at room temperature for 12 hours for NR growth. NR was isolated by centrifugation (7000 rpm) for 30 minutes, the supernatant was removed, and then the washing process was performed twice using non-ionic distilled water by repeating the centrifugation.

Diagnosis and characterization of GNP and GNR:

The characterization process was carried out using a (UV-visible spectrometer), (SEM), (XRD), zeta potential.

Bacteria used in the study: A group of bacteria was used *E. coli*, *Pseudomonas aeruginosa*, *S. typhmuri*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Enterococcus faecalis*.

Inhibitory effect of GNP and GNR:

A bacterial suspension of each type of bacteria used in the study was taken, containing 18×10^8 CFU/ml, according to McFarland. The surface of Mueller Hinton agar was planted with the prepared bacterial suspension by taking 50 microliters and spreading with a cotton swab. After that, holes were made Adding 20 microliters to the drill from the GNP solution of three

concentrations as well as adding 20 microliters of the GNR solution of three concentrations to the prepared bacteria separately (50, 100, 150) microliters/g. The plates were incubated in the incubator at a temperature of 37°C for 24 hours, and the results were recorded (**Harley & Prescott, 2002**).

RESULTS AND DISCUSSION

-Composition of GNP and GNR

The results shown in Figure (1) showed that the color changed from yellow to cherry red. As for GNR, the color changed from yellow to yellow-brown, as the seed solution was in the form of (2) (A). After that, a colorless growth solution (B) was prepared. The two solutions were stirred and left for 12 hours without stirring to obtain a pink color, evidence of the arrival of the gold nanoparticles (GNR) (C).



Figure (1) GNP



A



B



C

Figure (2): a- Preparation of the growth solution of GNR b- Preparation of the seed c - Preparation of GNR.

Solution of colorless GNR

The color change is attributed to surface plasmon resonance, which occurs in metals such as gold nanoparticles as a result of its particles reaching the magnification scale, and the color change is first evidence of the spherical difference value of gold nanoparticles (AL-Hadedee *et al*, 2019).

The electronic spectrum of the gold nano GNP showed a broad peak at 525 nm, Figure (3), which indicates the formation of the GNP. This result is consistent with (Al Dabbagh, 2022).

Since the absorption band is related to the shape and size of metal nanoparticles (Phillips *et al*, 2008), it affects the surface plasmon resonance, which is between 520 nm to 570 nm (Huang & El-Sayed, 2010). While the electronic spectrum of the gold nanorods (GNR) showed two regions of surface plasmon resonance: transverse and longitudinal at (528 and 775) nm, Figure (4), which indicates the formation of GNR.

The transverse surface plasmon resonance, resulting from electronic oscillation across the width of the rod, peaks at about 528 nm and is relatively weak. While the longitudinal mode leads to a much larger peak, this is due to the electrons oscillating in the longitudinal direction of the rod.

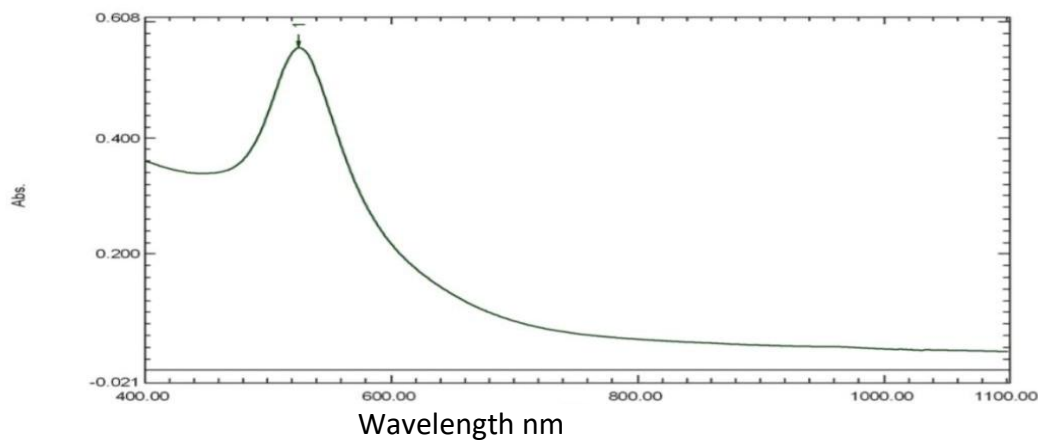


Figure (3): UV-Vis spectrum of GNP.

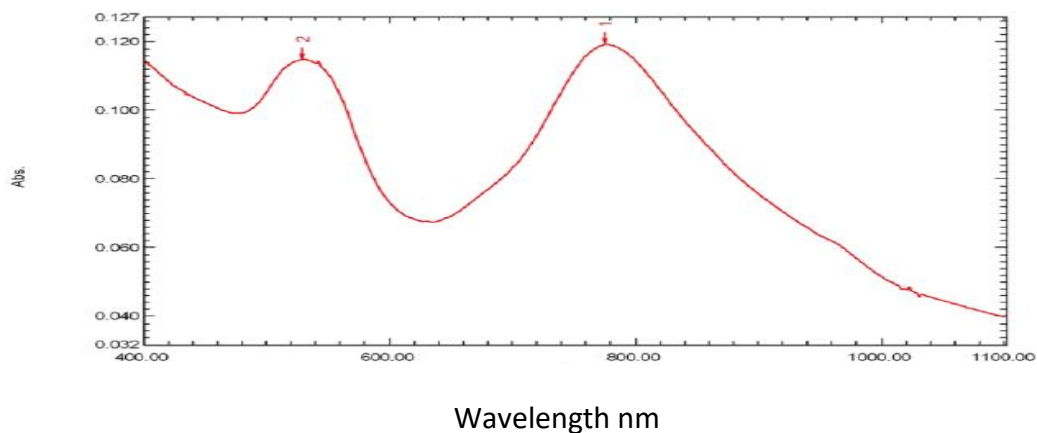


Figure (4): UV-Vis spectrum of GNR.

–Scanning Electron Microscopy

The SEM image of the nanoparticles showed a spherical shape with an almost homogeneous distribution, and sizes ranged between (16.75-26.5) nm, and the average was 21) nm, as shown in Figure (5).

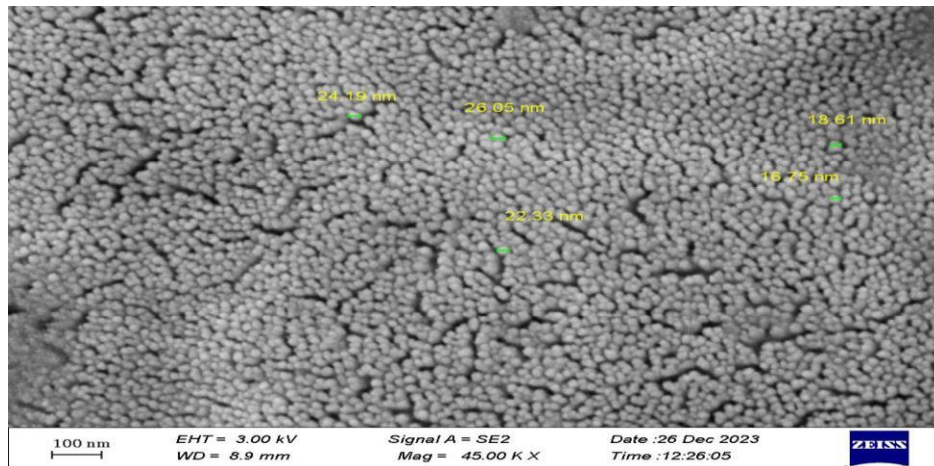


Figure (5): SEM of (GNPs).

In Figure (6): SEM images showed a rod-like shape for the gold nanoparticles, which were formed with a diameter ranging between (23.24 -51.96) nm and an average of (37) nm.

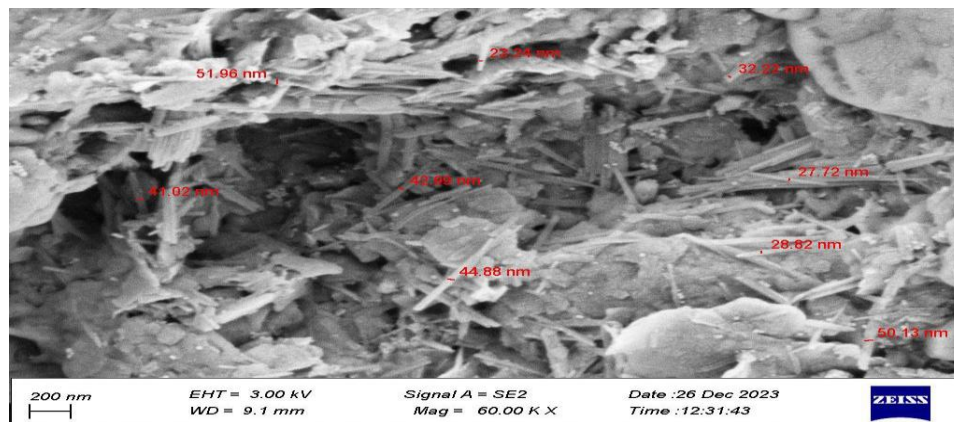


Figure (6): SEM of (GNRs).

X-Ray Diffraction (XRD)

Detecting the crystalline nature of the gold nanoparticle solution using X-ray diffraction (XRD). The results showed gold nanoparticles according to Figure (7), the characteristic peaks of the 2θ spectrum are at peaks 38.09° , 44.49° , 64.42° , and 77.89° . The results were consistent with a previous study (Syed *et al*, 2023).



It did not appear an additional peak in the diffraction peaks indicates that the synthesized GNP were of high purity without any contamination, and represents the XRD pattern of the spherical gold nanoparticles. It is manufactured by chemical reduction after complete reduction of Au^{3+} to Au^0 .

The average crystallite size of the gold nanoparticles obtained was according to the Scherrer's equation calculated one is an equation used to calculate the size of nanoparticles when knowing the angle of incidence and the peak width of one of the peaks of the diffraction pattern using the width at 2θ of the (510) peak of 38.09° (14 nm), the peak of 44.49° (460) is (11 nm), and the peak of 64.42° (378) is (17 nm) and the peak of 77.89° (271) is (17 nm). At a rate of 15 nanoparticles, according to Table (1). These results are consistent with (Qaddoori & Shamgani, 2022).

Table (1): XRD data.

Pos. [$^\circ 2\text{Th.}$]	Height [cts]	FWHM Left [$^\circ 2\text{Th.}$]	Rel. Int. [%]	Width [$^\circ 2\text{Th.}$]	Size nm
38.09	510	0.59	100	0.23	14
44.49	460	0.37	21	0.20	11
64.42	378	0.54	14.1	0.14	17
77.89	271	0.58	14.9	0.12	17

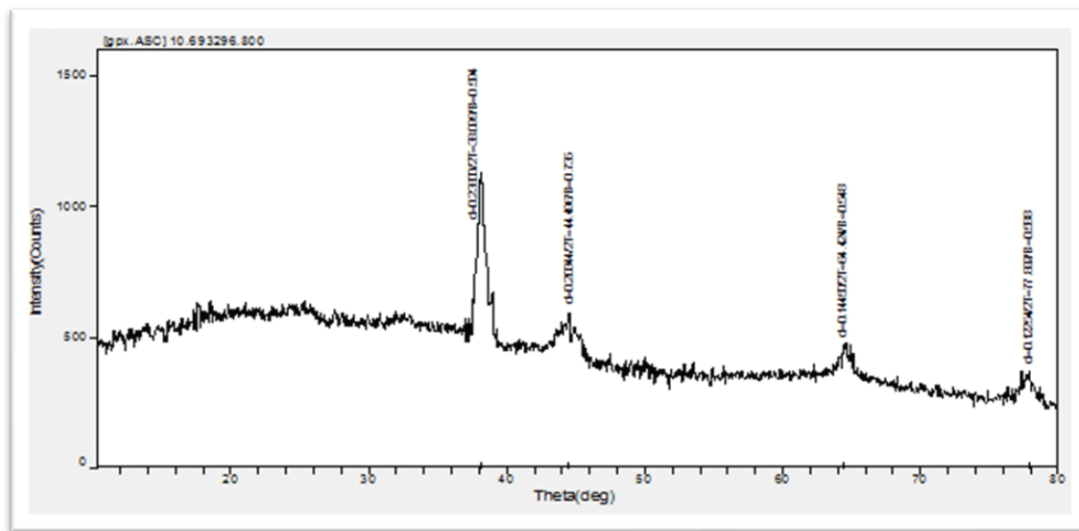


Figure (7): XRD of GNP.



The results of X-ray diffraction (GNR), it has atomic levels at diffraction angles (38.03, 45.09, and 65.62). According to the calculated Scherer equation, the size of the nanoparticles at 2 θ showed that the peak of 38.03° (422) is 18 nm, the peak of 45.09° (324) is 25 nm, and the peak of 65.62° (250) is 54 nm. With an average size of 32 nanometers for the rod nanoparticles, according to Table (2-4), which is consistent with (Qaddoori & Shamgani , 2022).

Table (2): XRD data.

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	Rel. Int. [%]	Tip Width [°2Th.]	Size nm
38.03	422	0.64	100	0.23	18
45.09	324	0.35	32	0.20	25
65.62	250	0.20	5.9	0.14	54

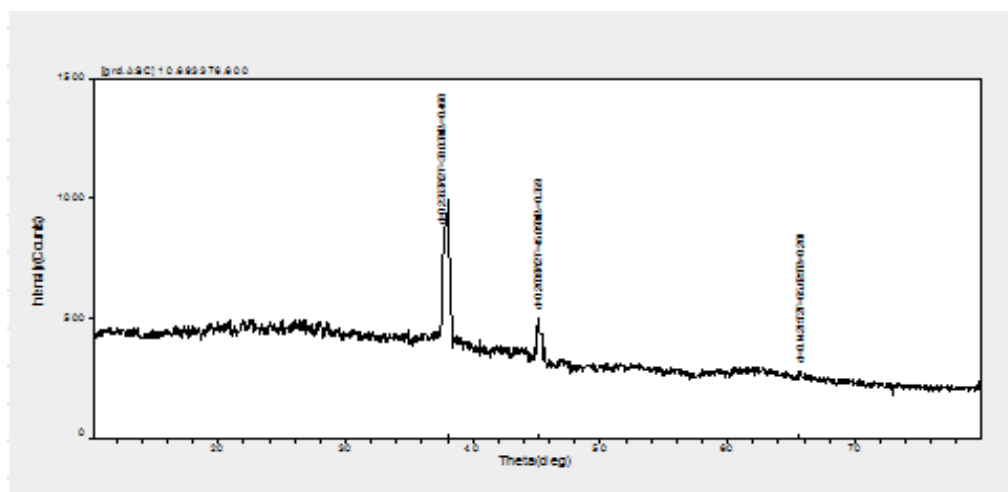


Figure (8): XRD of GNR.

-Zeta potential of GNP and GNR:

The zeta potential of the prepared GNP reached -26.91 mV, and this confirms that the negative charge is based on the chemical substance used as a reducing agent and stabilizing agent, and that repulsive forces between atoms are generated due to the negative charge (Fornaguera & Solans, 2018). If the value of the surface charge is less than- 25 They will clump due to the lack of repulsive force between the gold nanoparticles (AL-Hadedee *et al*, 2020), and the negative ions help to stabilize them because they cover the surface of the GNP, which is stabilized by the forces of electrostatic repulsion (Faried, *e t al.*, 2015), Figure (9).

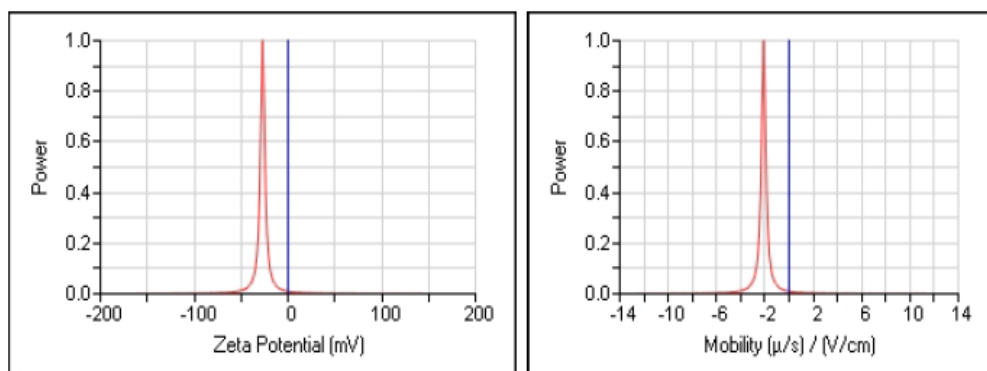


Figure (9): Zeta potential of GNP.

Zeta potential of GNR was positively charged (39.96) mV as a result of the preparation method and the chemicals involved in the reaction, and it is stable as a result of the repulsion occurring between the nanoparticles (Ann *et al*, 2017). Figure (10)

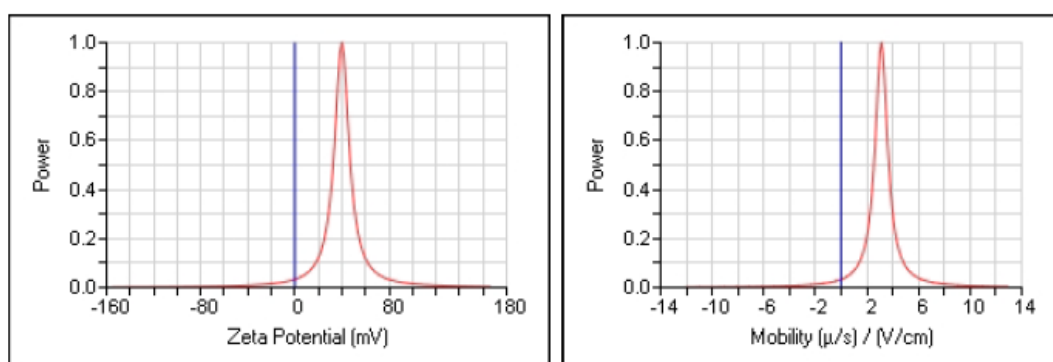


Figure (10): Zeta potential of GNR.

-Inhibitory concentration for GNP and GNR:

The results in Table (3) showed the highest zone of inhibition for spherical gold nanoparticles against positive bacteria. For *Streptococcus pneumoniae* gram stain, the concentrations (50, 100, 150) mg/ml were (12, 18, 24) mM, respectively. The smallest zone of inhibition was for the gram-positive bacteria *Klebsiella pneumoniae*, where it reached (9, 11, 16) mm, respectively. The difference in inhibition is due to the difference between the gram-negative and gram-positive bacteria, and this is consistent with what was found by from what has been studied, the positive bacteria damaged by food when exposed to spherical gold nanoparticles have been exterminated the cell wall of bacteria due to the negatively charged nanoparticles as well as the concentration of the nanomaterial led to the programmed death of bacterial cells (Ahmad *et al*, 2015).

Researchers found that GNP has an inhibitory effect on microorganisms by damaging the cell membrane in both Gram-negative and Gram-positive bacteria, causing the leakage of



cell components and their death. Its effect on negative bacteria was less than positive bacteria because the gold nanoparticles interact strongly with the lysine present on the membrane. The bacterial infection is caused by Gram-positive bacteria (Li *et al*, 2021), where pores appear in the bacterial membrane and cause bacterial death. In addition, it causes a decrease in adenosine triphosphate (ATP) levels, which leads to a decrease in metabolism (Cui *e tal*, 2012). Gold nanoparticles also work to enhance the photocatalytic activity of oxides and the production of peroxides, hydroxyl groups, and high concentrations of oxygen. (Xu *et al*, 2019). Which ultimately causes the bacteria to collapse.

Also, the reason for the inhibition is that the bacterial cells and GNP have an opposite charge, between them, which leads to a disturbance in the cell membrane and then its death (Bahjat *et al*, 2021).

Table (3): Inhibitory effect of GNP against types of Gram-positive and negative bacteria.

Bacteria		Inhibition zone (mm)		
		mg/ml 50	mg/ml 100	mg/ml 150
G-	<i>E .coli</i>	11	13	15
	<i>Pseudomonas aeruginosa</i>	0	15	21
	<i>S. typhmurium</i>	0	16	21
	<i>Klebsiella pneumoniae</i>	8	17	20
G+	<i>Staphylococcus aureus</i>	12	18	24
	<i>Streptococcus pneumoniae</i>	9	17	19
	<i>Enterococcus faecalis</i>	9	17	22

The results in Table (4) showed highest diameter of inhibition of GNR against gram-negative bacteria *Klebsiella pneumoniae* for concentrations (50, 100, 150) ml /mg, where it was (28, 22, 18) mm. As for the least diameter of inhibition, it was against the gram-positive bacteria *Staphylococcus auras*. The concentrations (50, 100, 150)ml/mg were (17.9.8) mm. The difference in diameter is due to the difference between the gram-negative and gram-positive bacteria, and this is consistent with what was found by (Ahmad *et al*, 2015). GNR has a different inhibitory effect on microorganisms, gold nanoparticles may work to change the ionic interaction in the bacterial cell membrane. The mechanism by which the gold nanoparticles interact with bacterial cells is that the bacterial cell carries a negative charge, while the nanoparticle metal oxides carry a positive charge, which creates an electromagnetic attraction between the bacteria and the surfaces of the particles. The minute's release ions that interact with the thio group of proteins transporting nutrients that protrude in the bacterial cell membrane and reduce the permeability of the membrane, leading to the death of the bacterial cell (Tauran *et al*, 2013). The mechanism of GNP inhibiting the ability of DNA to multiply the gene expression of proteins, as well as various cellular proteins and enzymes necessary in the production of the energy compound ATP, so they become ineffective, leading to the death



of the bacterial cell (Wang *et al*, 2017). It was also noted in the same table that the effect of GNR on the types of bacteria used in the study differed, and this may be due to the charge of GNR and the amount of surface charge of the bacteria (Lee-Anne *et al*, 2011). Also, the morphology of nanoparticles, such as their shape, surface charge, and total surface area of particles and ions, can explain many reasons for varying bacterial inhibition (panel Parijat *et al*, 2022)

Table (4): Inhibitory effect of GNR against types of Gram-positive and negative bacteria.

Bacteria		Inhibition zone (mm)		
		mg/ml 50	mg/ml 100	mg/ml 150
G-	<i>E .coli</i>	15	17	19
	<i>Pseudomonas aeruginosa</i>	12	18	23
	<i>S. typhmuri</i>	0	10	20
	<i>Klebsiella pneumoniae</i>	18	22	28
G+	<i>Staphylococcus aureus</i>	8	9	17
	<i>Streptococcus pneumoniae</i>	13	15	18
	<i>Enterococcus faecalis</i>	10	18	25

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