Iran. J. Ichthyol. (ICAEAS special issue 2021): 290-299

P-ISSN: 2383-1561; E-ISSN: 2383-0964
http://www.ijichthyol.org

Research Article

Preparation of Nanogold and binding of nisin to increase its effectiveness in inhibiting the positive and negative bacteria

Lamees Thamer AL-HADEDEE[1*], Hussein SAADOUN[2], Wurood F. AL-MASHHADANI[1]
[1]*Department of Food Science, College of Agriculture Engineering Science, University of Baghdad, Iraq.*
[2]*Department of Physics, College of Science, University of Misan, Maysan, Iraq.*
[*]*Email:* lames.thamer@coagri.uobaghdad.edu.iq

Abstract: Most pathogenic bacteria have the acquisition of antibiotic resistance. Therefore, continuous research to find a new antibiotic against pathogens is an urgent task to avoid the rapid spreading of diseases. The direction is to create nanomaterials instead of common antibiotics to inhibition some bacteria high effectively. The purpose of this study was to functionalize the gold nanoparticles (AuNPs) with nisin (N) and applied to a group of pathogenic bacteria to evaluate the effectiveness of them in killing the isolated bacteria, including *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Bacillus subtilis, Staphylococcus aureus*. The TEM study revealed the grain size of AuNPs ranging 1-14nm, whereas the grain size of AuNPs-N ranging 1-120nm. Also, we were measured the XRD of AuNPs and AuNPs-N to find out their structural properties. In addition to the evaluation of biologically active substances by FTIR system. The results through the biological activity showed that the nisin functionalized gold nanoparticles (AuNPs-N) exhibited perfect inhibitory activity against all the isolated bacteria.

Keywords: CMV, Leucopenia, Cancer patients, ELlSA, IgM, IgG.

Citation: Al-Hadedee, L.T.; Saadoun, H. & Al-Mashhadani, W.F. 2021. Preparation of nanogold and binding of nisin to increase its effectiveness in inhibiting the positive and negative bacteria. Iranian Journal of Ichthyology 8(ICAEAS 2021): 290-299.

Introduction

Lantibiotics are class peptide antibiotics that produced by a large numbers of Gram-positive bacteria such as *Streptococcus* and *Streptomyces* which are distinguished by the presence of several unusual amino acids. Due to their activity against germ pathogenic bacteria, they have been highlighted and significant progress in lantibiotics structure, function analysis, and regulation of biosynthesis (AL-Hadedee et al. 2019). The difference in the structure, and action patterns of antibiotics led to the diagnosis of two types of peptides, the first being A and the second B. Type A lantibiotic is a flexible peptide chain with a net positive charge, while, type B lantibiotics are rigid spherical molecules that carry either no net charge or a net negative charge, therefore, the prototype type A lantibiotic is nisin (Additives et al. 2017).

The nisin consist of 34 amino acids and make via fermentation by *Lactococcus lactis* subsp. *Lactis* (Al-Hadede & Hassan 2020). It is known that the first peptide that forms pores is nisin, so it has been used for a specific purpose as a lipid, due to its activity on bacterial, as nisin binds to lipids and creates pores in the membrane which leads to the breakdown of vital ionic gradients (Bhat & Vidya 2017). In many cases, at bacteria are resistant to antibiotics, therefore, new methods have appeared using nanoparticles as effective materials against a wide range of bacteria, and gold particles are one of the most important nanomaterial used in this aspect (Saha et al. 2007). AuNPs has been found to be an effective substance

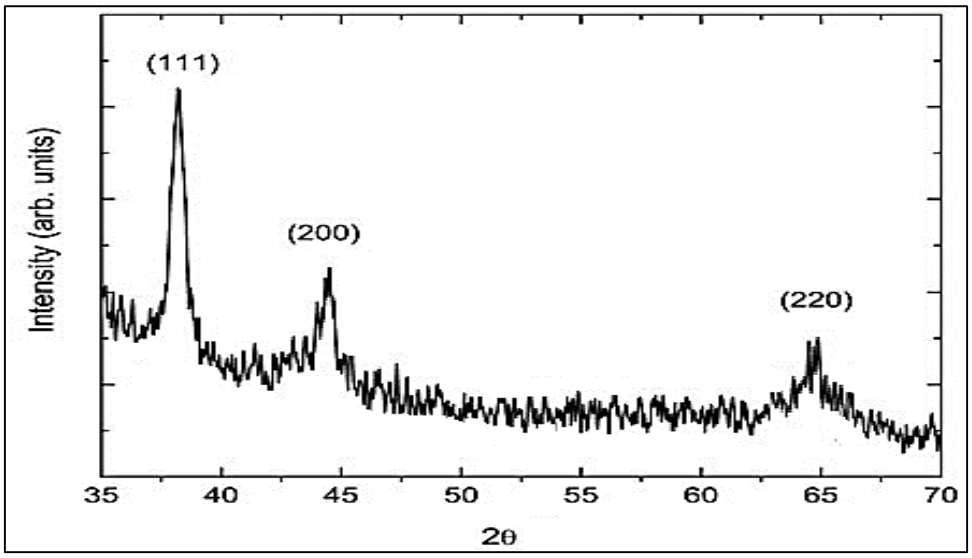

Fig.1. X-Ray diffraction pattern of AuNPs.

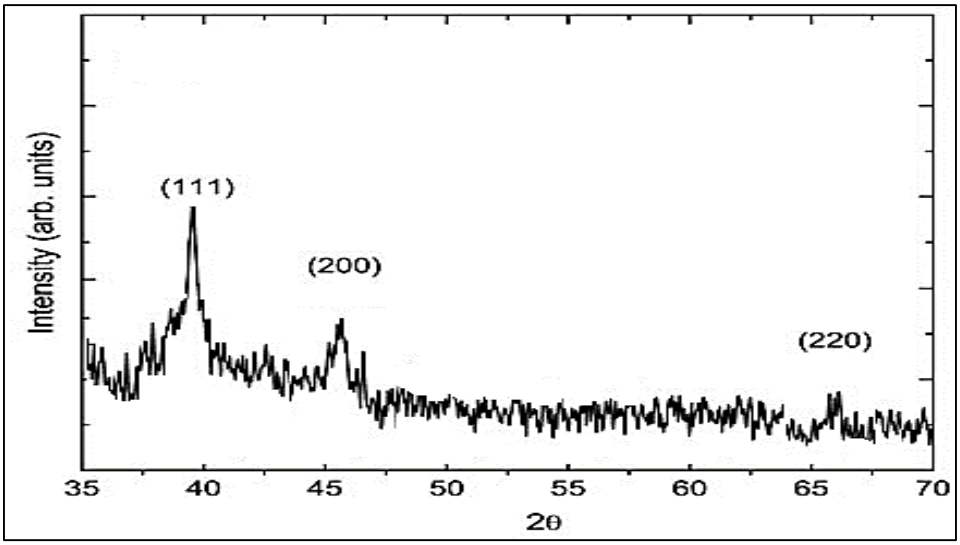

Fig.2. X-Ray diffraction pattern of Nisin-AuNPs.

to solve a multitude of pharmacological issues of cytotoxicity and antibiotic resistance (Sau & Murphy 2004). In this study, the well-known antimicrobial nisin peptide was used with golden nanoparticles to improve the potential of killing towards a wide range of bacteria.

Materials and Methods

Nisin product: The nisin was obtained from a subsidiary of Clerici-Sacco Group/Italian origin.

Collection of bacteria: The isolated bacteria (*E. coli, Pseudomonas aeruginosa, Bacillus subtilis, Salmonella typhimurium* and *Staphylococcus aureus*) were obtained from the University of Baghdad / Market Research Center and consumer protection.

AuNPs preparation: By the chemical reduction method, the gold nanospheres were constructed as: Preheat 50mL of 0.01% of wt. chloric acid solution ($HAuCl_4.3H_2O$) up to a boiling point, whereas, stirring in a 100mL flask. After that, a hundred microliters (μL) of 1 wt% sodium citrate dehydrate solution ($Na_3C_6H_5O_7.2H_2O$) was rapidly added to the ureic solution. The color of the solution changed during a few minutes, from yellow to black, and then

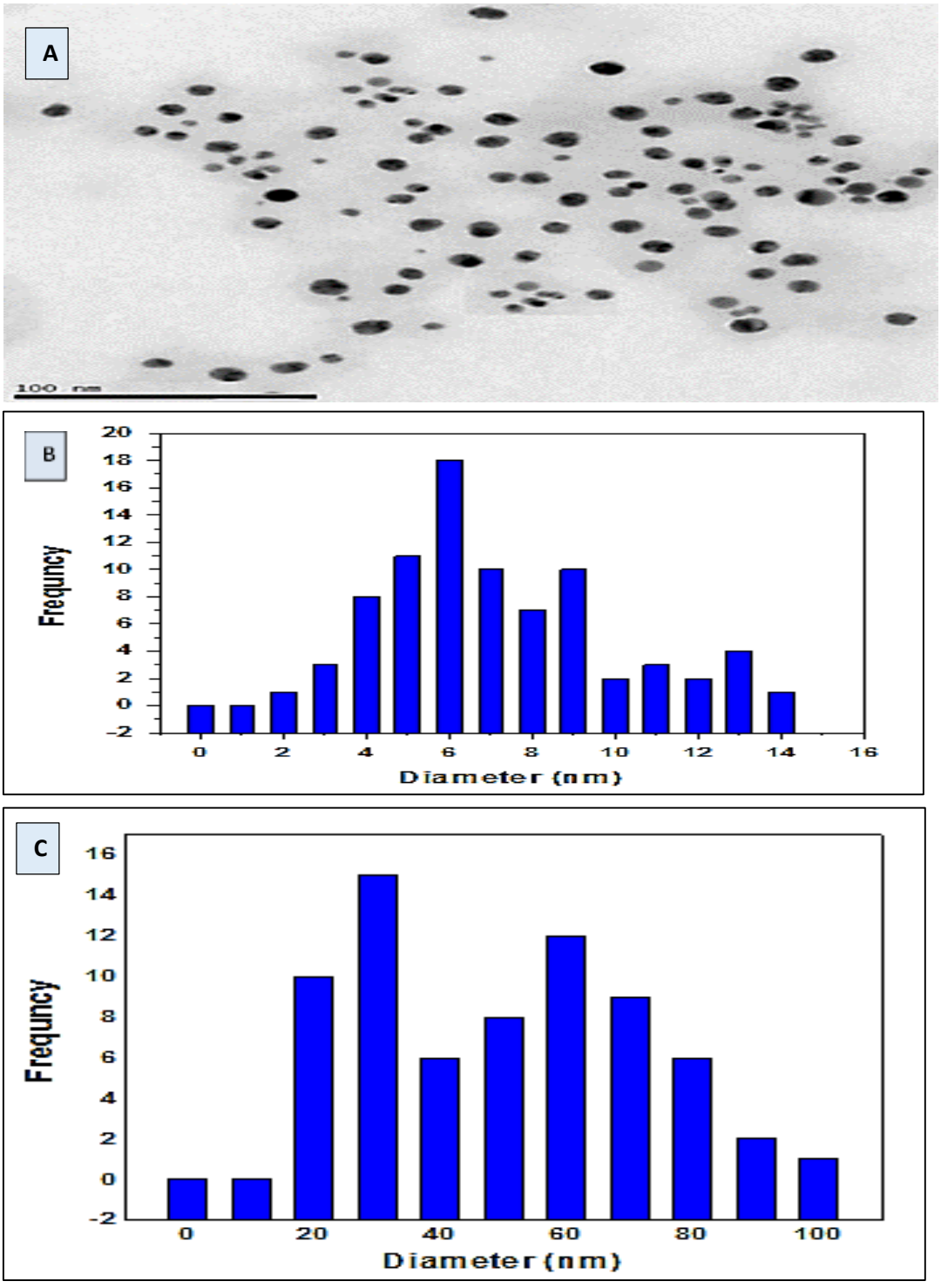

Fig.3. TEM of AuNPs: (A) AuNPs grain, (B) length of AuNPs and (C) area of AuNPs.

to red or purple that depending on the size of the nanoparticles (Zhang et al. 2020).

Conjugation of AuNPs with nisin (AuNPs-N): Gold nanoparticles were used for preparing different conjugates. Before combine, the PH of synthesized AuNPs solution was set at 7.4 using Na_2CO_3 (10mg/ml deionized water). 200µl of nisin (1mg/ml) and 100µl of doxorubicin (4mg/10ml) solution was prepared in phosphate buffer (10mM, pH=7.4), and then this solution was added dropwise to 1ml and

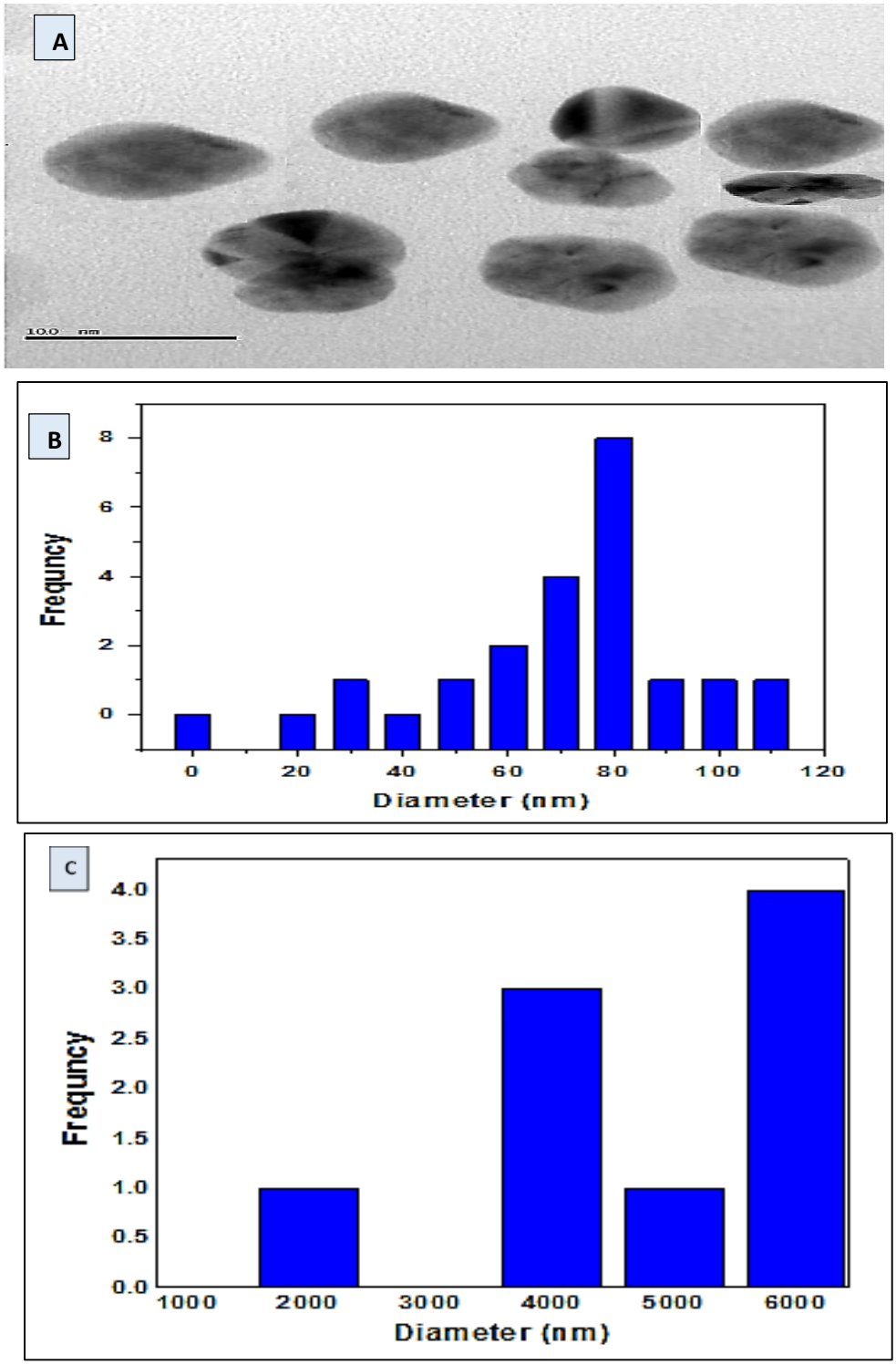

Fig.4. TEM of AuNPs: (A) AuNPs grain, (B) length of AuNPs and (C) area of AuNPs.

10ml of colloidal AuNPs solution respectively under mild stirring conditions. The mixture was incubated at 4°C in overnight, after that centrifuged for 30 in at 12000rpm to remove unconjugated nisin and doxorubicin (Dox) from the solution to obtain nisin-AuNPs and Dox-AuNPs. The pellet was suspended in phosphate buffer (10mM, pH=7.2) and stored at 4°C for later uses. For nisin-Dox-AuNPs synthesis,

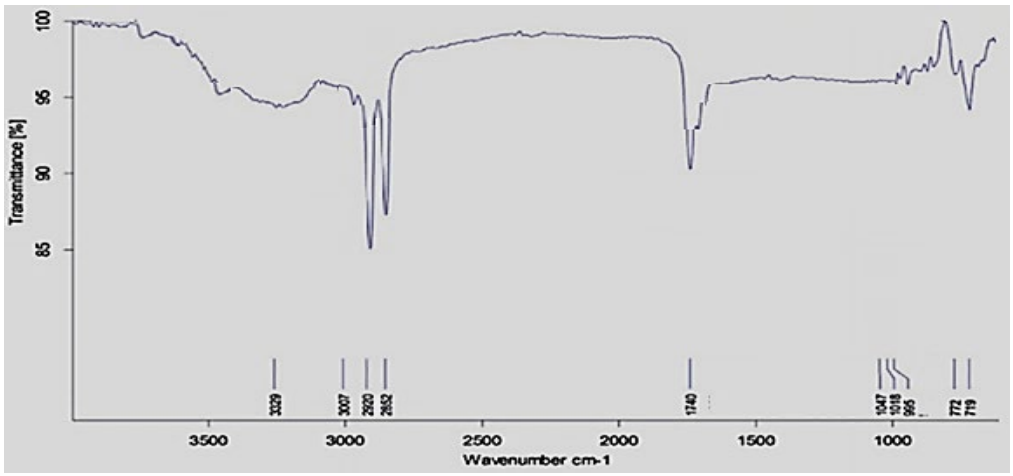

Fig.5. FTIR of Nisin.

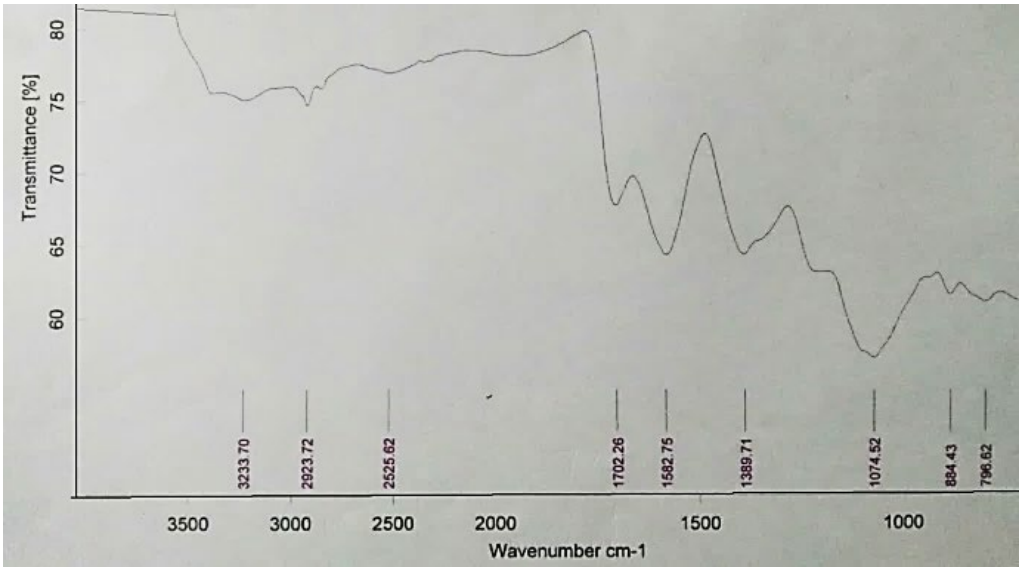

Fig.6. FTIR of AuNPs.

nisin (1mg/ml) was connected to doxorubicin (40µg/100µl) using EDC/s-NHS coupling reactions following a previously protocol published by the Protein Man, G-Biosciences using hydroxylamine as the linker. Thereafter, nisin-linker-doxorubicin was conjugated to AuNPs (200g) in 10 l AuNPs solution prepared in phosphate buffer, 10M, (pH=7.4) in overnight incubating at 4°C (Caswell & Spiro 1987).

Results

XRD of AuNPs: The XRD pattern was implemented to characterize the phase and purity of the as synthesized final product (Fig. 1). The GHNSs exhibited relatively strong diffraction peaks at 37.82, 64.40, 44.12 and 77.34º which were corresponded to four typical face-centered cubic (fcc) diffraction peaks i.e. 111, 200, 220, and 311 planes of the fcc lattice of Au, respectively (Wang et al. 2012). The peak corresponding to the 111 plane is much sharper and stronger compared with others. The ratio between the intensities of the 200 and 111 peaks is 0.31, which is much lower than the conventional value of 0.52 (Jena & Raj 2007), revealing the predominant orientation of the 111 planes. This has been proved by the formation of metal multipods, which is associated with the competitive growth

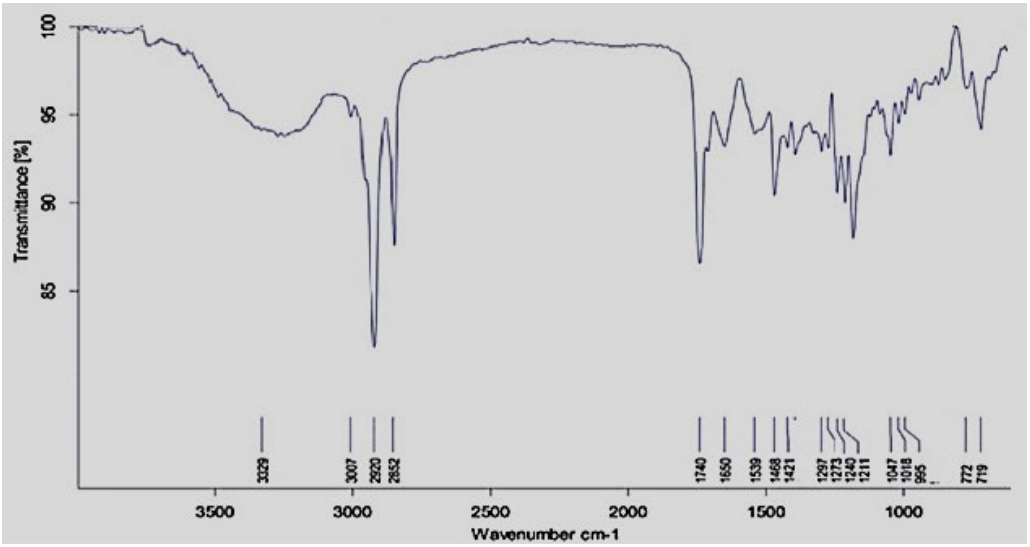

Fig.7. FTIR Nisin-AuNPs.

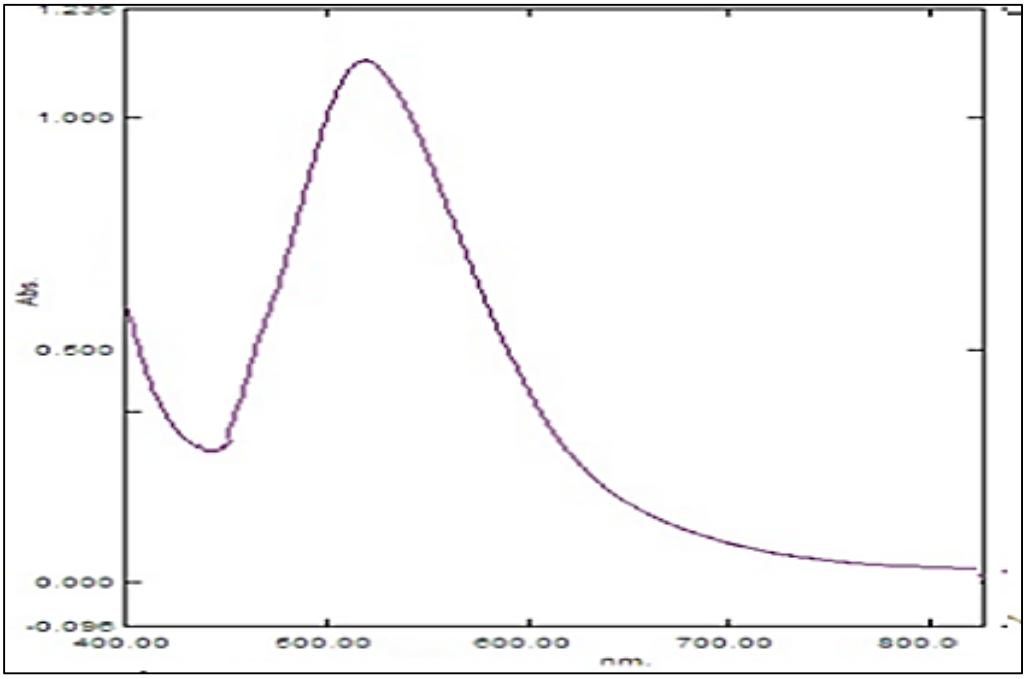

Fig.8. UV-Vis Spectroscopy of AuNPs.

between the 111 and 200 planes (Wu et al. 2006). Characteristic diffraction patterns of the fcc lattice planes of metallic Au 0 were observed for GHNSs (Xu et al. 2008).

XRD of nisin-AuNPs: The grain (length) of gold was 6 nanometers, which is the highest frequency equal to 18, as well as the distance between 20-80 nanometers which was the highest frequency and is equal to 16 (Fig. 2).

TEM of AuNPs: Length and area of the gold nanoparticles were calculated by the image program (Fig. 3).

TEM of nisin-AuNPs: Length and area of the gold conjugated with nisin were calculated by the imaging program (Fig. 4). The results showed that the size of the grain (length) of gold and nisin is 80 nanometers with the highest frequency equal to 8, and that the area of the gold nanoparticles with nisin is 6000

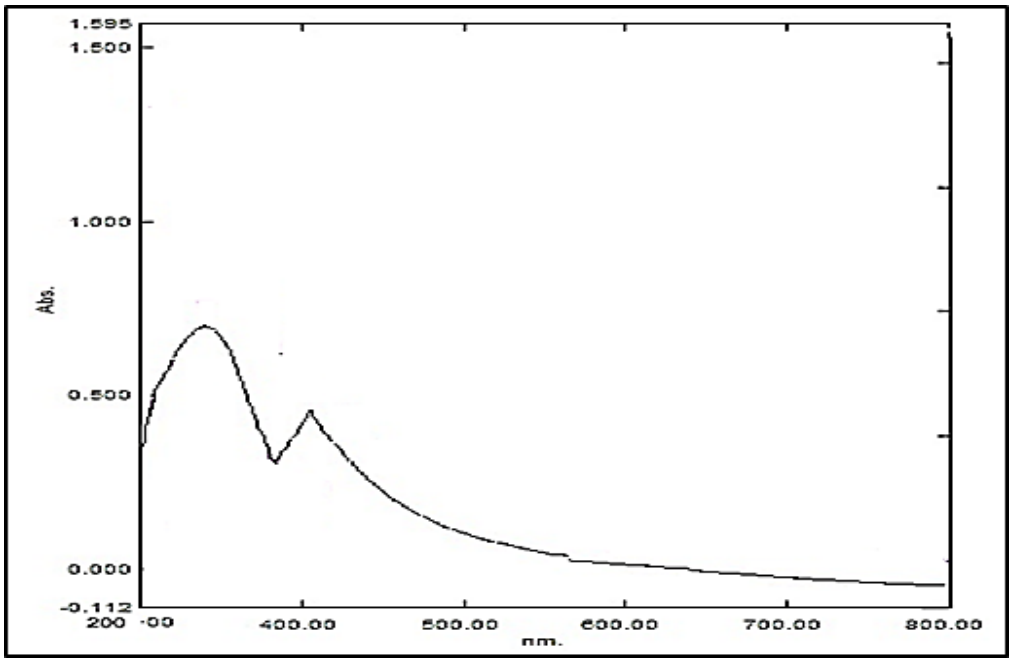

Fig.9. UV-Vis Spectroscopy of nisin.

Table 1. Effect of nisin (N) on growth of microorganisms.

Bacterial		Inhibition Zone (mm)	
		AuNPs	Nisin-AuNPs
G-	*E .coli*	10	21
	Pseudomonas aeruginosa	9	20
	Salmonella typhimurium	5	13
G+	*Bacillus subtilis*	9	15
	Staphylococcus aureus	8	19

Table 2. Effect of AuNPs and Nisin-AuNPs on growth of microorganisms.

Bacterial		Inhibition Zone (mm)			
		Nisin (25 mg/ml)	Nisin (50 mg/ml)	Nisin (75 mg/ml)	Nisin (100 mg/ml)
G-	*E .coli*	11	16	18	24
	Pseudomonas aeruginosa	15	18	21	27
	Salmonella typhimurium	-	-	15	20
G+	*Bacillus subtilis*	-	-	-	-
	Staphylococcus aureus	14	16	20	25

nanometers at the highest frequency is 4.

FTIR of nisin: FTIR spectrum of nisin showed hydroxyl group at site 3029cm^{-1} (Fig. 5). Signal appeared aromatic ring at site 3007cm^{-1}. Signal showed aliphatic group at site 2920cm^{-1}, and an aldehyde hydrogen atom at the site 285cm^{-1}. Also it showed a sharp beam at site 1740cm^{-1}. This indicates due to presence of carbonyl group, and the spectrum gave a clear beam at 1650cm^{-1} due to the aromatic double bundle.

FTIR of AuNPs: FTIR spectrum of AuNPs showed hydroxyl group at site 3223cm^{-1} and aliphatic group at site 2923cm^{-1} (Fig. 6). Signal showed carbonyl group at site 1702cm^{-1} and appeared beam at sit

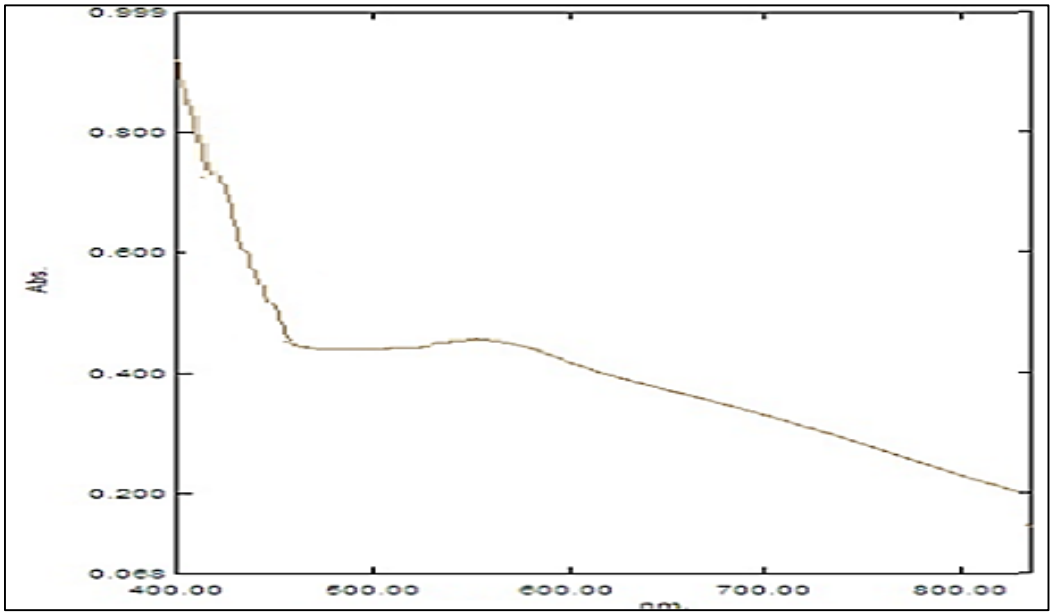

Fig.10. UV-Vis Spectroscopy of Nisin-AuNPs.

1582cm^{-1} due to presence of double bundle related to the aromatic ring which interaction with hydroxyl bundle at 3000cm^{-1}.

FTIR AuNPs-N: FTIR spectra of nisin and AuNPs showed presence of a broad band belonging to the hydroxyl group at 3324 cm^{-1} (Fig. 7) as well as aromatic ring at site 3007cm^{-1}. Signal appeared aliphatic group at site 2920cm^{-1}. Presence bundle at 2852cm^{-1} due to hydrogen atom which is related to aldehyde group. The signal at 1740cm^{-1} showed carbonyl group.

UV-Vis spectroscopy of AuNPs: The results of UV-Vis absorption spectra showed an individual absorption peak in the visible region for the colloidal AuNPs solutions which appeared at 523-529nm. Also, UV-Vis Spectroscopy results showed an increase in intensity of the absorption peaks and the concentrations of the materials (Fig. 8).

UV-Vis spectroscopy of nisin: The results of UV-Vis absorbance showed that the peak of nisin was at 235nm. The UV–vis absorbance spectra of nisin nanoparticles shifted toward a higher wavelength with a maximum peak at 315nm (Fig. 9).

UV-Vis spectroscopy of AuNPs-N: UV visible spectra of AuNPs-N were registered in UV spectrophotometer at 600nm scale (UV-3600, UV-VIS-NIRspectrophotometer, and Shimadzu) (Fig. 10).

Biological activity: Table 1 represents the biological activity of nisin when used alone at concentrations of 25, 50, 75 and 100µg/µl in inhibiting the growth of bacteria and Table 2 showed the biological activity of AuNPs separately and in another case when combine with nisin.

The inhibitory activity when combined the nisin with nanoparticles (AuNPs- Nisin) gave a high inhibition rate for microorganisms which reached 21, 20, 13, 15 and 19 (Table 2). These results were higher than the inhibitory activity when using nisin (N) and gold nanoparticles separatelya (Table 1), because the combination of (AuNPs-Nisin) increased the compounds concentration and active groups that increased the diameter inhibition of pathogenic bacteria.

Discussion

During the past years, AuNPs has been widely used in many electronic, chemical and biological fields (Zhang et al. 2020). Among them, gold nanoparticles are one of the most important as an inhibitor for a several of pathogenic bacteria. It is also found that AuNPs could form excellent compounds because of

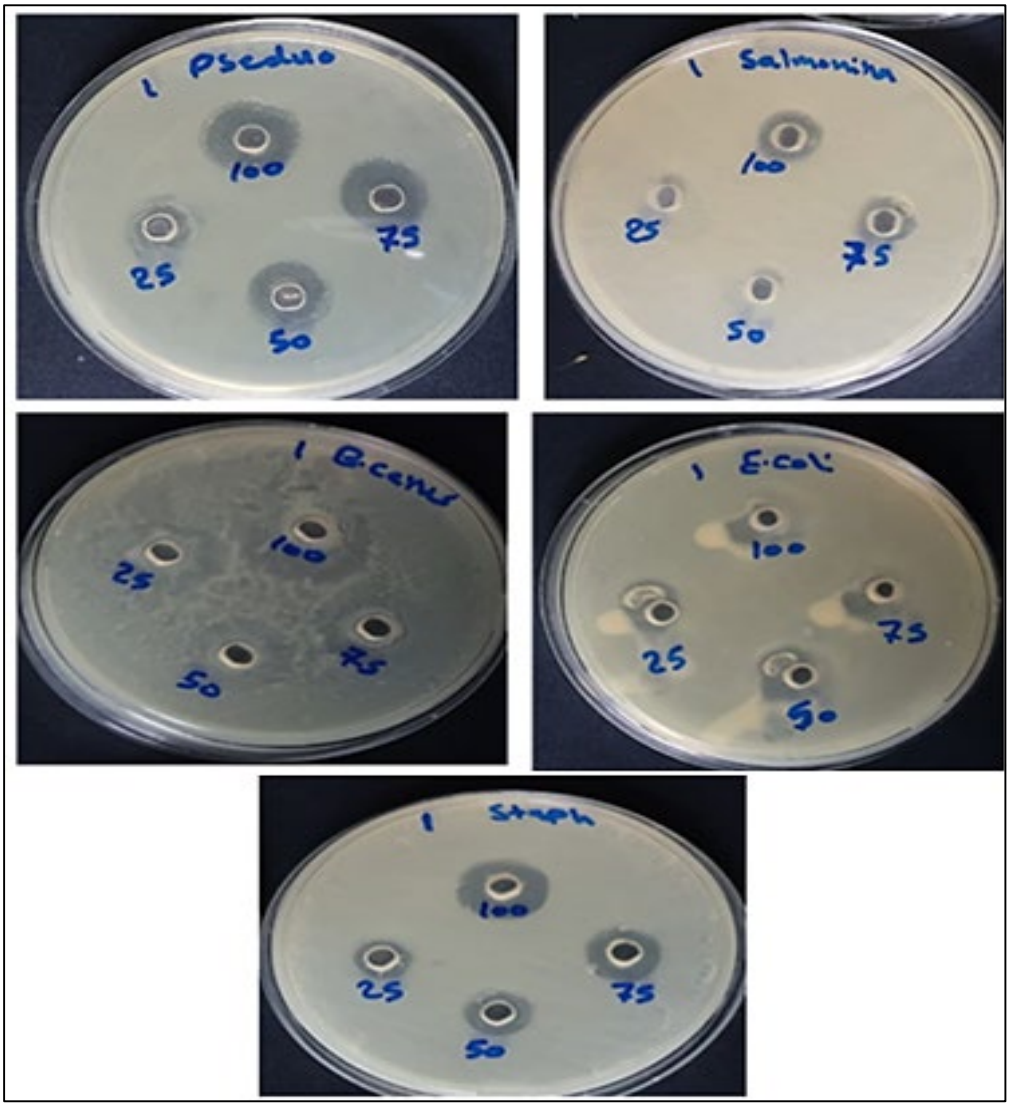

Fig.11. Effect of nisin at different concentrations on growth of microorganisms.

their small size, high surface area and special optical and electronic characters. The existence of capping layer around the gold nanoparticle plays important role in their biomedical applications and toxicity (Chen et al. 2013). Several methods have been used to prepare gold nanoparticles (AuNPs), and one of them is the chemical method, that the more sodium citrate used to prepare the gold nanoparticles lead to obtain smaller nanoparticles (Zhang et al. 2020).

Nisin has shown a good antimicrobial activity against common Gram-positive and negative bacteria, however, in present work, nisin was functionalized with AuNPs to get better antimicrobial therapy. Nisin consider a cationic antimicrobial agent that can interaction with the negative charged of Au NPs through electrostatic interaction (Bhat & Vidya 2017). Therefore, the nisin alone at different concentrations of 25, 50, 75 and 100mm showed poor inhibitory activity, whereas, free nanoparticles (NPs) shifts towards the positively after functionalized with nisin molecules. AuNPs-N showed potent bactericidal pathogenic activity in all different bacteria that used in this research. Most of isolates bacteria from intensive care units around the world are increasingly resistant to a large number of antimicrobial agents (Lowy 2003). Once the new antibiotics were introduced, these bacteria improved rapid resistance processes. Antibiotic resistance is

often obtained by horizontal gene transfer, chromosomal mutation as well as antibiotic selection (Chambers & De Leo 2009).

In a previous study, most of the nanoparticles were accumulated around the cell membrane of bacteria, after that, AuNPs-N promote the formation of huge pores which leads to leakage of cytoplasmic contents from cells (Bhat & Vidya 2017). The preparation pathway of nanoparticles, depended on their size, shape and surface charge, and these properties are critical factors for nanoparticle toxicity (Malugin & Ghandehari 2010). In this study, the efficacy of nisin bound with gold nanoparticles proved to be highly toxic against bacteria. The presence of negative charge on membrane of bacteria in high quantities than mammalian cells is a critical toxicity reason for AuNPs-N against bacteria.

References

Al-hadede, L.T. & Hassan, M.I. 2020. Silver Nanoparticles Synthisis by Green Method and Loading of the Enterosein to Study Its Antimicrobial Inhibition. In IOP Conference Series: Materials Science and Engineering 928(7): 072078.

AL-Hadedee, L.T.H.; Hasan, S.K.; Mousa, E.F. & Ahmed, W. 2019. Effect of magnetic field treatment on the milk characteristics with the presence of *Lactobacillus plantarum* and *Lactobacillus rhamnosus G*G bacteria. Plant Archives 19(1): 275-280.

Bhat, K.U. & Vidya, S.M. 2017. Nisin gold nanoparticles assemble as potent antimicrobial agent against *Enterococcus faecalis* and *Staphylococcus aureus* clinical isolates. Journal of Drug Delivery Science and Technology 37: 20-27.

Caswell, D.S. & Spiro, T.G. 1987. Proline signals in ultraviolet resonance Raman spectra of proteins: cis-trans isomerism in polyproline and ribonuclease A. Journal of the American Chemical Society 109(9): 2796-2800.

Chambers, H.F. & DeLeo, F.R. 2009. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nature Reviews Microbiology 7(9): 629-641.

Chen, H.; Dorrigan, A.; Saad, S.; Hare, D.J.; Cortie, M.B. & Valenzuela, S.M. 2013. In vivo study of spherical gold nanoparticles: inflammatory effects and distribution in mice. PloS one 8(2): e58208.

Jena, B.K. & Raj, C.R. 2007. Synthesis of flower-like gold nanoparticles and their electrocatalytic activity towards the oxidation of methanol and the reduction of oxygen. Langmuir 23(7): 4064-4070.

Lowy, F.D. 2003. Antimicrobial resistance: the example of *Staphylococcus aureus*. The Journal of Clinical Investigation 111(9): 1265-1273.

Malugin, A. & Ghandehari, H. 2010. Cellular uptake and toxicity of gold nanoparticles in prostate cancer cells: a comparative study of rods and spheres. Journal of Applied Toxicology: An International Journal 30(3): 212-217.

Saha, B.; Bhattacharya, J.; Mukherjee, A.; Ghosh, A.; Santra, C.; Dasgupta, A.K. & Karmakar, P. 2007. In vitro structural and functional evaluation of gold nanoparticles conjugated antibiotics. Nanoscale Research Letters 2(12): 614-622.

Sau, T.K. & Murphy, C.J. 2004. Seeded high yield synthesis of short Au nanorods in aqueous solution. Langmuir 20(15): 6414-6420.

Wang, A.J.; Li, Y.F.; Wen, M.; Yang, G.; Feng, J.J.; Yang, J. & Wang, H.Y. 2012. Melamine assisted one-pot synthesis of Au nanoflowers and their catalytic activity towards p-nitrophenol. New Journal of Chemistry 36(11): 2286-2291.

Wu, H.Y.; Liu, M. & Huang, M.H. 2006. Direct synthesis of branched gold nanocrystals and their transformation into spherical nanoparticles. The Journal of Physical Chemistry B 110(39): 19291-19294.

Xu, J.; Li, S.; Weng, J.; Wang, X.; Zhou, Z.; Yang, K. & Zhang, Q. 2008. Hydrothermal syntheses of gold nanocrystals: from icosahedral to its truncated form. Advanced Functional Materials 18(2): 277-284.

Younes, M.; Aggett, P.; Aguilar, F.; Crebelli, R.;, Dusemund, B. & Gott, D. 2017. Safety of nisin (E 234) as a food additive in the light of new toxicological data and the proposed extension of use. EFSA Journal 15(12): e05063.

Zhang, H.; Nie, P.; Xia, Z.; Feng, X.; Liu, X. & He, Y. 2020. Rapid Quantitative Detection of Deltamethrin in *Corydalis yanhusuo* by SERS Coupled with Multi-Walled Carbon Nanotubes. Molecules 25(18): 4081.