Synthesis Silver Nanoparticles as Antibacterial Against *Escherichia coli* and *Staphlococcus aurous* as a Model of Gram- negative and Gram- Positive Bacteria

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Abstract

Bacteria multidrug-resistance (MDR) pathogens led to increasing hospital and community – acquired infections, for which current antibiotic therapies are not effective which represent a growing problem, its well-known that the silver ion and silver nanoparticles have strong effect against microbial. In this study we prepare silver Np in chemical reduction methods and tested it on two type of multidrug resistance bacteria :E-coli and S.aureus. The result showed that the sliver Np has a strong effect against gram-positive and gram-negative bacteria.
Key word: Silver Np ,antibacterial , *E-coli* , *S.aureus*

الخلاصة

ان السلالات البكتيريه الممرضة والمقاومة لأنوع متعددة من المضادات الحيويه تؤدي الى زيادة العدوى الاصابات المكتسبة من خلال المستشفيات وعليه فان العلاجات المتوفرة من المضادات الحيوية الحالية ليست ذات فعالية كافيه ضد هذه السلالات وهذه بدورها تمثل مشكله صحيه متناميه، وكما عو معروف فان المواد النانوية ذات فعالية ضد البكتيريا حيث تم في هذا البحث تحضير سلفر نانوي بالطريقة الكيمائية وتم اختباره على نوعين من البكتيريا المقاومه للمضادات حيث اوضحت النتائج ان السلفر النانوي يمتلك تأثير مضاد للبكتيريا الموجه والسالبة الكرام.
الكلمات المفتاحيه: سلفر نانوي ,مضاد بكتيري ، بكتيريا الاشريكيا ، بكتيريا المكورات العنقوديه

Introduction

In recent years the research has focused on Nobel metal nanoparticles due to unique their chemical and physical properties such as electronic, magnetic, optical properties. Nanoparticles refer to the particles with nano scale size about minimum size 10nm to maximum size 100 nm ,this particle size exhibits large surface area to volume ratio ,the increase in this ratio has changed many properties compared to bulk materials [Marible *et al.* 2009].

Today Silver nanoparticle one of the most commonly nanoparticles used in medical science , Silver NP play important role in medicine and biological filed ,it has been used widely in many bio applications such as biomedicine ,biosensor, catalysis and used in medicine on burn treatment ,prevention of bacteria colonization on catheters, drug delivery ,etc [Aslan *et al.* 2005].

The applicability of silver nanoparticles as catalysis depends on the change of their size and stability ,The small surface area to the same ratio of volume means small nano size and that means larger active area of the catalyst . Due to high specific surface area , silver nanoparticles have high strong bacterial activity, against both gram positive and gram negative bacteria.There are many synthetic methods that have been developed to produce silver nanoparticles such as laser ablation, electrochemical method[Rodriguez *et al.*, 2000], chemical reduction methods [Shameli *et al.*,2011] etc,

Methicillin – Resistant Staphylococcus aureus (MRSA) and uropathogenic *E. coli* (UPEC) strain whichare regarded as the most common pathogen isolated from the patient with UTI. Those two strains are responsible for several difficult –to- treat infections in humans also these strains have been also developed through the process of natural selection.[Emody *et al.*,2003]

In this study, we prepare nano silver by chemical reduction methods, and we investigate the influence of silver nano particles against staph and E-coli bacteria

Materials and Methods

Silver nitrate ($AgNo_3$) ,was purchased from Reagent World ,tri sodium citrate,double –distilled deionized water

Silver nano particles preparation Method

Silver Npa was prepared using chemical reduction methods by using tri sodium citrate as a reduction agent ,0.001 M of $AgNo_3$ was heated to boil ,then 5 ml of tri sodium citrate was added to the solution drop by drop ,the solution were mixed and heated until color changed (pale-yellow) ,then it removed from heat device and it was stirred until cooled to the room temperature.

Below the mechanism of chemical reaction [Silva etal 2007and Hangxumx 2010]

$$4\,Ag^{+} + C_6H_5D_7Na + 2\,H_2O \longrightarrow 4\,Ag^{0} + C_6H_5O_7H_3 + 3Na^{+} + H^{+} + O_2 \uparrow$$

Bacterial strains

The *S.aureus* and *E. coli* bacterial cells were collected and selected from 70 patients (most founding strains were found samples 57 (81.4%)samples with *E.coli* while *S.aurus* in the 13(18.6%) samples) with urinary tract infection who were admittable in Al-Hilla teaching hospital, Babylon during a period from October2015 to January 2016. The two Bacterial isolates were identified as *E.coli* and *S.aureus* based on their morphology, Gram-staining. The Vitek 2 system was performed to identify the species level of *E. coli* isolates.

Antimicrobial Susceptibility Test:

The antimicrobial susceptibility patterns of isolates to different antimicrobial agents was determined and interpreted according to disk diffusion test that was used against 4 antibiotics, The following antimicrobial agents were obtained (from Oxoid, U.K) as standard reference disks as known potency for laboratory use:, trimethoprim-sulfamethoxazol (1.25/ 23.75mg), nitrofurantion (300mg), ciprofloxacin (5mg) and Ampicillin (l0mg).

Antibacterial activity

In vitro activity , silver Np against highly multi resistance strains gram – positive and gram- negative bacteria was evaluated by inhibition zone testing method by using (MHA) Mueller – Hinton agar[CLSI] for *Escherichia-coli* and blood agar for *Staphyl ococcus* aureus ,the inhibition zones were measured in cm after 24 hr of incubation at 35 C^0 .

Result and Discussion

Silver Np was prepared by using chemical reduction method by adding Tri Sodium citrate to aqueous solution of $AgNo_3$,the colorless solution change to pale- yellow color fig (1) ,this indicates the formation of nano particles .

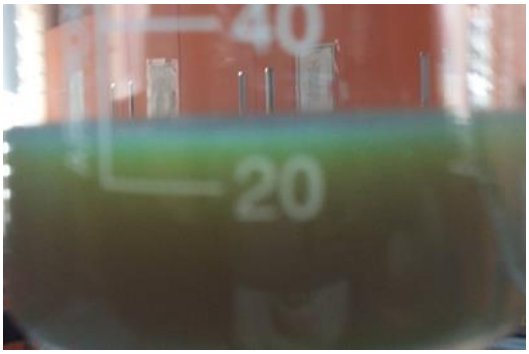

Fig (1):color of silver Np solution

The formation and characterization of silver Np was determine by using UV-Vis absorption spectrometer with wave length band about 300-600 nm ,the band of surface plasmon resonance (SPR) determined the morphology of the nanoparticles ,SPR bands are influenced by the morphology, size, and shape of the nanoparticles ,many studies have shown that the spherical silver Np contributes to the absorption band around 400 nm [Stamplecoskis, 2010.], absorption band in this study was around (417) as shown in fig (2) wich strongly suggests that silver Np were spherical in the shape.

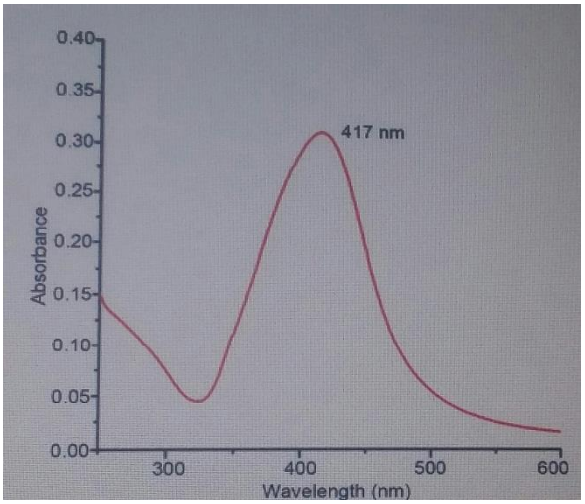

Fig (2):Absorbance band of silver Np

The size of nanoparticles determines its activity against bacteria because the binding of nanoparticles depends the surface area available for interaction ,when the particle is small this make large surface area available for interaction and this causes more activity of the nanoparticles against bacteria. table (1) and fig (3) show the inhibition zones of Bactria E-coli and S.aureus respectively

Table (1): Inhibition zones of bacteria

Typeof bacteria	Inhibition zone (cm)
E-coli	1.7
Staph	1.5

Fig (3): inhibition zones of bacteria

The results shows that the nanoparticles have strong activity against gram negative and gram positive bacteria and have more activity against *E-coli* than *Staph.* the activity of the Nanoparticles against bacteria have been reported by many studies ,but the mechanism of bactericidal effect of nanoparticles is not very well known, some studies have reported that the electrostatic attraction between positive charge of silver Np and negative charged cell membrane of microorganism [Hamouda, 2000; Dibror *et al.,* 2002; Dragieva *et al*., 1999],other studies reported that the oxidation react at the surface of nanoparticles silver ion diffusion ,thus causing structural change and finally bacterial will die. (Melinte *et al.*,2011).

Other reason for bacteria death is that the silver Np may attach to surface membrane and disturb its power function such as permeability and respiration, (Morible *et al.,* 2009)

In conclusion silver Np has strong activity against bacteria and this activity is influenced by the size of nanoparticles ,more small particles make large inhibition zones

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