

**SILVER NANOPARTICLES AS ANTIMICROBIAL AGAINST  
*SALMONELLA TYPHI*, *PROTEUS MIRABILIS* AND *BACILLUS  
CEREUS* 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 three types of multidrug resistance bacteria: *Proteus mirabilis*, *Bacillus cereus* and *Salmonella typhi*. The result showed that the silver Np has a strong effect against gram-positive and gram-negative bacteria.

**KEYWORD:** Silver Np, antibacterial, *Proteus mirabilis*, *Bacillus cereus*, *Salmonella typhi*.

**INTRODUCTION**

The field of nanotechnology is one of the most active research areas in modern materials science. In recent years the research has focused on noble metal nanoparticles due to unique their chemical and physical properties such as electronic, magnetic, optical properties. Nanoparticles can be termed as particles with nano scale size about minimum size 10nm to maximum size 100 nm, because of their size, nanoparticles have a 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 field, 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,

In this study we prepare nano silver by chemical reduction methods and we investigate the influence of silver nano particles against: *Proteus mirabilis*, *Bacillus cereus* and *Salmonella*

## MATERIALS AND METHODS

Silver nitrate ( $\text{AgNO}_3$ ), 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  $\text{AgNO}_3$  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 et al 2007 and Hangxumx 2010]



### Bacterial strains

*Proteus mirabilis*, *Bacillus cereus* and *Salmonella typhi* bacterial cells were collected and selected from 55 patients (most founding strains were found samples 20 (36.3%) samples with *proteus*, 18(32.7%) samples with *Salmonella*, 17(30.9%) samples *Bacillus*, with urinary tract infection who were admit table in Al-Hilla teaching hospital, Babylon during a period from October 2015 to June 2016. The Bacterial isolates were identified as *Proteus mirabilis*, *Bacillus cereus* and *Salmonella typhi* based on their morphology, Gram-staining. Vitek 2 system was performed to identify species level of bacterial isolated.

### Antimicrobial Susceptibility Test

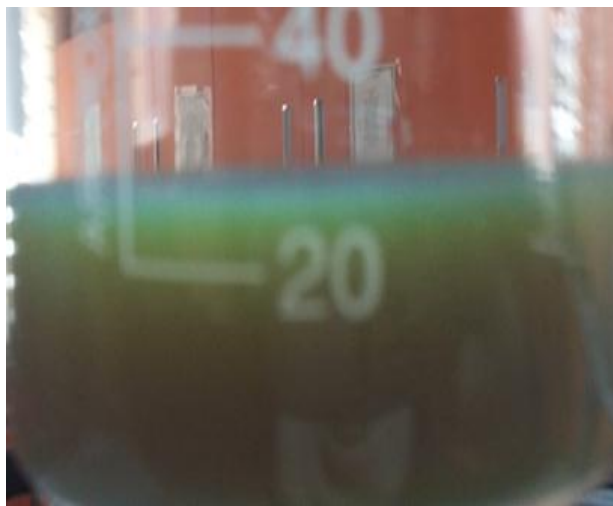
The antimicrobial susceptibility patterns of isolates to different antimicrobial agents was determined and interpreted according to disk diffusion test that was used against 4 antibiotics, The following antimicrobial agents were obtained (from Oxoid, U.K) as standard reference disks as known potency for laboratory use; trimethoprim-sulfamethoxazol (1.25/ 23.75mg), nitrofurantion (300mg), ciprofloxacin (5mg) and Ampicillin (10mg).

### 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], the inhibition zones were measured in mm after 24 hr of incubation at 35 C<sup>0</sup>.

### RESULT AND DISCUSSION

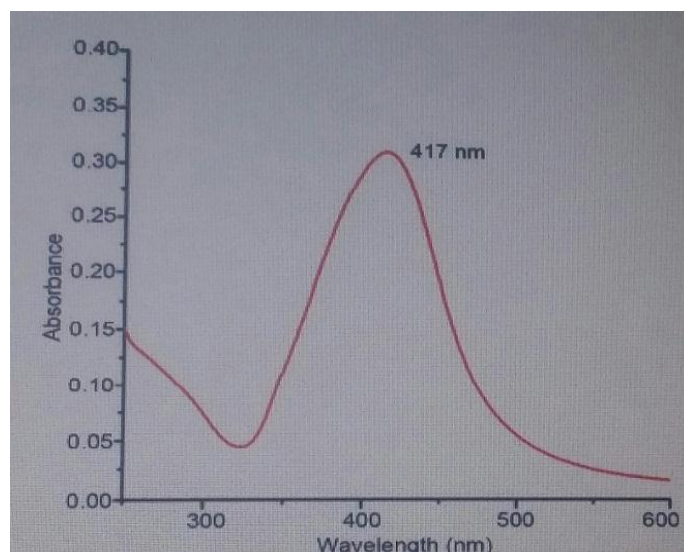
Silver Np was prepared by using chemical reduction method by adding Tri Sodium citrate to aqueous solution of AgNO<sub>3</sub>, the colorless solution change to pale- yellow color fig (1), this indicates the formation of nano particles.



**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 K.

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.

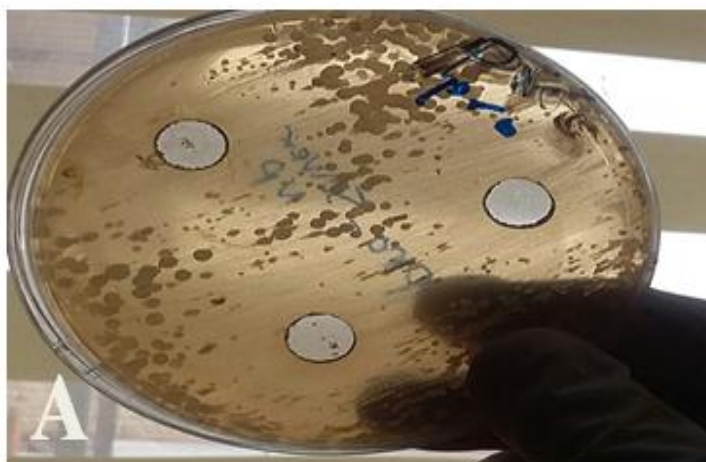


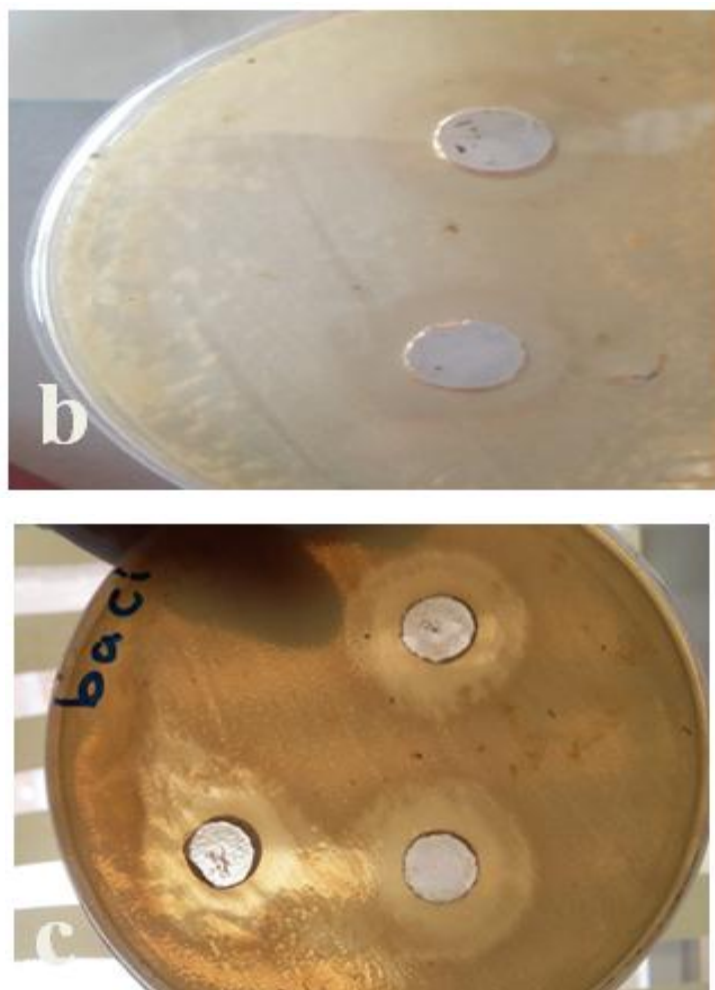
**Fig (2): Absorbance band of silver Np**

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 *Proteus mirabilis*, *Bacillus cereus* and *Salmonella typhi* respectively.

**Table (1): Inhibition zones of bacteria**

Typeof bacteria	Inhibition zone (mm)
<i>Proteus</i>	20
<i>Bacillus</i>	15
<i>Salmonella</i>	13





**Fig.(3): Apperanances of inhibitory zone with (A) *Proteus mirabilis* (B) *Salmonella typhi* (C) *Bacillus cereus***

The results shows that the nanoparticles have strong activity against gram negative and gram positive bacteria and have more activity against *Proteus* and *bacillus*. 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 T2000, 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 G2009.

In conclusion silver Np has strong activity against bacteria and this activity is influenced by the size of nanoparticles, because of their size it can easily reach the nuclear content of bacteria and they present the large and impressive surface area thus the contact with bacteria were the greatest, it can be reason behind the strong activity against bacteria and then more small particles make large inhibition zones.

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