

## Synthesis and characterization of silver nanoparticles for antibacterial activity

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### Abstract

We consider of the antimicrobial activity on the Ag-nanoparticles(Ag-NPs) aqueous solutions, which was prepared using a stabilizer, such as poly(*N*-vinyl-2-pyrrolidone) (PVP) , for *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E.coli*) by measuring the minimum inhibitory concentration (MIC). Antimicrobial effect of Ag-NPs for *S. aureus* and *E. coli* was investigated using disk diffusion method .Also, the Ag-NPs did show the antimicrobial activity for the test organisms. Antibacterial activity of Ag-NPs was found to be dependent on the shape of bacteria .

**Keywords :** Silver nanoparticles, Minimum Inhibitory concentration, Colony Forming Unit (CFU)

### 1. Introduction

Silver ions work against bacteria in a number of ways; silver ions interact with the thiol groups of enzyme and proteins that are important for the bacterial respiration and the transport of important substance across the cell membrane and within the cell <sup>[1]</sup>, and silver ions are bound to the bacterial cell wall and outer bacterial cell, altering the function of the bacterial cell membrane <sup>[2]</sup>, thus silver metal and its compounds were the effective preventing infection of the wound <sup>[3]</sup> .Silver can inhibit enzymatic systems of the respiratory chain and alter DNA synthesis <sup>[4, 5]</sup> .

Metal nanoparticles, which have a high specific surface area and a high fraction of surface atoms, have been studied extensively due to their unique physicochemical characteristics such as catalytic activity, optical properties, electronic properties, antimicrobial activity, and magnetic properties <sup>[6]</sup> .

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It can be expected that the high specific surface area and high fraction of surface atoms of nanosilver shapes will lead to high antimicrobial activity compared to bulk Ag metal. Recent, microbiological and chemical experiments implied that interaction of silver ion with thiol groups played an essential role in bacterial inactivation [7, 8]. Surface area involves the increase of contact surface, which is an important condition for the effects of silver nanoparticles.

We have recently developed a reduction method of converting Ag nanospheres into nanorods [9] and nanoplates [10]. This method offers great ease of control and a high yield of hexagonal particles. Herein we report an improved antibacterial effect of hexagonal Ag-NPs that involves a number of steps:

- 1) Synthesis of Ag-NPs by reduction of AgNO<sub>3</sub> in the presence of NaBH<sub>4</sub> [9, 10],
- 2) A study on the antibacterial effects of these Ag-NPs,

The purpose of this study was to examine the antibacterial activity of Ag-NPs against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*.)

## 2. Materials and methods

### 2.1. Materials, bacterial strain, and culture condition

Ag-NPs(0.074%) solution was prepared using chemical reduction method [9, 10]. We produced monodispersed, nonagglomerated particles with a uniform size distribution. For this experiment, nanoparticles have concentrations ranging from 0.0976 to 100 µg/mL. (*S. aureus*) ATCC 511539 and (*E. coli*) ATCC 35218 were used as a Gram-positive and Gram-negative bacterium, respectively. For the antimicrobial activity measurement, bacteria cultures were incubated at 38° C in Luria medium (tryptone 1.5%, yeast extract 0.75%, sodium chloride 1.2%, agar 1%, Difco). The concentration of the bacteria was controlled from 10<sup>5</sup> to 10<sup>6</sup> (CFU/mL).

### 2.2. Antimicrobial activity studies

#### 2.2.1. The disc diffusion method

The modified disc diffusion method [11- 14] was used to evaluate the antimicrobial activity of Ag-NPs against *S. aureus* and *E. coli*. This method was performed in Luria–Bertani (LB) medium solid agar Petri dish. The nanosilver shapes were cut into a disc shape with 1.5 cm diameter, sterilized by autoclaving 15 min at 120 °C, and was placed on *E. coli*-cultured agar plate and *S. aureus*-cultured agar plate which were then incubated for 24 h at 37 °C and inhibition zone was monitored. After incubation the presence of bacterial growth inhibition halo around the samples were absorbed and their diameter in millimeters was measured [15, 16].

Briefly, sterilized Luria–Bertani (LB) (broth was measured) 8 ml (into sterile tubes. Ten microliters) 5.4 ppm (of Ag-NPs was added in LB agar medium, respectively, which have 8mm diameter hole in the center.

[Growth suppression exchange = (Total growth suppression exchange – Diameter of the disk) 8mm]

#### 2.2.2. Measurement of minimum inhibitory concentration (MIC)

Ag-NPs with (0- 100 ppm), was added in LB medium, respectively. Each bacterium culture (*S. aureus* and *E. coli*) was controlled at 10<sup>5</sup>–10<sup>6</sup> CFU/mL and incubated at 37°C. To establish the antimicrobial activity of silver nanoparticles on the bacterial growth, the minimum inhibitory concentration of nanosilver shapes for *S. aureus* and *E. coli* were determined by optical density of the

bacterial culture solution containing different concentration of Ag-NPs after 24h. All of the experiments (MIC) were triplicated, on three different days .

### 2.2.3. Measurement of colony-forming unit (CFU)

Ag-NPs are content by weight in the spinning solution which contains about  $1.5 \times 10^5$  colony forming units (CFU) of *S .aureus* and *E .coli*, respectively .The mixtures were cultured at 37° C in a shaking incubator for 12 h .Silver nitrate was also tested as blank control and positive control, respectively .100 L of each of these cell solutions was seeded onto LB agar using a surface spread plate technique .The plates were incubated at 37° C for 24 h .Then the numbers of bacterial colonies (CFU) were counted .The counts were used to calculate the surviving number of bacteria<sup>17</sup> .The anti-bacterial efficacy ) ABE in (%of the specimen was calculated according to the following equation(1):

(Eq .1)

$$ABE = (\%V_c - V_t / V_c \times 100)$$

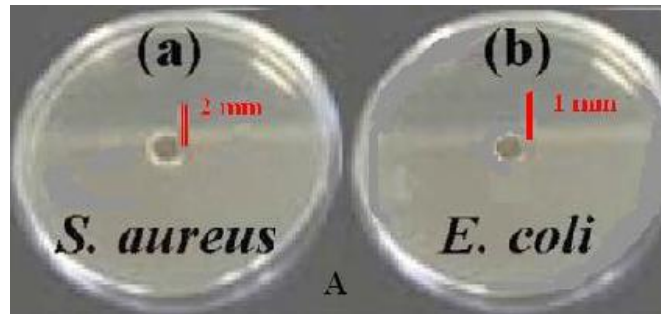
Where  $V_c$  and  $V_t$  stood for the numbers of viable bacterial colonies of the blank control )pure PBS buffer added )and test specimen, respectively. A bacterial culture medium containing Ag-NPs solution was diluted by adding sodium chloride )0.85 (%solution to control the osmotic pressure of bacteria, and then spared on LB medium .Each bacterium *S .aureu* and (*E .coli*) was incubated its cultivation temperature for 48 h after that colony-forming unit (CFU) was measured .The Ag-NPs) 0, 10 ppm) stabilized with (2.5 mM) PVP were added LB medium .Moreover, to compare the effect of Ag-NPs and PVP )2.5 mM) without Ag-NPs, was added to LB medium .The LB medium was incubated same method of above measurement of MIC .The decrease of bacterial at each LB medium was measured .

### 2.2.4. Scanning electron microscopy (SEM)

The morphological changes of *S .aureus* and *E .coli* by Ag-NPs were observed with a scanning electron microscope (SEM). Strains were prepared by cutting the agar, fixed for a minimum of 3 h in )2.5 %v/v) glutaraldehyde) 100mM phosphate buffer solution, pH 7.2), and then fixed in) 1%w/v) osmium tetra oxide for 1 h .The agar blocks were dehydrated through a graded series of ethanol) 30, 70, 80, 95, and 100%; each level was applied twice for 15 min each time and ethanol :isoamyl acetate )3:1, 1:1, 1:3, and 100 %isoamyl acetate twice for 30 min .(The agar blocks on grid were dried with a critical-point drier using liquid CO<sub>2</sub> and coated with gold-coater for 5 min .The coated samples were observed under LEO 440i electron microscopic with accelerating voltage of 10 kV .

## 3. Results and discussion

In this study, the antibacterial activity Ag-NPs for *Staphylococcus aureus*) *S aureus*, Gram-positive) and *Escherichia coli*) *E.coli*, Gram-negative) was measured by disk diffusion method .The Ag-NPs stabilized with PVP show high antibacterial activity .The growth inhibition ring of *S .aureus* and *E .coli* treated by Ag-NPs stabilized with PVP was 2 and 1 mm, respectively (Figure 1).



**Fig.1** Antibacterial activity of Ag-NPs solution stabilized with PVP, against *S. aureus* (ATCC 51153) (a) and *E. coli* (ATCC 35218) (b). All the concentration of silver nanoparticles are 10 $\mu$ L (5.4 ppm).

This clearly demonstrates that the antimicrobial activity is only due to nanosilver shapes impregnated inside bacterial and not due to individual bacterial. The mechanism for antibacterial action of silver nanoparticles is bacterial membrane disruption by the ions silver released from the PVP. The Ag<sup>+</sup> ions form insoluble compounds with sulphhydryl groups in the cellular wall of the microorganism that are responsible for the inhibition halo in the seeded culture media observed in (Figure 1). This result can be explained in terms of the presence of amin groups in the PVP chain and it's easy to induce Ag<sup>+</sup> motility. The Ag<sup>+</sup> release mechanism is not elucidated. However, is possible the amin group improve the Ag<sup>+</sup>/H<sup>+</sup> ionic exchange. All antibacterial activity tests were performed in triplicate and were done at least two different times to ensure reproducibility.

The inhibition kinetics of bacteria containing different nanosilver shapes, were investigated in *S.aureus* Figure 2 and (*E .coli*) Figure 3, respectively. The MIC of nanosilver shapes for Gram-positive and Gram-negative was 5 ppm (*S .aureus* ) and 10 ppm (*E .coli*), respectively. When *S . aureus* was exposed to Ag-Nps solution of 15 ppm was not enough to inhibit both *S .aureus* and *E . coli* within 5 h, respectively. From the MIC results, the Ag -Ag-Nps showed high inhibition kinetics against (*E .coli*) and (*S .aureus*.) The Ag-Nps solutions show good growth inhibition effect for both *S . aureus* and *E .coli*. The antibacterial efficacie (ABE) of the Ag-NPs solutions against *S .aureus* and *E . coli* showe in Table.1, respectively, and table data results indicating that the Ag-NPs solution is high ABE and this activity is quite strong. The silver is released steadily and thus the antibacterial activity is durable. Relatively, the ABE against *E .coli* is lower than that against *S .aureus*, probably because of the difference in cell walls between Gram-positive and Gram-negative bacteria. The cell wall of *E . coli*, which consists of lipids, proteins and lipopolysaccharides (LPS) , provides effective protection against biocides. However, the cell wall of Gram-positive bacteria, such as *S .aureus*, does not consist of LPS [18]. It is worthwhile mentioning that all these silver nanoparticle solutions were quit stable for several months without observable aggregation .

The surface of the cell walls of *S.aureus* was covered with substance resulted from the cell disruption after the nanosilver shapes treatment. The surface of the cell walls of *E .coli* treated with nanosilver shapes was severely disrupted compared to the non-treated *E .coli*. It indicated that the Ag-NPs have an antimicrobial activity against *E .coli* and *S .aureus* by disrupting cells and require a lower concentration to inhibit development of the *S .aureus* and *E.coli* strains.

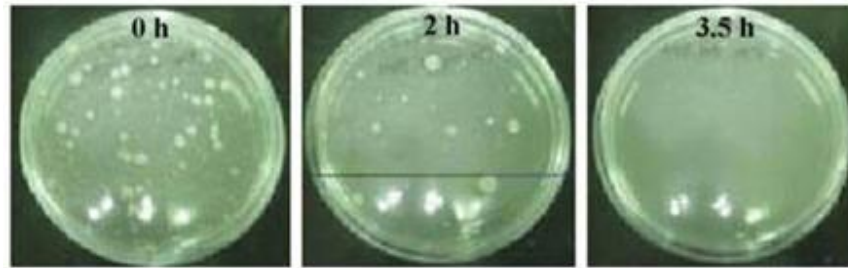


Fig.2. Growth inhibition curves of *E.coli* colonies, after Silver-Nps (10 ppm) treatment as the incubation time (0, 2, 3.5 h).

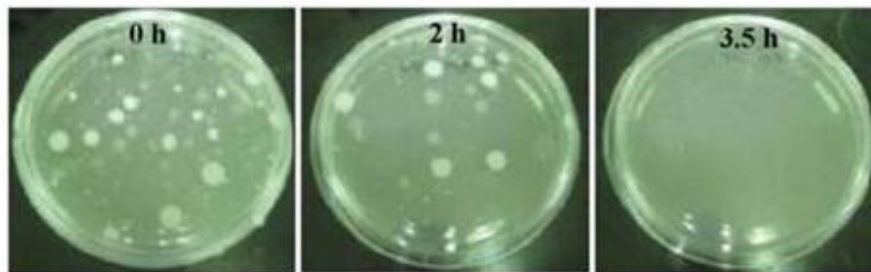


Fig.3. Growth inhibition of *S. aureus* colonies, after Silver-Nps (10 ppm) treatment as the incubation time (0, 2, 3.5 h).

#### 4. Conclusion

In summary, the Ag-NPs show strong antibacterial activity. The growth inhibition ring of *S. aureus* and *E. coli* treated by Ag-Nps were 2 and 1 mm, respectively. The significance of these results is a demonstration that the Ag-NPs solutions have a good antibacterial activity for both *S. aureus* and *E. coli*. The surfaces cell walls of *S. aureus* and *E. coli* were disrupted by nanosilver shapes. The antibacterial activity of Ag-NPs against of *S. aureus* and *E. coli* might be their adsorption on bacterial surface. The antibacterial activity of Ag ion is inhibition of intracellular enzyme activity. Therefore, the other possibility can be considered that, remaining Ag ions in nanosilver shape solutions or dissolved Ag ions might effect on bacterial growth. Our findings show that Ag-NPs, require a lower concentration to inhibit development of the *S. mutans* and *E. coli* strains, and this is probably due to the increasing surface area in Ag-NPs. The antibacterial efficacies (ABE) of the Ag- Ag-NPs solutions against *S. aureus* and *E. coli* indicating that, the Ag-NPs solution is high ABE and this activity is quite strong. This clearly demonstrates that the antimicrobial activity is only due to Ag-NPs impregnated inside bacterial and not due to individual bacterial.

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