

## RESEARCH ARTICLE

# Evaluation of Antibacterial and Cytotoxicity Properties of Zinc Oxide Nanoparticles Synthesized by Precipitation Method against Methicillin-resistant *Staphylococcus aureus*

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## ABSTRACT

A novel technique for nanoparticles with a chemical method and impact for resistance bacteria methicillin-resistant *Staphylococcus aureus* (MRSA), UV-visible analysis confirmed the by Fourier transform infrared spectroscopy (FT-IR) and Energy dispersive X-Ray (EDX), Scanning electron microscope (SEM) and X-ray diffraction pattern estimation antimicrobial excellent antibacterial activity against MRSA (with zone of inhibition of  $11 \pm 02$  mm,  $9 \pm 01$  mm,  $8 \pm 03$  mm and  $7.5 \pm 02$  mm and  $6.5 \pm 02$  mm) at different concentrations (0.5, 0.25, 0.125, 0.0625, 0.03125) mg/ml while good activity was  $16 \pm 03$  mm at  $17 \pm 02$  mm zone at 0.25, 0.125 mg/mL, respectively. The increase in microorganism resistance to antibiotics a couple of have caused Antimicrobial factors are widely recognized (ZnO NPs) and are less toxic and biological safety. evaluation of MRSA by minimum inhibitory concentration (MIC) (0.5, 0.25, 0.125, 0.0625, 0.03125) mg/ml of ZnO NPs. This research aims to study zinc oxide (ZnO) nanoparticles synthesis and antibacterial. *In vivo* evaluation precipitation method of cytotoxicity was determined WRL68 normal A375 cells.

**Keywords:** Chemical method, Zinc Oxide, Methicillin-resistant *Staphylococcus aureus*

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**Conflict of interest:** None

## INTRODUCTION

Microparticle (MPs) use extended to biomedical applications, including antibiotic drugs for conventional uses in clinics, implants, and cosmetics.<sup>1,2</sup>

Their particle size thereby influences antimicrobial activity against pathogenic microorganisms.<sup>3</sup> Delivery into tumor cells of ZnO NPs as carriers of antitumor drugs for targeted drug, on different types of cancers along with their mode of actions,<sup>4,5</sup> less cytotoxic metal oxide NPs of metallic oxide display promising prospects withinside the area of biomedicine.<sup>6</sup> There are many sorts of metallic oxides nanoparticles like ZnO, CuO, TiO<sub>2</sub>, Fe<sub>2</sub>O<sub>3</sub>, MgO, NiO, and ZrO<sub>2</sub> nanoparticles.<sup>7</sup> In scientific settings, this has a profound effect on the remedy of implant-related infections, as those are characterized by biofilm formation.<sup>8</sup> Different characterization nanocomposite materials such as physical and chemical NPs gave greater properties.<sup>9</sup> *Staphylococcus aureus*, even though commonly recognized as a commensal, human bacterial infection in which different gentle tissues include pores, skin, bones, and bloodstream.

Antistaphylococcal antimicrobial drugs have higher resistance to every new category.<sup>10</sup>

## MATERIAL AND METHODS

Zinc acetate heptahydrate (ZnSO<sub>4</sub>.7H<sub>2</sub>O) and (NaOH), used chemicals were employed without any treatment in this work.

### Synthesis of ZnO Nanoparticles

Directly by precipitation, The ZnO-NPs were prepared from two aqueous solutions, NaOH and ZnSO<sub>4</sub>.7H<sub>2</sub>O.<sup>11</sup> The precursor solwution with vigorous stirring for 20 minutes. A solution with pH=7 containing a white precipitate can be observed as shown in Figure 1. was collected the white precipitate filtered with distilled water and washed several times.

### Characteristics of Synthesis Samples

Techniques were used to conduct this work, such as Atomic absorption analysis, which was applied to estimate all ZnO NPs. Fourier transform infrared spectroscopy (FT-IR) and

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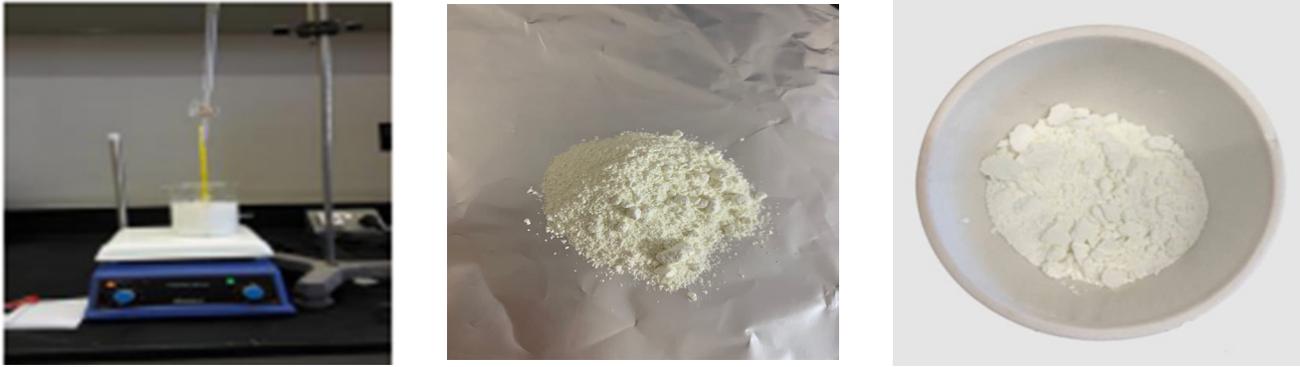


Figure 1: shows the precipitation of ZnO NPs, (white powder product).

X-ray powder diffraction (XRD) analysis and Atomic force microscopy (AFM) model, Transmission Electron Microscopy and Scanning Electron Microscopy.

#### Antimicrobial Activity

Evaluated for antibacterial activity synthesized ZNPs were activities against Gram-positive bacteria MRSA at different concentrations ZnO-NPs, by (WAD) assay, the working cultures at 0.125 mg/mL, were pest result with 32  $\mu$ L of ZnO-NPs on Müeller-Hinton Agar.

#### Broth Dilution

In tubes with Nutrient Broth (5 mL) and ZnO-NPs at final concentrations (0.5, 0.25, 0.125, 0.0625, 0.03125) mg/mL, each tubes were bacteria at  $10^5$  CFU mL and then incubated at 37°C, 48 hours.

#### Isolates and Culture Media

The isolate was sources of isolation (skin wounds) were obtained. It was diagnosed and identification by Department of Biology, College of Science, Baghdad University, Baghdad (Figure 2).



Figure 2: MRSA on MSA agar.

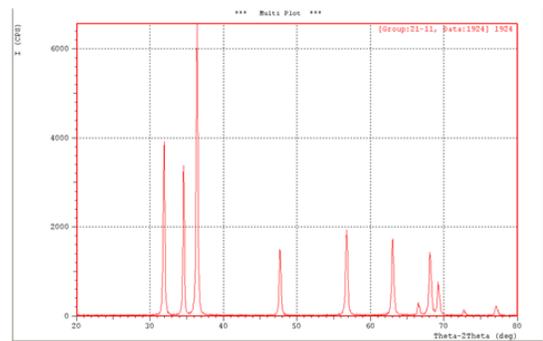


Figure 3: shows the X-ray diffraction of ZnO nanoparticles.

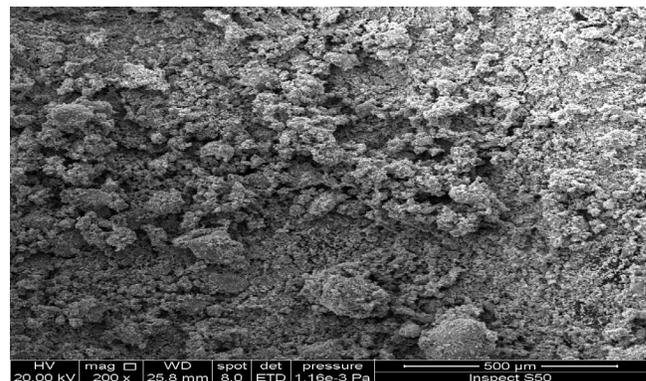
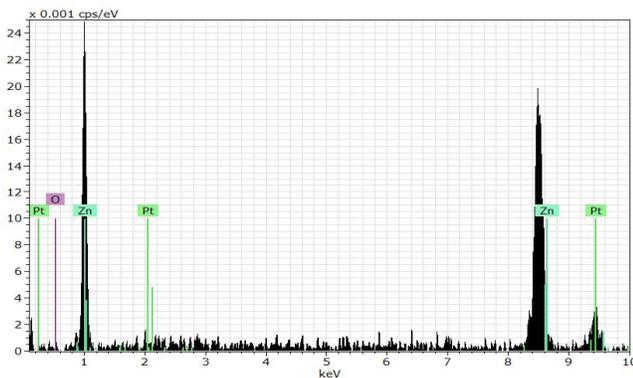


Figure 4: (A) EDX and (B) SEM of ZnO nanoparticles.

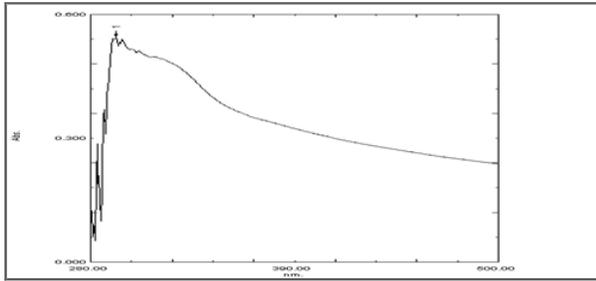
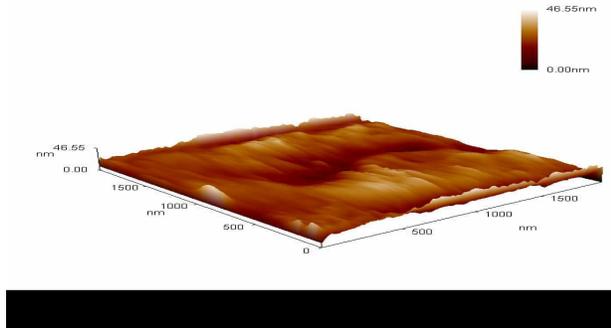


Figure 5: shows the UV-visible absorption of ZnONPs.



**Cytotoxic by MTT Assay**

Evaluation of the cytotoxic at three concentrations of chemical ZnO NPs anti-normal cell and WRL68 cells in 96-well plates, after 24 hours, ZnO NPs. to determine the equation was the IC50 value:  $Cell\ viability = \frac{Ab\ S}{Ab\ C} \times 100$

**RESULTS AND DISCUSSION**

**ZnO-NPs Characterization**

X-ray diffraction analysis was done in the range 25°–60° (2θ), diffraction results (Figure 3) demonstrated with the standard of ZnO. This result showed the hexagonal indicates to ZnO NPs.

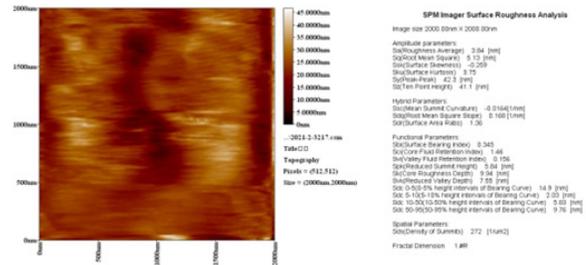


Figure 6: A, and B showed AFM of ZnO nanoparticles

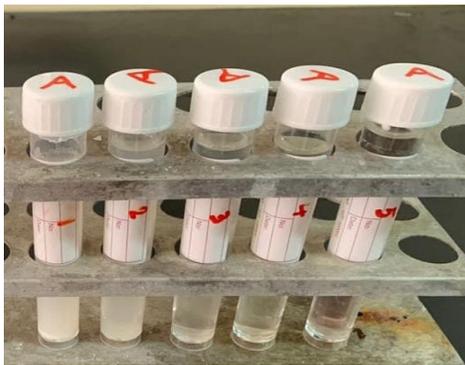


Figure 7: MIC of ZnO-NPs at final concentrations (0.5 ,0.25, 0.125, 0.0625, 0.03125) mg/m<sup>18</sup>

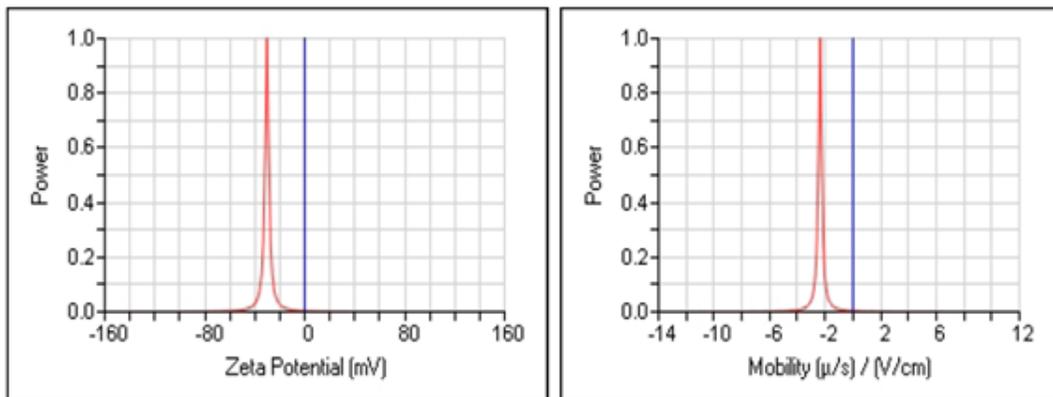


Figure 8: shows the ZnO nanoparticles.

This result almost agrees with other studies<sup>12</sup> synthesis shape crystallite size that found the average ZnO NPs.

Rod-like shape of ZnO-NPs by the SEM image (Figure 4). The NP average length and diameter were 47 nm. TEM and SEM of ZnO NPs indicate TEM image of the morphological characterization of ZnO NPs We observe a spherical shape. The result agrees with<sup>13</sup>, demonstrating that the ZnO NP's average size of approximately 20–25 nm and a spherical shape with an and EDAX the elemental composition of the metal oxide NPs.

Absorption spectroscopy UV is examined. It exhibits a strong absorption band at about 355 nm (Figure 5). Other research of ZNPs absorption UV is found rang 360 nm without calcined.<sup>14</sup>

In this work, a scanning probe microscope available at the department of Chemical Science, Al-Nahrain University AFM, measuring the granularity accumulation distribution, roughness, and grain size of the ZnO nanostructures range 40 nm shown in Figure 6. The result reported by Al-Taie is not consistent, which showed 125.77 nm as the average particle size.<sup>15</sup>

### Antibacterial Activity

For MRSA, the inhibition halo was present in concentrations 0.125 mg mL<sup>-1</sup>. This result detects the antibacterial effect of ZnO-NPs against MRSA, were performed as a qualitative AWD to observe and predict the ZnO-NPs.

The result disagrees with<sup>16</sup> other researchers. It is not able to determine the MIC as it is impossible and by diffusion of the antimicrobial agent in the agar

### Broth Dilution

The concentration effect of anti-MRSA was 0.125 mg/mL. The result broth media assay agrees on nanoparticle-bacteria interactions that can be considered confirmative.<sup>17</sup> Different particle morphologies for ZnO-NPs antimicrobial activity L are shown in Figure 7.

It measures the electrical charge of suspended particles in liquid and demonstrates the zeta potential of ZnO NPs (Figure 8). The device is located at the Ministry of Science and Technology. The ionic layer's electric potential is different across a charge.<sup>18,19</sup>

The concentration range between (0.5, 1, 1.5, 2, 2.5) mg/mL of ZnO NPs by chemical method resulted in a reduction in the number of A375 cells. The nano-ZnO is connected to the toxic oxygen species of photocatalytic activity.<sup>20</sup> The toxicity is very often correlated with apoptosis and a decrease in cell viability leads to cell death such as damage cell membrane LDH (Figure 9).<sup>20,21</sup>

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