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Preparation, characterization and analysis of zinc oxide nano-particles using a sol-gel technique as an inhibitor for bacteria

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ABSTRACT

In this work, zinc oxide nanoparticles (ZnO - NPs) were prepared using a sol-gel methodology and tested for their antibacterial activity against each of the following pathogenic species: *Escherichia coli*, *Klebsiella pneumonia,* and *Staphylococcus aureus* by well diffusion assay. The sample prepared was characterized by different techniques: Atomic Force Microscope AFM; Fourier Transform Infrared FT-IR; Scanning Electron Microscope SEM and X-Ray Diffraction Spectroscopy XRD. The XRD result showed that ZnO - NPs presence in wurtzite the structure of ZnO. The AFM and SEM of the surface analysis indicate that the most ZnO – NPs appear approximately in a spherical shape with some agglomeration. The average particle size for ZnO - NPs is nearly 37 nm. Volumes 25μl, 50μl, 75μl, 100μl, 125μl, and 150μl of 10 mg\ ml concentration of ZnO - NPs were used, the antimicrobial activity results showed that ability for ZnO - NPs to inhibit the growth of S.aureus increased as the solution volume increased, while the growing of (*K. pneumonia*) and (*E. coli*) was inhibited only with the volume 75μl where the inhibition zones diameters were 15mm and 10mm respectively.

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INTRODUCTION

The nanoparticles of Zinc oxide (ZnO - NPs) have high chemical stability, a wide range of radiation absorption and high photostability high coupling coefficient (Segets *et al.*, 2009; Xiangdong *et al.*, 1991). Also, they have many other technical and scientific benefits as a result to their high excitant binding energy and broad bandgap energy of 60 me.V. and 3.37 me.V. respectively, Besides the huge mechanical and thermal solidity at room temperature. The mentioned properties made it a good choice for future use in laser technology and electronics (Bacaksiz *et al.*, 2008; Wang *et al.*, 2005). Piezo-electrical and pyro-electrical characteristics of ZnO-NPs means that it can be used in the processing of H2 as an energy source, sensor, converter and photocatalyst (Wang, 2008; Chaari and Matoussi, 2012) because of its rigidity, stiffness and piezo-electrical constant. Because of its rigidity, hardness and piezo-electrical constancy, it is significant in the ceramics industry, whereas its biocompatibility, low toxicity and biodegradability make it an advantage for bio-medicine and pro-ecological systems (Bhattacharyya and Gedanken, 2008).

Deferent techniques such as nano-lithography, phys-

ical vapor deposition PVD, chemical vapor deposition CVD, sol-gel method, spray conversion processing SCP, and precipitation methods have been declared in the literature for the preparation of ZnO - NPs. The methods of precipitation are one of the most common methods of processing nanoparticles; the process decreases the temperature. The reaction in which homogeneous reagent mixtures precipitate. It is a simple method for synthesizing metal-oxide nanopowders, which are highly reactive at low temperatures (Al-Taweel, 2014; Zhang *et al.*, 2007; Al-Taweel, 2015).

The use of metal oxide-NPs as a possible antibacterial agent has been extensively studied. Deposition of NPs on the bacteria outer surface or aggregation of NPs in the cytoplasm in the peri-plasmic zone induces disruption of cellular function or disturbance of membranes and disorganization (Al-Taweel *et al.*, 2019; Zhang *et al.*, 2007).

Similarly, the ZnO - NPs expected to show a strong ability to slow bacteria growth due to bacteria membrane degradation, which makes the membrane more permeable that accumulates nanoparticles in the membrane tissue and bacterial cell cytoplasm. In addition, ZnO - NPs indicated behavior to shield intestinal cells from bacterial infection by hindering bacterial attachment by inhibiting increased permeability of the close junction and modulating cytokine (Roselli *et al.*, 2003). Moreover, for the activity of nanoparticles bactericidal materials, it's so important that the bacterial negatively charged cells attract electrostatically with the positively charged particles. This association not only inhibits bacterial growth, but it also triggers the production of ROS (a group of related living things) (causing reactions from other organisms or chemicals), which results in cell death (Stoimenov *et al.*, 2002; Jones *et al.*, 2008). ZnO - NPs have examined antimicrobial action versus Pseudomonas aeruginosa, which is a Gram-negative bacteria, funny lobacter jejuni, Escherichia coli, and Germ subtillis as a Gram-positive bacteria and (germs) aureus (Premanathan *et al.*, 2011; Kadhim and Al-Zaidy, 2019).

The work aims at preparing ZnO - NPs by sol-gel, characterization and antimicrobial action against positive and negative bacteria have been studied, *Staphylococcus aureus* (*S.aureus), Escherichia coli* (*E. coli*) and *Klebsiella pneumonia* (*K. pneumonia*) bacteria by using Muller Hinton media.

EXPERIMENTAL

Materials and Instrumentation

In the present search, the precursor of ZnO - NPs was made by Sol-Gelel Method. The chemical reagents used in this search were zinc sulphate heptahydrate [$ZnSO_4.7H_2O$] and NaOH powders of analytical grade purity. Instruments used for synthesis are Muffle furnace, Magnetic stirrer, FTIR Spectroscopy (Shimadzu-model 8400S), SEM (SEM, KYKY, EM 3200), XRD (Bruker AXS D_2 Phaser) and AFM (SPM-AA3000).

Synthesis methods (Sol-Gel Method)

The sol-gel technique was used for zinc salt reduction. The solution of [$ZnSO_4.7H_2O$] at a concentration of 0.5 M was reduced by using 1 M sodium hydroxide solution with a percent (1:2). The reducing agent was added dropwise under constant stirring for 30 min. At room temperature. After the complete addition of NaOH, the mixture was left on a magnetic stirrer for 12 hours, then drying at 70 °C, and the product was kept overnight, the result white powder grind and calcinated at 500 °C for 3 hours by muffle furnace. (Al-Taweel and Saud, 2016; Ewaid and Abed, 2017).

Methods of characterization

The ZnO - NPs, was characterized by the FT-IR; in the region for wavenumber 4000-400 cm^{-1}(Shimadzu FT-IR 8400s, Japan, KBr disc).

ZnO - NPs were analyzed and recorded using XRD (Bruker AXS Gmbh, Germany /D2 Phase) with CuK_α emission (0.1504nm), and the pattern was recorded from 20 to 70°. The average crystallite size of ZnO - NPs (D) was calculated from the Debye-Scherrer equation, as appear in Equation (1), (Mayekar *et al.*, 2014).

$$D = \frac{0.9}{\beta \cos\theta} \quad (1)$$

Where λ is the wavelength of the incident beam (1.5406 Å), D is mean for size crystallite, θ is scattering angle in degrees and β is full width at half-maximum (FWHM) in radians.

The morphological feature of the ZnO - NPs surface was determined using Scanning Electron Microscopy (SEM, KYKY, EM3200, China) with a speed up voltage of 25kV. The coarseness of the ZnO - NPs surface and the statistical data related to surface morphology was found by angstrom AFM (SPM-AA3000, USA).

Antibacterial activity test

Zinc oxide nanoparticles were tested for their antibacterial activity against each of the following pathogenic species (obtained from different clinical infections): Escherichia coli, Klebsiella pneumonia, and Staphylococcus aureus by well diffusion

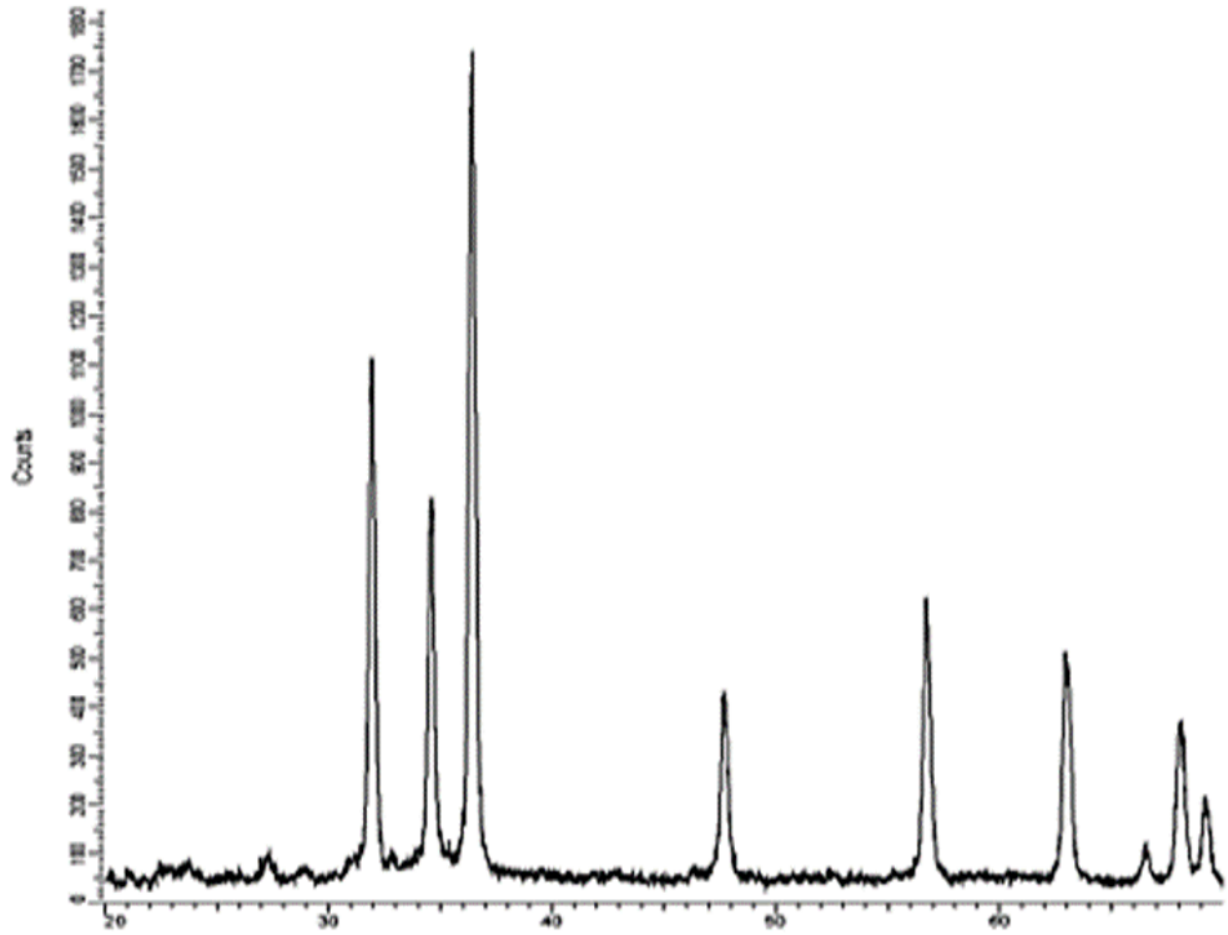


Figure 1: The XRD pattern of prepared ZnO - NPs

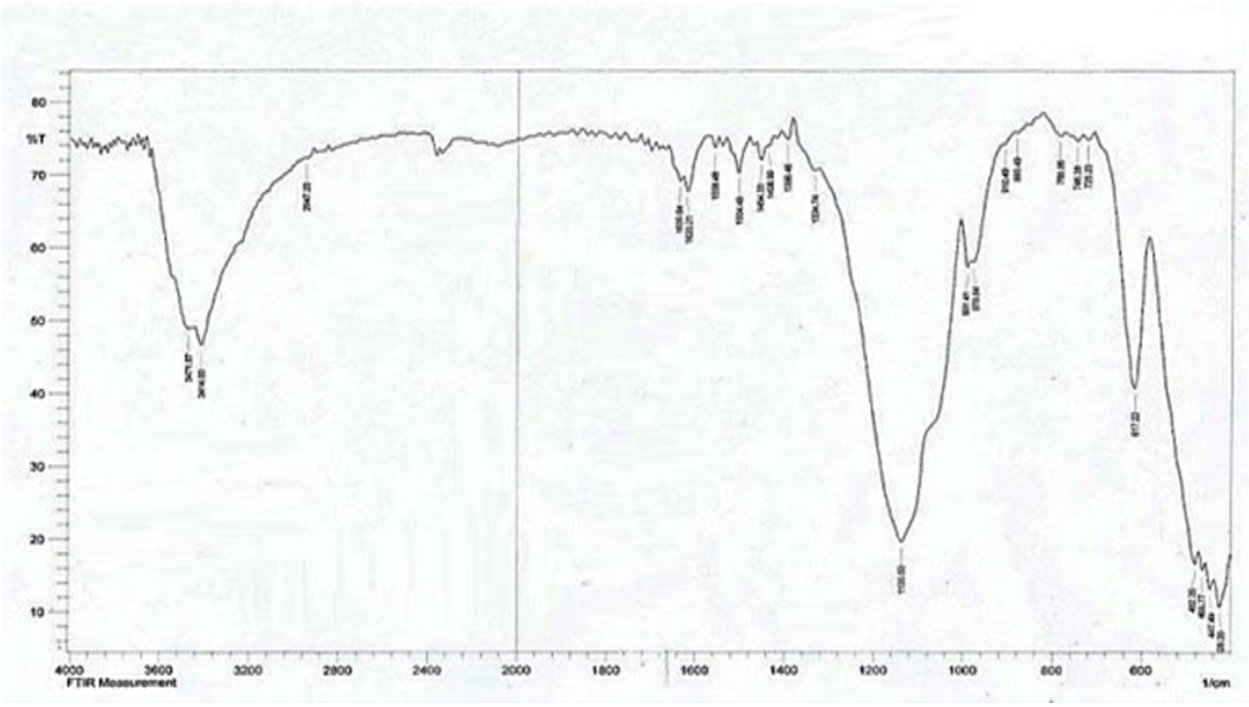


Figure 2: FT - IR spectrum of ZnO - NPs

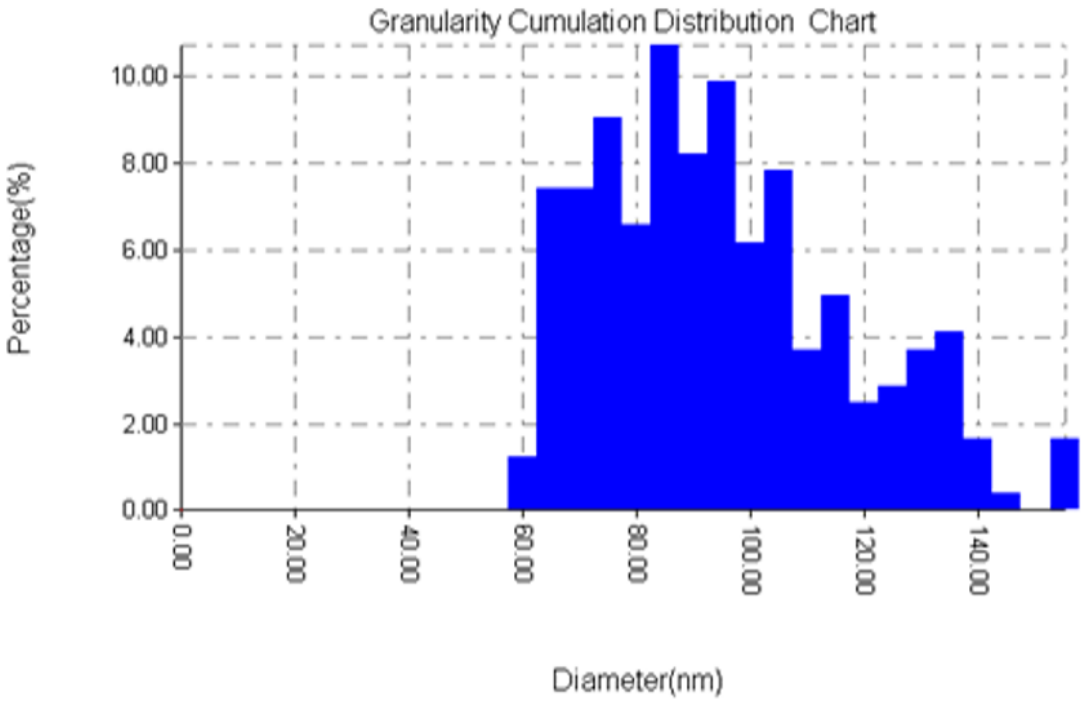


Figure 3: The granularity distribution of ZnO – NPs

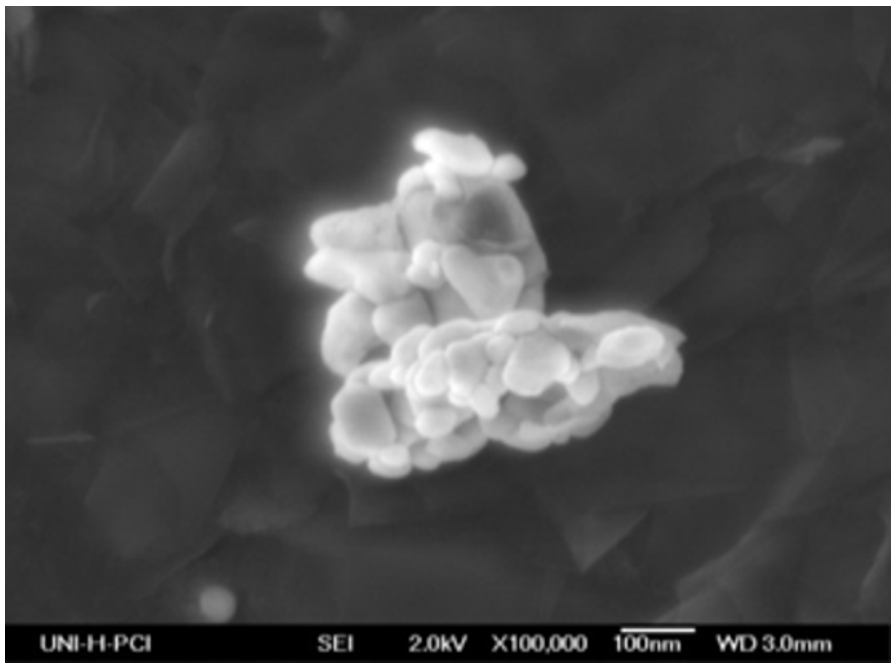
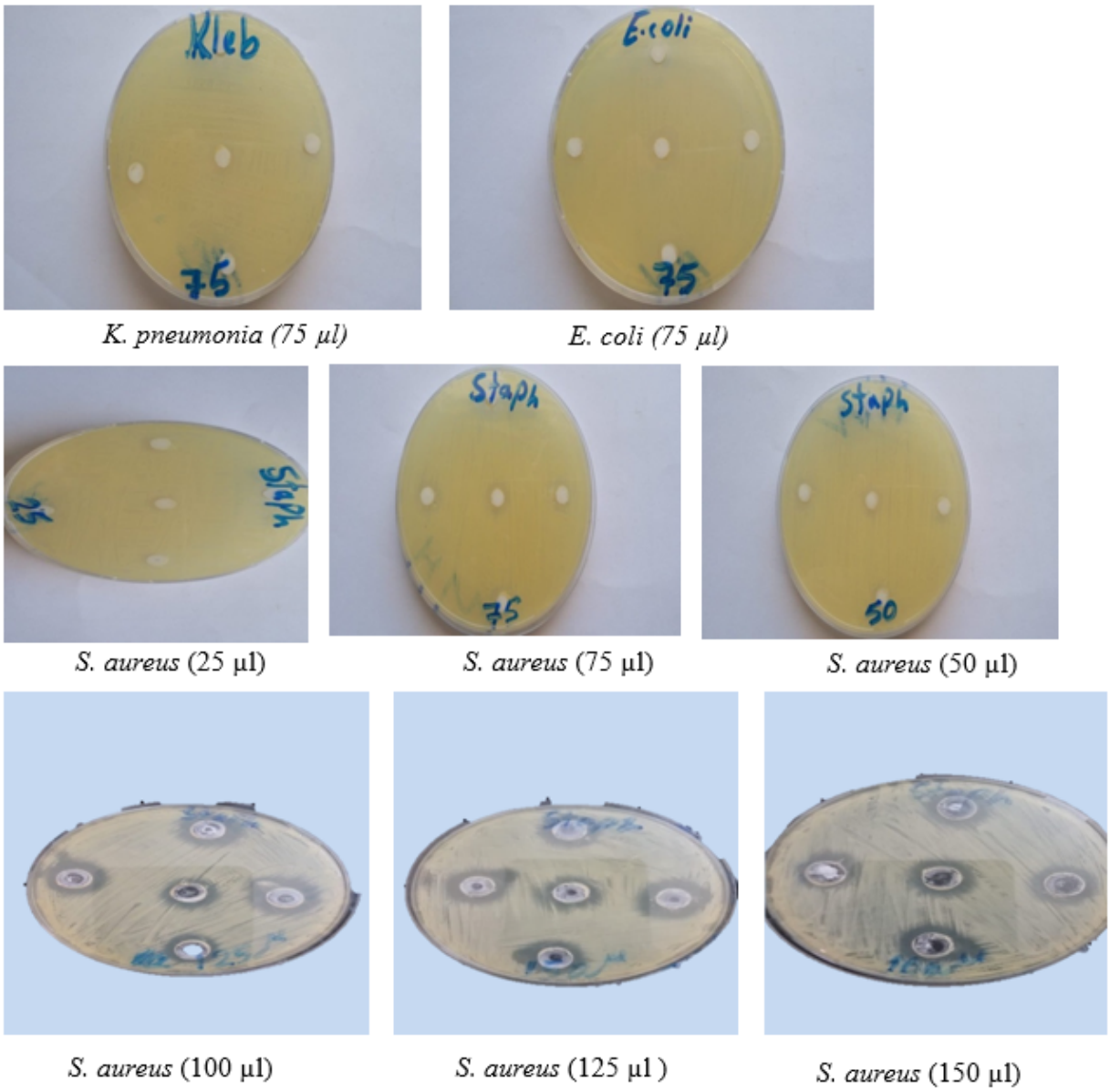


Figure 4: The SEM image of prepared ZnO – NPs

assay. The unpolluted cultures of the bacteria were obtained by subculturing them on Muller-Hinton broth at 35°C on a rotary shaker at 200rpm. Bacterial suspension of 0.5 McFarland density obtained from overnight cultures were swabbed uniformly on Muller-Hinton agar plates. Wells of size 6mm have been made on agar plates. Volumes 25μl, 50μl, 75μl, 100μl, 125μl, and 150μl of 10 mg\ ml concentration of nanoparticles solution were poured into wells of plates, and dishes were incubated for 18-24h at 35°C. The antimicrobial activity was measured by quantifying the diameters of growth inhibition regions in mm unit (Prakoso and Saleh, 2012; Sawai, 2003).

RESULTS AND DISCUSSION

XRD patterns of ZnO - NPs



K. pneumonia (75 µl) *E. coli (75 µl)*

S. aureus (25 µl) *S. aureus* (75 µl) *S. aureus* (50 µl)

S. aureus (100 µl) *S. aureus* (125 µl) *S. aureus* (150 µl)

Figure 5: The growth of *E.coli* and *K. pneumonia*

The XRD pattern of prepared ZnO - NPs in the range of 2θ= 20^o-70^o was appeared in Figure 1, for all that appeared Diffraction Peaks were indexed to the peaks of zinc oxide wurtzite structure (standard JCPDS Data -Card No 36-1415). The diffraction peaks at the angles 2θ equal to 31.8^o, 34.5^o, 36.8^o, 47.7^o, 56.7^o, 62.9^o, 68^o, which are corresponding to the reflection of crystal planes of wurtzite structure of ZnO, (100), (002), (101), (102), (110), (103), (112), respectively (Abdullah *et al.*, 2017). There's no other peaks appear in the x-ray pattern for another material than ZnO particles. The plane (101) was used to calculate the crystal size of ZnO - NPs, and it was found 16.36nm.

FT - IR spectrum for ZnO - NPs

Table 1: The inhibition zones (in millimeters) of bacterial species by ZnO- NPs

S.aureus	*K.pneumonia*	*E.coli*	*Volume (µl)*
8			25
9			50
10	10	15	75
18			100
19			125
20			150

The FT - IR spectrum for ZnO - NPs at 4000-400 cm^{-1}(Figure 2), shows main absorption peaks at

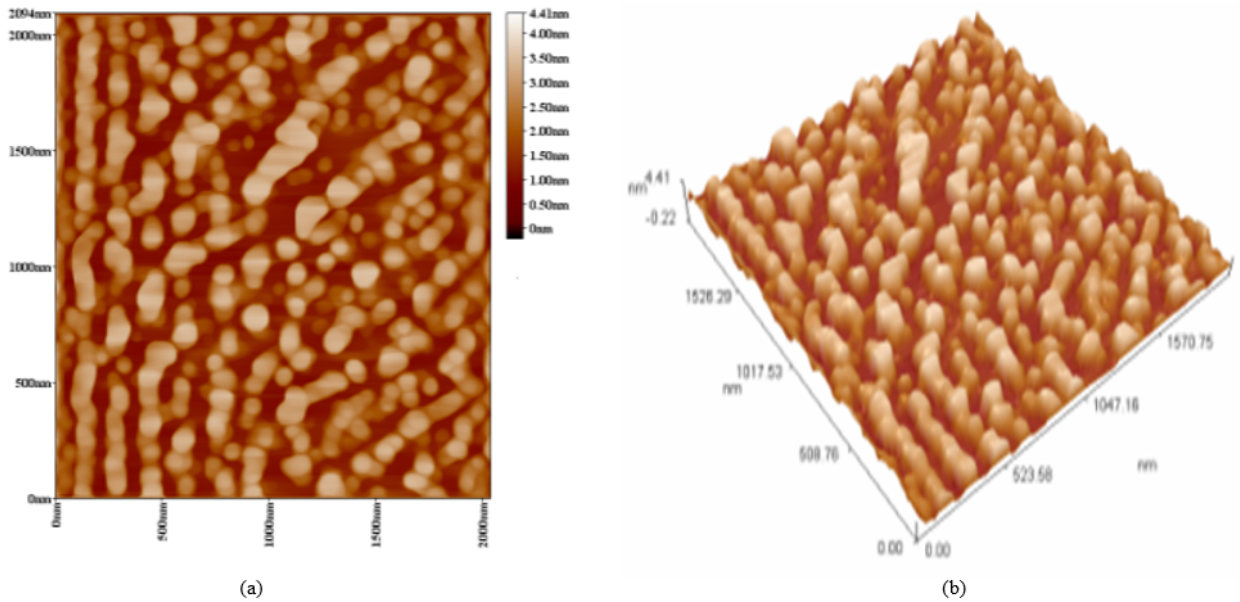


Figure 6: (a) and (b) shows, The 3D image of AFM analysis of ZnO– NPs

3414 cm^{-1} ,1635 cm^{-1} ,1620 cm^{-1} , 452-428 cm^{-1} . The peaks positioned around the range of 452-428 cm^{-1} are attributed to the stretch. Vib. Mode of Zn – O bond in ZnO. The broad pands at 3414 $cm^{-1}$and 1635 Cm^{-1} refer to the O-H stretching and bending vibration modes in adsorbed water. The other appeared peaks in the FTIR chart maybe refer to the adsorbed CO_2 from the atmosphere. (Ewaid and Al-Ansari, 2019; Ewaid *et al.*, 2019).

AFM Study of ZnO - NPs

The topography of surface for the prepared ZnO-NPs was investigated by AFM spectroscopy, the 3D images and the granularity cumulation distribution chart of ZnO - NPs were shown in Figure 6 (a) and Figure 6 (b) The 3D images show that particles of ZnO arranged in approximately uniform form and tend to agglomerate in larger particle size with an average diameter of particles, as appear from the granularity distribution chart, 92.8 nm. The roughness means, Sa Root average square (Figure 3).

Sq surface skewness Ssk, and surface kurtosis Sku were also calculated and found 0.655nm, 0.748nm, 0.125nm, and 1.74, respectively.

The SEM Study for ZnO - NPs

The SEM picture for ZnO - NPs Figure 4 appear it's surface morphology and which indicate that the most ZnO – NPs appear approximately in a spherical shape. In addition, the ZnO – NPs agglomeration was also indicated. The average particle size of ZnO - NPs is nearly 37 nm.

Antibacterial activity of ZnO - NPs

Antimicrobial activity of results showed that ability of ZnO - NPs to inhibit the outgrowth of S.aureus increased with the increasing of solution volume (Table 1), while that the outgrowth of E.coli and K. pneumonia was inhibited only with the volume of 75μl where the inhibition zones diameters were 15mm and 10mm respectively (Figure 5).

Gram-positive bacteria reveal a high susceptibility to different concentrations of nanoparticles in comparison with Gram-negative species, as it had been reported previously (Ewaid *et al.*, 2020). Two mechanisms was proposed for that the antimicrobial activity for ZnO - NPs :- the first one is the generation of reactivity O2 species (including H_2O_2, OH^- and $O_2{}^{-2}$), and the second is the induction of apoptosis (Pal *et al.*, 2018).

The NPs powder are actually disbursed in the solution of media and not dissolved. Thus they can't release the cations of Zn^{+2}. This may explain why the growth *of E. coli* and *Klebsiella pneumoniae* did not inhibit by some volumes of ZnO - NPs solution. The antibacterial activity of ZnO - NPs is influenced by the size of these nanoparticles, in addition to the concentration, where the smaller size particles have the larger surface area thus can easily penetrate into a bacterial membrane. Many studies shown that ZnO – NPs have good selective toxicity to the bacteria, but it exhibits minimal effects on the human cell (Siddiqi *et al.*, 2018).

CONCLUSIONS

The XRD, FTIR, AFM, and SEM analysis of prepared ZnO - NPs by sol-gel method show that prepared sample have wurtzite structure and approxi-

mately spherical shape with some agglomeration as appearing from SEM image, the particle size of the ZnO - NPs is approximately 37 nm. The antimicrobial activity of ZnO - NPs against bacteria, show the ability of ZnO - NPs to inhibit the growth of *S. aureus* increased as the solution volume of prepared ZnO – NPs increased, while the outgrowth of *E.coli* and *K. pneumonia* was inhibited only with the volume 75μl, and the inhibition zone diameters are15mm and 10mm respectively.

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