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 25 µl, 50 µl, 75 µl, 100 µl, 125 µl, and 150 µl 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 75 µl where the inhibition zones diameters were 15 mm and 10 mm respectively.

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 and , 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 meV. and 3.37 meV. 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 (Bhat-tacharyya and Gedanken, 2008).

Deferent techniques such as nano-lithography, physical vapor deposition PVD, chemical vapor deposition CVD, sol-gel method, spray conversion pro-
cessing 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 agglomeration 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, 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-Gel method. The chemical reagents used in this search were zinc sulphate heptahydrate [ZnSO$_4$.7H$_2$O] 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$_2$O] 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 calcinataed 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$_a$ 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).

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

Where $\lambda$ is the wavelength of the incident beam (1.5406 Å), D is mean for size crystallite, $\theta$ is scattering angle in degrees and $\beta$ 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 assay. The unpolulated cultures of the bacteria were obtained by subculturing them on Muller-Hinton media.
Figure 1: The XRD pattern of prepared ZnO - NPs

Figure 2: FT - IR spectrum of ZnO - NPs
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

The XRD pattern of prepared ZnO - NPs in the range of 2θ= 20°-70° 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 2\( \theta \) equal to 31.8\( ^\circ \), 34.5\( ^\circ \), 36.8\( ^\circ \), 47.7\( ^\circ \), 56.7\( ^\circ \), 62.9\( ^\circ \), 68\( ^\circ \), 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**

The FT - IR spectrum for ZnO - NPs at 4000-400 cm\(^{-1}\) (Figure 2), shows main absorption peaks at 3414 cm\(^{-1}\), 1635 cm\(^{-1}\), 1620 cm\(^{-1}\), 452-428 cm\(^{-1}\). The peaks positioned around the range of 452-428
Figure 6: (a) and (b) shows, The 3D image of AFM analysis of ZnO– NPs

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 AAnsari, 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$_2$O$_2$, OH$^-$ and O$_2$), 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 approximately 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 75 μl, and the inhibition zone diameters are 15 mm and 10 mm respectively.

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