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Research article

Zinc Oxide nanoparticles formation, characterization and biological approach

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Key words: Zinc oxide nanoparticles, Antimicrobial activity, Minimum inhibitory concentration (MIC), Characterization analysis.

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Abstract

In the present work we used two chemical methods for the synthesis of zinc oxide nanoparticles (ZnONPs). The synthesized ZnONPs was detected by precipitation methods and characterized using UV-vis spectroscopy and Transmission electron microscopy (TEM) to determine nanoparticles size and shape. The antimicrobial activity and minimum inhibitory concentration (MIC) of ZnONPs were carried out as biological approach. Our results showed that, the three concentrations of ZnO powder (0.1, 0.5 and 1.0 %) and 0.1 M $ZnSO_4$ synthesized ZnONPs are recorded as antimicrobial potential activity against tested pathogenic strains models (Gram positive, Gram negative and filamentous fungi). Characterization of ZnONPs revealed that, it was absorbed at rang of 373 to 374 nm. Also, the resulted showed that, ZnO nanoparticle from zinc powder at concentration of (0.1 %) had average size 15-42 nm while, other from zinc sulfate powder had average size 17-97 nm.

Introduction

For a long time, the antimicrobial medications have been utilized to restrain or kill microorganisms. However, microbial resistance to these drugs has developed on a very large scale over time, greatly reducing their effectiveness and is an ever growing problem [1]. A study says that, the drug resistant infections will kill an extra 10 million people a year worldwide - more than currently die from cancer by 2050 unless action is taken. Therefore, one of the most promising strategies for overcoming microbial resistance is the use of nanoparticles. Nanotechnology gave the solution to medicine because it has the ability to find materials in nanoscale diameter that have an enhanced bioactivity [2]. The main reason for their importance is the increased specific surface area of these nanoparticles in comparison to their volume, which enables their interaction with bio-organics present on the viable cell surface [3]. One of the famous nanoparticles is zinc oxide nanoparticle which is one of metal oxide nanoparticles. Zinc oxide (ZnO) is a polar inorganic compound. It appears as a white powder, nearly insoluble in water with many applications, such as antimicrobial, wound healing, UV filtering properties, high catalytic and photochemical activity, due to its unique combination of interesting properties such as non-toxicity, good electrical, optical and piezoelectric behavior, stability in a hydrogen plasma atmosphere and low price [4]. In the present work, the synthesis ZnO nanoparticles (ZnONPs) by two different methods were done, and the resulted nanoparticles were characterized using UV-vis spectroscopy and transmission electron microscopy (TEM). On the other hand, the antimicrobial activity and minimum inhibitory concentration (MIC) were also determined.

Experimental

Preparation of ZnO nanoparticles (ZnONPs) from zinc oxide powder

The preparation was occurred according to [5] with some modification. Different concentrations of zinc oxide powder (0.1, 0.5 and 1.0 g) were dissolved in 100 ml of 1% acetic acid for each one where it changed to zinc cations. Then the mixtures were sonicated for 30 min. After that, a magnetic stirring was used to add 1M NaOH drop by drop until the solution attained pH 10 and white precipitate was formed. The solutions were heated in water bath at (40–80°C) for about 3 hours. Then, they were centrifuged at 5000 rpm for 5 min and washed with distilled water several times then dried in an oven at 50°C for one hour and kept for further use

Preparation of ZnO nanoparticles (ZnONPs) from zinc sulphate powder

Zinc oxide nanoparticles (ZnONPs) were prepared according to [6] by wet chemical method with some modification, using zinc sulphate powder with sodium hydroxide as precursors and soluble exopolysaccharides (EPS) as stabilizing agent. This soluble exopolysaccharides (EPS) produced form *Lactobacillus sp* as mentioned before [7]. EPS 0.1% was dissolved in 500 ml of distilled water by using microwave oven. Then, 0.1 M of zinc sulphate was added to the above solution under continuous stirring to completely dissolving the zinc sulphate. After that, 0.2 M of sodium hydroxide solution was added drop by drop under continuous stirring until resulting in a white solution. The reaction was allowed to proceed for 2 hours after complete addition of sodium hydroxide then allowed to settle overnight. After that, the supernatant solution was the discarded carefully and the white precipitate was kept. The remaining white precipitate was washed three times by using distilled water, centrifuged at 5000 rpm for 5 min. Finally, the white precipitate was dried at 50°C and kept for further use.

Test microorganisms

The antimicrobial activity was done using various pathogenic microorganisms such as *Escherichia coli NCTC 10416* as models for Gram-negative bacteria; *Staphylococcus aureus ATCC 29213* as models for Gram-positive bacteria, *Aspergillus niger NRRL-363* as models for filamentous fungi.

Antimicrobial activity media

The media used for the antimicrobial activity of the strains under study have the following compositions (g/l):- Nutrient agar medium: - D-glucose, 5.0; peptone, 5.0; meat extract, 5.0; NaCl, 5.0 and agar, 20.0; the pH was adjusted to 7 [8]. Used for growth of bacterial strains and Czapek-Dox agar medium: - sucrose, 20.0; $NaNO_3$, 2.0; K_2HPO_4, 1.0; KCl, 0.5; $MgSO_4$. $7H_2O$, 0.001 and agar, 20.0; the pH was adjusted to 7 [9]. Used for growth of Filamentous Fungi.

Antimicrobial potential assay

The antimicrobial activity of (ZnONPs) was evaluated by the disc diffusion method by using the above test organisms. Samples were form into disc shapes of 5.0 mm in diameter, dried and subjected to UV sterilized for 2 hours. Then, they were placed on the surface of agar plates freshly inoculated with the test microorganisms. The petri-dishes were kept in a refrigerator for one hour to permit homogenous diffusion of the antimicrobial agent before growth of the test microorganisms and then plates were incubated at 37°C for 24 hours for Gram positive and Gram negative bacteria and at 28°C for 72 hours for filamentous fungi. The appearance of a clear inhibition zone around the sample in the inoculated petri-dishes is an indication of the antimicrobial activity [10].

Minimum inhibitory concentration test (MIC)

The minimum inhibitory concentration is the highest dilution which fails to show growth. In this test, the five double fold dilutions were prepared for each sample from a stock solution of 0.1 g/ml Zinc Oxide Nanoparticles. The nutrient agar medium was inoculated by 0.1 ml of the microbial culture spore suspension, poured in Petri- dishes and left to solidify. Wells of 9 mm diameter were made. Each well was inoculated with 0.1 ml from each of the sample dilutions separately. Dishes were incubated at 37°C for 48 hours and the inhibition zones produced were measured in mill meter.

Characterization of Zinc Oxide Nanoparticles

Transmission Electron microscopy (TEM)

This study was undertaken to know the size and shape of Zinc oxide nanoparticles. The TEM image was carried out using: Electron probe micro-analyzer JEOL – JXA 840A, Model Japan. Thin films of the sample were prepared on a coated copper grid by just placing a very small amount of the sample on the grid. Then the film on the TEM grid was allowed to dry and the images of nanoparticles were taken.

Ultraviolet (UV) spectrum

The UV spectrum analysis was carried out using: T80+UV/VIS Spectrometer, PG Instrument Ltd. Range: 190-1000 nm.

Results and Discussion

Antimicrobial activity

The disc diffusion experiment was carried out against *Escherichia coli NCTC 10416*, *Staphylococcus aureus ATCC 29213* and *Aspergillus niger NRRL-363*. The effect of different concentrations of zinc oxide nanoparticles (ZnONPs), which processed from zinc oxide powder, on the growth of the tested microorganisms mention above was illustrated in Table (1). The resulted proved that, all concentrations of zinc oxide nanoparticals (ZnONPs) have antimicrobial activity against the pathogenic tested strains under study. The zone of inhibition was decreased at the increased concentration of ZnO nanoparticles and the maximum inhibition of growth was obtained at conc. of 0.1 % which recorded 10, 20 and 22 mm of inhibition zone diameter for *Escherichia coli NCTC 10416*, *Staphylococcus aureus ATCC 29213* and *Aspergillus niger NRRL-363* respectively. On the other hand, the antimicrobial activity of zinc oxide nanoparticles (ZnONPs) which, processed from zinc sulphate powder was tested using the same pathogen strains mention before. The resulted showed that, *Escherichia coli NCTC 10416* as models of Gram negative bacteria give negative results. While, *Staphylococcus aureus*

ATCC 29213 as models of Gram positive bacteria and *Aspergillus niger NRRL-363* as models of Filamentous Fungi give positive results of 20 mm of inhibition zone diameter for each (Table 1).

Several researchers discussed the effect of zinc nanoparticals on the microorganisms and proved that, the activity of ZnO nanoparticals against the pathogen was due to a response of the surface of ZnO nanoparticals with water which led to formation of hoisted levels of receptive oxygen species, to be specific hydroxyl radicals and thus actuate as oxidative anxiety. Also, a presentation of microorganisms with ZnO nanoparticles results in an expanded cell disguise of the nanoparticles and microbial cell harm [11].

Minimum inhibitory concentration test (MIC)

The MIC test was carried out for each of the tested concentration of ZnO nanoparticles separately against the bacterial pathogen *Staphylococcus aureus ATCC 29213* and results demonstrated that: all tested ZnO nanoparticles were effective against tested bacterial pathogen as stock solutions (100%). Measuring the inhibition zones of tested bacterial pathogen when different concentrations of the ZnO nanoparticles were used showed that, the ZnO nanoparticles had an antibacterial effect expressed by the inhibition zones for ZnONPs (0.1%) and ZnONPs (0.5%) till a dilution of 50%. The inhibition zones for ZnONPs (1.0 %) and processed from zinc sulphate till a dilution of 25% (Table 2).

Several scientists investigated the effect of ZnONPs on different microorganisms and they proved that, the size of nanoparticals is very small, so it is easy to enter into microbial membrane and enables inhibition mechanisms to occur inside the cell. Also, ZnONPs produced H_2O_2 which interact chemically with lipid bilayers and membrane proteins [12]. On the other hand, the antimicrobial activity of these NPs contains both the aggregation of NPs in the cytoplasm on the outer membranes and the production of reactive oxygen species (ROS). ROS makes cell death by oxidizing the membrane lipids and membrane dysfunction [13,14].

Characterization of ZnO nanoparticals (ZnONPs)

Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) image shows as illustrated at Figure (1) ZnO nanoparticles (ZnONPs) which processed from zinc oxide powder at conc. of (0.1 %) with average size of 15.10-42.16 nm. While, Figure (2) showed that, the TEM of ZnO nanoparticles (ZnONPs) which processed from zinc sulfate powder with average size of 17.69-97.28 nm. It is obviously that nanoparticles have a larger grain size, uniform shape and polycrystalline in nature.

Ultraviolet spectrum (UV) of ZnO nanoparticals (ZnONPs)

This technique was used to demonstrate the presence of zinc oxide nanoparticals which produced either from zinc oxide powder or zinc sulphate powder. As illustrated in Figure (3), absorption peak of 373 nm was detected to confirm the presences of zinc oxide nanoparticals which processed from zinc sulphate powder. Also, zinc oxide nanoparticals which processed from zinc oxide powder at different concentrations showed nearly the same peak at 373-374 nm as illustrated in Figure 4.

Table 1. The antimicrobial activity of free ZnO nanoparticles processed from zinc oxide and zinc sulphate powders against tested pathogenic strains

	Tested pathogen organisms	Inhibition zone (mm)			
		Different Concs. of (ZnONPs) from zinc oxide powder (mm)			ZnONPs from zinc sulphate powder
		0.1 %	0.5 %	1.0 %	0.1 M
Grams negative	*Escherichia coli NCTC 10416*	10	10	10	-
Grams positive	*Staphylococcus aureus ATCC 29213*	20	15	15	20
Filamentous Fungi	*Aspergillus niger NRRL-363*	22	19	15	20

(-) mean negative results and disc diameter 5 mm

Table 2. The MIC of free ZnO nanoparticles prepeard from zinc oxide and zinc sulphate powders against *Staphylococcus aureus ATCC 29213*

	Different concentrations of ZnONPs %	Antimicrobial activity (mm)				
		100	50	25	12.5	6.25
ZnONPs from zinc oxide powder	0.1	23	20	0	0	0
	0.5	23	20	0	0	0
	1.0	28	25	20	0	0
ZnONPs from zinc sulphate powder	0.1 M	22	22	20	0	0

Well diameter = 9mm

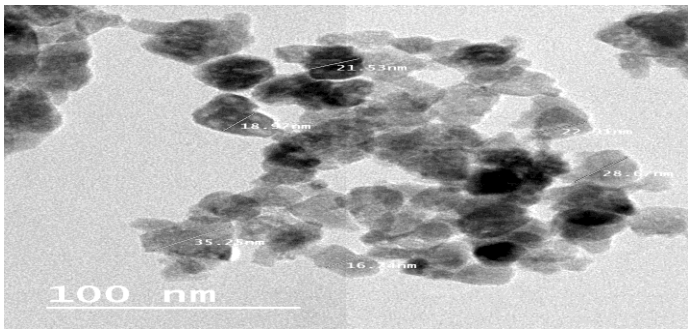

Figure 1. TEM of zinc oxid nanoparticals processed from zinc oxide powder

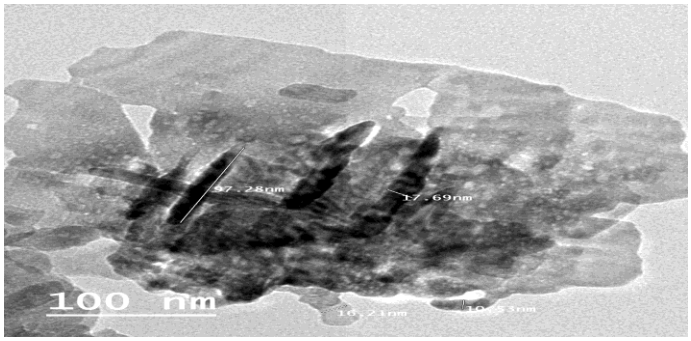

Figure 2. TEM of zinc oxid nanoparticals processed from zinc sulphate powder

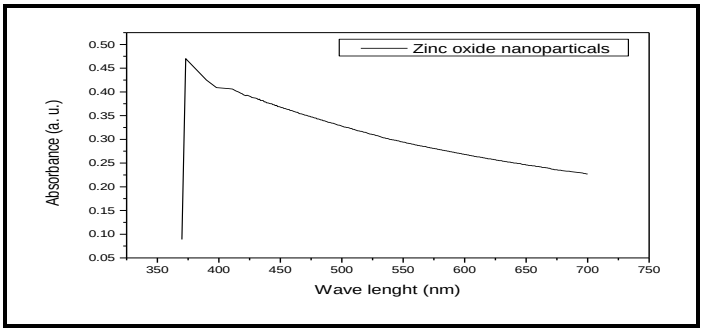

Figure 3. UV-spectrum of zinc oxide nanoparticals processed from zinc sulphate powder

The ZnONPs from two compounds (zinc oxide and zinc sulphate powder) were compared and results indicated that ZnONPs from zinc oxide was smaller than ZnONPs from zinc sulphate powder, but the ZnONPs from zinc sulphate powder was more active against pathogenic test organisms. In the last we can recommended the ZnONPs from zinc oxide in case of pharmaceutical industry.

Conclusion

The synthesis and characterization of ZnONPs from two compounds (zinc oxide and zinc sulphate powder) by precipitation methods was investigated. ZnONPs were characterized using UV-vis spectrophotometer at different concentrations and it gives the same absorption peak at 374 nm. Also, the Transmission Electron Microscopy (TEM) illustrated that, the nanoparticles have a larger grain size, uniform shape and polycrystalline in nature, with average

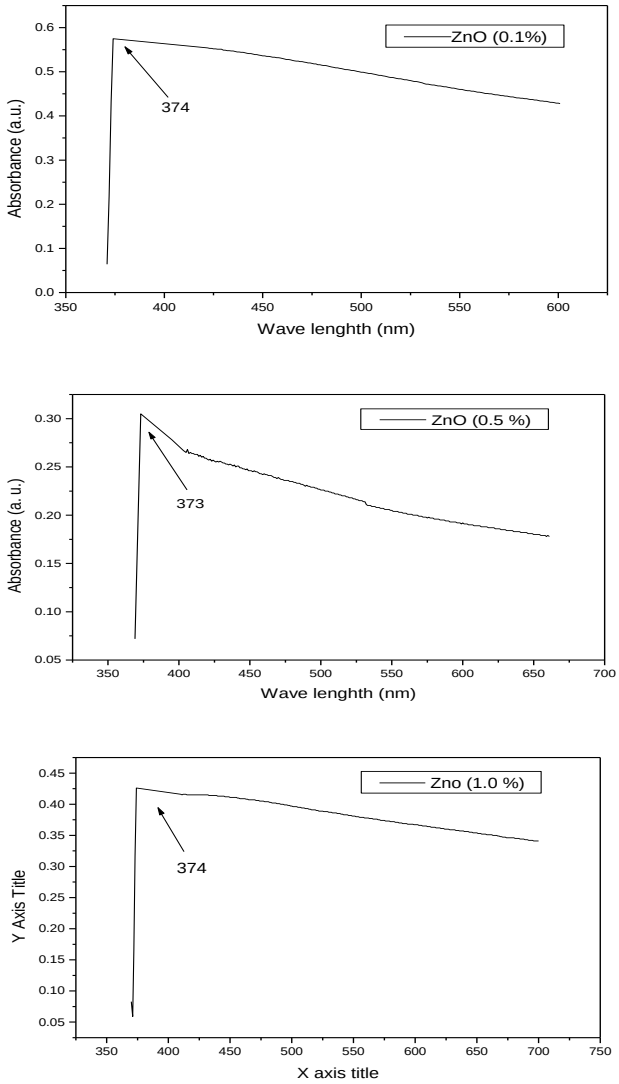

Figure 4. UV-spectrum of zinc oxide nanoparticals processed from zinc oxide powder.

size of 15.10-42.16 nm for ZnONPs which processed from zinc oxide powder while, ZnONPs which processed from zinc sulphate powder have average size of 17.69-97.28 nm. On the other hand, the results illustrated that, the ZnONPs have antimicrobial activity against the tested pathogens. The minimum inhibitory concentrations were 50% for concentrations of 0.1 and 0.5% and were 25% for concentrations 1.0 and zinc sulphate powder.

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