Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)
ISSN: 2349-2759
Available online at www.jipbs.com

Research article

Antibacterial effect of photo-activated zinc oxide nanoparticles capped with different polymers

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Key words: ZnO nanoparticles, PVP, X-ray diffraction, TEM, Bacillus.

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Abstract

In this paper, three types of zinc oxide nanoparticles (ZnO NPs) were prepared by a wet chemical method (precipitation method), the first type was ZnO NPs capped with Polyethylene Glycol (ZnONPs@PEG) and other ZnO NPs were capped with Polyvinylpyrroli-done (ZnO NPs@PVP), and the last type of ZnO NPs was provided without polymer. The samples were characterized via X-ray diffraction (XRD). The average crystal size and shape of the prepared ZnO nanopowder were determined by Transmission Electron Microscope (TEM). The antibacterial activity of the three types of ZnO NPs were tested against four types of bacteria that were *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus*, All ZnO NPs were photo-activated under the exposure to UV light at 254 nm before being applied against the four types of bacteria. The antibacterial activity of ZnO NPs was determined based on the appearance of zones of inhibition and the standard deviation was calculated for all appeared inhibition zones. The results obtained from TEM imaging revealed the formation of spherical, rods and nano flowers shapes for uncapped ZnO NPs, ZnONPs@PEG and ZnO NPs@PVP respectively. Also, it was found that the *Bacillus subtilis* inhabited using ZnONPs@PEG more than using the other types of ZnO NP.

Introduction

Resistant organisms including several microorganisms such as bacteria and viruses, in addition to parasites, they have the ability to survive attack produced by antimicrobial medicines, for example antibiotics, antivirals, and antimalarial, therefore traditional treatments become ineffective, useless and infections can be preserved and may spread easily to others. Antimicrobial resistance has an importance meaning of the use, mainly the waste of antimicrobial medicines and develops when a microorganism transforms or obtains a resistance gene. So we have to achieve and attainment of modern medicines [1] has been incorporated and integrated into the coatings of food cans, in packages for meat, fish, corn, and peas to protect colors and to inhibit spoilage due to its antimicrobial properties [2]. Nanotechnology tools are improving our understanding of how bacteria work, though providing new prospects to analyze the dynamic and physical features of molecules, molecular assemblies, and intact microbial cells, either through isolation or under *in vivo* settings [3]. As the complexity reduction of bacterial compared to eukaryotic cells, Nanotechnology tools applied to microbiology are possible to have a major influence on the developing fields of proteomics and systems biology also used to recognize and understand the complexity of biology. Through designing novel and new synthetic materials that will be applicable and suitable to non-biological systems[4].

ZnO nano-sized particles have more noticeable antimicrobial activities than large particles, since the small size approximately less than 100 nm and high surface to volume ratio of nanoparticles let for better interaction with bacteria. Current studies have exposed that these nanoparticles have selective toxicity to bacteria but display minimal effects on human cells, [5]. Zinc Oxide nanoparticles (ZnO NPs) have been presented to have a wide range of antibacterial activities against both Gram-positive and Gram-negative bacteria, including major foodborne pathogens such as *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*, and *Staphylococcus aureus* [6]. It is necessary to understand the mechanism of action of ZnO nanoparticles against bacteria in purpose to make better and improved use of ZnO nanoparticles in food products and other applications and to support the development of powerful, but nontoxic, antimicrobial derivatives, but up to now, the process underlying their antibacterial effect is not clear. Though, some studies have proposed that the primary cause of the antibacterial function might be from the disruption or interruption of the cell membrane activity [7]. It has similarly been

reported that (ZnO) can be stimulated and activated by UV and visible light to generate highly reactive oxygen species. The negatively charged hydroxyl radicals and super-oxides cannot enter into the cell membrane and are expected to remain on the cell surface, whereas H_2O_2 can penetrate and enter into bacterial cells [8]. The main aim of this research was to evaluate the antibacterial activity of ZnO nanoparticles either capped with different polymers (PEG) and (PVP) or uncapped ZnO nanoparticles.

Materials and methods

Chemicals and Substrates

All chemicals were reagent grade and were of highest available purity (A.P.); deionized water was used to prepare all the solutions. All glassware was thoroughly cleaned with aqua regia and rinsed with deionized water prior to use. Zinc acetate, Ammonium carbonate, Polyethylene Glycol with average molecular weight = 22,000, Polyvinylpyrrolidone (PVP) with average molecular weight = 22,000, Ammonia solution, Ethanol (95%) were purchased from Sigma-Aldrich. The prepared powder ZnO nanoparticles (PEG capped and PVP capped) were characterized to confirm the formation of ZnO nanoparticles by using X-ray diffractometer (SHIMADZU-MODEL XRD 6000), Cairo, Egypt. Particle shape and size measurements in this work were determined using the transmission electron microscopy (HR-TEM). The TEM images were carried out in Nanotech Company for photo-electronic, Dreamland, 6-October, Egypt. The HR-TEM is JOEL JEM-2100 operating at 200 kV equipped with Gatan digital camera Erlangshen ES500.

Bacterial strains and media

The following bacterial strains were used in this study: (collected, isolated and identified at Microbial Genetic Department, Genetic Engineering and Biotechnology, National Research Center, Giza, Egypt). Gram-positive *S. aureusBacillus subtilis* 168; and Gram-negative *E. coli* K12, All these strains were grown in tryptic soy broth (TSB) except for *E. coli*,*Pseudomonas aeruginos* and *B. subtilis* strains, which were grown in Luria–Bertani (LB) medium. All strains were grown aerobically at 37°C, in 10 mL of medium in 18 mm × 150 mm borosilicate glass culture tubes (Fisher Scientific) with shaking at 200 r.p.m. under normal laboratory lighting conditions unless specified.

Experimental

Synthesis of ZnO NPs@PEG, ZnO NPs@PVP and ZnO NPs

Powder PEG capped Zinc oxide nanoparticles were synthesized by precipitation of the surfactant solution (PEG 5%), 5 gm of (PEG) was poured into a conical flask (500ml), and then 2.1 gm of zinc acetate was dissolved in 100 ml of distilled water (H_2O) in order to make zinc acetate solution and then 0.96 gm of ammonium carbonate was dissolved in another 100ml of distilled H_2O to make ammonium carbonate solution, after that both of them were added into the conical flask containing (PEG) solution using droppers in order to be added through a slowly action drop by drop at the same time with dynamic stirring. After the process of dropping was finished, the produced suspension was kept under stirring for 2 hours at room temperature, precipitate was filtered using filtration system and washed with ammonia solution and ethanol with concentration of (95%) several times, and then the filtered was dried under vacuum for 15 minutes, after that it was calcinated using muffle furnace at 450°C for 3 hours. Then the powder of ZnO NPs@PEG was obtained.

The ZnO NPs@PVP was prepared by using the same method for ZnO NPs@PEG put 5 gm of (PVP) powder was used instead of PEG. On the other hand, ZnO NPs without polymers were synthesized by using the same method mentioned before in the synthesis of capped ZnO nanoparticles except the adding of polymers [9].

Characterization of ZnO NPs @ PEG, ZnO NPs @ PVP and ZnO NPs

X-ray diffraction (XRD) analysis

The crystalline size of pure and doped ZnO NPs has been determined from XRD spectra recorded using powder XRD (PAN-Analytic) set-up equipped with 3050/60 goniometer and Cu anode X-ray tube. The XRD scans for the powder samples were performed in the 2 (range 20-80A) keeping step size 0.001 for the Cu K X-ray radiation (λ D 1.5418 A) [10].

Transmission electron microscopy (TEM)

Formation and particle sizes of the synthesized materials were confirmed from TEM by placing a drop of the NPs dissolved in methanol on carbon coated grids and air drying. The TEM images were carried out in Nanotech Company for photo-electronic, Dreamland, 6-October, Egypt. The HR-TEM is JOEL JEM-2100 operating at 200 kV equipped with Gatan digital camera Erlangshen ES500 [10].

Photo-activation of Zinc Oxide nanoparticles by using ultra violet (UV) light

The 0.01g for each one of the three provided samples of ZnO nanoparticles, was weighted then dissolved in 10 ml of distilled water, and then the three samples were sonicated until a suspension solution of each sample was performed. After that, the samples were exposed to UV light for 1 hour at wavelength of 254 nm.

Antibacterial activity test of photo-activated ZnO nanoparticles

Antibacterial activity test of ZnO nanoparticles was achieved using well diffusion method. Antibacterial activities of the synthesized NPs were evaluated by the standard disc diffusion method described by Bauer, *et al.* [11] and modified according to clinical and laboratory standards institute guidelines. Four types of bacteria were cultured one day before the antibacterial activity test in order to be fresh at the time of incubation. The bacterial cultures were *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were inoculated on all agar plates by using sterile cotton swabs by moving it back and forth gently until the agar plate was completely inoculated by bacteria. After that by using sterile metal circles, wells were performed in all agar plates, approximately each plate was had 4 wells labeled by the name of ZnO nanoparticles that was added to formed wells. Then 100 µl of each sample of ZnO nanoparticles (photo-activated suspensions of ZnO NPs @ PVP, ZnO NPs @ PEG and ZnO NPs without polymer) was added to each well alone in all 8 inoculated agar plates. Each agar plate was labeled by the type of bacterial strain inoculated with. Finally, all petri-dishes were placed for incubation for 24 hours at 37°C.

Standard deviation calculation (std)

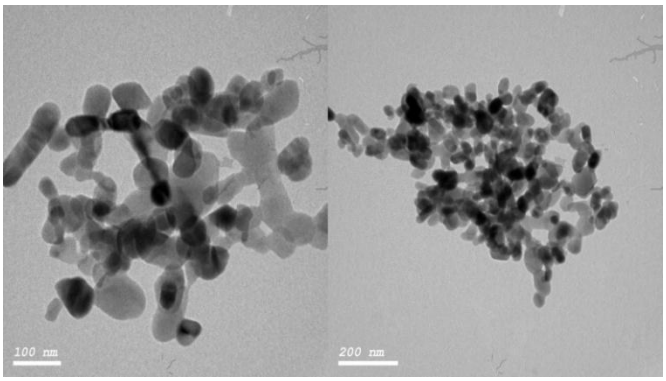
The standard deviation of inhibition zones was founded after incubation according to the positive effect of ZnO nanoparticles against bacterial strains. Standard deviation was calculated for each sample of ZnO nanoparticles. First the mean had to be calculated, the four dimensions for each inhibition zone were measured including the well using measuring ruler, and then the mean was calculated from the average of the values of four measured dimensions. Standard deviation was calculated by taking the square root of the total square differences dividing the number of dimensions minus one (n-1).

Results

Characterization of ZnO nanoparticle

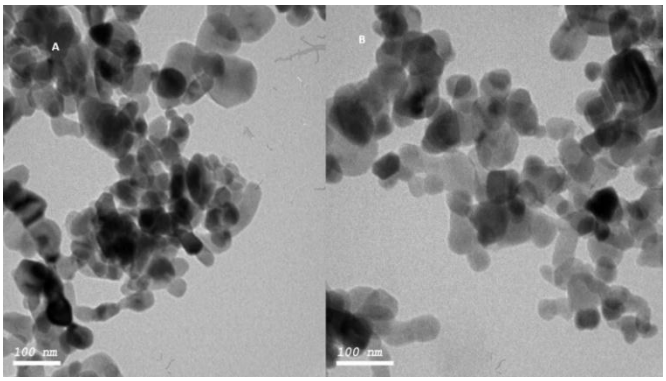
Transmission electron microscope (TEM) imaging

Particle sizes and shape measurements in this work were determined using the transmission electron microscopy (HR-TEM). Spherical and rods nanoparticles were observed in TEM images of PEG-capped ZnO nanoparticles as shown in figure (1) A and B. PVP-capped ZnO nanoparticles were approximately looking like flowers in their shape, thus they might be called as flowers as shown in figure (2, A and B). The average particle sizes of all prepared ZnO NPs were found to be up to 100 nm.



(A) (B)

Figure 1. (A) and (B) different spots of transmission electron microscope (TEM) imaging taken with different magnification scales (100 and 200 nm) for ZnO NPs @ PEG.



(A) (B)

Figure 2. (A) and (B) different spots of transmission electron microscope (TEM) imaging taken with different magnification scales (100 and 200 nm) for ZnO NPs @ PVP.

X-Ray diffraction (XRD)

X-ray diffraction is the most wide spread technique for determining the phase identification, crystal structure and lattice parameter of the crystalline solids. The X-ray diffraction graphs (XRD) were drew to confirm the purity of obtained powder ZnO nanoparticles. Figure (3) include the XRD- graph of PEG capped-ZnO nanoparticles, as shown there were 11 peaks observed and the crystallite size of nanoparticles was determined according to the position of noticed peaks, highest peak was the third one it was noticed at position of 36.18 and the crystallite size was equal 338.03 Å, while the shortest one was peak number 10 it was noticed at position of 72.50 and crystallite size was equal 490.61 Å. Figure (4) illustrates XRD- graph of PVP capped-ZnO nanoparticles, also 11 peaks were noticed to confirm the purity of ZnO nanoparticles and crystallite size, the highest peak was peak number three it was noticed at position of 36.01 and crystallite size was equal 362.12 Å, while the shortest peak was noticed at position of 72.32 and crystallite size was equal 507.93 Å. We were analyzed the XRD- graphs of ZnO nanoparticles depending on the number of noticed peaks, position and crystallite size.

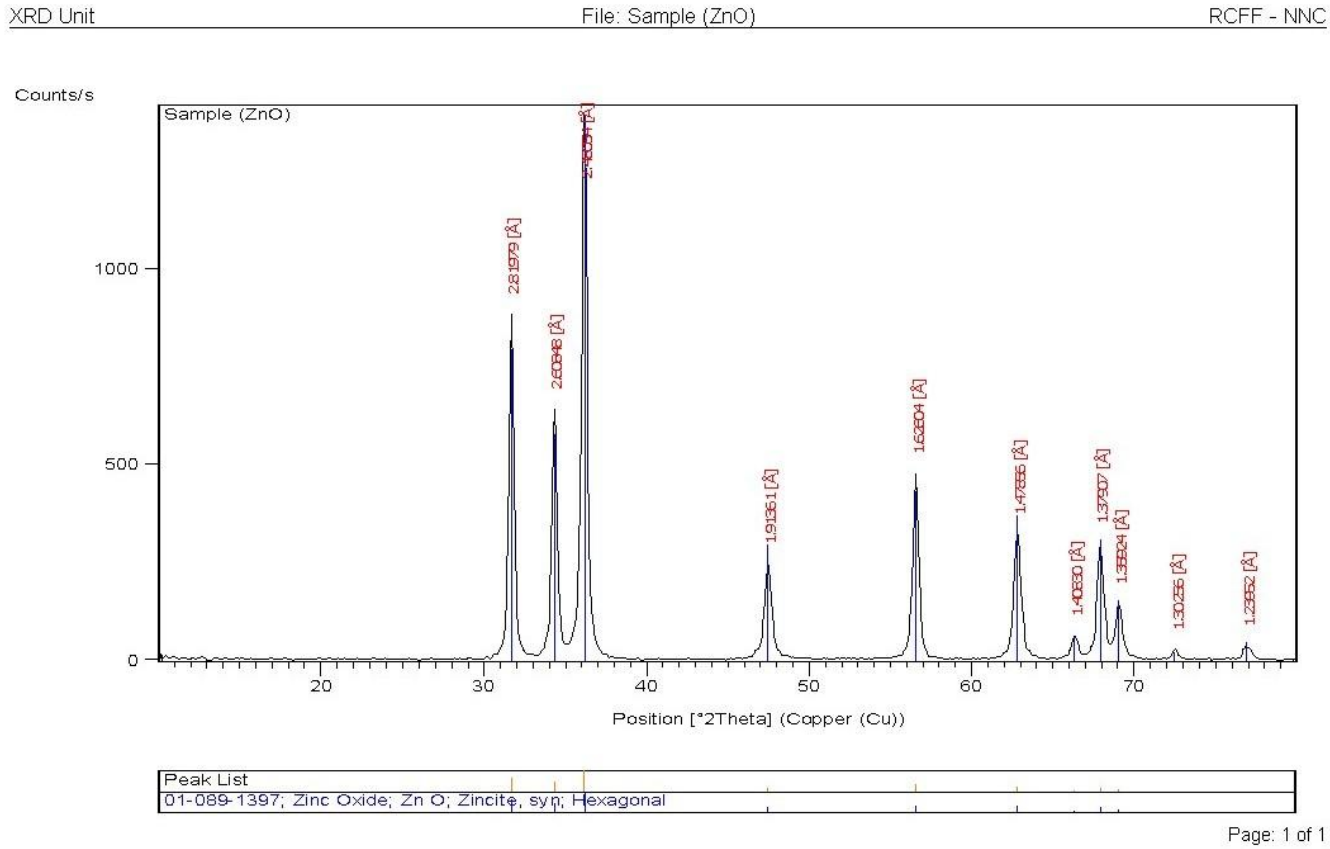


Figure 3. The X-ray diffraction graph refers the purity and crystallite size of ZnO NPs @ PEG.

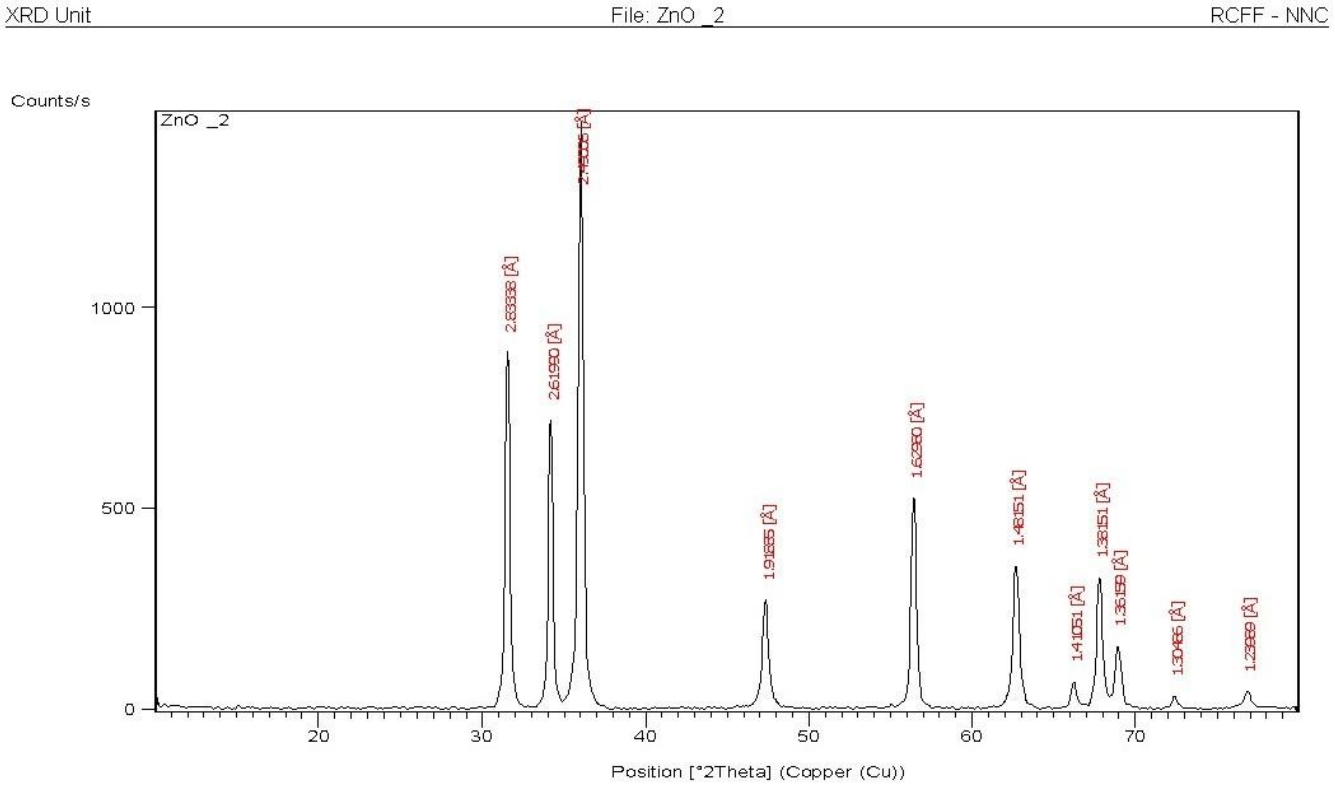


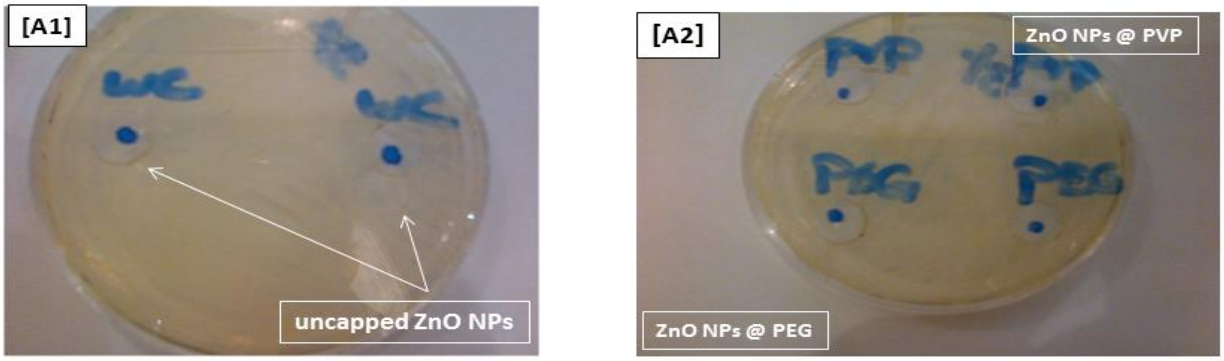
Figure 4. The X-ray diffraction graph refers the purity and crystallite size of ZnO NPs @ PVP.

Antibacterial activity test of ZnO NPs

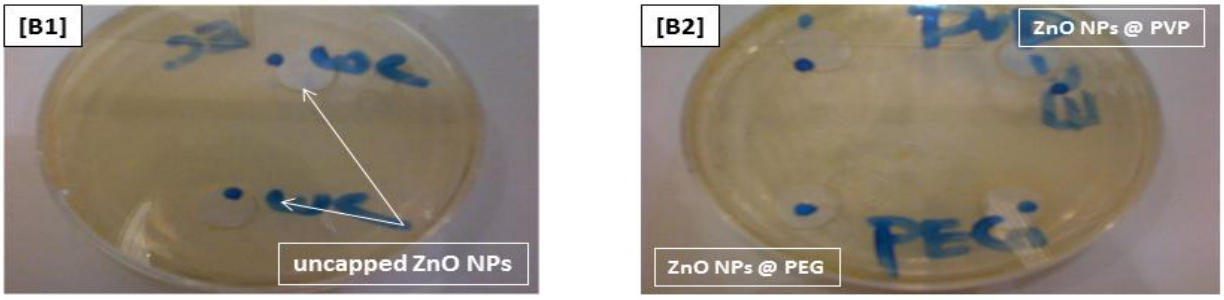
The antibacterial activity test in this research was tested against four types of bacteria that were *Bacillus subtilis*, *Escherichia coli* (*E. Coli*), *Pseudomonas aeruginosa* and *Staphylococcus aureus*. As showed in figure 5 and table 1 plate 1 which was inoculated with *Staphylococcus aureus*, after incubation show a negative result of antibacterial activity of PEG capped- ZNO nanoparticles and PVP capped- ZnO nanoparticles, there were no formed inhibition zones and *Staphylococcus aureus* was naturally grown on the nutrient agar medium. The same thing was observed in plate 2, uncapped- ZnO nanoparticles were also have a negative antibacterial activity against *Staphylococcus aureus*. Plates 3 and plate 4 were inoculated with *Escherichia coli* (*E. Coli*), and there were no observed inhibition zones caused by either capped ZnO nanoparticles (PEG capped or PVP capped) or uncapped ZnO nanoparticles. Figure 6 indicated that plates 5 and 6 were inoculated with *Pseudomonas aeruginosa* strain, and both of them were empty from observed zones of inhibition, all of ZnO nanoparticles, PEG capped-ZnO nanoparticles, PVP capped ZnO nanoparticles and uncapped ZnO nanoparticles were have no effect against *Pseudomonas aeruginosa* and it was naturally grown on nutrient agar media. Finally, that were plate 7 and plate 8 in figure 6 after incubation, there were observed zones of inhibition appeared surrounding all the wells formed either in plate 7 or plate 8 that were inoculated with *Bacillus subtilis*, it means that the growth of *Bacillus subtilis* was inhibited by PEG capped-ZnO nanoparticles, PVP capped-ZnO nanoparticles and uncapped ZnO nanoparticles.

Standard deviation calculation (std)

Standard deviation which is the possibility of increasing or decreasing of the area of inhibition zones against a certain bacterial strain was calculated for all ZnO nanoparticles samples used, as presented in table 2 according to the rule mentioned before in the methodology.

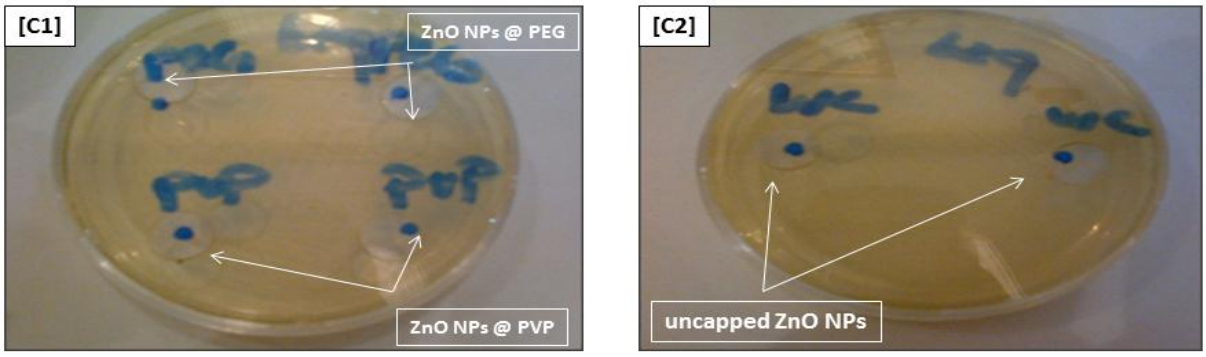


(A) *Staphylococcus aureus* grown on nutrient media: [A1] 2 wells containing 100 ul of uncapped ZnO nanoparticles, [A2] 4 wells containing 100ul of capped ZnO NPs with PEG and PVP.

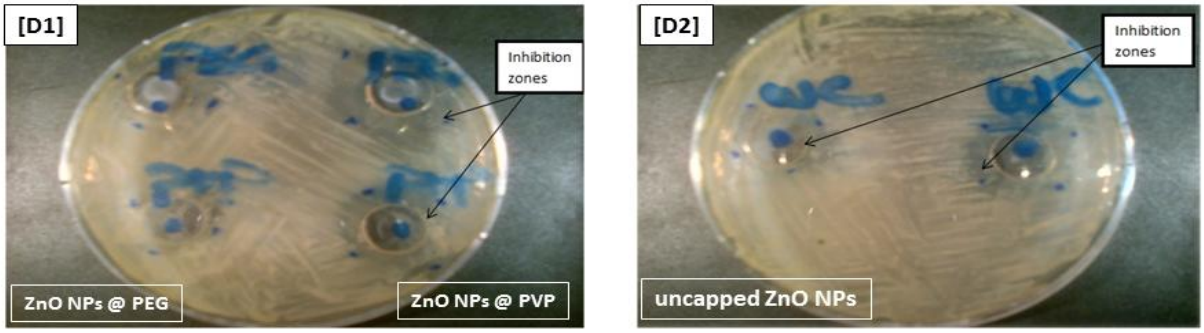


(B) *Escherichia coli(E.Coli)* grown on nutrient media: [B1] 2 wells containing 100 ul of uncapped ZnO nanoparticles, [B2] 4 wells containing 100ul of capped ZnO NPs with PEG and PVP.

Figure 5. (A) Represents the inhibition of *Staphylococcus aureus* by the effect of ZnONPs @ PEG and ZnONPs @ PVP and (B)represents the inhibition of *Escherichia coli* by the effect of ZnONPs @ PEG and ZnONPs @ PVP polymer.



(C) *Pseudomonas aeruginosa* grown on nutrient media: [C2] Two wells containing 100 ul of uncapped ZnO nanoparticles, [C1] 4 wells containing 100ul of capped ZnO NPs with PEG and PVP.



(D) *Bacillus subtilis* was inhibited by the effect of ZnO NPs @ PEG and ZnO NPs @ PVP, inhibition zones were formed and observed with all 4 wells and uncapped ZnO NPs.

Figure 6. (C) represents the inhibition of *Pseudomonas aeruginosa*by the effect of ZnONPs @ PEG and ZnONPs @ PVP and (D)represents the inhibition of *Bacillus subtilis* by the effect of ZnONPs @ PEG and ZnONPs @ PVP polymer.

Table 1. Antibacterial activity of PEG capped- ZnO nanoparticles, PVP capped- ZnO nanoparticles and Uncapped ZnO nanoparticles of bacterial isolates.

Sample	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Bacillus subtilis*
PEG capped - ZnO nanoparticles	-	-	-	+
PVP capped - ZnO nanoparticles	-	-	-	+
Uncapped ZnO nanoparticles	-	-	-	+

Table 2. Standard deviation values and mean of inhibition zone diameter of PEG capped- ZnO nanoparticles, PVP capped- ZnO nanoparticles and ZnO nanoparticles of *Bacillus subtilis*.

Sample	Mean	Std. deviation
PEG capped - ZnO nanoparticles	23.5	± 2.5
PVP capped - ZnO nanoparticles	20	± 2
Uncapped ZnO nanoparticles	19.7	± 1.8

Discussion

The development of infectious diseases produces a serious and danger threat to public health worldwide, and the increasing rate of the appearance of antibiotic-resistant strains through a short period of time within both Gram-positive and Gram-negative microorganisms are considered as a major and important public health concern. Actually, there are much alternative and different therapeutics used in order to control and prevent the spread of infections in both community and hospital environments and they are commonly required [12]. Recently, nanoparticle metal oxides represent a new class of important materials that are increasingly being developed for use in research and many other health-related applications. Highly ionic metal oxides are

considered as interesting materials for several reasons, such as their wide variety of physical and chemical properties and for their antibacterial activity. Even though the *in vitro* antibacterial activity and efficacy of regular zinc oxides (ZnO) have been investigated and explored, little is known about the antibacterial activity of ZnO nanoparticles.

Recently, nanoparticle metal oxides represent a brand-new category of vital materials that square measure progressively being developed to be used in analysis and lots of alternative health-related applications. Extremely ionic metal oxides square measure thought of as fascinating materials for many reasons, like their big variety of physical and chemical properties and for his or her medicament activity. Although the in vitro medicament activity and effectuality of normal metal oxides (ZnO) are investigated and explored, very little is thought regarding the medicament activity of ZnO nanoparticles.

Earliest growth analysis data suggest that ZnO nanoparticles have significantly higher antibacterial effects on *Staphylococcus aureus* than do other five metal oxide nanoparticles that were MgO, TiO_2, Al_2O_3, CuO and CeO_2. Moreover, several studies have clearly confirmed that ZnO nanoparticles have a wide range of antibacterial effects on a good number of other microorganisms. The antibacterial activity of ZnO may be based on the size and the presence of normal visible light. Further information suggests that ZnO nanoparticles have a powerful and potential application as a bacteriostatic agent in visible light and may have future applications in the development of derivative agents to control the spread and infection of a variety of bacterial strains. Although various classes of antibiotics such as penicillin and its derivatives were discovered in 1940s and 1950s, and in the last 40 years there were only two antibiotics that representing new chemical classes that were known as linezolid and daptomycin and have reached the market in the purpose of treating multiple antibiotic-resistant mainly Gram-positive infections. Actually, there are recent advances in the field of nanotechnology, mainly the ability to prepare highly ionic metal oxide nanoparticles of any size and shape, and it may lead to the development of new antibacterial agents. Several studies were discussed and have showed that ZnO among other many metal oxide nanoparticles analyzed produces a significant growth inhibition under normal laboratory lighting conditions. ZnO powders have been used for a long time and considered as an active element for dermatological applications in creams, lotions and ointments depending on its antibacterial properties [13].

In this research the antibacterial activity of powder zinc oxide nanoparticles (ZnO NPs) was determined after the photoactivation by the ultra violet (UV) light. However, ZnO nanoparticles are much more effective agents in controlling the growth of various microorganisms; moreover, the earliest studies show that the smaller particle size is the greater the efficacy in inhibiting the growth of bacteria. Photo-activated nanoparticles by using UV light such as TiO_2 was known to kill various bacteria [14], but non-activated TiO_2 was not able to inhibit significantly the growth of *Staphylococcus aureus*. Toxicological studies of ZnO nanoparticles against *E. coli* were performed by plate assays and transmission electron microscopy (TEM) analyses. The studies suggested that synthesized ZnO nanoparticles that were particles with average diameter 12 nm are able to slow down the bacterial growth as a result of inefficiency of the *E. coli* membranes, which increases the membrane permeability and therefore leading to the accumulation of nanoparticles in the bacterial membrane and cytoplasm regions of the cells [15].

While metals and metal oxides such as ZnO are known to be toxic to host human cells at moderately high concentrations as they are not expected to be toxic at very low concentrations. Actually, it has been shown that ZnO protects against intestinal diseases by protecting intestinal cells from *E. coli* infection by inhibiting the adhesion and internalization of bacteria [2]. And so, significant bacterial growth at lower concentrations of ZnO suggests that ZnO nanoparticles may not be toxic for various tested microorganisms. This may be due to that *E. coli* can metabolize ZnO as an oligo-element [2]. According to Gaballa and Helmann [16] studies, metal-ion homeostasis is important and essential for bacterial life due to their connection in the regulation of a wide array of metabolic functions as co-enzymes, cofactors and other catalysts, and additionally as structural stabilizers of enzymes and DNA-binding proteins. However, metal ions are toxic for bacterial cells. And as a result, there were certain bacteria have developed mechanisms in order to regulate and control the influx and efflux processes and therefore maintaining the stable intracellular concentration of metal ions, including the ZnO ion. The genes responsible for the transport of zinc ions have been characterized in several bacteria, including *Streptococcus pneumoniae, Haemophilusinfluenzae, Staphylococcus aureus, Synechococcus sp. strain PCC6803, Escherichia coli, and Bacillus subtilis* [16]. In *Staphylococcus aureus*, *zntA* and *zntR* genes have been characterized, and it has been shown that *ZntA*, that is a trans-membrane protein, and it is mainly responsible for the efflux of zinc ions while *ZntR* worked as regulatory encoding protein for a Zn-responsive [17]. Initial results of further studies suggest that ZnO nanoparticles can be used externally in purpose of controlling the spreading of bacterial infections. Actually, it would be interesting to determine if any other derivatives and products of ZnO nanoparticles with various chemical groups or bio-agents are more effective at eliminating and reducing various microorganisms. The cell wall structure was the approved to be the main target for the prevention and control of bacterial spreading and

infections. The cell wall of most pathogenic bacteria is composed of surface proteins for sticking together and colonization, and other components such as polysaccharides and teichoic acid that mainly protect against host defenses and other harmful environmental conditions [18].

Cell wall compartments are actually charged macromolecules and for that there are specific linkages to interrupt their main function as the location may be activated by introducing specific groups on the surface of the nanoparticles. It has been stated that certain long-chain polycations coated onto surfaces can strongly kill on contact both Gram-positive and Gram-negative bacteria [19].

Additionally, these researches have showed that families of unrelated hydrophobic groups are similarly efficient and powerfully killing bacteria. Therefore, in the future, it is expected that ZnO nanoparticles that are containing formulations may be utilized for external uses as antibacterial agents in gels, lotions, mouthwashes, and surface coatings on various substrates in order to prevent microorganisms from attaching, colonizing, spreading, and also forming biofilms in medical devices.

In this research all of ZnO nanoparticles were photo-activated by using UV light. The main rule of UV light in the photo activation of ZnO nanoparticles is to enhance their antibacterial activity, UV activate the Zn^{+} ions by move the electrons from the lowest energy level to the highest level and according to this they become activated, therefore, photo-activated ZnO nanoparticles must be applied against bacterial strains directly after the photo activation done. Figure 7, art figure that represent the release for electrons after photo-activation of ZnO NPS then used to damage bacteria.

In this study, standard deviation of ZnO nanoparticles was calculated to determine the possibility of increasing or decreasing of observed zones of inhibition. It was calculated by taking the square root of total square differences of the measured dimensions of observed inhibition zones (mean). Table 2 illustrated the standard deviation calculation done for all observed zones of inhibition caused by the positive effect of PEG capped-ZnO nanoparticles, PVP capped- ZnO nanoparticles and uncapped ZnO nanoparticles. As shown in raw number 1, inhibition zones caused by PEG capped- ZnO nanoparticles were have a standard deviation of ± 2.5m while in raw number 2 the standard deviation of inhibition zones caused by PVP capped-ZnO nanoparticles were equal ± 2, and finally in raw number 3, the standard deviation of zones of inhibition caused by uncapped ZnO nanoparticles was equal ±1.8.

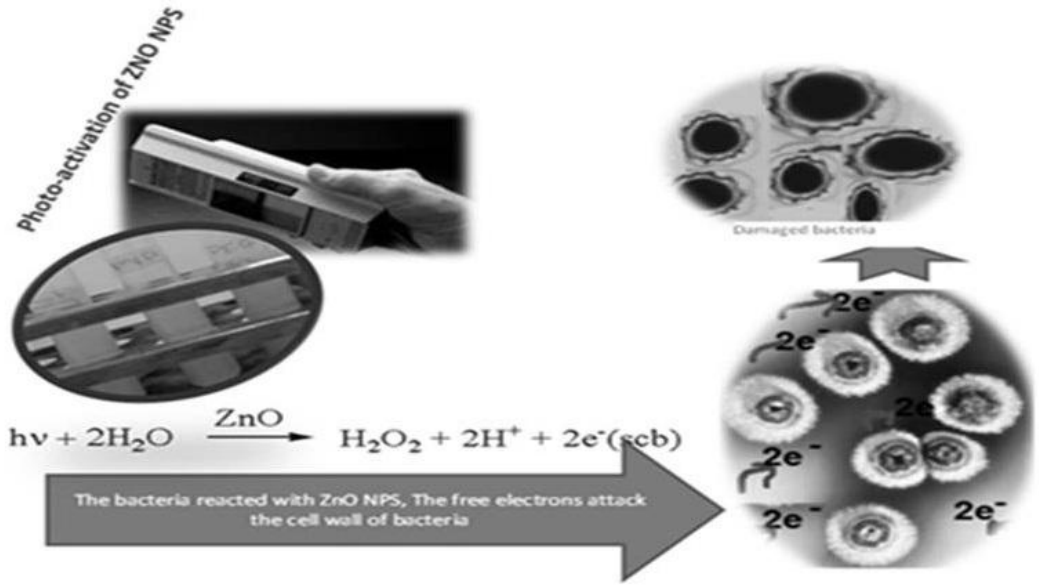


Figure 7. Art figure that represent the release for electrons after photo-activation of ZnO NPS then used to damage bacteria.

Conclusion

Finally, as a conclusion the target of this research was to evaluate the anti-microbial activity of ZnO nanoparticles either capped with different polymers (PEG) and (PVP) or uncapped ZnO nanoparticles. All of samples of powder ZnO nanoparticles were obtained and characterized, and to be applied easily against the bacterial strains involved in this study, they were prepared in a suspension form by dissolving the powder particles in distilled water in order to be diffused easily through nutrient agar media. One type of bacteria which was *Bacillus subtilis* was inhibited to be grown normally on nutrient agar media by the effect of ZnO nanoparticles (capped with PEG, capped with PVP and uncapped ZnO NPs), The negative effect occurred or observed within other bacterial strains might

be was according to several reasons, such as may the cell wall structure of the bacteria, or the bacterial strains were needed to be incubated for longer time more than 24 hours to be growth inhibited, or other reason that the size of particles themselves, may the nanoparticles were needed to be more smaller in size, and also the time of the photoactivation process may supposed to be longer in order to activate the energy and therefore the effect of ZnO nanoparticles will be activated against those bacterial strains that were not inhibited. However, further studies approved that ZnO nanoparticles have an inhibitionaction mostly against *Escherichia coli* (*E. coli*) and *Bacillus subtilis* bacterial strains.

Acknowledgment

Great thankful for Dr. Ayman Diab and Dr. Gehan Safwat, Faculty of Biotechnology, MSA University for their great guidance, and extreme encouragement. Special thanks for all staff members and workers in Nanotech Company for helping us in the practical work.

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