Comparative Studies Between Zinc Oxide and Manganese Oxide Nano-Particle for their Antimicrobial Activities

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The biosynthesis of metal nanoparticles is an expanding research area due to the potential applications for the ecofriendly development of novel technologies. Present study reported the antimicrobial properties of zinc oxide and manganese oxide nano-particles were investigated using: Gram positive bacteria namely (Bacillus megaterium, Bacillus subtilis, Sarcina lutea and Staphylococcus aureus), Gram negative bacteria (Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae and proteus vulgaris) and the pathogenic yeast (Candida albicans and fungi Aspergillus niger). The average sizes of the zinc and manganese nanoparticles were 111nm and 157 nm, respectively, as determined through transmission electron microscopy. The microbial effect of zinc oxide and manganese oxide nano-particles were compared based on diameter of inhibition zone in well diffusion tests and minimum inhibitory concentration (MIC). Bacterial sensitivity to nanoparticles was found to vary depending on the microbial species. Well diffusion studies with E. coli and B.subtilis revealed greater effectiveness of the zinc nanoparticles compared to the manganese nanoparticles. P. aeruginosa depicted the highest sensitivity to nanoparticles compared to the other strains and was more adversely affected by the manganese than zinc nanoparticles. Good correlation was observed between MIC and minimum bactericidal concentration (MBC) measured in liquid cultures. For zinc nanoparticles a good negative correlation was observed between the inhibition zone observed in disk diffusion test and MIC/MBC determined based on liquid cultures with the various strains.

Key words: Nanoparticles, zinc, Manganese, Antimicrobial, MIC, MBC.

In recent years, there has been an increasing interest in developing materials with low dimensional nanostructure such as nanoparticles, nanotubes and nanorods due to their potential technological application in nano scale devices. Also, it has been obvious that their properties depend sensitively on their size and shape. Therefore, the challenges in nanocrystal synthesis are to control not only the crystal size but also the shape and morphology. In order to produce the desired nanostructural materials, various method have been developed, such as electrodeposition1. The effect of nanoparticles on bacteria is very important since they constitute the lowest level and hence enter the food chain of the ecosystems 2. Recent studies have demonstrated that specifically formulated nanoparticles demonstrate good antibacterial activity and constitute the antimicrobial formulations3. Antibacterial agents are divided into two parts: the organic and inorganic matters. The organic antibacterial materials have been used as insecticides and bactericides for many years. Unfortunately, high temperatures in manufacturing
process reduce their antibacterial properties. However, inorganic antibacterial agents show excellent resistance against the bacterial and thermal stability.

Heavy metals such as Co, Cu, Fe, Ni, Mn and Zn exist in trace amounts as essential elements in biological systems and play important roles in biochemical reactions of living systems [5]. Such essential trace metals as well as others with no essential biological function (e.g. Al, Cd, Cs, Hg, Pb and Sn) are all increasingly being found at very high concentrations in biological systems of humans, animals and even microbes due to increased industrial use accompanied by improper disposal [6]. Although there have been reports on the activity of drug-metal complexes, most researchers used presynthesized complexes requiring special conditions (e.g. high temperatures and refluxing for long periods) for their studies. Such requirements may however not be met in living systems such as the human body. Also, little or no work has been done on the specific effects of Cd, Cr, Mn, or Zn on the activity of chloramphenicol in solution on the growth of the microorganisms used in this study [7].

Manganese has attracted the attention of several researchers owing to the fascinating physical and biochemical characteristics. The utility of manganese is quite diversified such as an alloying agent for aluminium, in dry cell batteries (carbon-zinc Leclanche type), oxidizing agent etc [8]. The best known complexes of manganese with sulfur ligands are dithiophosphinates the dithiocarbamates [9].

Inorganic materials such as metal and metal oxides have attracted lots of attention over the past decade due to their ability to withstand harsh process conditions [10-11]. Of the inorganic materials, metal oxides such as TiO$_2$, ZnO, MgO and CaO are of particular interest as they are not only stable under harsh process conditions but also generally regarded as safe materials to human beings and animals [12]. The use of nanoparticles of silver and zinc oxide has been seen as a viable solution to stop infectious diseases due to the antimicrobial properties of these nanoparticles. The intrinsic properties of a metal nanoparticle are mainly determined by size, shape, composition, crystallinity and morphology [13].

Reddy [14] report selective toxicity of nanoparticles, including metal oxides, to prokaryotes and eukaryotes. Studies on eukaryotes, primarily involving mammalian cells, indicate that nanoparticles of ZnO cause higher levels of oxidative stress resulting in inflammation and cytotoxicity. In prokaryotes, oxidative stress can induce cell death due to interactions between reactive oxygen species and proteins, DNA or the cell membrane [15-16].

The objective of this study was to compare the microbial effect of Zinc oxided and manganese nanoparticles using various microbial strains. Such a comparative study would reveal strain specificities and would eventually lead to better utilization of nanoparticles for specific application. Ten representative microbes typically recommended for use in antimicrobial assays. The antimicrobial effect was quantified based on the inhibition zone measured in the agar well diffusion tests conducted in plates and by determining the minimum growth inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of nanoparticles in liquid batch cultures.

**MATERIALS AND METHODS**

Commercial quality of nano MnO and ZnO nanoparticles, were obtained from local suppliers. Surface morphologies of the specimens were observed with a scanning electron microscope (SEM, PHILIP XL-30). Sample preparation and characterization Semi-quantitative analysis of nanoparticles was carried out by energy-dispersive X-ray spectroscopy (EDS, FEI Quanta 200, Holland). The crystallinity of the nanoparticles was further characterized using a X-ray diffractometer (XRD, Philips PW3040/60 X'pert PRO, The Netherlands) employing Cu Kα radiation [17].

**Electron spin resonance spectroscopy**

Structural investigation by recording their powder X-ray diffraction patterns transmission electron microscopy (TEM) of ZnO and MnO nanostructures. The patterns were recorded on a Bruker D8 Advance X-ray diffractometer at room temperature (300 K) using monochromatic Cu Kα radiation (λ=0.15406 nm). For this purpose, a fine drop of ZnO nanoparticles and MnO nanoparticles dispersed in hexane were placed on carbon-coated copper grids and the hexane was allowed to evaporate slowly at room temperature. The
elemental concentration of ZnO and MnO nanoparticles in the core–shell nanostructures was determined by inductively coupled plasma atomic emission spectroscopy (ICP AES). Microorganisms

The organisms used were: Gram positive bacteria namely Bacillus megaterium ATCC 25848, Bacillus subtilis NRRL B-543, Sarcina lutea ATCC27853 and Staphylococcus aureus; NRRL B-313, Gram negative bacteria Escherichia coli; NRRL B-210, Pseudomonas aeruginosa NRRL B23 27853, Klebsiella pneumoniae ATCC 27736 and proteus vulgaris NRRL B-123. The pathogenic yeast was Candida albicans NRRL Y-477 and fungi Aspergillus niger NRRL-3. These microorganisms were obtained from Natural Research center, Department of Chemistry of Natural and Microbial product, Cairo, Egypt.

Antimicrobial Activity

ZnO and Mn nanoparticles were tested in vitro for their antimicrobial activities against pathogenic strains by the agar diffusion technique. The tested nanoparticles were dissolved in dimethyl sulfoxide (DMSO) to prepare chemicals of stock solutions of 50 ìg/ml. The pathogenic bacteria and fungi were maintained on nutrient agar and Czapek’s-Dox agar media, respectively in Petri dishes with an inner diameter 9 cm to provide thin agar plates after solidification of thickness 3.4-3.5 mm. After solidification, hollows of 10 millimetre diameter wells were cut from the agar using a sterile cork-borer, and 100ìl of each of the tested solutions were poured into the wells. The Petri dishes were incubated at 5-8°C for 2-3 h to permit good diffusion and then incubated at 30°C for 24 h in case of yeast and fungi. After the incubation the diameters of inhibition zone (mm) were measured.

Minimum Inhibitory Concentration (MIC)

The antimicrobial activities of the samples were evaluated through the determination of the (MIC) by the method of micro dilution in culture broth. For both the antibacterial and the antifungal assays, the particles were dissolved in DMSO (50 mg/ml). Further dilutions were prepared at the required quantities of 50, 45, 40, 35, 30, 25, 20, 15, 10, 5, 1 and 0.5 ìg/ml concentrations. The values of (MIC) values were determined using the method of twofold serial dilutions.

RESULTS AND DISCUSSION

Electron spin resonance spectroscopy

The scanning electron microscopy (SEM) images of ZnO and MnO nanoparticles indicating the homogeneous size and high purity of the product (Fig1 a and b respectively), these fig are contrast with this obtain by Zawrah and Sherein [24]. On the other hand (Fig. 2 a and b) confirmed that the metal particles of ZnO and MnO nanoparticles are in the nanorange and that they are approximately spherically in shape. Subsequent image analysis revealed that the ZnO nanoparticles are relatively smaller (mean±SD: 111 to 122 nm; size range: 176–214 nm for a scan of n=2582 particles) than the MnO nanoparticles (157 to 162 nm; range: 176–214 nm for a scan of n=683 particles). Considerable asymmetry was observed in the particle size distribution profile.

Antimicrobial Activity

The antibacterial activity of nanoparticles was compared for various microorganisms using the diameter of inhibition zone in well diffusion,
The diameter of inhibition zone (DIZ) reflects the magnitude of susceptibility of the microorganism. The strains susceptible to disinfectants exhibit larger DIZ, whereas resistant strains exhibit smaller DIZ. The well with ZnO nanoparticles were surrounded by a larger DIZ compared to the MnO nanoparticles for all E. coli and B. subtilis strains selected for this. While the DIZ was affected by the type of microorganisms, it was essentially invariant across the various strains.

Similarly, for S. aureus the ZnO nanoparticles impregnated wells were found to be more effective compared to manganese nanoparticle impregnated wells, however the difference in the DIZ was merely 20%. In contrast, using P. aeruginosa and B. megaterium, the wells impregnated with manganese nanoparticles showed a significantly larger DIZ, almost 10% greater compared to that observed with ZnO nanoparticles. This effect is due to the generation of high rate of oxygen species from the surface of ZnO nanoparticles, which penetrate in the cell membrane and lead to the death of the bacteria. The generation of highly reactive species such as OH, H₂O₂, and O₂⁻. Since DIZ was measured on agar plates using a ruler with 1mm resolution, the possibility of measurement errors exist; however, the method illustrates the potential biocidal effect of nanoparticles to different microbial strains. As for the well diffusion tests, the batch studies also reveal differences in sensitivity to ZnO and MnO nanoparticles for the various microbial strains. These results correspond with the results of Rajendran as ZnO nanoparticles showed antibacterial activity was much higher against S. aureus.

The antibacterial data reveals that MnO nanoparticles in solution show prominent inhibition capacities antibacterial properties against the bacterial species under study. The results also confirmed with Tweedy and Loeppky, that the antimicrobial activity of the metal chelates show more inhibitory effects than the parent ligand. Furthermore, the MnO nanoparticles enhanced activity, due to chelating the polarity of the metal ion will reduce to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups.

Fig. 1. (1a and b) Electron spin resonance spectroscopy of ZnO and MnO nanoparticles

Fig. 2. (2a and b) TEM images of ZnO and MnO nanoparticles
Minimum Inhibitory Concentration (MIC)

MIC and MBC were determined by the standard microbrial method. ZnO and MnO nanoparticles suspensions with different concentrations were incubated with E. coli and P. aeruginosa in aqueous NB as shown in fig (5 a and b) respectively. In batch studies, a greater lag phase and lower maximum absorbance were observed as the concentration of nanoparticles increased. Similar observation was reported by Sondi in their studies on effect of silver nanoparticles on a single strain of E. coli. As concentration of nanoparticles increased to MIC of the respective strains, no growth was observed in the flask. The bactericidal effect of nanoparticles is dependent on the concentration of nanoparticles and the initial bacterial concentration.

Bacterial growth was studied by using a spectrophotometer inspecting the NB for turbidity. If the material being tested does not kill but instead inhibits the growth of bacteria (bacteriostatic agent), the bacteria will grow when it is removed from the solution containing the material, and colonies will be observed upon plating an aliquot. If the material being tested is bactericidal, the absence of bacterial colonies will be observed upon plating. To establish whether the suspensions were bacteriostatic or bactericidal, 100µl aliquots were taken from the incubated NB, each containing ZnO or MnO nanoparticles and E. coli, or P. aeruginosa.

![Fig. 3. Agar plates containing ZnO and MnO nanoparticles impregnated wells and DIZ for (a) E. coli and (b) P. aeruginosa](image)

![Fig. 4. The diameter of inhibition zone (DIZ) surrounding ZnO/MnO nanoparticles impregnated wells in presence of various microorganisms.](image)
were placed on nutrient agar plates and incubated for 18–20h. The results are showed that ZnO nanopathicals suspension with a concentration in the range of 0.5 to 50ìg effectively inhibits bacterial growth of E. coli and P. aeruginosa on using ZnO nanoparticles. this results are appears because of ZnO nanoparticles strongly resisted to microorganisms. There are some reports on the considerable antibacterial activity of CaO, MgO and ZnO, which is attributed to the generation of reactive oxygen species on the surface of these oxides.

The antibacterial activity was observed at concentrations mor than 5ìM for all the ZnO and MnO nanoparticles on using P. aeruginosa. Nanoparticles can act as antibacterial and antifungal agents, due to their ability to interact with microorganisms. Exerting their antibacterial properties, nanoparticles attach to the surface of the cell. This interaction causes structural changes and damage, markedly disturbing vital cell functions, such as permeability, causing pits and gaps, depressing the activity of respiratory chain enzymes, and finally leading to cell death.

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REFERENCES


