Research Article

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COMPARATIVE STUDIES OF SYNTHESIS, STABILITY AND ANTIBACTERIAL ACTIVITY OF ZINC OXIDE NANO-PARTICLES

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Abstract: Research related to the antibacterial activity of nanoparticles will enable new developments in the field of nanomedicine. Targeted drug delivery, diagnostics, magnetic resonance, imaging etc., are possible because of the biological cell interactions of the nanoparticles. The present study focuses on one such interaction of ZnO nanoparticles. The antibacterial activity and stability of ZnO nanoparticles synthesised using different methods was investigated. The inhibitory effect of ZnO nanoparticles against *Escherichia coli, Proteus vulgaris, Pseudomonas, Propionibacterium acnes, Bacillus, Klebsiella and Staphylococcus aureus* was tested by agar well diffusion method. The zones of inhibition for all the selected bacterial strains were measured. The ZnO nanoparticles synthesised by the different methods were effective against all the organisms tested and the nanoparticles were quite stable.

Keywords: Zinc oxide nano-particles, Antibacterial Activity, Agar Well Diffusion.

INTRODUCTION

The term nanotechnology was defined by the National Nanotechnology Initiative as the manipulation of matter with at least one dimension sized from 1 to 100nm (1nm = 10^{-9}m). The materials synthesized by the different Nano technological techniques, possess unique properties that decide their applications in various fields of science including surface science, organic chemistry, semiconductor physics, molecular biology, micro-fabrication, etc.

The applications of nanotechnology are countless. Quantum dots can be used in display technology, lighting, solar cells and biological imaging. Atomic force microscope tips can be used in nanolithography. DNA nanotechnology helps us construct well-defined structures out of DNA and other nucleic acids. Bionics or bio-mimicry seeks to apply biological methods and systems found in nature, to the study and design of engineering systems and modern technology. Bio nanotechnology is the use of biomolecules for applications in nanotechnology, including use of viruses and lipid assemblies. Nano-particles are used to reduce environmental pollution. Several nano-particles are used in the medical field as biomarkers to detect diseases, treat malignant tumours, and deliver drugs for chemotherapeutic purposes.

Nano Scale particles have emerged as novel antimicrobial agents owing to the high surface area to volume ratio, which is coming up as the current interest in the researchers due to the growing microbial resistances against metal ions, antibiotics and the development of resistant strains. The recent growth in the field of porous and nanometric materials prepared by non-conventional processes has stimulated the search of new applications of ZnO nanoparticles.

ZnO is a polar inorganic crystalline material with many applications 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

Zinc oxide nanomaterials are used in the preparation of substances possessing medically as well as cosmetically useful properties. Due to its antibacterial properties, zinc oxide is applied on the skin, in the form of powders, antiseptic creams, surgical tapes and shampoos, to relieve skin irritation, diaper rash, dry skin and blisters. Zinc oxide nano-particles increase the antibacterial efficiency of Ciprofloxacin. Zinc oxide is used along with iron oxide to prepare calamine lotion, and with eugenol to prepare zinc oxide eugenol which is used for dental applications[1, 2]. It is also used in rectal suppositories to provide relief against the discomfort and irritation caused by hemorrhoids'. Zinc oxide is used in sunscreens, since it is capable of absorbing ultraviolet light, and thus protects the skin from sun damage. Zinc oxide nano-particles are used industrially as a protective coating against photo- destruction[3].

Thus the present study focuses on the comparison of various methods involved in the synthesis of ZnO nanoparticles and their effectivity as antimicrobial agents.

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MATERIALS AND METHODS

Preparation of Zinc Oxide Nano-Particles:

Synthesis of Zinc oxide nano-particles-I:[4] 100ml of 1M zinc nitrate (27.948gm in 100ml) was mixed with 250ml of 1M sodium hydroxide (10gm in 250ml), leading to the neutralization of the two solutions. The pH was then set at 12 using NaOH. The mixture was centrifuged at 3000 rpm for 3 minutes. The pellet obtained after centrifugation was washed thrice with de-ionised water and twice with standard ethanol. The cleansed pellet was heated overnight at 80°C. It was ground to a fine powder and weighed.

Synthesis of Zinc oxide nano-particles-II:[4] 500ml of 0.5% starch and 14.874gm of zinc nitrate were mixed together, and the solution mixture was neutralised for 2 hours with 300ml of 1N sodium hydroxide (10gm in 250ml). The mixture was centrifuged at 3000 rpm for 3 minutes. The pellet obtained after centrifugation was washed thrice with de-ionised water and twice with standard ethanol. The cleansed pellet was heated overnight at 80°C. It was ground to a fine powder and weighed.

Synthesis of ZnO nanoparticles:III[5] Zinc oxide nanoparticles were prepared by suspending 0.2 M zinc acetate in 20 ml of Dimethyl sulfoxide. It was stirred for about 30 minutes. 1.2 M of KOH prepared in 10 ml of ethanol was added drop wise to zinc acetate suspended in DMSO. After stirring for 5 minutes 0.12 ml of thioglycerol was added and stirring continued for an hour till the solution turned milky. The particles were then washed with methanol thrice and were later dispersed in methanol. The absorption spectrum using UV-VIS spectrophotometer was measured.

Preparation of the Test Inocula:

A loop full of culture of each of the test organisms was transferred to flasks containing 100 ml of nutrient broth and were grown 37°C for 24h.

Antibacterial activity of ZnO nanoparticles against aerobic oganisms:

Antibacterial activity of ZnO nanoparticles against *Escherichia coli, Proteus vulgaris, Pseudomonas, Klebsiella,* (Gram negative), *Bacillus,, Staphylococcus aureus* (Gram positive) was tested by agar diffusion method (well method). Agar diffusion method is a means of measuring the effect of an antimicrobial agent against bacteria. The cultures were inoculated over the dried surface of nutrient agar. Wells bored in the agar were impregnated with ZnO nanoparticles I, II and III. ZnO I and II were dispersed in ethanol and ethanol was used as control. Whereas ZnO III was dispersed in Methanol solvent and methanol was used as control. The compound diffuses from the well into the agar. The concentration of the compound will be highest near the well and will decrease as distance from the well increases. If the compound is effective against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration creating a zone of inhibition. Thus, the size of the zone of inhibition is a measure of the compound's effectiveness: the larger the clear area around the well, the more effective is the compound.[6,7]

Antibacterial activity of ZnO nanoparticles against Propionibacterium acnes - Facultative anaerobic oganism:

The agar plates were inoculated with the culture and the wells impregnated with ZnO nanoparticles were incubated at 37°C in anaerobic chamber for 48 hrs. The Zones of Inhibition were measured.

RESULTS AND DISCUSSION

Table.1: Antibacterial activity of ZnO nanoparticles

Organisms	Zone diameter of Inhibition (mm)								
	ZnO I			ZnO II			ZnO III		
	First Week	Second week	Third Week	First Week	Second week	Third Week	First Week	Second week	Third Week
Bacillus	20	20	25	23	23	22	20	20	20
Escherichia coli	22	22	20	22	22	25	20	22	26
Klebsiella	20	25	25	21	21	25	15	15	20
Proteus vulgaris	23	27	22	20	20	25	20	20	20
Pseudomonas	15	13	22	22	21	15	25	18	22
Propionibacterium	13	20	20	16	18	16	21	20	20
Staphylococcus aureus	20	17	21	20	22	22	15	18	20

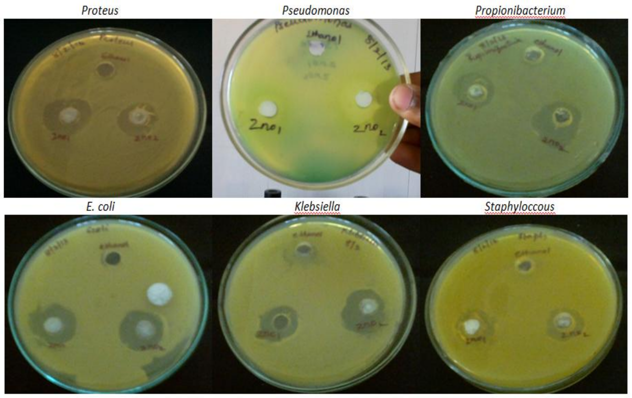

Fig.1: Antibacterial activity of ZnO I and ZnO II tested during first week of their synthesis

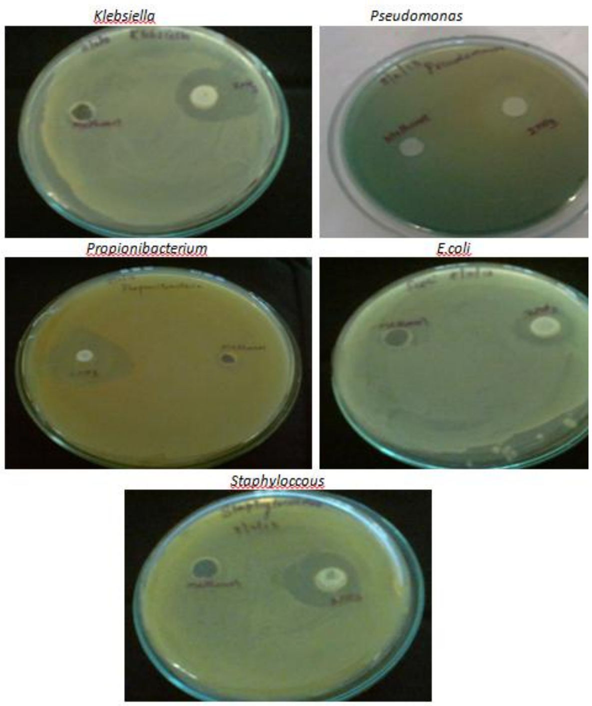

Fig.2: Antibacterial activity of ZnO III tested during first week of their synthesis

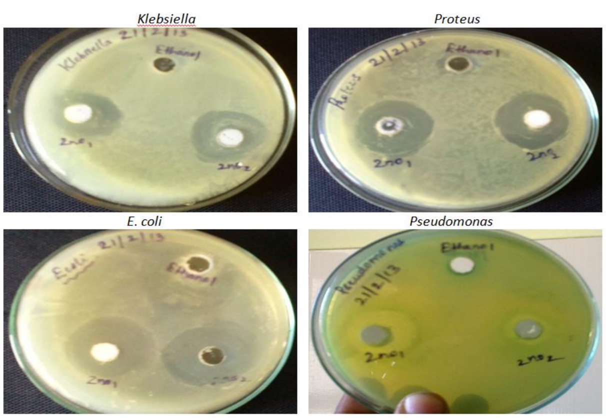

Fig.3: Antibacterial activity of ZnO I and ZnO II tested during third week of their synthesis

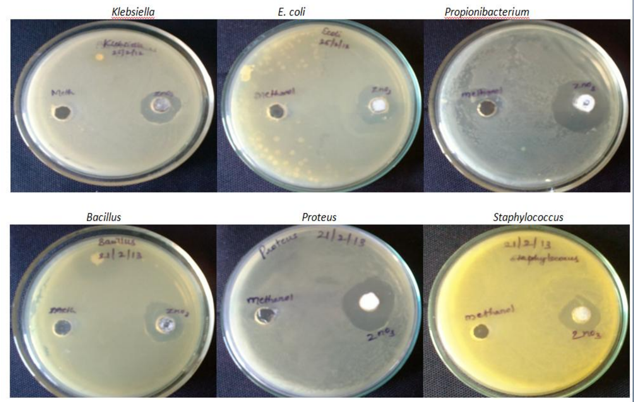

Fig.4: Antibacterial activity of ZnO III tested during third week of their synthesis

The antibacterial activity of Zinc oxide nano-particles was investigated against various Gram positive and Gram negative strains of bacteria using agar well diffusion technique. The diameter of inhibition zones around each well is represented in Table-1**.** *P. acnes* isolated from acne is a relatively slow growing typically aero tolerant anaerobic gram positive bacterium that colonizes sebum rich follicles, causing acne vulgaris commonly called as acne. Because of resistance of *P. acnes* to antibiotics[8] An alternate method of treatment would be by employing the nanoparticles. ZnO nanoparticles I, II and III synthesized using different methods were effective against *P. acnes.* But of all the three ZnO nanoparticles ZnO III was more effective against *P. acnes* and was quite stable.

Staphylococcus aureus, a gram positive microorganism, one of the four causes of nosocomial infections was inhibited by ZnO nanoparticles. All the three nanoparticles were effective against the organism. These results were in accordance with the result obtained by others[9,10].

ZnO nanoparticles inhibited the growth of *E. coli, Klebsiella, Pseudomonas, Proteus and Bacillus species.* The antibacterial effect may be due to the destructive effect of ZnO nanoparticles on the bacterial cells and increased production of active Oxygen such as H_2O_2 which leads to the cell death[10]. Ethanol and Methanol used as control did not show any activity. Bulk ZnO did not show any antimicrobial activity against the organisms tested.

The surface modification of ZnO nanoparticles causes an increase in the membrane permeability and the cellular internalization of these nanoparticles. This causes changes in the level of proteins[9]. Nano-particles are better anti-bacterial agents than the bulk material of the same composition, since they possess greater surface area, which ensures more interactions with the bacteria. Studies suggest that zinc oxide nano-particles bring about bacterial cell mortality by increasing the permeability of the bacterial cell membrane, leading to a defect in the membrane transport systems. On entry into the bacterial cells, the zinc nano-particles react with the proteins (enzymes, cellular proteins) and nucleic acids (especially DNA), and denature them, thus inhibiting the replication. One of the main advantages of employing zinc oxide nano-particles as anti-bacterial agents is that there are very few chances of the bacteria developing resistance, since their activity is non-specific. However, the inhibitory effect of zinc nano-particles depends upon the concentration of nano-particles used, the number of bacteria in the sample and the type of bacteria in the sample. It has also been found that zinc oxide nano-particles prevent the attachment of bacteria to the host cell surface and the formation of bio-films. ZnO is the current focus of the Nanotechnology Safety Initiative under National Institute of Environmental Health and Safety. These features of zinc oxide nano-particles suggest that they would make excellent anti- microbial agents for treatment of various infections.

CONCLUSION

ZnO nanoparticles synthesised by all three methods showed activity against all the organisms tested (Gram positive and Gram negative), indicating a wide range of activity. ZnO I and II were effective against *Bacillus, Staphylococcus, E. coli, Pseudomonas, Klebsiella* and *Proteus* whereas ZnO III was more effective against *Propionibacterium acnes*. The nanoparticles were found to be stable exhibiting more or less same activity till the third week of their synthesis, thus making them potential antimicrobial agents.

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