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COMPARATIVE STUDY ON EFFECT OF DIFFERENT CAPPING AGENTS ON THE ANTIMICROBIAL ACTIVITY OF ZNO NANOPARTICLES

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ABSTRACT: The present study focuses on the synthesis of ZnO nanoparticles by chemical method in alcohol base using three different capping agents- Thioglycerol, oleic acid and Triethanol amine (TEA). The effect of concentrations was analyzed for their effectiveness in particle growth and antibacterial activity. The antibacterial activity of ZnO nanoparticles using these capping agents at different concentrations was tested against six laboratory microorganisms namely *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus*, and *Klebsiella* by agar well diffusion method. Zone of inhibitions produced by ZnO nanoparticles with different capping agents was tabulated. The quantitative measurement of zones of inhibition led to the conclusion that all the capping agents were effective in inhibiting growth of different organisms of which the maximum activity was shown by Thioglycerol, capped ZnO nanoparticles at a concentration of 0.12% against all organisms.

INTRODUCTION: Nanotechnology research has gained momentum in the recent years by providing nanoparticles that exhibit antibacterial, anti-corrosive, antifungal and UV filtering properties. Innovative solutions in the field of biomedical, materials science, optics and electronics. ZnO is used in a host of creams and ointments that are used to treat skin diseases. The antimicrobial activity of nanoparticles has been studied with human pathogenic bacteria, mainly *Escherichia coli*. Several natural and engineered nanomaterials have demonstrated strong antimicrobial properties through diverse mechanisms including photo catalytic production of reactive oxygen species that damage the cell components of bacteria and viruses (e.g.: TiO₂, ZnO), and also the bacterial cell

envelope (e.g. ZnO and Ag Nanoparticles¹. ZnO nanoparticles exhibit strong antibacterial activities on a broad spectrum of bacteria and do not induce any cytotoxicity. Zinc oxide nanoparticles are used in the preparation of substances possessing medically as well as cosmetically useful properties (Jones et al).

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 blisters². Zinc oxide Nanoparticles increase the antibacterial efficiency of Ciprofloxacin³. These Nanoparticles are an important source of zinc, which is needed to carry out various essential biochemical reactions in the body in addition to helping in maintaining a healthy immune system. Zinc oxide Nanoparticles are used industrially as a protective coating against photo- destruction⁴. 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

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interest among the researchers due to the growing microbial resistances against metal ions and antibiotics. The recent growth in the field of porous and Nano metric materials prepared by non-conventional processes has stimulated the search of new applications of ZnO nanoparticles.

The present study focuses on the effect of the different capping agents on the antibacterial activity of ZnO nanoparticles.

MATERIALS AND METHODS:

Preparation of Zinc Oxide Nano-particles:

Materials:

Zinc acetate, Potassium hydroxide, dimethyl sulphoxide (DMSO), ethanol, Acetone, methanol, Thioglycerol, Tri ethanol amine, Oleic acid.

(i) Synthesis of Zinc oxide Nanoparticles-I: (Thioglycerol as capping agent) ^{5,6}

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 0.06ml/0.12 ml/0.24ml 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.

(ii) Synthesis of Zinc oxide nano-particles-II:

(Triethanol amine as capping agent)⁶

The same procedure as above was followed for the synthesis of ZnO nanoparticles but instead of Thioglycerol, 0.06ml/ 0.12ml/ 0.24ml of Triethanol amine was used as a capping agent.

(iii) Synthesis of Zinc oxide Nanoparticles-III : (Oleic acid as capping agent) ⁶

The same procedure as above was followed for the synthesis of ZnO nanoparticles but instead of Thioglycerol, 0.06ml/ 0.12ml/ 0.24ml of Oleic acid was used as a capping agent.

Antibacterial Activity of ZnO Nanoparticles:

Antibacterial activity of ZnO nanoparticles against *E coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus* and *Staphylococcus aureus* were tested by agar diffusion method (well method). The cultures were placed on Nutrient agar. Wells bored in the agar were impregnated with ZnO nanoparticles. 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 ^{1, 7, 8}. The zones of inhibition were measured.

RESULTS AND DISCUSSION:

TABLE: 1: ANTIMICROBIAL ACTIVITY OF ZnO NANOPARTICLES (MM IN DIAMETER):

Organism	Thioglycerol			Triethanol amine			Oleic acid		
	0.06ml	0.12ml	0.24ml	0.06ml	0.12ml	0.24ml	0.06ml	0.12ml	0.24ml
<i>Bacillus</i>	15	30	20	15	17	13	13	16	12
<i>Staphylococcus</i>	42	44	24	12	32	20	11	20	24
<i>E. coli</i>	20	24	20	16	21	20	15	12	15
<i>Proteus</i>	20	22	20	12	20	16	11	14	12
<i>Pseudomonas</i>	24	26	18	17	19	17	13	15	13
<i>Klebsiella</i>	28	32	26	20	30	17	14	24	22

The ZnO nanoparticles synthesized using different capping agents was very effective against all the cultures. From **Table 1** and **Fig. 1**, we can infer that among the different capping agents used, Thioglycerol capped ZnO nanoparticles were more effective than Triethanol amine and Oleic acid. Thioglycerol when added at a concentration of

0.12ml was more effective than 0.06 and 0.24ml concentration. Thioglycerol is used as a component of corrosion inhibitors and ore floatation agent. In biochemistry, studied in the activity of immune system or denatures the proteins due to its strong reducing properties. Low molecular weight thiols or their esters such as 2-mercaptoethanol, 1-

thioglycerol and dithiothreitol denatures the proteins by reducing disulphide linkages leading to tautomerization and breaking up quaternary protein

structure. They have been studied as anti-cancer agents by acting as alkylating agents to damage the cancer cell DNA⁹.

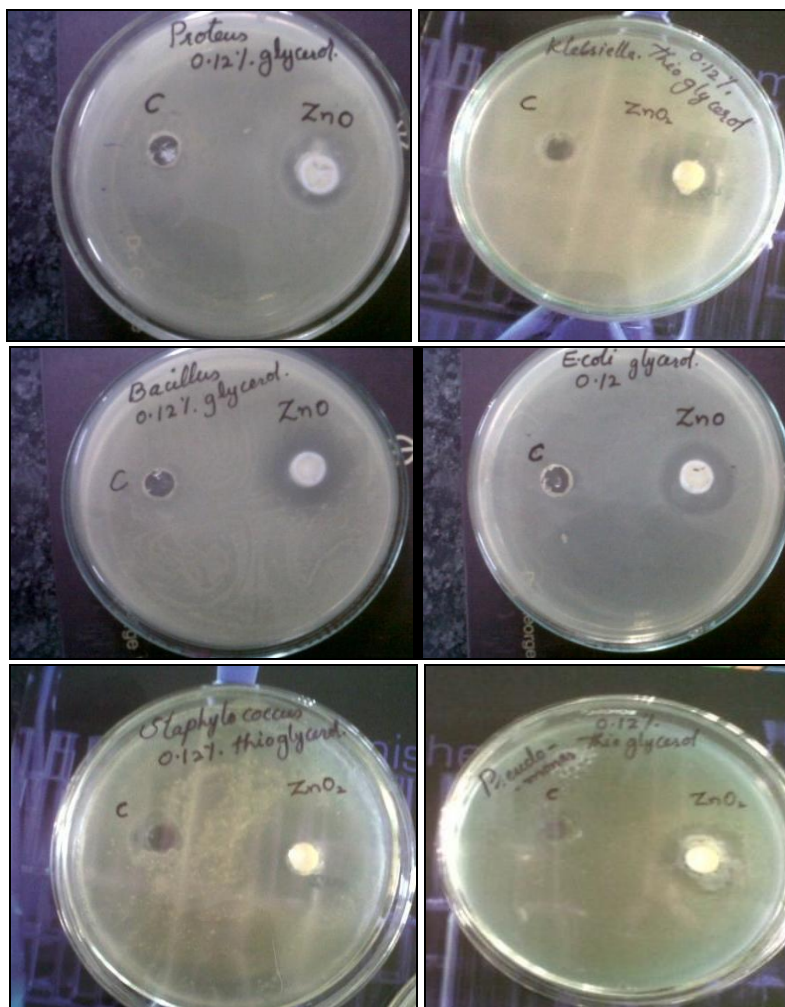


FIG. 1: ANTIMICROBIAL ACTIVITY OF THIOGLYCEROL CAPPED ZINC OXIDE NANOPARTICLES AGAINST VARIOUS MICROORGANISMS.

In his famous and often cited talk given to the American Physical Society in 1959, Richard Feynman challenged scientists across all disciplines to consider the possibilities that could be achieved by miniaturization and atomic level control. In the ensuing fifty years, significant progress has been made to this end, affording scientists the ability to reproducibly create nanometer-sized inorganic structures. As the chemical and physical properties of nanomaterials are intimately linked to its size and shape, significant effort has been placed toward the synthesis of novel nanomaterials^{1, 10, 11}. The ability to modify physical and chemical properties such as light scattering, absorption and emission, magnetic properties, electrical properties and others toward a specific application have made inorganic nanomaterials suitable for a wide variety of

applications. Traditionally, these applications have included sensors, catalysis, electronics, surface enhanced Raman spectroscopy, biology and diagnostic imaging^{1, 12, 13}. Nanotechnology has attracted global attention because nanoparticles (NP) have properties unique from their bulk equivalents. Nanoparticles of Ag, CuO and ZnO are being used industrially for several purposes including amendments to textiles, cosmetics, sprays, plastics and paints¹⁴. A common feature of these three Nanoparticles is their antimicrobial activity. The antimicrobial activity of NP largely has been studied with human pathogenic bacteria, mainly *Escherichia coli* and *Staphylococcus aureus*. Nano-Ag is inhibitory to *E. coli* and *S. aureus*^{1, 15, 16}. These microbes also are sensitive to nano-CuO and nano-ZnO^{1, 17}.

Nanoparticles action may be due in part to their release of free ions. Heavy metal ions have diverse effects on bacterial cell function. Zn is an essential element for cells; levels of Zn above the essential threshold level inhibit bacterial enzymes including dehydrogenase¹⁸ and certain protective enzymes, such as thioperoxidase, and glutathione reductase¹⁹. Zn inhibition of NADH oxidase is proposed to impede the respiratory chain of *E. coli*. Additionally, loss of membrane potential is associated with inhibition by Zn ions at cytochrome oxidase.

CONCLUSION: ZnO nanoparticles have been successfully synthesized using zinc acetate, DMSO and KOH in ethanol at room temperature using three capping agents, i.e. TEA, oleic acid and Thioglycerol at different concentrations. It has been found that thioglycerol is more effective capping agent as compared to oleic acid and TEA. Thioglycerol was very effective when its quantity was 0.12ml than at 0.06ml and 0.24ml (2 times). This could be due to the effect of Thioglycerol on the particle size of ZnO nanoparticles.

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