COMPARATIVE STUDY OF ANTIBACTERIAL APPLICATIONS OF ZINC OXIDE NANOPARTICLES AND POLYMER NANOCOMPOSITE.

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ABSTRACT

In the present work we report the synthesis of zinc oxide nanoparticles(ZnO-NPs) and its composite with the the polymer. Zinc oxide nanoparticles were prepared using zinc nitrate and sodium hydroxide by wet chemical synthesis. These nanoparticles were characterised by FTIR, XRD, SEM and EDAX. The particle size determined from XRD by using Debye Scherrer's formula was compared with the particle size obtained by SEM. These particles were checked for its antimicrobial activity for two Gram negative bacteria i.e. Proteus mirabilis and E coli; two Gram positive bacteria S albus and S faecalis and two fungi viz. A niger and Candida albicans. The nanoparticles were found to be inhibitory for E coli and S albis. The polymer considered was Polyaniline. Polyaniline was prepared from the monomer aniline by chemical oxidative method using ammonium persulphate as oxidant. These nanoparticles were used to prepare Polyaniline-ZnO nanocomposite. This composite of ZnO nanoparticles and polyaniline was studied for its potential as antimicrobial agent to be applied in dentistry and lotions.

The polyaniline nanocomposite filled with ZnO nanoparticles were prepared by chemical oxidative method in acidic medium using ammonium persulphate as an oxidant. The composition, morphology and structure of the nanoparticles and nanocomposite were characterised by FTIR, XRD, SEM and TGA. Scanning electron microscopy (SEM) characterization of the composite morphology reveals a homogeneous distribution of the ZnO NP throughout the film for applied NP/polymer ratio. The optical properties of the embedded NP are not affected by the matrix The characteristic FTIR peaks of PANI were found to shift wave number in PANI–ZnO composite due to formation of H-bonding. These observed effects have been attributed to interaction of ZnO nanorods with PANI molecular chains. These nanoparticles and nanocomposites were then studied for its antimicrobial applications against both the Gram positive and Gram-negative species. The nanocomposite exhibits bactericidal behaviour towards Escherichia coli (E. coli), S faecalis, S albis and the fungii, A niger, Candida albicans.

Index Terms - Zinc oxide, polyaniline, nanocomposite, SEM, TEM, XRD, Antimicrobial

1. Introduction

Zinc oxide has unique physical and chemical properties, like high chemical stability, high electrochemical coupling coefficient, broad range of radiation absorption and high photostability, which make it a multifunctional material. In materials science, zinc oxide is classified as a semiconductor II-VI whose covalence is on the boundary between ionic and covalent semiconductors. A broad energy band (3.37 eV), high bond energy (60 meV) and high thermal and mechanical stability at room temperature make it attractive for its use in electronics, optoelectronics and laser technology. The piezo- electric and pyroelectric properties of ZnO make it useful as a sensor, converter, energy generator and photocatalyst in hydrogen production Because of its hardness, rigidity and piezoelectric constant it is an important material in the ceramics industry, while its low toxicity, biocompatibility and biodegradability make it a material of importance for biomedicine.

The various structures of zinc oxide nanoparticles indicate that ZnO can be classified among the materials with potential applications in the fields of nanotechnology. Zinc oxide can occur in one, two, three dimensional structures.

In this paper, the method used for synthesis of nanoparticle and nanocomposite alongwith its antimicrobial application is discussed. The zinc oxide occurs in various structures and offers a wide range of properties. The variety of methods for ZnO production, such as vapour deposition, precipitation in water solution, hydrothermal synthesis, the sol-gel process, precipitation from microemulsions and mechanochemical processes are available and make it possible to obtain products with particles differing in shape, size and structural features. The methods adopted by us are discussed in the following sections.

Polyaniline is considered as a material of promising applications because of its environmental and thermal stability,good elecrical conductivity and low-cost of preparation. Polyaniline exists in various forms which depend on its oxidation states as leucoemeraldine(fully reduced form), emeraldine and pernigraniline(fully oxidised form).The properties of polyaniline depend on the reaction conditions. The conducting polymer has good electrical, electrochemical and optical properties.

2. MATERIALS AND METHODOLOGY

2.1 Preparation of ZnO nanoparticles

In the preparation by **wet chemical method,** zinc oxide nanoparticles were synthesized by using 0.2 M solution of $Zn(NO_3)_2$ and 0.4M NaOH. Zinc nitrate was dissolved in water to which sodium hydroxide was added dropwise with continuous stirring at room temperature leading to generation of metal hydroxides. The stirring was continued for 6 hrs at 85ºC. The reaction mixture was then filtered and dried in oven at 60ºC. These hydroxides are calcined in furnace at 600ºC and zinc oxide nanoparticles were obtained. The product was characterised by SEM, EDAX and XRD

2.2 Preparation of Polymer ZnO nanocomposites

These nanoparticles were used to prepare Polyaniline-ZnO nanocomposites. The polyaniline nanocomposite filled with ZnO nanoparticles was prepared by chemical oxidative method in acid medium using ammonium persulphate as an oxidant. The ZnONPs were added before the initiation of the polymerisation.

2.3 Antimicrobial activity

2.3.1 Procurement of cultures-
All the microbial cultures were procured from National Collection of Industrial Microorganisms (NCIM), NCL, Pune. Four bacteria [***Proteus mirabilis***, **(NCIM2388),** ***E. coli*** **(ATCC2574*)***, ***S. albus*** **(NCIM2178*)*** and two fungii ***A. niger,*** **(ATCC504)** ***Candida albicans*** **(NCIM3100)**

2.3.2 Maintenance of cultures-
The pure cultures were maintained by routine sub-culturing at one month interval on nutrient agar and potato dextrose agar slants for bacteria and fungi respectively. (Hi-Media laboratories private limited, Mumbai, India).

2.3.3 Disc Diffusion Assay by Kirby-Bauer Method.
All the compounds were subjected to anti-microbial activity by measuring the diameter of zone of inhibition using disc-diffusion assay by Kirby-Bauer method. Potato-Dextrose and Mueller Hinton agar plates were prepared by pouring 20 ml each in sterile petri- plates for fungal and bacterial assay respectively and allowed to solidify. During the assay, standard bacterial cultures were grown in Nutrient broth and fungal cultures were grown in Potato-Dextrose broth respectively. 24-48 hr old fresh bacterial culture and 48-72 hr old fresh fungal spore suspension was used. The cotton swabs were used for the inoculation of the cultures. The swabs were lightly dragged across the agar surface in a zigzag manner so that the surface is inoculated uniformly. Pre-sterilized filter paper discs of 5 mm were dipped into the individual compounds separately and placed on the inoculated agar plates. The plates were incubated for a period of 24-48 hrs. at 37 ^{o}C for bacteria and 48-72 hrs at 20 for fungi. After the incubation period the plates were observed for a clear zone of inhibition. The zones of inhibition were measured in mm using a measuring scale and the average was calculated. The experiments were carried out in 4 replicates.

1. The compounds were dissolved in DMSO
2. 100 ug /ml of concentration were used for the study.
3. The antibiotics Ciprofloxacin and Fluconazole were used as standards for the bacteria and the fungii respectively.

3 CHARACTERISATION

3.1 XRD ZnO
The synthesized ZnO nanoparticles were characterized by XRD and SEM.Figure-1 shows the XRD of the synthesized ZnO nanoparticles. All the peak positions and relative peak intensities of the ZnO product agree well with those of the standard XRD pattern (JCPDS data (file 36-1451) and

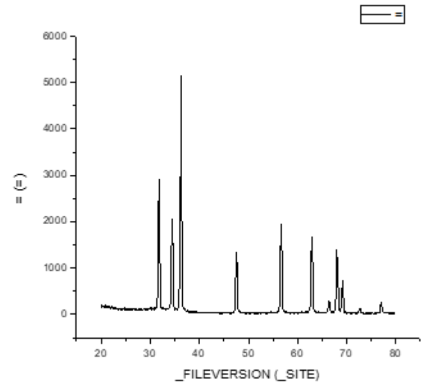


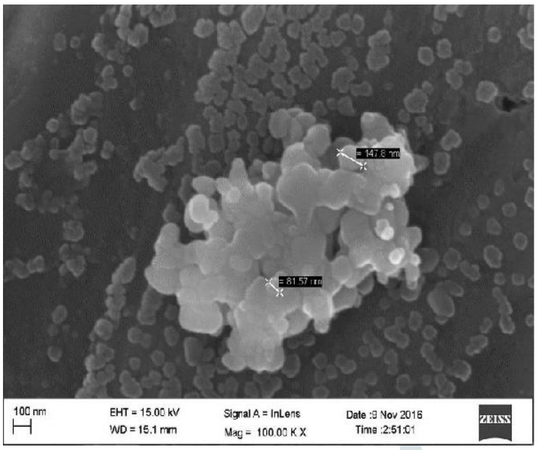
Figure-1**. XRD images of synthesized ZnO Nanoparticles**

no characteristic peaks of impurities. The purity of thus synthesized ZnO nanoparticles was indicated. The phase structure of ZnO nanoparticles belongs to a wurtzite structure (hexagonal phase). The ZnO nanoparticles have an average grain size of about 16-29 nm as calculated by the Debye-Scherrer's equation.

The XRD shows the peaks corresponding to the standard XRD peaks observed in JCPDS file and show the particle size in the nanometric range. XRD pattern showed both sharp and broad peaks indicating the mixtures to be of micro- and nanoparticle sizes

3.2 SEM image and EDX

Figure-2 (A) and (B), show the SEM image and EDX results of the ZnO nanoparticles. The shape and morphology of ZnO nanoparticles were studied from SEM. SEM analysis showed agglomerated nano-sphere morphology of ZnO nanoparticles of size 18-35 nm, well in agreement with the XRD analysis. The atomic weight percentage of the constituent elements was nearly 1:1 in the formed ZnO as given by the EDX result. SEM shows two different types of entities. Polymer in the central region show agglomeration state whereas zinc oxide nanoparticles with average particle size less than 100nm.
EDAX ZnO peaks corresponding to zinc and oxygen which show the purity of the sample.



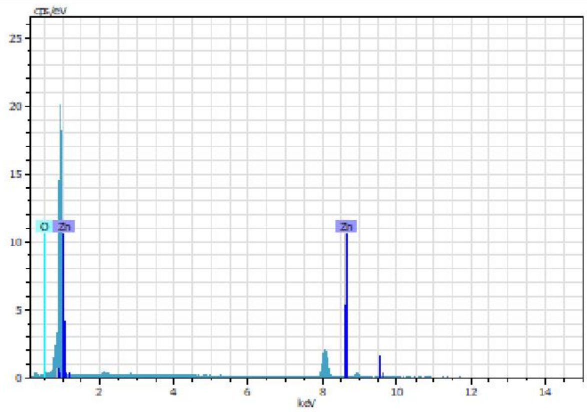


Fig 2(A) SEM of ZnO nanocomposites **Fig 2(B)EDAX** of ZnO nano

SEM shows two different types of entities. Polymer in the central region show agglomeration state whereas zinc oxide nanoparticles with average particle size less than 100nm. EDAX ZnO peaks corresponding to zinc and oxygen which show the purity of the sample.

3.3 ANTIMICROBIAL ACTIVITY

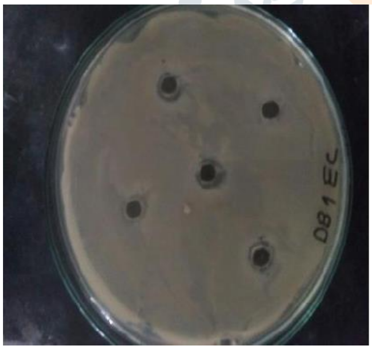


Fig 3(A) Antibacterial activity of ZnONPs for *E coli* 3(B)Antibacterial activity of ZnO nanocomposite

3.4 Report of analysis of the sample**

No	Sample Name	Zone of Inhibition (mm)					
		Microorganisms studied					
		A*	Mean	B*	Mean	C*	Mean
1	ZnO nanoparticles	**Nil**	**-**	**10,11,11,10**	**10.5**	**11,11,10,11**	**10.75**
2	ZnO and Polyaniline nanocomposites	**12,13,12,12**	**12.25**	**11,12,11,11**	**11.25**	**11,12,11,11**	**11.25**
3	Std. antibiotic	**20**		**18.8**		**19.5**	
4	**Solvent DMSO**	**Nil**	**-**	**Nil**	**-**	**Nil**	**-**

***Proteus mirabilis*, (NCIM2388), B-*E. coli* (ATCC2574), C-*S. albus* (NCIM2178)**

No	Sample Name	Zone of Inhibition (mm)					
		Microorganisms studied					
		D	Mean	E*	Mean	F*	Mean
1	ZnO nanoparticle	Nil	-	Nil	-	Nil	-
2	ZnO + polyaniline nanocomposite	Nil	-	11,12,11,11	11.25	11,12,13,13	12.25
3	Std. antibiotic	23.5		25		30	
4	**Solvent DMSO**	Nil	-	Nil	-	Nil	-

D-*S.faecalis*, E-*A.niger*, (ATCC504) F-*Candida albicans* (NCIM3100)

Concentration of the compound used-500 µg/ml
Concentration of the antibiotic used-100 µg/ml

4. RESULT AND DISCUSSION

- The XRD pattern obtained is in agreement with the standard JCPDS and the particle size in the range between 16-29 nm. The crystal shows wurtzite structure.
- SEM confirms the size in the nanometric range.
- EDAX confirms the presence of zinc and oxygen elements and the purity of the sample.
- The nanocomposite seems to be microbicidal for the bacteria Proteus mirabilis and the fugii Aspergillus niger and Candida albicans whereas the nanoparticle is ineffective against all the three.
- The antibacterial activity against both the bacteria Escheria coli and Staphylococcus albus is almost comparable with both the nanoparticles as well as nanocomposite.
- The bacteria S faecalis seems to be resistant to both the nanoparticles as well as nanocomposite.

The study shows that the nanocomposite films exhibit bactericidal behaviour towards *Escherichia coli* (*E. coli*) for a ZnO concentration as low as ≈ 0.74 µ g cm^{-2} (1.33 mmol cm^{-3}) and to be carried out for the remaining.

5. CONCLUSIONS:

I. The Zinc oxide nanoparticles were found to be in the nanorange upto 100nm in size.
II. The Zinc oxide nanoparticles were homogeneously distributed within both the polymer matrices as the addition was prior to the process of polymerisation and it was revealed by SEM. The crystal lattice structure and particle size were confirmed by XRD.
III. The polymer-zinc oxide nanocomposites were found to be more effective against microorganisms than only zinc oxide nanoparicles.as Zinc oxide nanoparticles were effective against only *E coli* and *S albis* whereas the polyaniline zinc oxide nanocomposite was effective against all the tested species except *S faecalis* and is found to be more microbicidal .

6. SIGNIFICANCE:

- The Method used by us is a simple and easy to follow.
- The particle size of the zinc oxide nanoparticles is in the range 16-100 nm
- The zinc oxide nanoparticles as well as the polymer zinc oxide nanocomposite are showing antibacterial activity
- Such a comparative study of the nanoparticles and its nanocomposite is carried out for the first time.

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