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Enhanced Antimicrobial Activities of Hybrid ZnMgAlO Nanocomposite by Soft Chemical Method

V.Jeevanantham[1],Dr.K.V.Hemalatha[2],S.Satheeskumar[3],
[1]Assistant Professor, Department of Chemistry, Vivekanandha College of Technology for Women, Tiruchengode– 637205, Tamilnadu, India
Email : jeere06@gmail.com
[2]Assistant Professor, Department of Chemistry, Coimbatore Institute of Technology, Coimbatore-641014, Tamilnadu, India.
Email : hemaac@gmail.com
[3]Assistant Professor, Department of Physics, KSR Institute of Engineering and Technology, Tiruchengode637215, Tamilnadu, India.
Email : satheesphysics@gmail.co

ABSTRACT
In the present investigation, ZnMgAlO nanoparticles were prepared by soft chemical method. The synthesized NPs were analyzed by XRD and SEM EDAX. ZnMgAlO crystal structure was confirmed through powder XRD technique as hexagonal wurtzite structure.The surface morphology was analyzed from SEM images. Finally, antimicrobial activity of all the synthesized samples was tested against Bacillus subtilis and Chlamydia trachomatis bacteria and Xylaria hypoxylon, Fistulina hepatica fungus.The observed results showed good anti-bacterial and anti-fungal activities.

Indexing terms/Keywords

Soft chemical method, ZnMgAlO nanoparticles, anti-bacterial and anti-fungal activities.

Academic Discipline And Sub-Disciplines

Chemistry ; Nanochemistry

SUBJECT CLASSIFICATION

Metal oxide synthesis

TYPE (METHOD/APPROACH)

Research in Chemistry; Nano-Technology Experimental Investigation; Literature Studies; Results and Discussion; Conclusions.

1. INTRODUCTION

Zinc Oxide is a wide energy band-gap (Eg ≈ 3.37 eV) semiconductor (II–VI) material [1]. It is an important material due to unique physical, chemical, biological and excellent electronic properties. Zinc oxide NPs are prepared by various methods such as hydro thermal, direct precipitation, chemical reduction method and pulsed LASER deposition etc., [2,3] Hydrothermal synthesis method has been adopted as a simple mode for processing of metal oxides[4,5]. Currently, green synthesis method for preparing NPs is gaining significance due to its simplicity and eco-friendly. The impurities doped with zinc oxide improve the chemical and physical properties. The elements such as Al, Mg, Ni, Ag, Mn are doped/co-doped with zinc oxide for improvement of properties.[6]

Antimicrobial activities of ZnMgAlO NPs against bacteria Bacillus subtilis (gram positive), Chlamydia trachomatis(gram-negative), Xylaria hypoxylon and Fistulina hepatica fungi were quantitatively evaluated in culture media [7]. It is considered that the detected active oxygen species generated by these metal oxide nanoparticles could be the most important mechanism of their antibacterial activity. TiO2 and the oxides of other nanoparticles like CdO and ZnO have also been reported for antibacterial activities [8]. The properties of antimicrobial activity are used to killing or prevent the growth of microbial like bacteria and fungi [9, 10]. The present work aimed to prepare ZnMgAlO nanoparticles by soft chemical method. The structure, morphological and antibacterial activity of the prepared NPs was investigated. To the best of our knowledge, this is the first time comparative toxicity estimate study of biological and chemically synthesized a mixture of doping with ZnO Nps against bacterial and fungal pathogens.

2. EXPERIMENTAL DETAILS

2.1. Materials and Synthesis

Analytical grade reagents zinc nitrate hexahydrate $Zn(NO_3)_2.6H_2O$, Aluminium nitrate nona hydrate $Al(NO_3)_3.9H_2O$, Magnesium nitrate hexahydrate [$Mg(NO_3)_2.6H_2O$] and sodium hydroxide [NaOH] obtained from Merck chemicals were used without further purification. Pure de-ionized water was used for preparing solutions and purification of the prepared nanoparticles. To prepare the $Zn_{1-x-y}Mg_xAl_yO$ nanoparticles, a soft chemical method was used. The precursor solution was allowed for 24 h reaction time at room temperature. The resulting precipitate was purified with de-ionized water several times and it was dried at air for 3 h for the preparation of samples. The growth temperature was chosen as 120°C. The different doping concentration of Magnesium and Aluminum [x = 0, 0.10, 0.05, y = 0, 0.10, 0.15] were added.

2.2. Test micro organism

The following clinical isolates of bacteria and fungi were used for the study: Bacillus subtilis, Chlamydia trachomatis and Xylaria hypoxylon, Fistulina hepatica fungus. Microbial cultures were grown on nutrient agar and potato dextrose agar (PDA) for bacteria and fungi respectively and maintained the temperature at 4°C in refrigerator. Throughout this study, the same nutrient was used for all culture.

2.3. Antimicrobial activity

Antibacterial activity of ZnMgAlO nanoparticles were tested by agar well disc diffusion method. The microorganisms used for this study were Bacillus subtilis and Chlamydia trachomatis and fungus, Xylaria hypoxylon, Fistulina hepatica. The concentration of nanoparticles was used as 10, 30, 50, 100 and 150 μg/ml. The diameters of minimum inhibition concentration zones were determined.

3. RESULTS AND DISCUSSION

3. 1. XRD analysis

XRD analysis was used to examine the phase and crystalline size of the prepared samples. Figure 1 shows the XRD pattern of the prepared $Zn_{1-x-y}Mg_xAl_yO$ nanoparticles by soft chemical method for the samples (S1, S2, S3, S4). All the diffraction peaks were readily indexed for the hexagonal wurtzite structure of ZnO. The diffraction peaks were in good agreement with the standard JCPDS No: 80-0075. By using Debye-Scherer's formula particle sizes were calculated [11].

$$D = 0.9\,\lambda / (\beta \cos\theta) \qquad (1)$$

The full-width at half-maximum was measured with help of Gaussian fitting program. The calculated particles size of prepared samples found 11 – 32 nm.

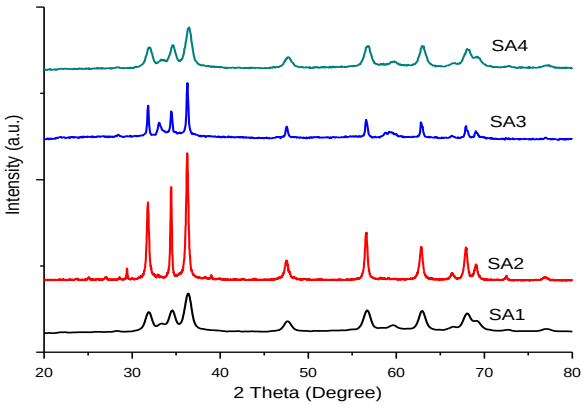

Fig. 1 XRD spectra of ZnMgAlO nanopaticles of different doping concentration of Magnesium and Aluminum.

3. 2. UV-Vis absorption spectra analysis

The UV-Vis absorbance spectra of prepared samples were shown in Figure 1. The absorption peaks present samples and calculate the band gap (Eg) values were listed in Table 1. The absorption peak values were in close agreement with the reported values [3, 7]. The band gap values calculated were larger than that of bulk and substantiated the formation of small size particles. The decrease in particle size originates from the quantum confinement effect. The blue shift was observed in the absorbance spectra of nanoparticles. The absorption peak was observed at 364.9 nm in sample S1. The incorporation of aluminum decreased the value of absorption peak at 327 nm. The band gap of the nanoparticles were calculated by Eg= [(hc)/(λe)] eV.

Table 1. Absorption wavelength and Band gap of synthesized nanoparticles

Sample	Absorption wavelength(nm)	Band gap (eV)
SA1	325.1	3.82
SA2	364.9	3.40
SA3	327.7	3.79
SA4	319.6	3.88

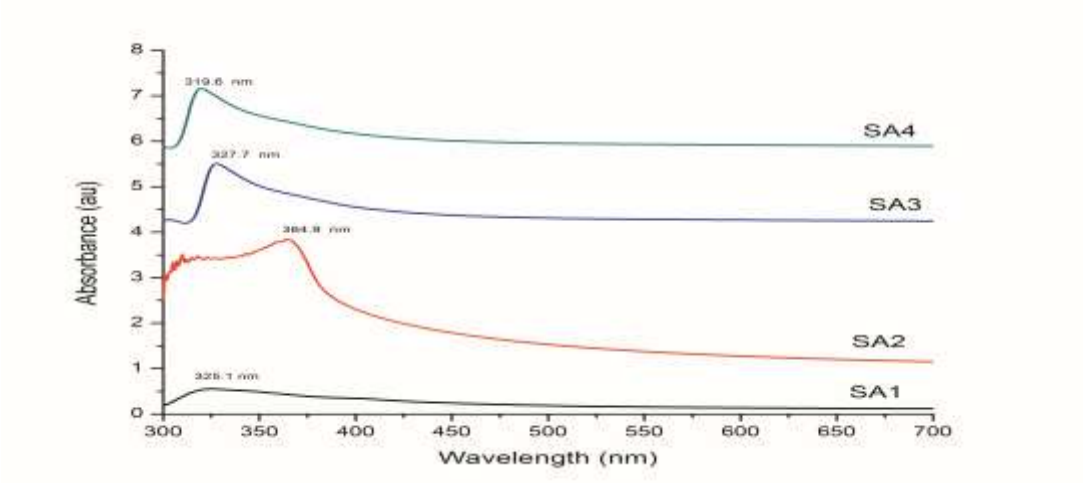

Fig. 2 PL spectra of ZnMgAlO nanopaticles of different doping concentration of Magnesium and Aluminum

3. 3. TEM ANALYSIS

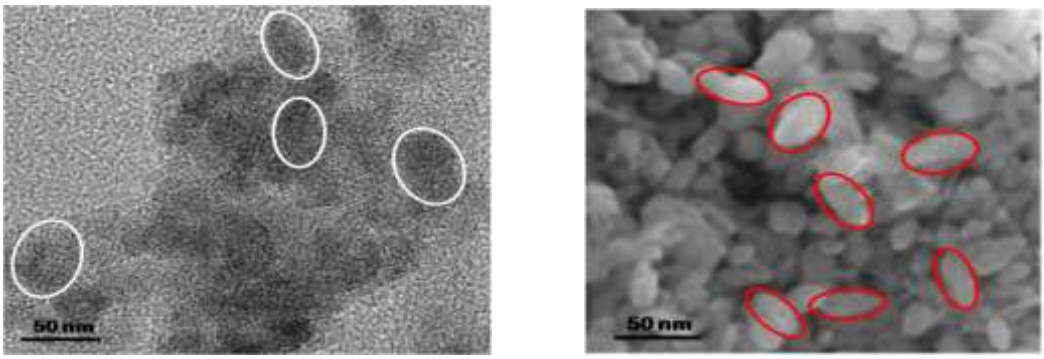

Fig. 3 TEM analysis of ZnMgAlO nanopaticles - SA4

3. 4. EDAX analysis

EDAX spectrum of Magnesium doped and aluminum co-doped zinc oxide nanoparticles was shown in Figure 3. The peaks for Zn, Mg, Al and O were clearly seen in the EDAX analysis. The EDAX analysis confirmed the incorporation of Mg and Al in the prepared nanoparticles. There was no impurity peaks in samples.

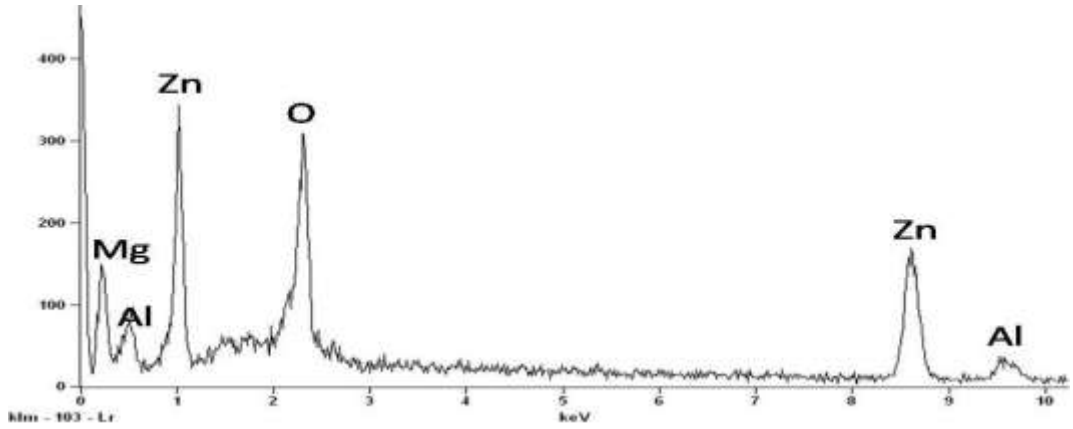

Fig. 4 EDAX analysis of ZnMgAlO nanopaticles - SA4

3. 5. Antimicrobial studies

In vitro antimicrobial activities of the synthesized ZnMgAlO nanoparticles were determined using agar well diffusion method. Approximately 20 ml of stertile molten and cooled media LB and SDA were poured in sterilized petri-dishes, and the plates were left overnight at room temperature. After inoculation and cultivation of different target bacteria on top of nutrient agar, wells were placed in selected area on different plates. Each standard disk was impregnated with freshly prepared ZnMgAlO NPs and agar wells of 5 mm diameter were prepared with a sterilized stainless steel cork borer and were properly labeled. About 0.05 and 0.1 ml of various concentrations (10, 30, 50, 100, 150 μg/ml for bacteria and fungi)

of ZnMgAlO NPs as S1, S2, S3 and S4 were added in the wells. The plates containing the ZnMgAlO NPs microbes and were incubated at 36^{o}C and the observed activities were compared. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the wells in the discs. The diameter of such zones was measured using a meter scale and the mean value for each organism was recorded and expressed in mm. The antimicrobial activity of ZnMgAlO NPs was checked by determining the MIC (minimum inhibitory concentration). To evaluate the MIC, an appropriate volume of pathogens in LB and SD broth was added to ZnMgAlO NPs suspensions whose concentrations varied from 0.01 to 10 mM for bacteria and from 2 to 20 mM for fungi. The chosen nanoparticles were prepared with dimethyl sulphoxide (DMSO) and mixed with 450 ml/ml of broth and 50 ml of fresh microbial inoculums and the whole setup was allowed to grow overnight at 36^{o}C for 24 h and 72 h respectively. Compounds were tested six times and the results were averaged. The visual turbidity of the tubes was noted before and after incubation. The pictures were taken by an Olympus C2020Z digital camera. The data obtained in all tests were compared with the control. Statistical t-test was used to evaluate the significance of experimental results ($P< 0.05$).

The microorganisms used for this study were B.subtilis, C.trachomatis, X.hypoxylon and F.hepatica. The diameters of MIC zones were determined with various concentration 10, 30, 50, 100 and 150 µg/ml of ZnMgAlO NPs and were listed in Table 2 and Table 3.

Table 2. Minimum Inhibition Concentration of ZnMgAlO nanoparticles with B.subtilis, C.trachomatis bacteria

Sample	Minimum Inhibition Concentration (mm)									
	Bacillus subtilis (Gram +)					Chlamydia trachomatis (Gram -)				
	10	30	50	100	150	10	30	50	100	150
S1	7.3	8.1	8.3	9.5	10.3	14.2	14.8	15.4	15.9	16.2
S2	7.5	8.2	8.5	9.8	10.4	14.6	15.1	15.5	15.9	16.3
S3	6.4	7.0	7.1	7.8	8.2	14.9	15	15.3	15.7	16.1
S4	6.4	7.1	7.3	7.9	8.5	15.8	16	16.2	16.5	16.9

Table 3. Minimum Inhibition Concentration of ZnMgAlO nanoparticles with X. hypoxylon and F. hepatica

Sample	Minimum Inhibition Concentration (mm)									
	Xylaria hypoxylon					Fistulina hepatica				
	10	30	50	100	150	10	30	50	100	150
S1	6	6	6	6	6	6.3	6.6	6.9	7.2	7.6
S2	6	6	6	6	6	6.7	7.1	7.4	7.7	7.9
S3	6	6	6	6	6	6.9	7.4	7.7	7.8	8.1
S4	6	6	6	6	6	7.3	7.5	7.7	7.9	8.3

B. subtilis was a gram-positive bacterium. It was widely found in soil and water, and it may infect food poison. The sample (S2) was effective against B. subtilis, the diameter of MIC increased with increase in the concentration. The sample S2 was highly efficient (MIC = 10.4 mm) for 150 µg/ml concentration.

C. trachomatis was a gram negative bacterium, infections are the mostly blindness of humans. The prepared NPs showed superior activity against C. trachomatis. The sample (S4) is observed to be effective against C. trachomatis. The diameter of MIC values 15.8, 16, 16.2, 16.5 and 16.9 mm 10, 30, 50, 100 and 150 µg/ml concentration respectively.

X. hypoxylon was an ascomycetous fungus. It was commonly found growing on dead wood. All samples S1, S2, S3 and S4 were effective against X. hypoxylon Fungus. The diameter of MIC values same for all concentrations. The highly efficient (MIC = 6.0 mm) for all the concentration.

F. hepatica was an unusual bracket fungus classified in the agaricales. The prepared NPs showed superior activity against F.hepatica. The sample (S4) is observed to be effective against F.hepatica. The diameter of MIC values 7.3, 7.5, 7.7, 7.9 and 8.3 mm 10, 30, 50, 100 and 150 μg/ml concentration respectively.

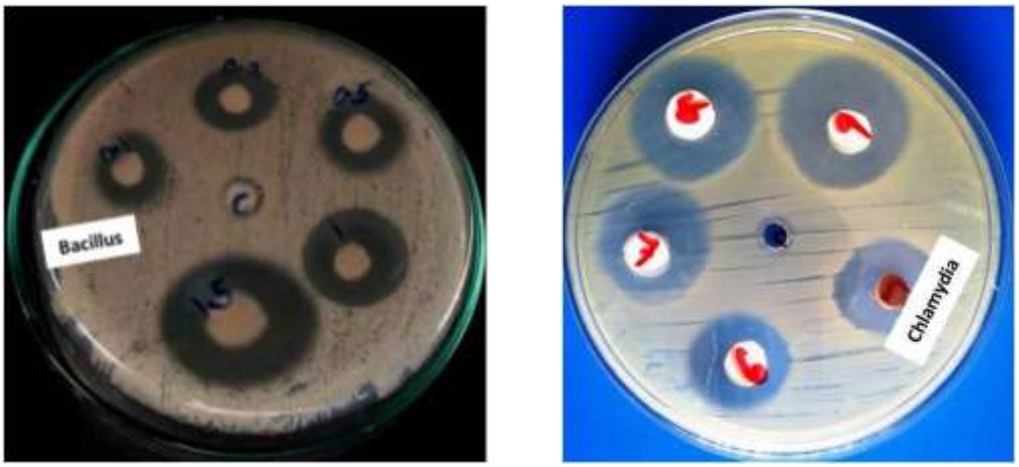

Figure 5. Antibacterial activity against (a) Bacillus subtilis (b) Chlamydia trachomatis

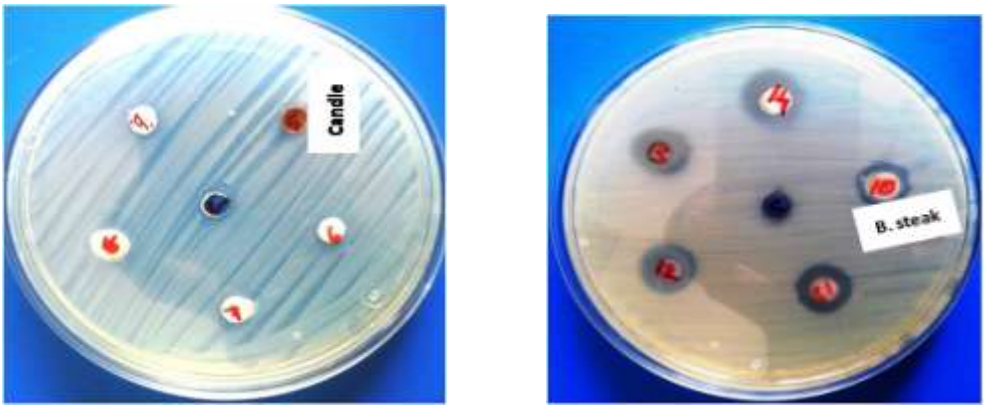

Figure 6. Antifungal activity against (a) Xylaria hypoxylon (b) Fistulina hepatica

Gram positive bacteria have one cytoplasmic membrane and the thick cell wall was composed of peptidoglycan and Gram negative bacteria have peptidoglycan between inner and outer membrane which is thinner. Since gram negative bacteria are killed more quickly than gram positive bacteria which tends to withstand the inhibition. The ZnMgAlO NPs were effective antibacterial agents. Increase in concentration of NPs increased in the diameter of MIC. All the samples were excellent antibacterial agents. The sample S2 and S4 exhibited valuable antibacterial effect against the test strains. Similarly, ZnMgAlO NPs were also shown significant antifungal activities against F.hepatica. More number of mechanism have been proposed for the antibacterial activity above synthesized nanoparticles as i) release of Zn_{2+} ion from ZnO materials (ii) damaging the cell membrane due to the size of the nanoparticles (iii) and the oxygen generated from the nanoparticles.

The reason of cell death was the leakage of genetic materials due the damage in cell membrane. The surface-to-volume ratio of prepared NPs was high and the prepared NPs were highly reactive with microorganisms. When the NPs were placed on the surface of bacterial strains, then reactive oxygen species (ROS) were formed. These ROS were responsible for the increase in the permeability of the cell membrane. This confusion in the activity of cell membrane directs cell death. Hence all the prepared ZnMgAlO NPs showed good growth inhibition against the bacterial strains.

All the prepared nanoparticles shows antimicrobial activity against selected pathogens and MIC was observed the stains sample C.trachomatis and B.subtilis. So, the activity of NPs was observed in samples S4 and S2 was more killing effect of pathogens of C.trachomatis and B.subtilis respectively. For the fungal X. hypoxylon zone of MIC were observed 6 mm for all concentration of NPs. In this case there is no change in MIC with change in concentration of NPs. Among the F.hepatica pathogens MIC was noted 8.3 mm for the sample S4.

4. CONCLUSION

ZnMgAlO nanoparticles were prepared by soft chemical method. The nanoparticles crystal structure was confirmed using XRD analysis and the sizes of the particles were found in the range of 11 nm - 15 nm. The SEM micrographs demonstrated the surface morphology of the prepared ZnMgAlO nanoparticles and rod shaped morphology were formed. Anti-bacterial and anti-fungal activities of ZnMgAlO nanoparticles were demonstrated against B. subtilis, C. trachomatis, X. hypoxylon and F. hepatica micro organisms. The ZnMgAlO nanoparticles show an increased permeability of cell membrane which leads the cell death.

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Author' biography with Photo

Mr.V.Jeevanantham completed M.Tech from Anna University in 2009, M.Sc degree from Bharathiar University in 2005 and he persuing ph.d in anna university Currently working as assistant professor in Vivekanandha College of Technology For Women. His research interests include Nano Technology, Metal Oxide Synthesis, Photodegradation in Nanoparticles.