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Synthesis and Characterization of ZnO-MMT Nanocomposite for Antibacterial Activity Studies

ARTHI FEIONA, T; SABEENA, G; SAKTHI BAGAVATHY, M; PUSHPALAKSMI, E; JENSON SAMRAJ, J; *ANNADURAI, G

[1]*Division of Nanoscience, Sri Paramakalyani Centre for Excellence in Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi - 627 412, India.*
**Corresponding Author Email: gannadurai@msuniv.ac.in*

ABSTRACT: ZnO oxide Nanoparticle and ZnO oxide with Montmorillonite nanocomposite were prepared by an environmentally friendly, efficient, and inexpensive method that was synthesized using the chemical method. ZnO nanoparticles as an effective antibacterial material were immobilized on the surface of montmorillonite (MMT). The objectives of this paper are to summarize our research activities in (a) developing processes to disperse nanomaterials (undoped and doped zinc oxide powders) in the polymers matrix, (b) using X-ray diffraction (XRD), Fourier Transform Infra-Red Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM) and Thermo Gravimetric Analysis (TGA) techniques to characterize polymer matrix structures, (c) studying structure-property relationship of these types of new materials, and (d) evaluating the antibacterial performance of these materials for different applications. The results showed that the ZnO nanocomposite was uniformly dispersed in the polymer matrix and the particles remained their average size (20 - 150 nm) before incorporation into the polymer matrix.

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Nanomaterials are the most studied materials of the century that gave birth to a new branch of science known as "nanotechnology." Nanomaterials are prepared from bulk size materials, but the smaller size and shape of these particles differentiate their chemical actions from those of their parent material (Brunner and Wick Manser, 2006). Nanomaterial can be applied in various fields such as cosmetics, paints, displays, batteries, medicine, catalysis (Sk *et al.,* 2020), gas sensor, food engineering (production, processing, safety, and packaging), agriculture, energy (storage and conversion) and construction. Engineered nanomaterials manufactured with nanoscale dimensions are generally grouped into four types: carbon, metals, metal oxides, dendrimers, and composites (Ju-Nam and Lead, 2008). Polymers are host materials for metal nanoparticles (Arivalagan *et al*., 2011). The polymer acts as a surface topping specialist when nanoparticles are implanted in them. The nanocomposites obtained display of upgraded optical properties. Nanocomposites are a special class of materials having unique properties and the wide application potential in diverse areas. Novel properties of nanocomposites can be obtained by successfully joined characteristics of parent constituents in a single material. These materials are different as both materials like pure polymers and inorganic nanoparticles (Matei *et al.,* 2008). Recently, nano-crystalline inorganic oxides exhibited enormous opportunities and attracted the interest of several research groups because of their different topical characteristics and wide range of particle sizes. The catalysis by nanomaterials has become an area of interest, as these materials exhibit better catalytic activity compared to their bulk-sized counterpart. ZnO is one of the multifunctional inorganic nanoparticles that has drawn increasing, attention in recent years due to its many significant physical and chemical stability, high catalysis activity, effective antibacterial and bactericide function, intensive ultraviolet and infrared adsorption. The advance of ZnO nanoparticles could improve the properties of the polymer matrix. Many reports have been published about the good physicochemical properties using ZnO in composites. Recently, composites of ZnO with MMT have attracted much attention because of their excellent properties as a semiconductor material, especially for the degradation reactions of recalcitrant organic pollutants. Moreover, it acts as a good antibacterial, antifungal with the aim of numerous bacterial and fungal species (Basri *et al.,* 2020). The enhancement in photocatalytic activity of ZnO with MMT

**Corresponding Author Email: gannadurai@msuniv.ac.in*

composites has been associated with the changes in their structural, textural, and optical properties, such as surface area, particle size, the formation of a specific crystalline phase, and low bandgap energy. The main difference between gram-positive and gram-negative bacteria is that the protein Gram-positive bacteria have thicker cell walls containing many layers (consisting of peptidoglycan and acid teichoic) while protein Gram-negative bacteria have a thinner cell wall that contains many layers (consisting only of peptidoglycan) (Singh and Khanna, 2007). These cause the difference between the gram-positive and gram-negative reaction to an antibiotic. Although the cell walls are thicker, Gram-negative bacteria are more resistant to antibiotics than Gram-positive bacteria because the bacteria can penetrate the lipid layer on the outer membrane of the bacteria. In this study, stable Zinc oxide nanocomposite was synthesized by the reduction of Zinc acetate with sodium alginate solution. The ZnO oxide Nanoparticle was characterized by Fourier Transform Infra-Red spectroscopy. The ZnO oxide Nanoparticle and ZnO oxide with Montmorillonite nanocomposite were characterized via FTIR and XRD, and its thermal stability was assessed using TGA. Besides, the antibacterial effect of ZnO oxide Nanoparticle and ZnO oxide with Montmorillonite nanocomposite were investigated.

MATERIALS AND METHODS

Materials: Zinc acetate [Zn $(CH_3COO)_2.6H_2O$], Sodium Alginate was purchased from Sigma-Aldrich, USA. All the chemicals were of analytical grade. Water was purified by passing deionized water through a double demonized system.

Preparation of Zinc oxide beads: Zinc solution was prepared both from zinc acetate and by dissolving the appropriate amounts of the respective salts in ultra-pure water. 1g of calcium chloride and by dissolving the appropriate amounts of the respective salts in ultra-pure water. Both two solutions were mixed. Alginate solutions at a concentration of 1% w/w were prepared by dissolving the appropriate amount of sodium alginate in ultra-pure hot water under magnetic stirring.

The zinc alginate beads were produced by dropwise addition of 10 ml of alginate solution into 20 ml of zinc solution through a 0.49mm inner diameter stainless steel needle. The formed gel beads were maintained in the gelling medium for 30 min under gentle stirring and then separation from the solution through the stainless-steel grid placed in a porcelain crucible and heated at 450 or 800^{0}C for 24 h with a heating rate 10^{0}C/min.

Synthesis of Montmorillonite based Zinc oxide nanocomposite: Zinc oxide nanoparticles were prepared by the reaction between zinc acetate and sodium alginate. The aqueous solution of the prepared zinc acetate was mixed with a solution of sodium alginate and vigorously stirred at 40^{0}C for 30 min. The nanocomposite was prepared by the addition of the aqueous mixture of zinc acetate solution of sodium alginate into the aqueous suspension of Montmorillonite and stirred for 24 h. The resulting solid phase was separated by decantation, washed several times with distilled water, and then dried at 105^{0}C for 24 h. The resulting Zinc Oxide with Montmorillonite Nanocomposite was calcined at 500^{0}C for 1 h.

Antibacterial activity: The antibacterial activities consist of an agar well diffusion method where the zone of inhibition determines the extent of antibacterial activity for all the three solutions. The MIC of the Zinc oxide nanoparticle and zinc oxide with Montmorillonite nanocomposite was identified to determine the lowest concentration that inhibits the visible growth of the test organisms. Suspensions of the test organism were swabbed on the culture medium. Wells were then carved on the plates. Different concentrations of the Zinc oxide nanoparticle and zinc oxide with Montmorillonite nanocomposite were added to the wells. All the plates were incubated at 37^{0}C for 24 h to determine the inhibitory growth of the Zinc oxide nanoparticle and zinc oxide with Montmorillonite nanocomposite on the particular pathogen. The procedure was repeated three times and the mean value was taken into consideration.

RESULT AND DISCUSSIONS

Fourier Transform Infra-Red Spectroscopy: FTIR spectroscopy is a very useful and convenient technique to detect the interaction developed between two or more components of a composite material. FTIR spectra of selected samples are shown in Fig 1 and 2. The broad peak ranging between 440 cm^{-1} and 550 cm^{-1} (Fig 1 and 2) can be assigned to the ZnO group. The IR study gives information about phase composition as well as the way oxygen is bound to the metal ions. The technique is used to identify the functional groups present in the synthesized od Zinc oxide nanoparticle. Fig.1 shows the IR transmittance spectra of all the samples in the wavenumber range of

4000-400 cm^{-1}. Broadbands of 3377cm^{-1} are assigned to O-H stretching at and bending vibrations of H_2O. The band at 1401 cm^{-1} corresponds to the stretching vibration of the CH_2 ethylene group. Finally, the bands located at 871 cm^{-1} corresponds to the stretching vibrations of the C-C groups (Vacate and Ghamsari, 2007).

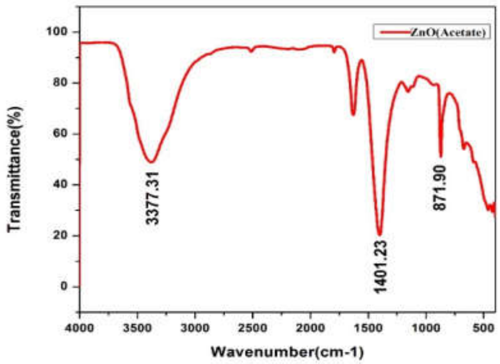

Fig 1: FTIR analysis of ZnO oxide Nanoparticle

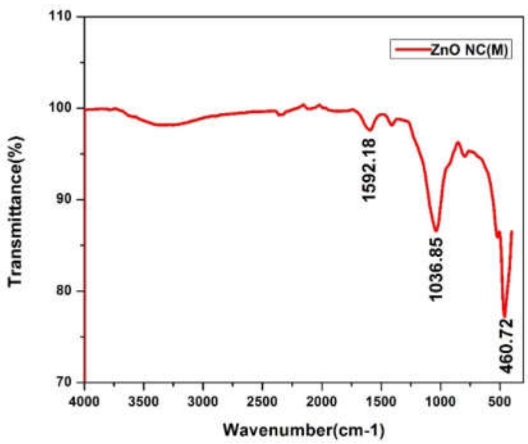

Fig 2: FTIR analysis of ZnO oxide with montmorillonite Nanocomposite

The spectra of FTIR for ZnO oxide with montmorillonite nanocomposite in Fig. 2. In Fig. 2, it can be seen the absorption bands of the ZnO with montmorillonite nanocomposite at 460 cm^{-1} wavenumber, which is the identical absorption band of the Zn-O bond, similar to the research conducted by (Amna *et al.,* 2015). 1036 cm^{-1} are the C-O stretching vibration of uronic acid. This result is similar to the previous research conducted by (Fertah *et al.,* 2017). The absorption band of the asymmetric O-C-O vibration at 1400-1650 cm^{-1} wavenumber is the vibration of the symmetric carboxylate stretching. FT-IR studies of the pure Montmorillonite, Zinc oxide nanoparticle, and zinc oxide with Montmorillonite nanocomposite samples are carried out in the region 4000–400 cm^{-1} to find out the changes in the Montmorillonite and ZnO structures after immobilization. The characteristic bands of obtained FT-IR spectra as well as assigned functional groups were investigated.

X-Ray Diffraction Analysis: The coexistence of the characteristic peak of Zinc Oxide with Montmorillonite Nanocomposite confirmed the immobilization of ZnO nanoparticles on the surface of Montmorillonite. The average crystallite size of ZnO was found to be about 25 nm in Zinc Oxide with Montmorillonite. X-ray patterns are depicted in Fig 3, the samples containing Zinc Oxide with Montmorillonite Nanocomposite exhibited sharp and strong peaks at 26.8°, 36.1°, 45.6°,61.8° (2θ) which correspond to (100), (101), (101), (103) reflections, respectively, and agree with the characteristic peaks of ZnO wurtzite - type hexagonal crystalline structure (JCPDS 36-1451). Comparison of the Zinc oxide nanoparticle and zinc oxide with Montmorillonite nanocomposite patterns indicates that the ZnO nanoparticles in both pure and immobilized form have the same structure with all major peaks matching well with the standard pattern of bulk ZnO. Also, low intense peaks in nano ZnO may be attributed to the deeper penetration of ZnO onto the lamellar structures of clay. Based on the experimental findings presented here, a structural model is proposed for the stabilization of ZnO nanomaterials in the layer of montmorillonite as illustrated in Fig 3. In this model, two kinds of ZnO species exist. One type exists in the interlayer space and the other in the mesoporous formed by the house-of-cards stacking of exfoliated clay crystallites. The high intensity of the (100) peak suggests anisotropic growth and orientation of the crystals (Ba-Abbad *et al.,* 2013).

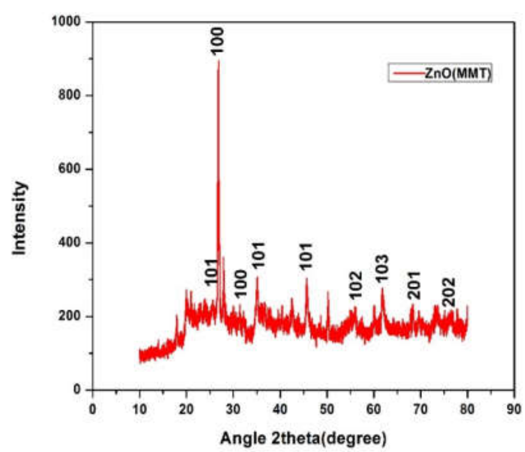

Fig 3: XRD Analysis of ZnO (acetate) with montmorillonite Nanocomposite

Scanning Electron Microscopy: The morphology and size of raw Zinc oxide nanoparticle and zinc oxide with Montmorillonite nanocomposite were investigated using SEM analysis and the results are shown in Fig.3. Besides, the SEM image of as-synthesized Zinc oxide nanoparticle and zinc oxide with Montmorillonite nanocomposite showed a flaky-shaped structure for ZnO nanoparticles (Fig.4). Fig.4 shows the presence of ZnO nanoparticles on the surface of Montmorillonite, indicating the successful synthesis of zinc oxide with Montmorillonite nanocomposite SEM studies were carried out to find out the surface morphology of zinc oxide-based montmorillonite nanocomposite resulted from the annealing of 400. SEM micrographs of the zinc oxide with montmorillonite Nanocomposite have been represented in Fig 4. SEM studies show zinc oxide with montmorillonite nanocomposite is in pure form and particles are beautifully white colored nanocomposite. Fig 4 shows that the Scanning Electron Microscopy image demonstrates a uniform structure and size for Zinc oxide-based montmorillonite nanocomposites (Yang Wang *et al.,* 2007). SEM images show that the samples with medium concentrations have a better size distribution Fig 3. This is because the particles are less aggregated in medium concentrations of the samples, and therefore the particles' sizes are smaller than those of higher concentrations of ZnO NPs. Also, this fact confirms the results of XRD analyses. However, in some places, the size of the particles is bigger, and they are agglomerated by different groups. Also, it demonstrates the Zinc oxide nanocomposite is a well-dispersed spherical shape in the powder sample. The particle sizes of ZnO based montmorillonite nanocomposite are very sensitive to the calcination's temperature. The zinc oxide-based montmorillonite nanocomposite appears slightly aggregated due to the absence of strong surface protecting ligands and found to be spherical. The particle was crystalline as revealed by the XRD analysis.

Thermo Gravimetric Analysis: TGA estimations were carried out on ZnO based montmorillonite nanocomposite. The samples of settled weight were heated at the rate of 100°C/min from room temperature to 800°C, which was in between the boiling point of the solvent and degradation temperature of the polymer. Figure 4 demonstrates ZnO based montmorillonite nanocomposite respectively, from room temperature to 300°C. The TGA thermogram for ZnO based montmorillonite nanocomposite is shown in Fig 4. It should be noted that the observed TGA data of Fig 4 show that the weight loss proceeds in successive stages with increasing temperature (Wu and Xie, 2004; Ahangar *et al.,* 2017).

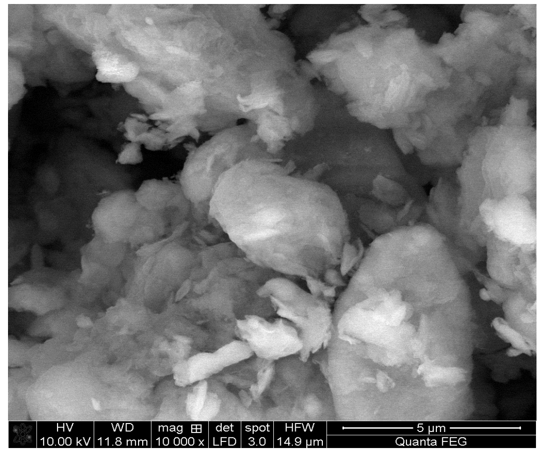

Fig 4: SEM Analysis of ZnO (acetate) with montmorillonite Nanocomposite

The first step is in the range of 65.6°C to about 133.7°C, demonstrating the dehydration of surface-adsorbed water. The second step, which is a major weightlessness tape, occurs in the range of 133.7°C to about 482°C with no further weight loss up to 600°C temperature. As can be seen from the TGA curve, the precursor can be completely decomposed to ZnO after annealing at ~ 500°C. It is observed that the pure peak has a degradation temperature of 600^{0}C. It was observed that the nanocomposites increase significantly with increasing ZnO content and also the char yield at 800^{0}C increased significantly from 65.6°C to about 133.7°C nanocomposites. Therefore, the incorporation of ZnO in the polymer matrix improved the thermal stability of the nanocomposites. The increase in thermal stability could be due to strong interaction and/or interfacial bonding between the matrix and ZnO nanoparticles, which hindered the segmented movement of the Peak. Figure 5 shows the derivative curve of the nanocomposites as a function of temperature. When major weight loss begins, there is a sharp increase in the derivative which then reaches a maximum rate of weight loss. After the maximum, the derivative curves tend to level off somewhat. It shows that the maximum decomposition of the nanocomposites increases from 500^{0}C for the pure matrix to 500^{0}C for zinc oxide with Montmorillonite nanocomposite.

Antibacterial activity: Antibacterial activity of zinc oxide nanoparticles has received significant interest worldwide particularly by the implementation of nanotechnology to synthesize particles in the nanometer region. Many microorganisms exist in the range from hundreds of nanometers to tens of micrometers.

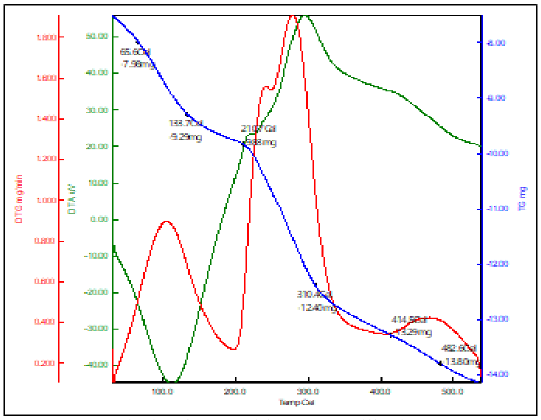

Fig 5: TGA Analysis of ZnO with montmorillonite Nanocomposite

zinc oxide nanoparticles exhibit attractive antibacterial properties due to increased specific surface area as the reduced particle size leading to enhanced particle surface reactivity. zinc oxide is a bio-safe material that possesses photo-oxidizing and photocatalysis impacts on chemical and biological species. The ability of ZnO to inhibit bacterial growth by the generation of radical oxygen species is well documented (Peng *et al.,* 2013; Zhang *et al.,* 2007; Icli, 2004; Applerot *et al.,* 2009; Yamamoto *et al.,* 2002). ZnO is a semiconductor with a wide bandgap. As with other semiconductors, radiation of ZnO with higher photon energy than its bandgap causes the movement of electrons from the valence band to the conduction band of the particle. The result of this process is the formation of a positive area that can be described as a hole (h^+) in the valence band and a free electron in the conduction band. On the surface of the ZnO particles, these holes react with hydroxyl groups and absorb water to create a hydroxyl radical. In the presence of oxygen, the lone electron in the conduction band creates a superoxide ion, which can also become a hydroxyl radical, and so forth. Derivatives of this active oxygen damage the bacterial cell. The well diffusion method was used for the assessment of antibacterial activity.

Antimicrobial activity of zinc oxide nanoparticle and zinc oxide with Montmorillonite nanocomposite have very strong inhibitory action against Gram-positive and Gram-negative bacteria. The Gram-negative bacteria such as *Pseudomonas, Escherichia coli,* and then the Gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus is* shown based on the inhibition zone (mm) size in Table 1. Here the zone of inhibition is more for both zinc oxide nanoparticle and zinc oxide with Montmorillonite nanocomposite and antibiotics like tetracycline. The concentrations of zinc oxide nanoparticle and zinc oxide with Montmorillonite nanocomposite (0.05g) were prepared and applied against bacterial species (Ricardo *et al.,* 2009). The enhanced antimicrobial activity of nanoparticles can be attributed to their increased surface area available for interactions, which enhances the bactericidal effect than the large-sized particles. Recently,

Table 1. The antibacterial activity of zinc oxide nanoparticle and Zinc oxide with Montmorillonite nanocomposite

S. No	Name of Bacteria	ZnO NPs (mm)	ZnO/MMT NC (mm)
		0.05g	0.05g
1	*Escherichia coli*	17	20
2	*Bacillus subtillis*	19	22
3	*Staphylococcus*	20	25
4	*Pseudomonas*	29	31
5	*Enterobacter*	15	19

Fig 5. Antibacterial Activity of ZnO (acetate) with Montmorillonite nanocomposite

Conclusion: In this investigation, biodegradable active films based on polymer zinc oxide nanoparticle and zinc oxide with Montmorillonite nanocomposite were successfully prepared using a simple eco-friendly approach. The nanocomposite films showed significant antibacterial activity bacteria. Based on our research, it can be concluded that the incorporation of the zinc oxide with Montmorillonite nanocomposite increased the antibacterial activity against both Gram-positive and Gram-negative bacteria, significantly. Thus, zinc oxide with Montmorillonite nanocomposite film could be a promising novel active food packing.

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