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Synthesis and characterization of poly(lactic acid) - zinc oxide nanocomposites and their synergistic effect as antibacterial agent

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Abstract

The objective of this research is to develop hybrid nanocomposites that present a novel approach to combat bacteria. The nanocomposites were created through microwave-assisted direct polycondensation of lactic acid with varying concentrations of magnesium oxide nanoparticles (ZnONps). The study investigated the structural properties and antiseptic effects of ZnONps and PLA-ZnO nanocomposites. Compared to pure lactic acid, the PLA-ZnO nanocomposites exhibited higher average molecular weight and thermal stability. Fourier transform infrared (FTIR) spectroscopy identified the formation of hydrogen bonding between the PLA chain and ZnONps. The particle size of ZnONps was analyzed using dynamic light scattering (DLS). The antiseptic activities of the nanocomposites were assessed against various bacterial strains, including gram-negative Escherichia coli (E. coli) and Salmonella typhi (S. typhi), as well as gram-positive Staphylococcus aureus (S. aureus) and Bacillus subtilis (B. subtilis), using the disc diffusion method. The nanocomposites demonstrated exceptional antiseptic activity against all tested strains at significantly lower concentrations than standard antiseptic agents.

Keywords: PLA, MgO, nanoparticles, Antibacterial activity

Full length article **Corresponding Author*, e-mail:kuldeep.joshi@cug.ac.in

1. Introduction

Biocompatible and biodegradable polymers have gained significant attention over the past decade, attracting interest from both biomedical and ecological perspectives [1]. The synthesis of biodegradable polymers has been particularly emphasized due to their approval for essential biomedical applications by the US Food and Drug Administration. An example of such a polymer is poly lactic acid (PLA), derived from renewable resources like cornstarch. PLA, a linear aliphatic thermoplastic, is readily biodegradable through hydrolytic and enzymatic pathways. It has demonstrated potential in producing textiles, films, vehicle interiors, appliance components, service utensils, and packaging materials. Moreover, PLA is immunologically inert, making it a suitable choice for designing tissue engineering scaffolds. Inorganic oxide nanoparticles, including magnesium, aluminum, iron, titanium oxides, have garnered attention for their diverse applications in separation, catalysts, environmental remediation, sensing, and biomedical fields [2-3]. Nanosized metal oxide particles, such as zinc, copper, and iron oxides, are used in medical practices and exhibit antibacterial activity, finding applications in cosmetics, sunscreens, toothpaste, and more [4]. However, some studies have indicated adverse effects on skin and dermal tissues due to the large surface area of metal oxide nanoparticles [6-7]. Chemical stability is also a concern, which can be addressed by dispersing these nanoparticles into synthetic and naturally occurring polymers. Polymer-supported metal or metal oxide nanoparticles represent a novel class of hybrid materials that enhance nanoparticle properties. The synthesis of such hybrid materials involves two pathways: dispersing nanoparticles within pre-formed polymers (ex-situ) or synthesizing nanoparticles within a pre-formed polymer framework (in-situ) [8]. The ex-situ procedure involves physically trapping metal or metal oxide nanoparticles within the polymer or biopolymer network

through casting and solvent evaporation, chemical polymerization, or co-precipitation. This encapsulation method not only aids in stabilizing the nanoparticles by preventing agglomeration and the formation of larger particles but also enhances their dispersion. In contrast, the in-situ process entails the synthesis of metal and metal oxide nanoparticles within a pre-existing polymer framework or matrix. Recent studies have highlighted the effectiveness of biopolymer-metal nanocomposites as antibacterial agents [9-11].In metal oxide-polymer nanocomposites, the polymer matrix serves to hinder the direct interaction between metal oxide nanoparticles and formative cells, mitigating the toxic effects associated with bare metal oxide nanoparticles. Conversely, these nanocomposites play a significant role in enhancing the fundamental properties of pristine polymers [12-19].The present study focuses on PLA-ZnO nanocomposites synthesized using a microwave-assisted direct polycondensation method. The nanocomposites were characterized using various physicochemical methods. Antibacterial activities were assessed against both gram-positive and gram-negative bacteria, comparing the results with standard antiseptic agents.

2. Materials and Methods

All the reagents utilized in this study were of analytical grade and employed in their original state without any further purification. Magnesium chloride crystals (MgCl2·6H2O), butanol, and sodium hydroxide (NaOH) pellets were procured from Merck (Mumbai, India), while methanol and isopropyl alcohol were obtained from CDH (New Delhi, India). Lactic acid (85% aqueous solution) was sourced from National Chemicals (Vadodara, India). The bacterial strains, Escherichia Coli (ATCC25922), S-Typhi (ATCC6539), Staphylococcus aureus (ATCC25923), and Bsubtilis (ATCC 6633), as well as the reference antiseptic agents Streptomycin, Penicillin-G, Tetracycline, and Ampicillin, were supplied by Bio-Disc and Hi-Media (Mumbai, India).

2.1 Synthesis of ZnO nanoparticles

Microwave-assisted sol-gel methodology was employed for the synthesis of ZnO nanoparticles (Nps). Initially, 4 g of MgCl2·6H2O was dissolved in methanol, followed by the gradual addition of an aqueous solution of NaOH (0.1 M) until the pH reached 10.0. Concurrently, as NaOH was added, spontaneous formation of $Mg(OH)_2$ precipitates occurred, resulting in the development of a sol that subsequently transformed into a gel. The gel was then placed into a microwave reactor and subjected to synthesis in a CEM Discover microwave operating at a continuous power of 2.45 GHz. The gel underwent heating at a 100-watt output power for 10 minutes, resulting in the production of a crystalline white powder. Subsequently, this powder underwent calcination at temperatures of 300°C, 650°C, and 800°C for 3 hours in a muffle furnace, followed by slow cooling in the furnace to yield white amorphous ZnO nanoparticles. Various physicochemical techniques were employed to characterize the synthesized nanoparticle powder.

2.2 Synthesis of nanocomposites

PLA-ZnO nanocomposites were synthesized through the direct polycondensation of lactic acid with 0.5%, 1.0%, and 1.5% by weight loading of ZnO nanoparticles (Nps) utilizing microwave irradiation. The synthesis procedure took place in a CEM Discover microwave, employing continuous power at 2.45 GHz. After 8 minutes of microwave irradiation at 200 watts, a nanocomposite latex was obtained. The product was then dissolved in chloroform and added dropwise to isopropyl alcohol, resulting in the precipitation of PLA-ZnO nanocomposites. The precipitate was filtered, and the solid was subsequently dried under vacuum conditions. Structural characterization of these nanocomposites was conducted using FTIR and gel permeation chromatography (GPC). The diameter of the nanocomposites were analyzed through dynamic light scattering (DLS).

2.3 Characterization of ZnONps and PLA-ZnO Nanocomposites

To ascertain the interaction between ZnO nanoparticles and PLA biopolymer, FT-IR spectra were recorded using a FTIR spectrometer in the range of 4000-400 cm^{-1}. The molecular weight (Mw) and number-average molecular weight (Mn), along with the polydispersity index (PDI, equal to Mw/Mn), were determined via GPC (Waters Alliance separation module auto injector coupled with Waters 2414 RI detectors). The measurements were conducted at room temperature with a flow rate of 1 mL/min, using DMF as the carrier solvent. Molecular weight calibration was performed based on the polystyrene standard, and samples were dissolved in dimethylformamide at a concentration of 1 mg/mL. The dimensions of ZnO nanoparticles (Nps) and PLA-ZnO nanocomposites were assessed using Dynamic Light Scattering (DLS). DLS, performed using a Microtrac-NanotracT™ 252 instrument, was utilized to determine the particle diameters. All measurements were conducted in triplicate, and the final value represented the average. Dilution of the samples was carried out prior to measurement to prevent multiple light scattering effects.

2.4 Biological Activities

The in vitro antiseptic activity of the samples was assessed using the disc diffusion method on tripton soy agar, and the results were quantified based on the inhibition zone in millimeters (mm). The antiseptic efficacy of PLA-ZnO nanocomposites, ZnONps, and lactic acid (LA) was tested against four bacterial strains: E. coli and S. typhi as gram-negative bacteria, and S. aureus and B. subtilis as gram-positive bacteria. Reference antiseptic agents, namely Streptomycin, Penicillin-G, Tetracycline, and Ampicillin, were employed for comparison. Nanocomposites were dissolved in chloroform, and their impact on gram-negative and gram-positive bacteria was examined by culturing the organisms on tripton soy agar (TSA) plates (106 colony forming units (CFU) of each strain per plate). Discs with a diameter of 5 mm were prepared from Whatman filter paper-1, sterilized by dry heat at 140°C for 1 hour, previously soaked in a known concentration of the test compounds and control for 1 hour, and then placed on the surface of TSA, which was seeded with 1.0 ml of microorganism culture. The plates were incubated at 37°C for 24 hours. The diameters of the inhibition zones around the disc specimens were measured to evaluate the antimicrobial activity of each disc sample, and the average of three replications was recorded. Antibiotics (streptomycin 10 µg/ml, penicillin-G 10 µg/ml,

tetracycline 30 µg/ml, and ampicillin 10 µg/ml) were added to different media as appropriate.

3. Result and Discussion

The FTIR spectra presented in Fig. 1 compare the characteristics of pure Lactic Acid (LA) with those of the nanocomposites. In the spectrum of pure LA, a prominent absorption band at 1720 cm^{-1} corresponds to the stretching vibration of the carbonyl group (-C=O). The C-O stretching vibration of the acid is observed at 1080 cm^{-1}, while the in-plane bending of C-O-H is noticeable at 1400 and 1438 cm^{-1}, and the O-H peak is identified at 2800 cm^{-1}.In the spectra of ZnO nanoparticles (ZnONps), strong bands in the low-frequency region are attributed to metal-oxygen stretching. Conversely, in the FTIR spectra of nanocomposites, these strong bands in the low-frequency region disappear, indicating the taking place of polymerization of LA by ZnONps. Additionally, the stretching vibration of a carboxyl group (-C=O) undergoes a shift around 1730 cm^{-1}, signifying the presence of repeated ester units. In the nanocomposite spectra, all peaks near the -C=O exhibit lower frequencies, with intensity linearly increasing with the amount of ZnONps. This shift is attributed to the formation of intermolecular H-bonding, involving the C=O group of PLA and the remaining -OH groups from the ZnONps surface. In this scenario, the -OH group from ZnO surface acts as a proton donor, and the carboxylic group of PLA acts as a proton acceptor, were facilitating the formation of H bonding. Table 1 provides a summary of the molecular weights for both PLA and bulk nanocomposites. The GPC analysis indicates an increase in the molecular weight of nanocomposites within the loading ratio range of ZnONps between 70K to 90K. This finding aligns with the results obtained from the DSC analysis. Notably, the Mw and Mn values of PLA-ZnO nanocomposites surpass those reported in a previous study [22]. The polydispersity index, measured from GPC, ranged from 1.3 to 1.8 for both PLA and nanocomposites. This observed variation in molecular weights could be attributed to the grafting of ZnONps at different points along the polymer chains, resulting in enhanced strength and molecular weight. Additionally, the elevated temperature during the MW-assisted reaction may facilitate dehydrative polycondensation and etherification processes, ultimately contributing to chain extension. Fig. 2 shows dynamic light scattering comparison plot of Nps and nanocomposites. The mean particle diameter of Nps obtained from DLS graph is about 100 nm. .In the case of PLA-ZgO nano composites size of the material increasing around 700 nm.

3.1 Antibacterial activity

The in vitro antiseptic activity of ZnONps in PLA-ZnO nanocomposite samples and standard agents was assessed using the disc diffusion method. In this method antiseptic activities of material confirms through inhibition zones in different bacterial strains. for PLA-ZnO nanocomposites and standard antiseptic agents across all four strains. The findings, summarized in Fig. 3, it indicates that the antiseptic efficacy of ZnONps and PLA-ZnO nanocomposites is more potent against E. coli than S. aureus, likely due to differences in cell wall composition. One potential reason for the effective antiseptic activity against bacterial strains is the size of ZnONps and PLA-ZnO nanocomposites. Their nano size allows easy access to the bacterial nuclear content, and the large surface area facilitates increased interaction with bacteria. The antibacterial activity of PLA-ZnO nanocomposites is attributed to the release of ZnONps from the nanocomposites. In the case of nanocomposites, ZnONps interact with bacteria through the polymeric matrix, enabling the accumulation of ZnO at the bacterial cell surface for an extended period. The steady and prolonged release of ZnONps from PLA-ZnO nanocomposites inhibits bacterial growth for an extended duration. In contrast, bare ZnONps directly interact with the cell membrane, inhibiting bacterial growth, and the generation of reactive oxygen species could be a contributing factor to bacterial inhibition.

Table 1: GPC of PLA-ZnO Nanocomposites

Sample	Mw	Mn	PDI
PLA-0.4%ZnO	70621	54673	1.36
PLA-0.8%ZnO	83715	57331	1.63
PLA-1.2%ZnO	89460	60439	1.77

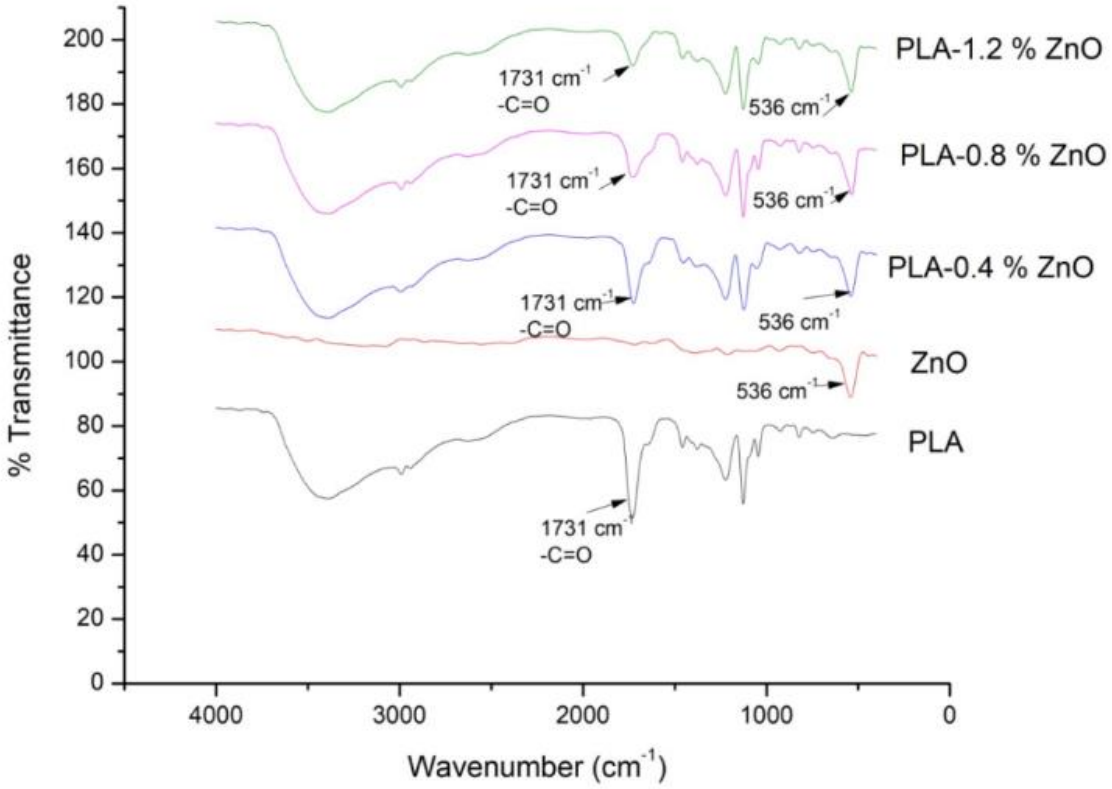

Figure 1: FT-IR Spectra

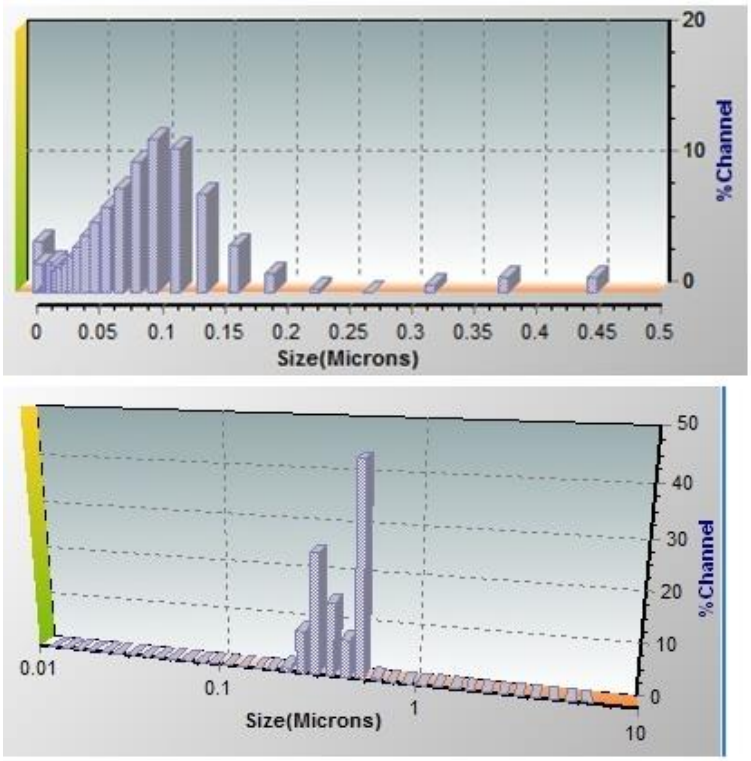

Figure 2: DLS of ZnO Nps and PLA-MgO Nanocomposites

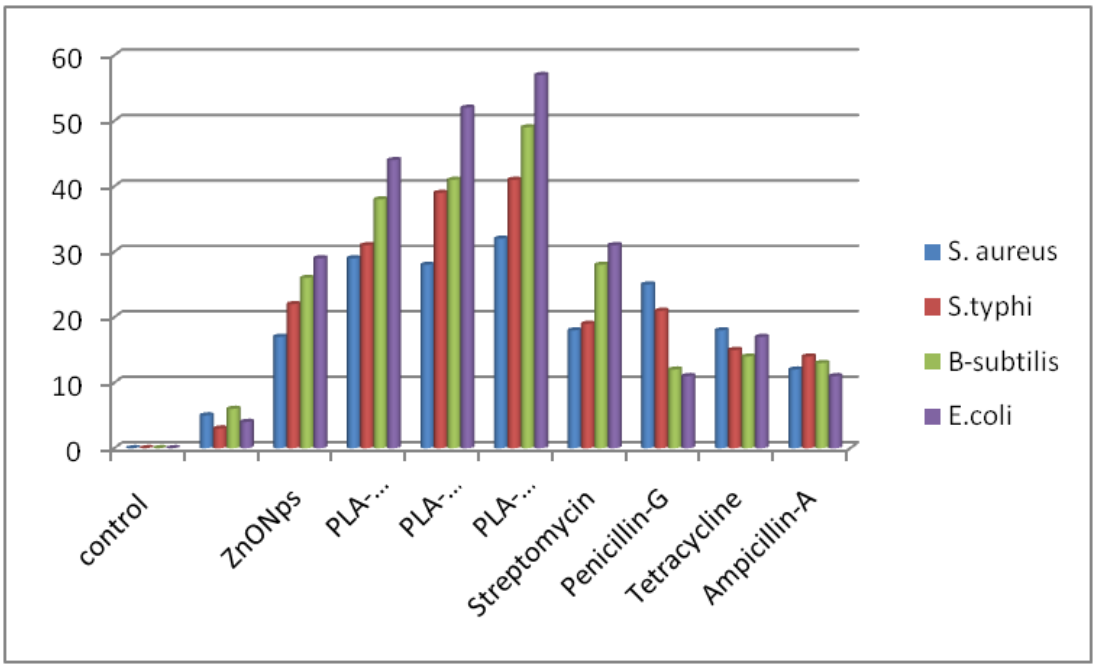

Figure 3: Antibacterial activity of ZnO Nps and PLA-ZnO nanocomposites

4. Conclusion

In this study, we synthesized nanocomposites of PLA-Zno through microwave-assisted, in situ polycondensation of lactic acid and ZnO nanoparticles. The resultant nanocomposites exhibited significantly higher efficacy against gram-positive and gram-positive bacteria as compared to commercial antibiotics. The nanocomposites effectively mitigated the toxic effects associated with bare MgO nanoparticles. Therefore, their use in applications such as abrasion dressings, scaffolds for tissue engineering, surgical devices, cosmetics, and other biomedical applications appears promising. This study underscores the potential of PLA-MgO nanocomposites for future in vivo applications within the biomedical field.

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