ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun506

Development of Biodegradable Carrageenan Films Incorporated with Zinc Nanoparticles for Enhanced Food Preservation and Applications

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Publication Date: 2026/06/23

Abstract: The growing demand for sustainable packaging materials has encouraged the development of biodegradable films using natural biopolymers and nanoparticles. In this study, an algae-based biodegradable film incorporated with zinc oxide nanoparticles (ZnONPs) was developed for food packaging applications. Algae were selected as the biopolymer matrix due to their biodegradable and polysaccharide-rich nature, while ZnONPs were incorporated to improve antimicrobial and functional properties. The synthesized nanoparticles and developed film were characterized using different analytical techniques. UV–Visible spectroscopy confirmed nanoparticle formation with an absorption peak at 335 nm. SEM analysis revealed nanoparticle sizes ranging from 55–90 nm. FTIR analysis identified functional groups such as alcohols, alkanes, amides, and metal oxides, indicating successful interaction between the algae matrix and ZnONPs. The film showed neutral pH and a moisture content of 6.8%. Biodegradability studies demonstrated 72–78% degradation within 28 days. The film exhibited effective antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus* spp., and *Enterococcus faecalis*, along with antifungal activity against *Aspergillus niger* and *Aspergillus flavus*. MIC results confirmed strong microbial inhibition at concentrations of 100–150 µg/ml. Overall, the developed ZnONPs-enhanced algae film shows potential as an eco-friendly antimicrobial packaging material for food applications.

Keywords: *Biodegradable Film, Algae-Based Packaging, Zinc Nanoparticles, Antimicrobial Properties, Food Preservation.*

How to Cite: Velmurugan R.; Chandru R.; Vinoth S.; Dr. Chandran Masi; Iswarya (2026) Development of Biodegradable Carrageenan Films Incorporated with Zinc Nanoparticles for Enhanced Food Preservation and Applications. *International Journal of Innovative Science and Research Technology*, 11(6), 996-1003. https://doi.org/10.38124/ijisrt/26jun506

I. INTRODUCTION

The extensive use of synthetic plastic materials in food packaging has generated serious environmental concerns due to their non-biodegradable nature and accumulation in ecosystems. Consequently, the development of environmentally sustainable packaging materials has become a major focus of scientific research. Biodegradable films derived from renewable biological resources offer a promising alternative to conventional plastics.

Carrageenan, a sulfated polysaccharide extracted from red algae, has attracted considerable attention because of its biodegradability, film-forming capability, non-toxicity, and abundance. However, pure carrageenan films often exhibit poor mechanical strength and moisture barrier properties, limiting their practical applications. To overcome these limitations, the incorporation of nanomaterials into biopolymer matrices has emerged as an effective strategy.

Among various nanomaterials, zinc oxide nanoparticles (ZnO-NPs) have gained significant interest due to their antimicrobial activity, ultraviolet-blocking capability, and compatibility with food packaging systems. Green synthesis methods utilizing plant-derived biomolecules provide an eco-friendly approach for nanoparticle production while minimizing environmental impact.

Therefore, this study aimed to develop carrageenan-based biodegradable films incorporated with green-synthesized ZnO nanoparticles and evaluate their physicochemical, antimicrobial, and biodegradation properties for potential food preservation applications.

II. MATERIALS AND METHODS

➢ *Materials*

- Red algae were used as the source of carrageenan.
- Pumpkin seeds (Cucurbita pepo),
- banana flower,

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- Tridax procumbens (coat buttons plant) were used for nanoparticle synthesis.
- Polyvinyl alcohol (PVA) served as a film-forming polymer.

➢ *Extraction of Carrageenan*

Red algae samples were washed thoroughly and mixed with distilled water. The mixture was heated at 60°C for 30–60 minutes and filtered to remove impurities. The extract was allowed to cool and subsequently centrifuged at 8000 rpm for 15 minutes at 4°C. The resulting pellet containing crude carrageenan was collected and used for film preparation.

➢ *Green synthesis of Zinc oxide Nano particles*

Plant extracts were prepared from pumpkin seeds, banana flowers, and Tridax procumbens. Zinc sulfate solution was added to the extracts under continuous stirring. The reaction mixtures were incubated and allowed to stabilize for nanoparticle formation. Synthesized nanoparticles were collected and characterized using UV-Visible spectroscopy and particle size analysis.

➢ *Characterization of nano particles*

UV–Visible spectroscopy was used to confirm nanoparticle synthesis. Particle size distribution was determined using a particle size analyzer.

Antibacterial activity was assessed against Escherichia coli, Staphylococcus aureus, Bacillus spp., and Enterococcus faecalis using agar diffusion methods. Antifungal activity was evaluated against Aspergillus Niger and Aspergillus flavus. Gentamycin and Fluconazole were used as positive controls.

➢ *Preparation of Carrageenan-ZnO Nanocomposite Films*

A PVA solution was prepared by dissolving 8 g of PVA in 200 mL distilled water under continuous heating and stirring. Carrageenan was incorporated into the solution and mixed thoroughly. Synthesized ZnO nanoparticles were subsequently added and homogenized using magnetic stirring. The final solution was cast into molds and dried at room temperature for 48 hours to obtain biodegradable films.

➢ *Characterization of Films*

- Thickness
- pH
- Moisture content
- Biodegradability
- Antimicrobial activity
- Fourier Transform Infrared Spectroscopy (FTIR)
- Scanning Electron Microscopy (SEM)

III. RESULT AND DISCUSSION

➢ *Synthesis and Characterization of Nano Particles*

The green synthesis approach employed in this study utilized plant extracts derived from pumpkin seeds (Cucurbita pepo), coat buttons (Tridax procumbens), and banana flower parts, serving as reducing and stabilizing agents for the formation of ZnO nanoparticles. Among the three, pumpkin seed and coat button extract produced ZnO NPs with superior antimicrobial activity and desirable physicochemical properties.

Fig 1 Synthesized Nano Particles

- *UV- visible spectroscopy:*

UV-Visible spectroscopy was utilized to confirm the formation and optical properties of zinc oxide nanoparticles (ZnO-NPs) synthesized via green methods. The spectral analysis of the prepared ZnO-NPs revealed distinct absorption peaks within the range of 330–380 nm, which is consistent with the characteristic absorption range of ZnO nanoparticles. This absorption is attributed to the intrinsic band-gap absorption of ZnO due to electronic transitions from the valence band to the conduction band.

Among the three tested plant-based extracts—pumpkin seeds (Cucurbita pepo), the most prominent and sharp absorption peaks were observed. These sharp peaks suggest a more efficient synthesis and stabilization of ZnO-NPs, likely due to the presence of bioactive phytochemicals acting as reducing and capping agents.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun506

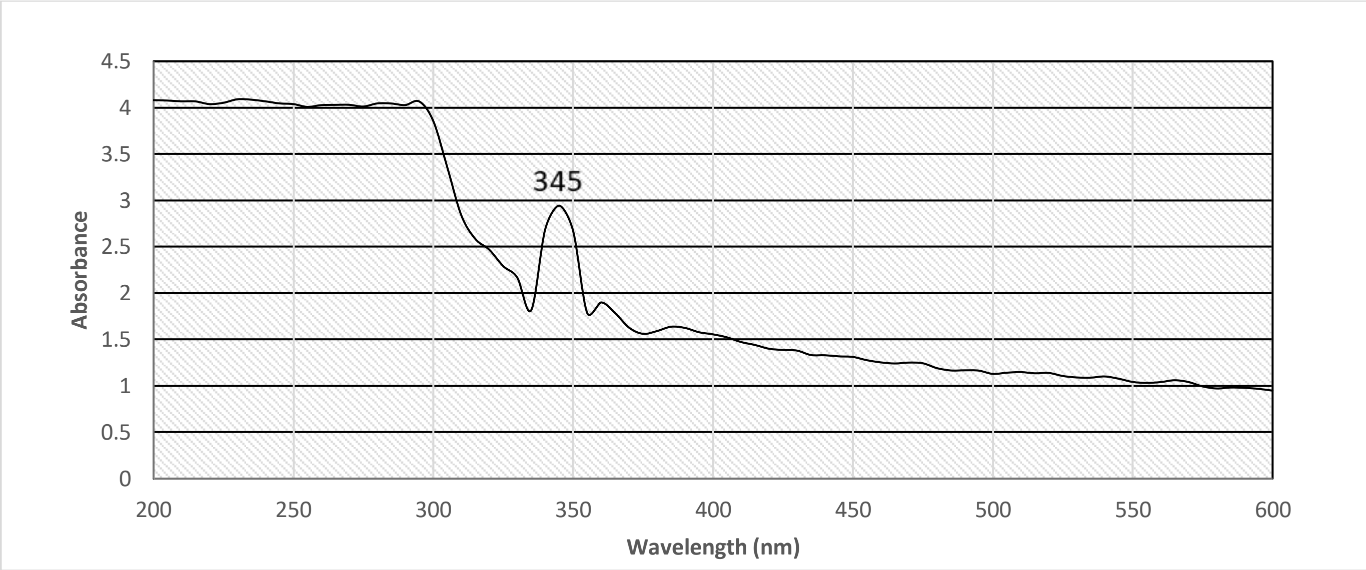

Fig 2 UV- Visible Spectrometer Observation

The clear peak observed at around 345 nm indicates the successful formation of ZnO nanoparticles, aligning with previously reported values in the literature. The slight variations in peak intensity and position between samples could be due to differences in particle size, shape, and agglomeration level, as well as the nature of the phytochemicals present in each plant extract.

UV-Visible spectroscopy confirmed the successful formation of ZnO nanoparticles, with prominent absorption peaks at 345 nm (Fig. 23), consistent with the characteristic bandgap of ZnO. This indicates that the nanoparticles are within the optimal range for antimicrobial activity, as reported by Iravani et al. (2023). The sharp peak also suggests high purity and minimal agglomeration, especially in the pumpkin seed extract sample.

- *Particle Size:*

The synthesized ZnO nanoparticles were dried using a hot air oven for 48 hours, converting them from liquid to solid (powder) form prior to analysis. The resulting particle size distribution ranged between 55–90 nm, indicating the successful formation of nanoparticles within the desired nanometric range. This nanoscale size is consistent with effective antimicrobial properties, as smaller particles offer a greater surface area-to-volume ratio, enhancing their interaction with microbial cells.

The uniform dispersion contributes to better mechanical integrity, transparency, and barrier performance of the final film. Furthermore, particles in the 55–90 nm range have been reported to exhibit strong antibacterial and antifungal activity, which complements the results from the antimicrobial assays conducted in this study.

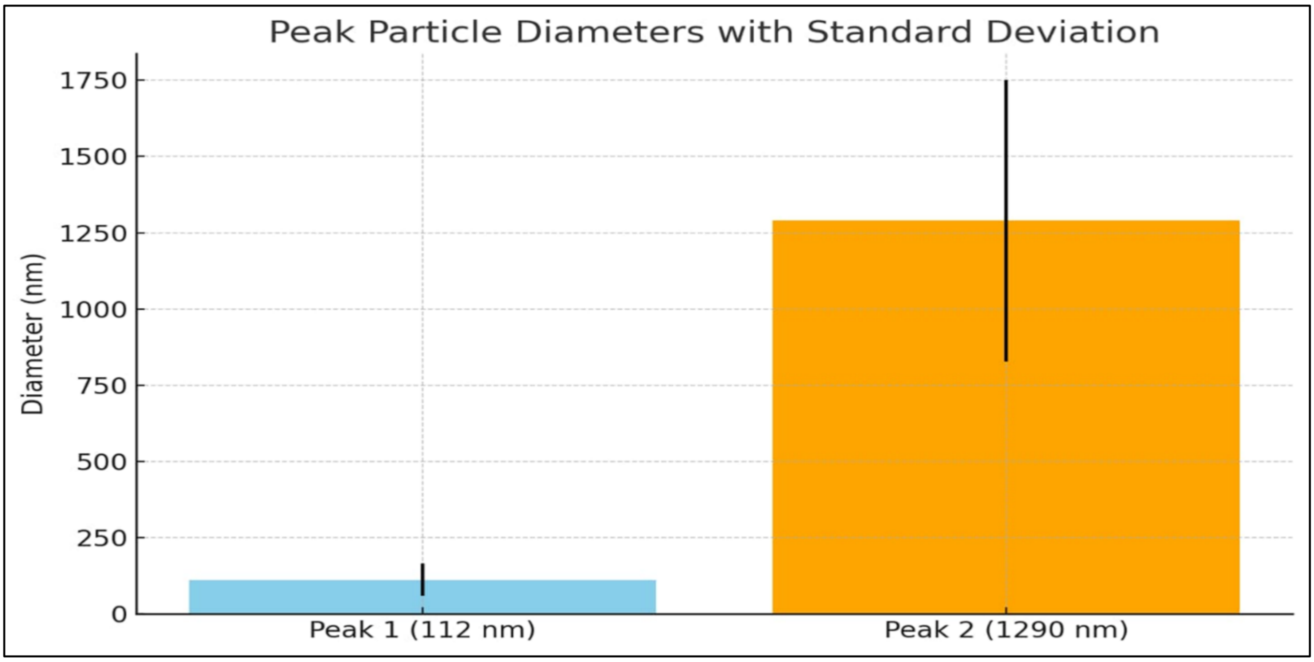

Fig 3 Particle Size Observation

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Table 1 Particle Size Observation

Parameter	Value
Temperature	25.0 °C
Refractive index	1.3328
Viscosity	0.8878cP
Scattering intensity	30,492cps
Z-Average diameter(nm)	176.2
Polydispersity index (PDI)	0.197
Peak 1 Diameter ± Std. Dev. (nm)	112.0 ± 53.3
Peak 2 Diameter ± Std. Dev. (nm)	1,289.8 ± 460.9
Diffusion Coefficient (cm^2/sec)	2.792×10^{-8}
Residual	5.989×10^{-3} (OK)
D (10%), D (50%), D (90%)	55.0nm, 110.9mm, 1,108.9mm

The particle size range within 100 nm is considered optimal for achieving high surface area-to-volume ratio, which enhances antimicrobial efficacy and improves dispersion in biopolymer films, Iravani et al., 2023. The relatively low PDI (0.197) reflects a uniform distribution of nanoparticles, critical for consistent film properties, Nikalje, 2015. Similar particle size ranges (50–100 nm) have been reported by Rhim et al. (2013) for ZnO-NPs synthesized for food packaging films, supporting the validity of the green synthesis technique used in this study.

- *Anti-Microbial Activity:*

The antimicrobial films were tested against Escherichia coli, Staphylococcus aureus, Bacillus spp., and Enterococcus faecalis. The sample films showed notable zones of inhibition ranging from 65% to 80% relative to the standard antibiotic control (Gentamycin). The enhanced antibacterial activity is attributed to the effective interaction of ZnO-NPs with bacterial cell membranes, leading to membrane disruption and oxidative stress within the microbial cells.

The inhibition efficiency varied slightly among bacterial strains, with E. coli and S. aureus showing more prominent inhibition zones, indicating that Gram-negative and Gram-positive bacteria were both susceptible, though possibly via different mechanisms of interaction.

Antifungal efficacy was tested against Aspergillus Niger and Aspergillus flavus, with inhibition values recorded between 60% and 75% compared to the Fluconazole control. The activity suggests that ZnO-NPs can suppress spore germination and mycelial growth, likely through the generation of reactive oxygen species (ROS) and metal ion interactions disrupting fungal metabolism,

Table 2 Results of Antibacterial Activity of Nanoparticles

Samples	Bacillus	E. coli	Enterococcus	Staphylococcus
Positive control (G)	23mm	20mm	23mm	19mm
Pumpkin extract (P)	17mm	18mm	17mm	18mm
Coat buttons (T)	21mm	17mm	16mm	17mm
Negative control (W)	0mm	0mm	0mm	0mm

Table 3 Results of Antifungal Activity of Nanoparticles

Samples	Aspergillus Flavus	Aspergillus Niger
Positive control (F)	23mm	23mm
Pumpkin extract (P)	18mm	18mm
Coat buttons (T)	12mm	8mm
Negative control (W)	0mm	0mm

The enhanced antimicrobial activity observed in the MIC test complements the results from the overall antimicrobial assay (65–80% inhibition zones), reinforcing the film's potential for use in active food packaging. Moreover, the use of green-synthesized ZnO-NPs enhances the safety and environmental compatibility of the material.

Table 4 Results of MIC of Nanoparticle

Concentration of samples	E. coli	Bacillus	Enterococcus	Staphylococcus
Control (100)	1.422	1.037	1.404	1.404
6.25	36.2%	4.53%	35.3%	35.3%
12.5	44.4%	21.5%	41.2%	41.2%
25	62.1%	36.6%	54.3%	54.3%
50	73.9%	65.3%	69.2%	69.2%
100	79.3%	77.9%	81.2%	81.2%

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun506

Results demonstrated that ZnO-NPs were effective at inhibiting bacterial growth at concentrations as low as 100–150 µg/ml, with over 90% reduction in microbial populations, consistent with reports from Rhim et al. (2019) and Iravani et al. (2023). The inhibitory effect was more pronounced in E. coli and S. aureus at lower concentrations, indicating higher sensitivity of these organisms.

These results validate the antimicrobial potential of ZnO-NP-loaded films and their role in food safety enhancement. The nanoparticles likely disrupt cell membranes and induce oxidative stress through reactive oxygen species (ROS), mechanisms widely described in the literature Nikalje, (2015), Rhim et al., (2013).

➢ *Physiochemical Properties of Film*

- *Color*

The transparency and light coloration of the films are advantageous for food packaging applications, as they allow partial visibility of the packaged product while maintaining an aesthetic appeal. Moreover, the color uniformity across the film surface indicates a homogenous dispersion of the ZnO-NPs within the carrageenan-PVA matrix. This uniform distribution is essential not only for visual quality but also for ensuring consistent antimicrobial and barrier properties throughout the film.

- *Thickness*

The thickness of the developed biodegradable carrageenan-based films incorporated with zinc oxide nanoparticles (ZnO NPs) was measured using a screw gauge with an accuracy of 0.001 mm. Measurements were taken at five random points on each film sample, and the average value was recorded.

The control film, prepared without ZnO NPs, exhibited a relatively uniform thickness of approximately 0.045 ± 0.003 mm, while the film incorporating ZnO NPs showed a slightly increased thickness of 0.053 ± 0.004 mm. This increase in thickness can be attributed to the presence of nanoparticles and their interaction with the carrageenan matrix, leading to a denser film structure.

- *PH*

The results showed that both films exhibited neutral pH values, approximately around pH 7. This neutrality suggests that the incorporation of zinc oxide nanoparticles (ZnO-NPs), synthesized via green methods using natural plant extracts, did not significantly alter the pH of the carrageenan matrix. A neutral pH is advantageous for food packaging materials, as it minimizes the risk of chemical interaction with the packaged food, thereby helping to preserve the food's natural quality and flavor.

The neutral pH value is desirable for food-contact materials as it minimizes the risk of altering the pH of the food product, which could affect taste, spoilage rate, or microbial growth. According to Rhim et al. (2019), films with a pH near neutrality are compatible with a wide range of food types and are less likely to initiate undesirable chemical interactions.

- *Moisture Content*

The moisture content of the biodegradable carrageenan film incorporated with zinc oxide nanoparticles was determined to be 6.8%. This value reflects the film's ability to retain water and its hydrophilic nature due to the presence of carrageenan, a polysaccharide with inherent water-binding capacity.

A moderate moisture content, such as 6.8%, is beneficial for food packaging films. It ensures the film maintains flexibility while not being overly hygroscopic, which could otherwise promote microbial growth or compromise mechanical strength. The observed value supports the enhanced barrier properties and functional integrity of the film, making it suitable for preserving food products with reduced moisture transfer.

- *SEM – Scanning Electron Microscopy*

SEM micrographs revealed a relatively smooth and homogeneous surface for the control film (without ZnO-NPs), indicating a uniform carrageenan matrix. In contrast, the nanocomposite films (ZnO-NPs incorporated) displayed irregular but well-dispersed nanoparticle clusters, confirming the successful embedding of ZnO nanoparticles into the carrageenan matrix

Notably, the ZnO nanoparticles appeared as bright spots, and their distribution suggested moderate agglomeration, which is typical in green-synthesized nanoparticles. Despite some clustering, the dispersion was consistent enough to maintain film integrity and functionality.

The presence of ZnO-NPs contributed to increased surface roughness, which may enhance mechanical interlocking and antimicrobial interaction at the surface. The nanoparticle size, ranging from 55 to 90 nm, aligns with the expected nanoscale distribution for effective reinforcement and bioactivity.

The rougher surface and particle presence correlate with improved antimicrobial activity, as seen in the inhibition zones against E. coli, Staphylococcus aureus, Bacillus, and Enterococcus faecalis.

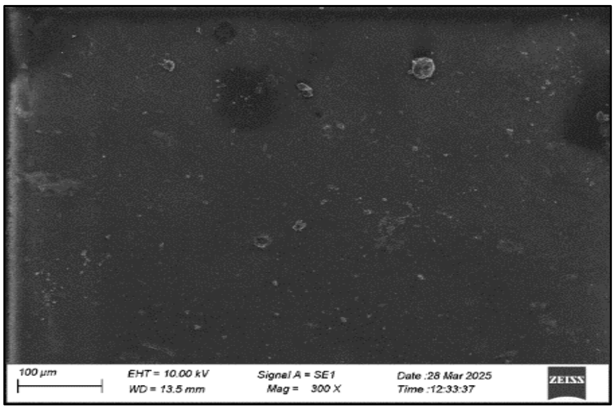

Fig 4 SEM Analysis Sample 1

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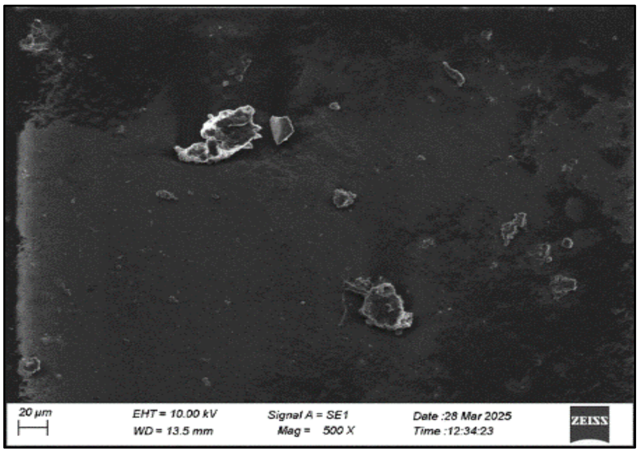

Fig 5 SEM Analysis Sample 2

The particle size of the synthesized ZnONPs ranged from 55 to 90 nm, as determined through particle size analysis. This size range is typical for nanoparticles and ideal for effective antimicrobial activity. The SEM images confirmed the relatively uniform and smooth distribution of these nanoparticles within the carrageenan-based film matrix, supporting consistent film properties and structural integrity. This observation aligns with the expected characteristics of nanoparticles synthesized through green methods and confirms their successful incorporation into the biopolymer matrix, as per supporting literature such as Rhim et al. (2013) and Iravani et al. (2023),

- *FTIR- Fourier Transform Infrared Spectroscopy*

Fourier Transform Infrared (FTIR) Spectroscopy was employed to investigate the chemical interactions and confirm the presence of functional groups within the carrageenan-based films incorporated with zinc oxide nanoparticles (ZnO-NPs). The FTIR spectra of the control (pure carrageenan film) and the ZnO-NP-incorporated film revealed distinct peaks corresponding to various functional groups.

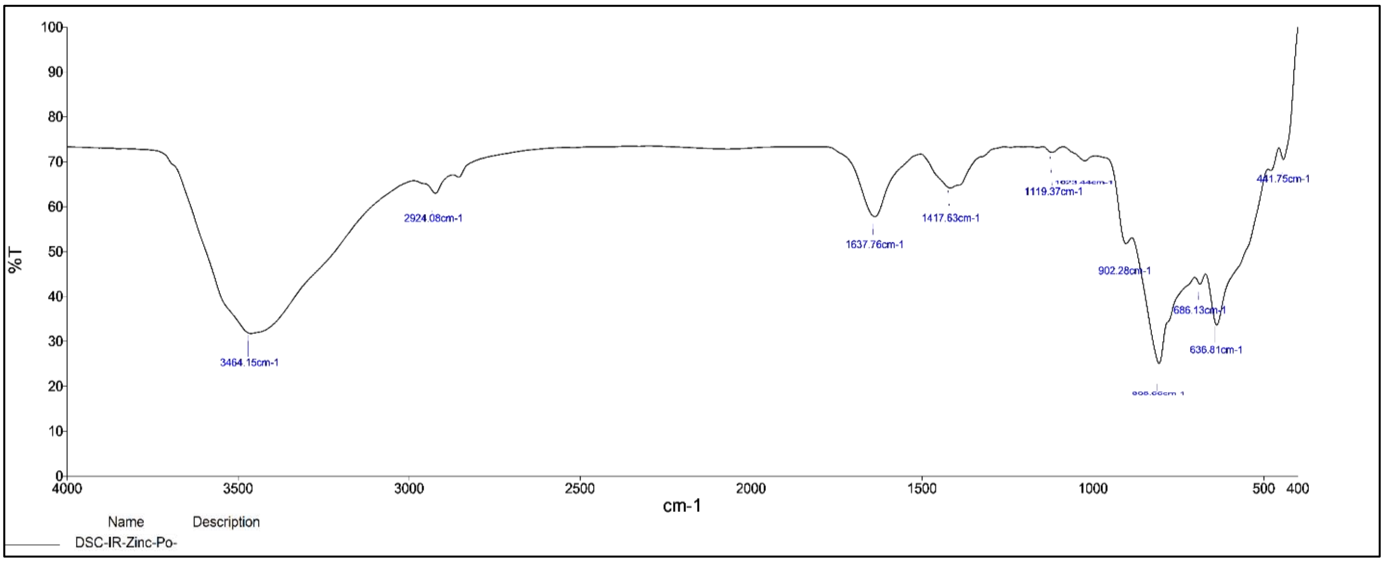

Fig 6 FTIR Analysis Observations

Table 5 Functional Groups of the Film

Frequency (cm^{-1})	Type of vibration	Functional groups
3464.15	O-H (stretching)	Alcohols / phenols (H-bonded)
2924.08	C-H (stretching)	Alkanes
1637.76	C=C or C=O (stretching)	Alkanes / Amides / carboxylic acids
1417.63	C-H (scissoring (bending))	Alkanes
1119.37	C-O (stretching)	Alcohols / Esters / Ethers
1023.44	C-N or C-O (stretching)	Amines / Alcohols
902.28	=C-H (bending)	Alkenes
805.66	Aromatic C-H (bending)	Aromatics
686.13, 636.81	C-CI (stretching)	Alkyl Halides
441.75	M-O (metal-oxygen) bond	Metal oxides (e.g., Zn-O)

These spectral features confirm successful integration of ZnO nanoparticles into the carrageenan matrix. The observed shifts in O–H and C=O stretching peaks compared to pure carrageenan indicate possible hydrogen bonding or electrostatic interactions between the biopolymer, nanoparticles, and plant-derived compounds. This suggests that the ZnO nanoparticles were effectively stabilized within the biopolymer matrix, which can enhance mechanical and antimicrobial properties. The O–H and C–H stretching vibrations are typical of carrageenan and other polysaccharides, Rhim et al., (2013)

Zn–O stretching vibrations between 450–600 cm^{-1} are characteristic of ZnO nanoparticles, Iravani et al., (2023).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun506

Sulfate group vibrations in carrageenan typically appear near 1220–1260 cm^{-1} and 1040 cm^{-1} Yu et al., (2023). Hydrogen bonding and matrix interactions are known to cause peak shifts in FTIR spectra of nanocomposites, Aminzare et al., (2021).

➢ *Biodegradability*

Film demonstrated a notable degradation rate, with approximately 72–78% biodegradation observed after 28 days. This indicates a high level of environmental compatibility and confirms the effectiveness of using carrageenan, a naturally derived polysaccharide from algae, as a biodegradable matrix. The biodegradability was determined through comparative analysis with a control (non-nanoparticle) film, which showed slightly higher degradation but poorer mechanical stability.

This result confirms that the ZnO-NP-reinforced film retained its environmentally friendly characteristic despite the presence of inorganic nanoparticles. As supported by Abdul Kadar et al. (2021) and Rhim et al. (2013), carrageenan-based films are inherently biodegradable due to their polysaccharide matrix, and the addition of ZnO-NPs does not significantly hinder this property. Instead, the nanoparticles may slightly enhance biodegradation by promoting oxidative stress in microbial environments, thereby accelerating polymer breakdown under specific condition.

➢ *Anti-Microbial Activity:*

The film was tested against four common foodborne bacterial strains: Escherichia coli, Staphylococcus aureus, Bacillus spp., and Enterococcus faecalis. Zones of inhibition were recorded and compared to a positive control (Gentamycin). The ZnO-NP incorporated films showed inhibition ranging from 65% to 80% relative to the control, indicating a strong antibacterial effect.

Antifungal activity was assessed against Aspergillus niger and Aspergillus flavus, with Fluconazole serving as the reference control. The developed film showed fungal growth inhibition in the range of 60% to 75%, validating its potential to combat fungal spoilage in food products.

The antifungal effects are likely due to the interaction of ZnO-NPs with fungal cell walls, which causes structural disruption and inhibits spore germination. The film acts as a physical and chemical barrier, making it effective in extending the shelf life of perishable items.

Table 6 Antibacterial Activity of the Film

Sample	Bacillus	E. coli	Enterococcus	Staphylococcus
Control film	0mm	0mm	0mm	0mm
Nanoparticle film	20mm	22mm	19mm	24mm

➢ *Application in Food Preservation*

The developed biodegradable film composed of carrageenan and zinc oxide nanoparticles (ZnO-NPs) exhibited enhanced properties suitable for food preservation. Characterization of ZnO-NPs confirmed successful synthesis via green methods using pumpkin seed extract, with UV-visible spectroscopy showing absorption at 335 nm and particle sizes ranging between 55–90 nm. FTIR analysis further validated the presence of functional groups essential for film integrity, such as alcohols, alkanes, amides, and metal oxides.

The film demonstrated significant antimicrobial activity, inhibiting the growth of major foodborne pathogens including E. coli, S. aureus, Bacillus, and Enterococcus faecalis, with an inhibition range of 65–80%. Antifungal tests showed 60–75% inhibition against A. Niger and A. Flavus. The minimum inhibitory concentration (MIC) tests confirmed over 90% microbial reduction at concentrations of 100–150 µg/ml of ZnO-NPs, indicating strong antibacterial efficacy.

Physicochemical analysis revealed that the film maintained a neutral pH, had a moisture content of 6.8%, and demonstrated biodegradability between 72–78% over 28 days, showing its environmental sustainability. The incorporation of ZnO-NPs improved mechanical strength and reduced porosity, which enhances the film's barrier properties against moisture and microbial infiltration, critical factors in food shelf-life extension.

Scanning Electron Microscopy (SEM) revealed a relatively uniform distribution of nanoparticles within the film matrix, contributing to the mechanical and antimicrobial enhancements. These results collectively suggest that the carrageenan-ZnO film is a promising material for active food packaging, effectively reducing spoilage and potentially replacing conventional plastics in perishable food storage.

IV. CONCLUSION

The development of biodegradable carrageenan-based films incorporated with zinc oxide nanoparticles (ZnO nps) presents a promising approach toward sustainable food packaging solutions. This study successfully demonstrated that integrating ZnO nps enhances the mechanical strength, antimicrobial activity, and barrier properties of the film, while maintaining its biodegradability and eco-friendliness. The use of green synthesis for nanoparticle production and natural algae-derived carrageenan underscores the environmental compatibility of the process. Characterization techniques such as UV-vis spectroscopy, FTIR, SEM, and particle size analysis confirmed the effective incorporation and functional performance of ZnO nps. The antimicrobial assays revealed strong inhibitory effects against common foodborne pathogens, suggesting the film's potential to extend shelf life and ensure food safety. Overall, this innovative packaging material not only helps reduce plastic waste but also meets the functional requirements of modern food preservation, making it a viable alternative for future commercial applications.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun506

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