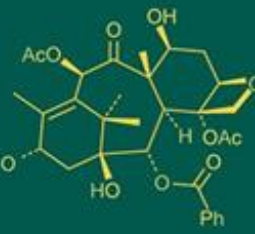
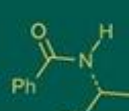
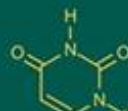
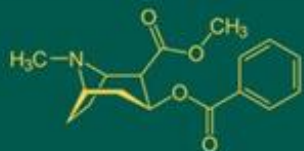


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Zinc oxide nano particle integrated biodegradable film in food quality monitoring

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Abstract

Nano technology deals with the synthesis and usage of materials with nano scale (1-100nm). Zinc Oxide Nano Particle (ZnO NP) has potential application like antibacterial, antifungal, antioxidant, UV absorption and Gas-barrier properties, making it highly suitable for active and smart packaging applications. ZnO can be produced by physical methods such as laser absorption, high energy ball milling and thermal evaporation whereas chemical methods use solgel, solvothermal, hydrothermal, microemulsion and coprecipitation methods the other method used is the biological methods which includes green synthesis, plant extracts, bacteria and fungi. The ZnO infusion can be done by solvent/solution casting method, melt extrusion method, spray coating and dipping and electrospinning method. Biodegradable or synthetic polymer matrices like Chitosan, Soy protein isolate, Cellulose, Sodium caseinate and Cellulose nano fibrils can be used as packaging materials. When the ZnO particles incorporated into ZnO NPs enhance mechanical strength, thermal stability, and shelf-life extension of packaged foods by inhibiting microbial growth and oxidative degradation. Furthermore, ZnO NP based packaging systems show potential in food quality monitoring through their optical and sensing capabilities.

Keywords: Zinc oxide, smart packaging, nano particles, nano technology, quality monitoring

Introduction

Various preservation methods have been used to extend the shelf of food products and the use of ZnO NP in the food industry serve as an effective agent in extending the shelf life and maintaining the quality of food products. In food industry these particles serve as effective agent in extending the shelf life and maintaining the quality of food products. Due to their antimicrobial properties, these nano particles especially in food packaging are capable of preventing the penetration of contaminants and pathogens into the packaging and prevent the transmission of foodborne diseases to humans. The ZnO has beneficial properties like good transparency, antimicrobial agents, high electron mobility, wide band gap, high thermal and mechanical stability (Zhou *et al.*, 2023) ^[1].

Physical methods such as laser absorption, high energy ball milling and thermal evaporation whereas chemical methods uses solgel, solvothermal, hydrothermal, microemulsion and coprecipitation methods the other method used is the biological methods which includes green synthesis, plant extracts, bacteria and fungi have been used with advantages and disadvantages the most commonly used method is chemical method the Sol- gel, Solvothermal, co-precipitation, micro-emulsion, hydrothermal processes and other processes are some of the ways ZnO NP can be made. The method that is most frequently used among these is sol gel synthesis. Solvothermal method: Widely used technique that usually uses non-aqueous solvents under controlled temperature and pressure higher than atmospheric pressure. (Li *et al.*, 2016) ^[2].

The films used for the infusion of ZnO NP such as Chitosan, starch, cellulose, soy protein isolate, carboxymethyl chitosan, polylactic acid, gelatin, chitosan – polyvinyl alcohol, cellulose nano fibres, sodium caseinate, carboxycellulose-starch, Alginate. The amount of ZnO NPs generally incorporated in biopolymers is in the range between 0.1 and 10%. (Yadav *et al.*, 2021; Alizadeh-Sani, *et al.*, 2022) ^[3, 32].

The infusion of ZnO NP films by Solvent Casting, also known as solution mixing, is a process in which the nanoparticles in a polymer are vigorously stirred or ultrasonicated before casting into a mold and then the solvent is evaporated. The solvents which are used generally includes water and ethyl alcohol (Soumili Banerjee *et al.*, 2024) ^[4]. Solution casting is the most commonly used method for producing ZnO-infused biopolymer films, primarily due to its cost-effectiveness and ease of preparation (Kim, *et al.*, 2022 ; Luo 2021) ^[5, 6].

This method involves preparing a film-forming solution by directly incorporating 0.5–5.0 % (w/w) ZnO, along with a plasticizer into a biopolymer solution, followed by thorough homogenisation.

Synthesis of zinc oxide nano particles

Zinc oxide nanoparticles (ZnO NPs) can be synthesized using two main approaches: the bottom-up (Fig1) and top-down (Fig2) methods. In the bottom-up approach, nanoparticles are formed by building up from atoms or molecules, and this can be achieved through physical (Fig3), chemical (Fig4), and biological (Fig5) methods. In contrast, the top-down approach involves breaking down bulk materials into nanoparticles using techniques such as sputtering, etching, mechanical milling, and electro-explosion methods. (Dey et al., 2025) ^[7]

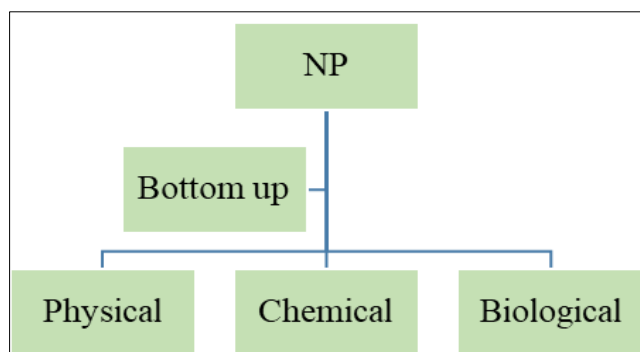


Fig 1: Classification of Nanoparticle Synthesis Methods

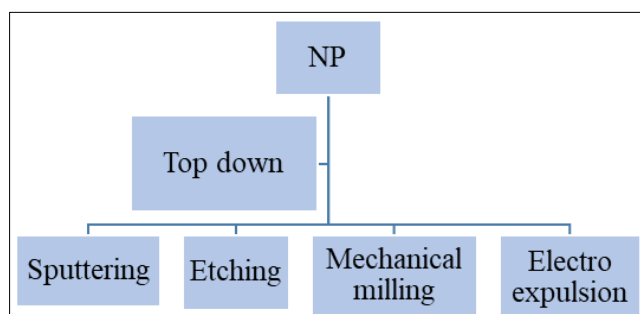


Fig 2: Top-Down and Bottom-Up Approaches for Nanoparticle Synthesis

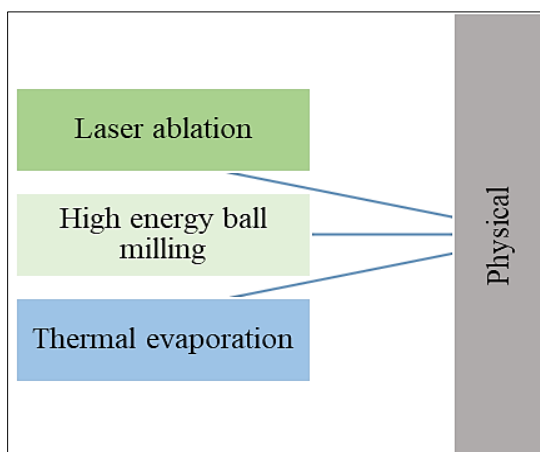


Fig 3: Chemical Methods for ZnO Nanoparticle Synthesis

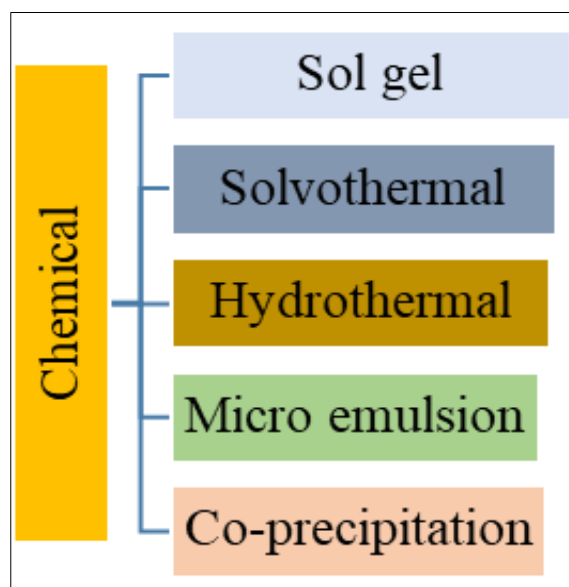


Fig 4: Physical Methods for ZnO Nanoparticle Synthesis

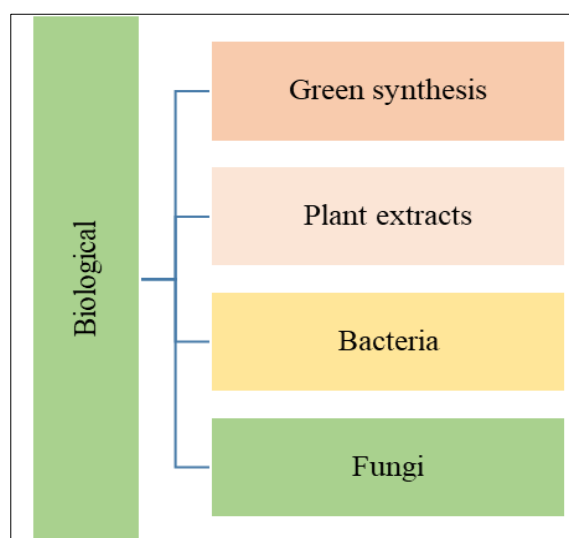


Fig 5: Biological (Green) Synthesis of ZnO Nanoparticles

Characterization of ZnO NP

Zinc (Zn) and oxygen (O) belong to groups 12 and 16 of the periodic table, respectively. Zinc oxide (ZnO) is a well-known II–VI semiconductor widely studied in materials science due to its unique properties. It exhibits high optical transparency, antimicrobial activity, high electron mobility, a wide band gap (~3.37 eV), and excellent thermal and mechanical stability at room temperature. Additionally, ZnO shows strong room-temperature luminescence. Its wide band gap places it at the borderline between ionic and covalent semiconductors.

The crystalline of ZnO has a wurtzite (B4) crystal structure, having a hexagonal unit cell with two lattice parameters $a = 0.325\text{nm}$ and $c = 0.521\text{ nm}$. With its hexagonal structure each anion is surrounded by four cations at the corner of the tetrahedron, which displays the tetrahedron coordination and hence exhibits the $s p^3$ covalent bonding. The tetrahedral shape of ZnO gives rise to non-centrosymmetric structure.

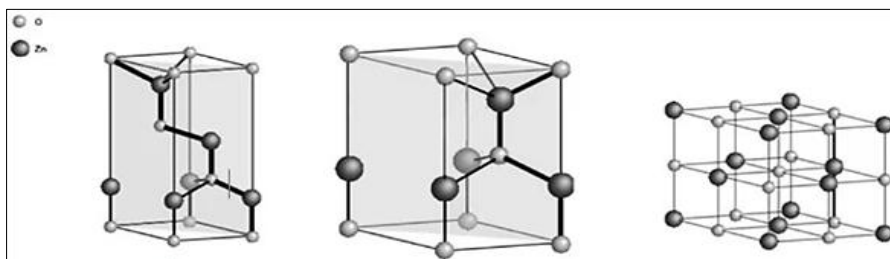


Fig 6: Crystal structure models of ZnO, a) zinc blend, b) wurtzite and c) rock salt

Fig 6 (Zhou et al., 2023).

Physical methods

1. Pulsed Laser ablation in the liquid method:

ZnO nanoparticles were synthesized by immersing a high-purity ZnO target (99.99%, 2.5 cm diameter and 6 mm thickness) in 15 mL of distilled water contained in a cylindrical glass beaker at room temperature. Laser ablation was performed using a Q-switched Nd: YAG laser operating at a wavelength of 1064 nm, with a pulse duration of 10 ns and a repetition rate of 10 Hz. (Dey et al., 2025)^[7]

Laser ablation was carried out using pulse energies of 70, 90, and 130 mJ to investigate their effect on nanoparticle formation.

The laser beam was focused onto a small spot ($\sim 70\ \mu\text{m}$ in diameter) using a convex lens, producing fluences of 18.2, 23.3, and 33.7 J/mm² for pulse energies of 70, 90, and 130 mJ, respectively. The ablation process was performed on the target surface for 30 minutes.

To prevent laser beam trapping within cavities formed during target ablation, the glass beaker was mounted on a rotating stage. Additionally, a magnetic stirrer was used to maintain continuous circulation and homogenization of the colloidal solution, ensuring effective interaction between the laser beam and the metal target.

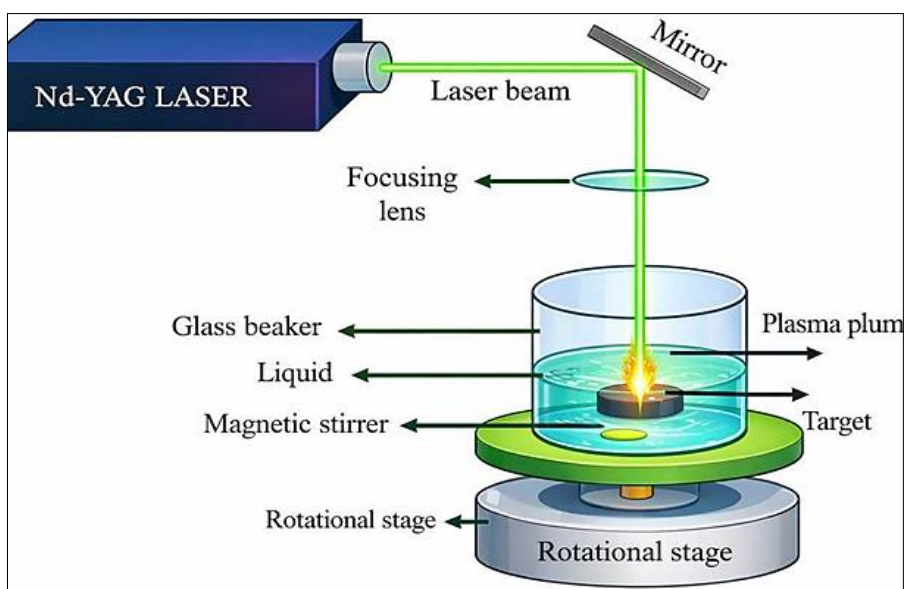


Fig 7: Pulsed Laser ablation

The conventional setup for this process is shown in Fig 7. (Flemban et al., 2023)

Results

The synthesis of ZnO nanoparticles (ZnO NPs) at different laser ablation energies was systematically investigated. The pulse energy significantly influences both particle size and concentration; increasing the laser energy results in smaller particle sizes and higher nanoparticle concentrations. The average particle sizes obtained were 12.2, 9.7, and 8.7 nm at pulse energies of 70, 90, and 130 mJ, respectively. The lattice parameter c was calculated as 5.12 Å at 70 mJ and 5.08 Å at both 90 and 130 mJ, showing a slight decrease with reduction in particle size. The morphology and structural characteristics were analyzed using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). UV-visible absorption spectra revealed a blue shift in the band gap at higher laser energies, attributed to quantum confinement effects and

reduced structural defects. The zeta potential values indicate that the synthesized nanoparticles are physically stable. Furthermore, the formation of ZnO NPs was confirmed by the characteristic Zn–O stretching vibration observed at 450–500 cm⁻¹ in the FTIR spectra. (Flemban et al., 2023)^[8]

1. High-energy ball milling (HEBM): is a top-down technique used for the synthesis of fine metal nanoparticles, where bulk materials are mechanically ground using zirconia balls in a high-energy shaker mill. A major advantage of this method is its ability to produce large quantities of nanoparticles in a single process. In this study, the samples were milled for durations of 2, 10, 20, and 50 hours using a horizontal oscillatory mill operating at 25 Hz. The results indicate that particle size decreases with increasing milling time, demonstrating that prolonged milling leads to finer nanoparticles. (Dey et al., 2025)^[7]

2. **Process:** In high energy ball milling, metal powders are ground in a ball mill to reduce particle size and induce nano particle formation as shown in Fig 8 (Dey et al., 2025)^[7].
3. **Results:** The milled sample consists of approximately 30 nm spherical ZnO nanoparticles, as estimated from SEM analysis, while TEM measurements indicate an average particle size of about 22 nm. An increase in the lattice parameter *c* from 5.204 to 5.216862 Å is associated with new photoluminescence (PL) emission band around 365 nm. Prior to milling, the micro sized material exhibited a single intense emission peak at 384 nm; however, after 2

hours of milling, an additional emission band appeared near 365 nm. (Salah and Habib et al., 2011)^[9]

Chemical method: Sol- gel, Solvothermal, co-precipitation, micro-emulsion, hydrothermal processes and other processes are some of the ways ZnO NP can be made. The method that is not frequently used among these is sol gel synthesis.

- Solvothermal method: Widely used technique that usually uses non-aqueous solvents under controlled temperature and pressure higher than atmospheric pressure (Li et al., 2016)^[2].

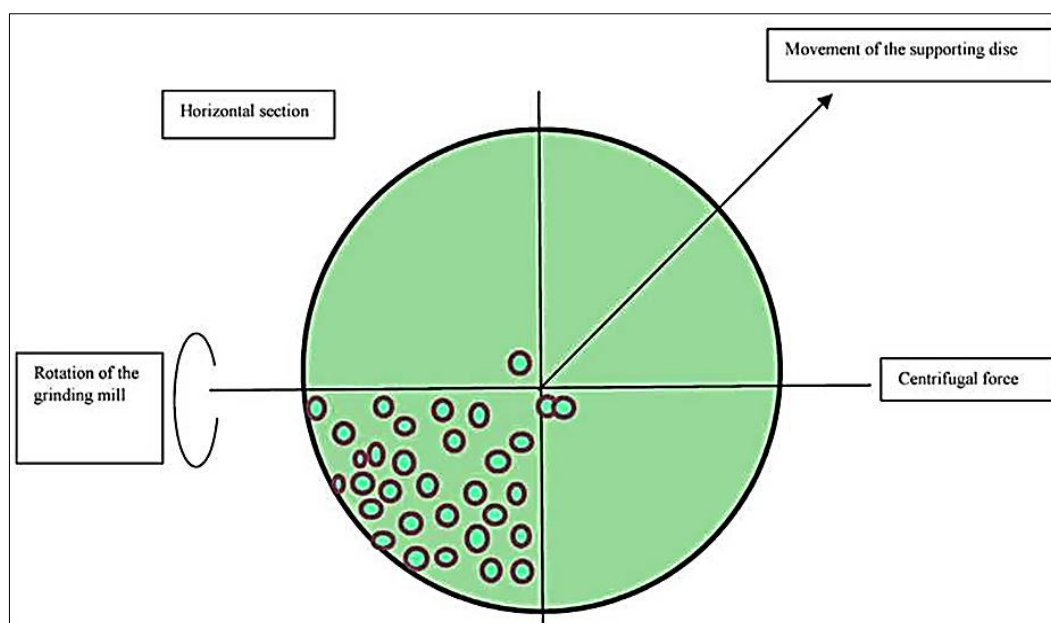


Fig 8: Process of high energy ball milling

Table 1: Physicochemical Synthesis Methods of ZnO Nanoparticles and Their Applications

Method of synthesis	Precursor/reactant	Synthesis condition	Properties	Applications	Ref
Solvothermal with conventional heating	Zinc salt, dimethyl sulfone, KOH	Reaction temp: 60 °C for 3 and 12 h, Drying temp. 65 °C for 12 h.	Spherical, crystallite size : 4 nm and 10 nm Particle size (TEM) : 4 nm and 10 nm	Antibacterial agent	Xiangyang et al., 2015
Solvothermal with conventional heating	Zn(Ac ₂) · 2H ₂ O, ethanol, NaOH, CTAB, wood samples, FAS-17	Reaction temp. 2 h at room temp, Autoclave temp. 90 °C for 4 h, Drying temp. 45 °C for 24 h.	Rod like, particles size: 85 nm and 1.5 μm (dia and length)	UV resistor	Yao et al., 2016

Two different solvothermal synthesis methods using conventional heating have been reported for the preparation of ZnO nanostructures with distinct properties and applications. In the first method, zinc salt, dimethyl sulfone, and KOH were used as precursors. The reaction was carried out at 60 °C for 3 and 12 hours, followed by drying at 65 °C for 12 hours. The synthesized ZnO particles exhibited a spherical morphology with crystallite sizes of 4 nm and 10 nm, which was consistent with the particle size observed through TEM analysis. These nanoparticles demonstrated effective antibacterial activity. In the second method, Zn(Ac)₂ · 2H₂O, ethanol, NaOH, CTAB, wood samples, and FAS-17 were employed as reactants. The reaction was initially conducted at room temperature for 2 hours, followed by autoclaving at 90 °C for 4 hours and drying at 45 °C for 24 hours. This process resulted in the formation of rod-like ZnO structures with particle dimensions of approximately 85 nm in diameter and 1.5 μm in length. These

nanostructures were found to possess UV-resistant properties, making them suitable for protective applications.

Hydrothermal process

This process involves the synthesis of ZnO nanoparticles under high temperature (100-1000 °C) and pressure (1–10,000 atm) conditions in an autoclave, using water or other solvents such as ethanol or polyols. Heating can be achieved either by conventional or microwave methods. Conventional heating relies on conduction and convection within the reaction medium, which may result in non-uniform temperature distribution. In contrast, microwave heating provides uniform and rapid heating, leading to improved purity and homogeneity of the synthesized nanoparticles. (Droepenu et al., 2022)^[17]
 Advantages: Produces highly crystalline nano particles with controlled size and shape. It is cost effective and environmentally friendly. (Dey et al., 2025)^[7]

Table 2: ZnO-Based Nanocomposites Prepared by Hydrothermal Synthesis and Their Applications

Method of synthesis	Composite	Precursor /reactant	Conditions	Properties	Applications	Ref
Hydrothermal	ZnO-CdS-Ag	Zn(NO ₃) ₂ ·6H ₂ O, C ₂ H ₄ O ₃ , KOH, Cd(NO ₃) ₂ ·4H ₂ O, C ₂ H ₅ NS, AgNO ₃ , N ₂ H ₄	Reaction temp (ZnO): 60 °C for 2 h; Autoclave temp (ZnO): 120 °C for 48 h; Drying temp (ZnO): 120 °C for 8 h; Reaction time (ZnO–CdS): 1 h; Drying temp (ZnO–CdS): 70 °C; Reaction time (ZnO–CdS–Ag): 1 h; Drying temp: 70 °C	Rod-like; Crystallite size: 23 & 15 nm (ZnO & CdS); Surface area: 18.9 m ² /g (ZnO–CdS–Ag)	Photocatalyst against E.coli	Gupta <i>et al.</i> , 2017 [18]

A ZnO–CdS–Ag nanocomposite was synthesized using the hydrothermal method with multiple precursors, including Zn (NO₃)₂ · 6H₂O, C₂H₄O₃, and KOH for ZnO formation; Cd (NO₃)₂ · 4H₂O and C₂H₅NS for CdS incorporation; and AgNO₃ with N₂H₄ for silver deposition. The synthesis involved several stages: ZnO was first prepared at a reaction temperature of 60 °C for 2 hours, followed by hydrothermal treatment in an autoclave at 120 °C for 48 hours and drying at 120 °C for 8 hours. Subsequently, ZnO–CdS was formed with a reaction time of 1 hour and dried at 70 °C. Finally, Ag was introduced to obtain the ZnO–CdS–Ag composite, with an additional reaction time of 1 hour and drying at 70 °C. The resulting nanocomposite exhibited a rod-like morphology with crystallite sizes of approximately 23 nm for ZnO and 15 nm for CdS, along

with a surface area of 18.9 m²/g. This material demonstrated effective photocatalytic activity against *E. coli*, indicating its potential application in antimicrobial and water treatment systems (Gupta *et al.* 2017) [18].

Green synthesis of Zinc oxide nano particles

From plants: Phytochemicals found in plants are used in the green synthesis process as a reducing agent to convert metal ions or metal oxides into metal nanoparticles. These include things like polyphenolic substances, proteins, alkaloid compounds, terpenoids, and polysaccharides.

Green synthesis from different plant species is written in the table:

Table 3: Plant-Mediated Green Synthesis of ZnO Nanoparticles and Their Biomedical Applications

S no	Plant source	Precursor	Description	Size and shape	Applications	Ref
1	<i>Grewia flavescens</i> Malvaceae	Zinc nitrate hexahydrate	Leaf extract	20–30 nm Spherical	Antibacterial activity, Drug delivery, Cytotoxicity	Sana <i>et al.</i> , (2023) [19]
2	<i>Lupinus albus</i> and <i>Lupinus pilosus</i> Fabaceae	Zinc nitrate hexahydrate	Aerial parts	19–26 nm Crystalline	antibacterial and photocatalysis applications	Mirza <i>et al.</i> , (2023) [20]
3	<i>Cassia sieberiana</i> Fabaceae	Zinc acetate	Methanolic root bark extract	12.9 ± 3.1 nm Spherical	Anti-inflammatory, Antioxidant, Antimicrobial, Drug delivery	Kyene <i>et al.</i> , (2023) [21]
4	<i>Moringa oleifera</i> Moringaceae	Zinc acetate di-hydrate	leaf extract	119 ± 36 nm Agglomerated spherical	Anticancer agent, Biomedicine	Rafique <i>et al.</i> , (2023) [22]
5	<i>Brassica oleracea</i> Brassicaceae	Zinc nitrate hexahydrate	Leaf extract	52 nm Flower-like	Photocatalytic dye degradation, Antimicrobial, Larvicidal	Manojkumar <i>et al.</i> , (2023) [23]
6	<i>Neem (Azadirachta indica)</i>	0.1M zinc nitrate	Leaf extract	20-40nm Quasi-spherical	Antibacterial, antioxidant, cytotoxicity	Shyam gupta <i>et al.</i> , 2025

Several studies have reported the green synthesis of ZnO nanoparticles using various plant sources, highlighting their diverse properties and applications. *Grewia flavescens* (Malvaceae) leaf extract has been utilized with zinc nitrate hexahydrate as a precursor to produce spherical nanoparticles of 20–30 nm, which exhibited antibacterial activity, drug delivery potential, and cytotoxic effects (Sana *et al.*, 2023) [19]. Similarly, aerial parts of *Lupinus albus* and *Lupinus pilosus* (Fabaceae) were used to synthesize crystalline ZnO nanoparticles ranging from 19–26 nm, demonstrating antibacterial and photocatalytic applications (Mirza *et al.*, 2023) [20]. In another study, *Cassia sieberiana* (Fabaceae) methanolic root bark extract with zinc acetate yielded spherical nanoparticles of 12.9±3.1 nm, showing anti-inflammatory, antioxidant, antimicrobial properties, and suitability for drug delivery (Kyene *et al.*, 2023) [21]. Leaf extract of *Moringa oleifera* (Moringaceae), when combined with zinc acetate

dihydrate, produced agglomerated spherical nanoparticles with a size of 119±36 nm, which were explored for anticancer and biomedical applications (Rafique *et al.*, 2023) [22]. Additionally, *Brassica oleracea* (Brassicaceae) leaf extract facilitated the formation of flower-like ZnO nanoparticles of approximately 52 nm, exhibiting photocatalytic dye degradation, antimicrobial, and larvicidal activities (Manojkumar *et al.*, 2023) [23]. Furthermore, *Azadirachta indica* (Neem) leaf extract with 0.1 M zinc nitrate resulted in quasispherical nanoparticles of 20–40 nm, demonstrating antibacterial, antioxidant, and cytotoxic properties (Gupta *et al.*, 2025) [24].

Synthesis by bacteria

The employment of bacteria to synthesize NPs has several drawbacks, such as the requirement for monitoring the culture medium and the size and form of the NPs, as well as the cost of the medium in which the bacteria are grown.

Table 4: Bacteria-Mediated Biosynthesis of ZnO Nanoparticles

Family	Bacterial strain	Method of purification	Shape and size	Ref
<i>Bacillaceae</i>	<i>B.licheniformis</i>	Drying at 120 °C and annealing at 700 °C for 5 h	200 nanoflowers with 400 long and 40 wide(TEM)	Alghuthaymi <i>et al.</i> , 2022 [10]
<i>Pseudomonadaceae</i>	<i>Aeromonas hydrophila</i>	Centrifugation, washing, lyophilization and storage at 0 °C	Round, oval 57.72 (AFM), and 42–64 (XRD)	Fakhar <i>et al.</i> 2022 [45]
<i>Staphylococcaceae</i>	<i>Staphylococcus aureus</i>	Centrifugation, washing, lyophilization and storage at 0 °C	Acicular (10–50 nm)	Rauf <i>et al.</i> , 2017 [11]

Different bacterial families have been explored for the biosynthesis of nanostructured materials, with variations in purification methods, morphology, and size. Members of the Bacillaceae family, such as *Bacillus licheniformis*, have been reported to produce nanoflower-like structures following drying at 120 °C and annealing at 700 °C for 5 hours. These structures exhibited dimensions of approximately 200 nm, with lengths of about 400 nm and widths of 40 nm as observed through TEM analysis (Alghuthaymi *et al.*, 2022) ^[10]. In the case of the Pseudomonadaceae family, *Aeromonas hydrophila* was used for nanoparticle synthesis, followed by purification steps

including centrifugation, washing, lyophilization, and storage at 0 °C. The resulting particles were round to oval in shape, with sizes of approximately 57.72 nm measured by AFM and 42–64 nm determined by XRD (Fakhar *et al.*, 2022). Similarly, *Staphylococcus aureus* from the Staphylococcaceae family produced acicular (needle-like) nanoparticles with sizes ranging from 10–50 nm, using comparable purification techniques involving centrifugation, washing, lyophilization, and cold storage (Rauf *et al.*, 2017) ^[11].

Synthesis using fungi

Table 5: Fungi-Mediated Biosynthesis of ZnO Nanoparticles

Family strain	Fungal strain	Precursor	Size and shape	Ref
Saccharomycetaceae	<i>Candida albicans</i>	zinc oxide	hexagonal phase that resembles a sphere (wurtzite structure) 15–25 (SEM), 25 (XRD), and 20 (TEM).	Ilkhechi <i>et al.</i> , 2021 ^[12]
Trichocomaceae	<i>Aspergillus</i> strain	Zinc sulfate	Spherical forming aggregates 50–120(SM).	Parveen <i>et al.</i> , 2016 ^[13]

Fungal strains have also been widely investigated for the biosynthesis of ZnO nanoparticles, offering controlled morphology and size distribution. For instance, a member of the Saccharomycetaceae family, *Candida albicans*, has been utilized with zinc oxide as a precursor to synthesize nanoparticles exhibiting a hexagonal wurtzite structure with a morphology resembling spherical particles. The particle size was reported to be in the range of 15–25 nm as observed by

SEM, approximately 25 nm by XRD, and around 20 nm by TEM analysis (Ilkhechi *et al.*, 2021) ^[12]. Similarly, fungi from the Trichocomaceae family, particularly *Aspergillus* strains, have been employed using zinc sulfate as a precursor to produce spherical nanoparticles forming aggregates, with sizes ranging from 50–120 nm as determined by SEM (Parveen *et al.*, 2016) ^[13].

Comparison of physical/chemical synthesis and green synthesis

Table 6: Comparison of Conventional and Green Synthesis Methods for ZnO Nanoparticles

Physical/chemical methods	Green synthesis method
Costly and inefficient production	Cost-effective due to the accessibility of plant materials and the capping and lowering effects of active bio-components. Production of nanoparticles on a large scale.
Require inert environment, high or low temperature, and pressure.	At normal temperature and pressure, nanoparticles can be synthesized.
A Significant amount of energy is consumed during synthesis process	need no complicated energy system for synthesis
A highly restricted laboratory environment is required.	Nanoparticle production doesn't need to be done under controlled circumstances or in a well-equipped lab.
When toxic chemicals are utilised in chemical processes to create or stabilise nanoparticles, the resultant product may be contaminated or have a faulty surface because the toxic compounds can absorb on the surface of the nanoparticles.	Because natural secondary metabolites of plants are used, the finished product is often secure and stable.

Methods of infusion of zinc oxide nano particles into biodegradable film:

- Solvent/solution casting method
- Spray coating and dipping
- Electrospinning method
- Melt extrusion method

1. Solvent casting, also known as solution mixing, is a technique in which nanoparticles are dispersed in a polymer solution through vigorous stirring or ultrasonication. The homogeneous mixture is then cast into a mold, followed by solvent evaporation to form a solid film. Commonly used solvents include water and ethanol (Banerjee *et al.*, 2024) ^[4]. Solution casting is the most widely used method for fabricating ZnO-incorporated biopolymer films, owing to its simplicity, low cost, and ease of processing (Kim, *et al.*, 2022 ; Luo 2021) ^[5, 6]. Flow chart of synthesis through solvent casting method is shown in Fig 9 and Fig 10) In this method, a film-forming solution

is prepared by incorporating 0.5– 5.0% (w/w) ZnO nanoparticles and a suitable plasticizer into a biopolymer matrix, followed by thorough homogenization. The resulting mixture is cast onto Petri dishes and dried under controlled temperature conditions to allow solvent evaporation. After drying, the formed film is carefully peeled from the casting surface. However, this method presents certain limitations, including the loss of volatile compounds during drying and the tendency of ZnO nanoparticles to agglomerate, which can affect film uniformity and functional properties. These challenges can be mitigated by encapsulating volatile or bioactive compounds using nanotechnology to reduce evaporation losses, and by employing stabilizers or dispersing agents to minimize nanoparticle aggregation and ensure a more homogeneous film structure. (Luo 2022 and Wang *et al.*, 2019) ^[25, 26].

2. Coating has become a crucial active packaging technique for preserving quality and extending the shelf life of fresh

food products. Among the most widely used methods, spray coating and dipping are employed to form protective layers on food surfaces. Spray coating (Fig11) involves the uniform application of a treatment solution using an aerosol sprayer, whereas dipping involves immersing the food product in a pre-formulated solution, typically containing ZnO-infused micro/nanocomposites along with antioxidant agents and plasticizers to enhance functionality. After dipping, excess solution is removed, and the samples are dried under controlled temperature and humidity conditions. The dipping method is particularly favored due to its simplicity and effectiveness in producing active packaging without the need for specialized equipment (Bai Qu *et al.*, 2025)^[27]. Nanotechnology has advanced the use of ZnO nanoparticles in beverage packaging coatings, enhancing both antimicrobial protection and overall packaging functionality. ZnO-infused nanocomposite films offer antimicrobial protection and enhance the overall functionality of packaging (Bu *et al.*, 2023)^[28].

3. Electrospinning (Fig 12) is a cutting-edge technique used to produce nanofibers containing nanoparticles by applying a high-voltage electric field. It is widely employed in the fabrication of nanomaterial films, allowing precise control over the properties of nanobased packaging materials. This method utilizes electrostatic forces to achieve effective

encapsulation of nanomaterials within the fibers. Results have shown that the resulting membrane can achieve a porosity exceeding 70%, and when the polyaniline mass fraction is 5%, the antibacterial activity reaches 75.2% against *Staphylococcus aureus* and 80.08% against *Escherichia coli*. (Zhou *et al.*, 2024).

The application of electrospinning for the production of nanoparticle (NP) films offers several advantages, including simplicity of operation, strong controllability, and relatively low cost. Films produced by this method exhibit a large surface area, high loading capacity, and high porosity, thereby enhancing the overall performance of packaging materials. However, this method also presents certain challenges, such as high equipment cost and the complexity of parameter settings. (Sameen *et al.*, 2022)^[29].

ZnO NP incorporated Biodegradable film: The amount of ZnO nanoparticles (ZnO NPs) incorporated into biopolymers typically ranges from 0.1% to 10%. Common biopolymers used for this purpose include chitosan, starch, cellulose, soy protein isolate, carboxymethyl chitosan, polylactic acid, gelatin, chitosan–polyvinyl alcohol blends, cellulose nanofibers, sodium caseinate, carboxymethyl cellulose–starch composites, and alginate.

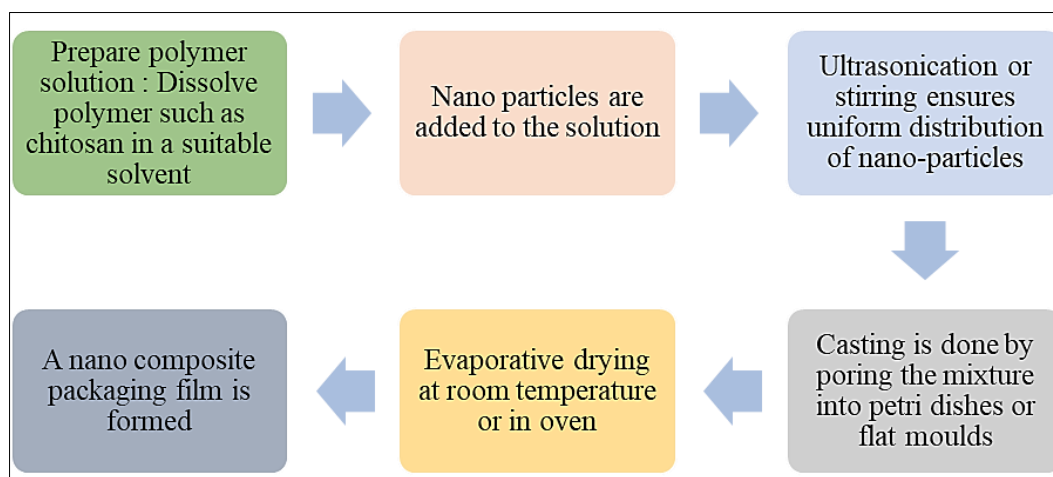


Fig 9: Process of solvent casting method

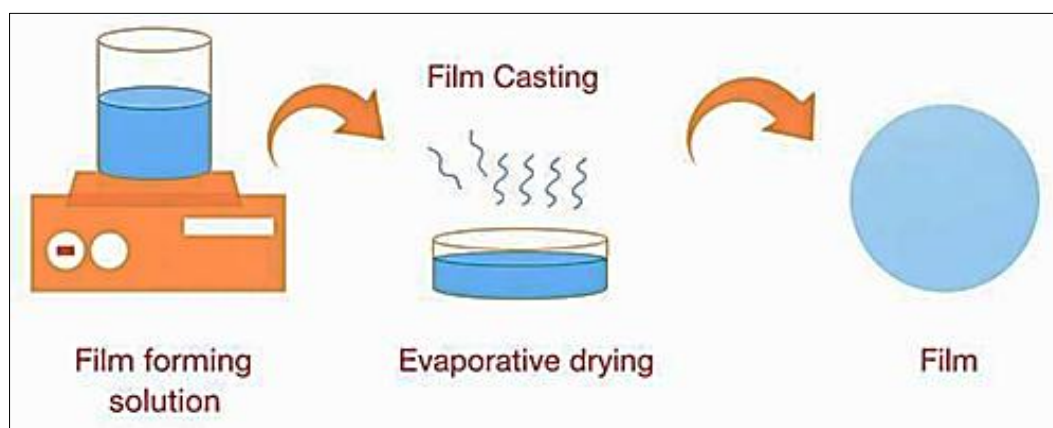
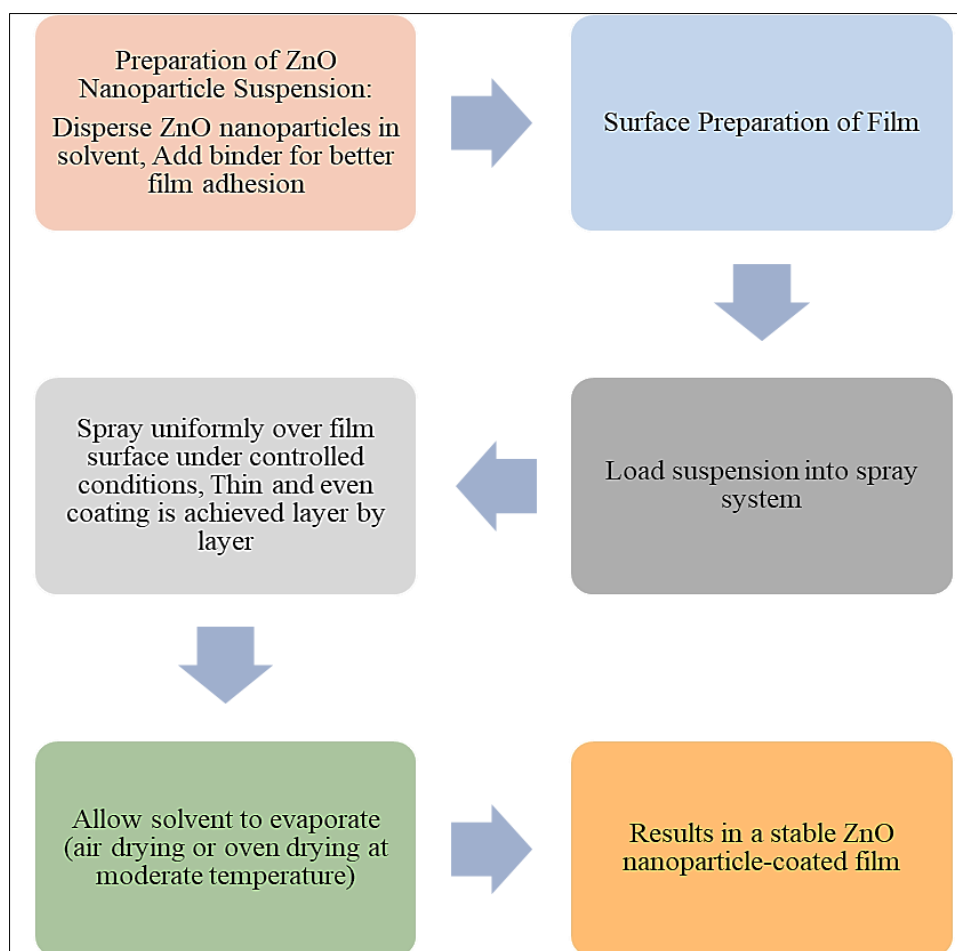
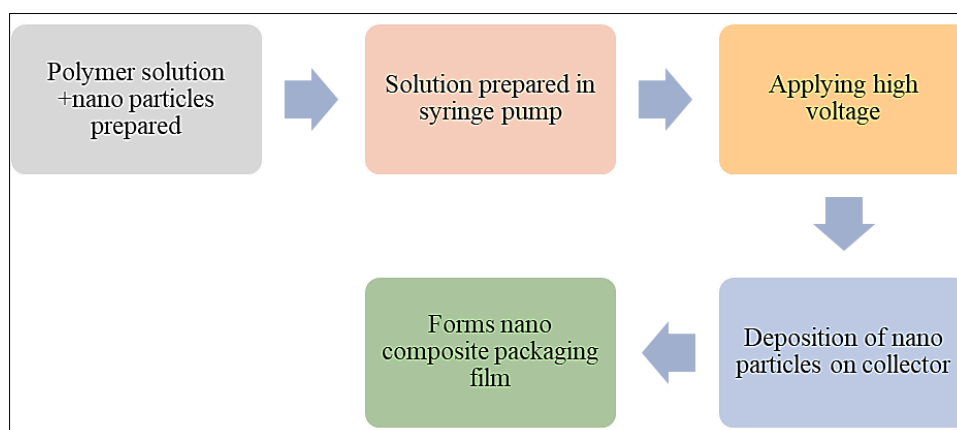


Fig 10: Solvent casting method

**Fig 11:** Process of spray coating method**Fig 12:** Process of electrospinning method**Table 7:** ZnO Nanocomposite Packaging Films and Their Biofunctional Properties

Types of packaging material	ZnO source	ZnO particle size	Prepare method	Biofunctional benefits	Ref
Chitosan	ZnO NP loaded with galic acid	15-30 nm	Solution casting	Antioxidant and antibacterial activity	Yadav <i>et al.</i> , (2021) ^[3]
Soy protein isolate	Commercial ZnO	30±10nm	Solution casting	Antioxidant and antibacterial activity	Huang <i>et al.</i> , (2021) ^[30]
Cellulose	ZnO NP loaded with cellulose	100-200 nm	Solution casting	Antibacterial	Xie (2022) ^[31]
Sodium caseinate	Commercial ZnO	10-30 nm	Solution casting	Antibacterial	Alizadeh-Sani, <i>et al.</i> , (2022) ^[32]
Cellulose nano fibrils	Commercial ZnO	<100 nm	Solution casting	Antioxidant and antibacterial activity	Wu <i>et al.</i> , (2021) ^[33]

Various types of biopolymer-based packaging materials incorporated with ZnO nanoparticles have been developed to

enhance bio functional properties such as antioxidant and antibacterial activity. Chitosan-based films loaded with ZnO

nanoparticles and gallic acid, with particle sizes ranging from 15–30 nm, have been prepared using the solution casting method and demonstrated both antioxidant and antibacterial properties (Yadav *et al.*, 2021)^[3]. Similarly, soy protein isolate films incorporated with commercially available ZnO nanoparticles of size 30 ± 10 nm, also prepared via solution casting, exhibited enhanced antioxidant and antibacterial activity (Huang *et al.*, 2021)^[30]. In another study, cellulose-based films containing ZnO nanoparticles of 100–200 nm synthesized through solution casting showed significant antibacterial properties (Xie, 2022)^[31]. Sodium caseinate films with commercial ZnO nanoparticles of 10–30 nm also displayed antibacterial activity when prepared using the same method (Alizadeh-Sani *et al.*, 2022)^[32]. Furthermore, cellulose nanofibril-based films incorporated with ZnO nanoparticles of size less than 100 nm demonstrated both antioxidant and antibacterial activities, highlighting their potential application in active food packaging systems (Wu *et al.*, 2021)^[33].

Role of Zinc oxide NP in food packaging

Antimicrobial property: ZnO is recognized as a nanoparticle with strong antimicrobial properties, capable of exhibiting significant antibacterial and antifungal effects. ZnO nanoparticles (ZnO NPs) are effective against both Gram-

positive and Gram-negative bacteria, and the differences in their susceptibility can be attributed to variations in the levels of intracellular antioxidants. (Manoj Kumar *et al.*, 2025).

This material attacks the surface of microorganisms by generating reactive oxygen species (ROS) and releasing metal ions, leading to the degradation of the cell wall and ultimately cell death. ZnO also has the ability to penetrate the cell membrane and disrupt essential cellular functions, including enzymatic activity, metabolism, and protein synthesis, thereby exerting its antimicrobial effects. The accumulation of ZnO nanoparticles on the surface of microbial cells leads to the destruction of cellular structures and damage to the cell membrane and cell wall. These changes result in severe disruption of cellular function and ultimately cell death. (Abolfazl Jahani *et al.*, 2025)

Mechanisms of zinc oxide (ZnO) materials used in antimicrobial applications: Particular interactions of (a) metal ion release, (b) adsorption, and (c) reactive oxygen species generation based on their physicochemical properties; and following intracellular responses of (d) energy metabolism inhibition, (e) lipid peroxidation and membrane damage, and (f) DNA replication disruption and DNA break Fig.13 (Jin *et al.*, 2021)^[34]

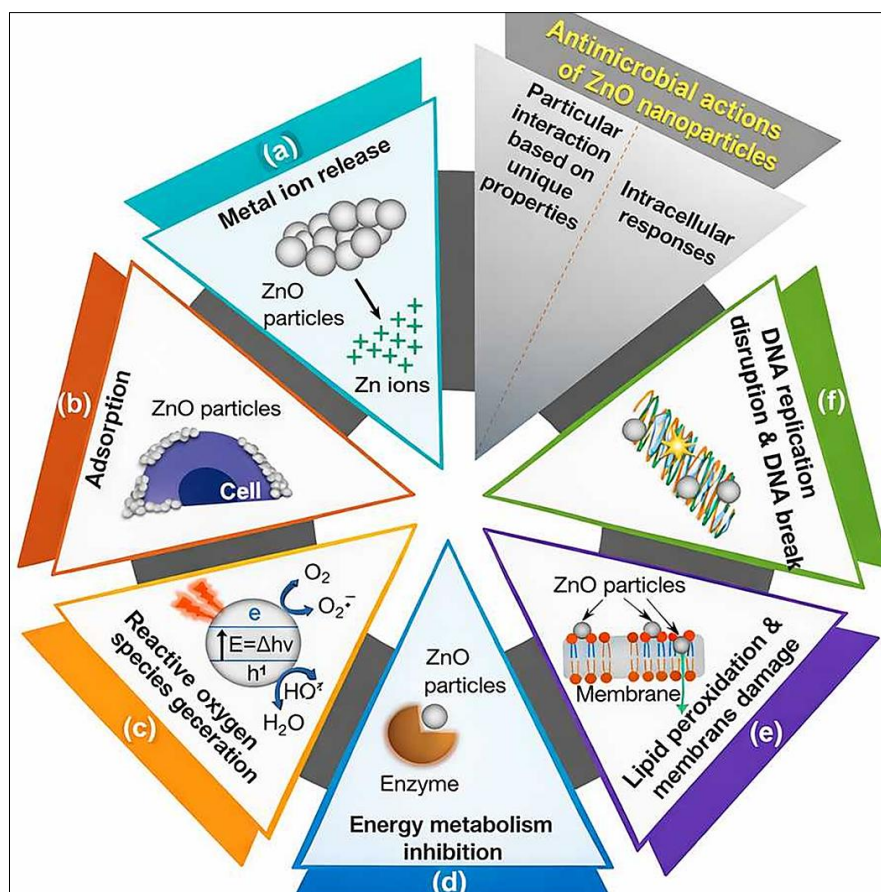


Fig 13: Antimicrobial property of Zinc NP

Barrier properties: Resistance to oxygen, moisture, and UV radiation: When ZnO nanoparticles are uniformly dispersed within a polymer matrix, their high surface-to-volume ratio enables them to act as absorbents, attracting polymer chains to their surface. This interaction reduces the entropy of the polymer chains, while adsorption interactions lead to a decrease in enthalpy. Consequently, the free volume is reduced, and the mobility of the polymer chains is restricted. Additionally, due

to the relatively hydrophobic nature of ZnO, moisture absorption by the polymer matrix is decreased, thereby minimizing the adverse effects of moisture. (Mohammed *et al.*, 2022)^[35]. Uniformly dispersed ZnO nanoparticles create tortuous pathways that hinder the diffusion of gases such as oxygen. This characteristic enhances the barrier properties of the nanocomposite against moisture and oxidizing gases. Furthermore, due to its crystalline structure, ZnO is capable of

absorbing UV radiation. When UV rays strike ZnO, the energy is absorbed and converted into heat, preventing the radiation from reaching the underlying surface. In this way, ZnO effectively provides both scattering and absorption of UV rays. (Channa *et al.*, 2022) ^[36].

Additionally, their ability to absorb UV radiation can prevent the degradation of food products caused by UV exposure, providing an extra layer of protection for perishable items. (Ezati *et al.*, 2023) ^[37].

Photocatalytic activity: ZnO nanoparticles have attracted significant attention due to their photocatalytic properties, making them highly effective in the degradation of environmental contaminants. Upon exposure to UV light, ZnO nanoparticles generate electron-hole pairs, which initiate redox reactions capable of breaking down organic pollutants in water and air. (Zeid *et al.*, 2024) ^[38]. The photocatalytic activity of ZnO is attributed to its wide bandgap, which allows it to absorb UV radiation and create highly reactive species, such as hydroxyl radicals and superoxide anions. (Baibara *et al.*, 2021) ^[39]. ZnO's high surface area, combined with its low toxicity and stability, enhances its performance as a photocatalyst. (Lu *et al.*, 2008) ^[40]. In food packaging, the photocatalytic properties of ZnO nanoparticles can be utilized to improve the safety and shelf-life of packaged goods. By degrading harmful microorganisms and contaminants that may affect the food, ZnO helps maintain food quality and extend its freshness. (Ezati *et al.*, 2023) ^[37].

Antioxidant property: The antioxidant activity of the films was evaluated by measuring the free radical scavenging activity

of compounds using the DPPH method (Huang *et al.*, 2021) ^[30]. ZnO NPs exhibit antioxidant properties primarily through their ability to scavenge reactive oxygen species (ROS), thereby reducing oxidative stress in cells. The particles can interact with free radicals, neutralizing them before they cause cellular damage (Mohammad *et al.*, 2023) ^[41]. Additionally, ZnO NPs are known to influence various cellular antioxidant defence mechanisms, such as enhancing the activity of antioxidant enzymes like superoxide dismutase (SOD) and catalase (Khan *et al.*, 2021) ^[42].

Antimicrobial activity of Zinc oxide NP incorporated biopolymers:

Biopolymers such as chitosan, starch, cellulose, gelatin, and alginate have been widely studied using different microbial strains. Chitosan-based films incorporated with nano zinc oxide, tested against *Bacillus subtilis* and *Escherichia coli*, exhibited stronger bactericidal activity compared to pure chitosan films (Priyadarshi *et al.*, 2017) ^[14]. Starch-based films evaluated against *Staphylococcus aureus* and *E. coli* also demonstrated notable antimicrobial properties.

Cellulose-based films tested against *E. coli* and *Listeria monocytogenes* showed higher antibacterial activity specifically against *E. coli* (Roy *et al.*, 2020) ^[15]. Similarly, gelatin films studied against *Streptococcus pneumoniae*, *B. subtilis*, and *E. coli* exhibited an increased zone of inhibition against *E. coli* and *B. subtilis* (Yadav *et al.*, 2020) ^[16].

In contrast, alginate-based films tested against *B. subtilis* and *E. coli* did not demonstrate any significant antibacterial activity against the tested microorganisms (Salama *et al.*, 2019) ^[43].

Table 8: Antimicrobial Activity of ZnO Nanocomposite Packaging Materials

Nature	Materials	Microbes used	Observation
Biopolymers	Chitosan	<i>B. subtilis</i> and <i>E. coli</i>	Nano zinc oxide integrated CS films have stronger bactericidal activity against <i>B. subtilis</i> and <i>E. coli</i> than pure CS films (Priyadarshi, <i>et al.</i> , 2017) ^[14] . The bactericidal activity of cellulose-based films against <i>E. coli</i> is higher (Roy <i>et al.</i> , 2020) ^[15] .
	Starch	<i>S. aureus</i> and <i>E. coli</i>	
	Cellulose	<i>E. coli</i> and <i>Listeria monocytogenes</i>	The gelatin film showed an increased zone of inhibition against <i>E. coli</i> and <i>B. subtilis</i> (Yadav <i>et al.</i> , 2020) ^[16] . The alginate-based film did not demonstrate any antibacterial activity against the tested bacteria Salama <i>et al.</i> , 2019) ^[43] .
	Gelatin	<i>B. subtilis</i> and <i>E. coli</i>	
	Alginate	<i>Streptococcus pneumoniae</i> , <i>B. subtilis</i> and <i>E. coli</i>	

Migration and safety: The migration of ZnO from polypropylene food containers into different food-simulating solutions under varying storage time and temperature conditions was quantified using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), and migration was observed. Results revealed significant migration of nano-sized zinc oxide into oily, acidic, and aqueous simulants. The amount of zinc oxide migrated increased with storage time and temperature; however, overall, ZnO exhibited a relatively low tendency to migrate into food simulants. The Zn²⁺ ions were quantified by ICP-MS, with migration levels ranging from 0.15 to 0.56 µg L⁻¹. Compared to aqueous simulants, higher migration levels of zinc oxide were observed in 4% acetic acid and hexane simulants. (Liu *et al.*, 2016) ^[44]. The CEF panel (European food safety authority panel) recommended that SML (safety migration limit) for zinc is 25mg/kg (EFSA, 2016).

Conclusion

Zinc oxide (ZnO) nanoparticles exhibit significant potential for enhancing food packaging materials due to their unique functional properties, including antibacterial activity,

antioxidant capacity, and resistance to ultraviolet (UV) radiation. These characteristics contribute to improved physical and chemical properties of packaging films, thereby extending the shelf life of food products and reducing microbial contamination.

Moreover, the incorporation of ZnO nanoparticles into packaging systems offers a sustainable and environmentally friendly approach to food preservation. Despite these advantages, further research is necessary to evaluate the scalability, long-term safety, and regulatory aspects of their application in industrial food systems.

Future developments may focus on combining ZnO nanoparticles with other advanced materials to create intelligent and highly efficient packaging solutions. Such innovations hold great promise for widespread application across the food industry, ultimately ensuring improved food quality, safety, and shelf-life extension.

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