

GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING LEMON PEEL EXTRACT FOR ANTIBACTERIAL ACTIVITIES

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Abstract: Three distinct extracts of citrus peel waste were used to make zinc oxide (ZnO) nanoparticles utilizing a simple green synthesis approach in an alkaline medium. UV-vis spectroscopy, x-ray powder diffraction (XRD), and Fourier-transform infrared spectroscopy were used to characterize the produced Nano-crystalline materials (FTIR), while antibacterial activities of ZnO nanoparticles. In this work, scanning electron microscopy (ESEM). The lattice Parameters calculated from the (100) diffraction peak are a = 3.23241 Å, c = 5.98162 Å. The bond at 1377 cm -1 corresponds to the asymmetric stretching mode of C=O. The particles also seem to be highly agglomerated in nature. Antibacterial For 5000 µg / well- add 50 µL from stock; for 1000 µg / Well – Add 10µL (Final Volume 50 µL or desired volume) and similarly for the other tested concentrations the working solution was prepared and final volume was made up to 50 µL.

Index Terms: - Citrus peel waste, Zinc oxide nanoparticles, Green synthesis, and Antibacterial activities

1. INTRODUCTION

Nanotechnology involves manipulating matter at the atomic and molecular scale (1-100 nanometers), unlocking unique properties distinct from bulk materials. Stemming from Richard Feynman's 1959 lecture, nanotechnology spans nanomaterials, Nano electronics, Nano medicine, and Nano biotechnology. Nanomaterials, like carbon nanotubes and nanoparticles, revolutionize aerospace, automotive, and construction with lighter, stronger materials. They enhance energy storage in batteries and super capacitors, extending life and improving charging speed.

Nano medicine employs nanoscale particles for precise drug delivery, diagnostics, and tissue engineering, enhancing treatment efficacy while minimizing side effects. Nano biotechnology merges nanotech and biology, enabling targeted drug delivery, biosensors for early disease detection, and gene editing. Despite its promise, ethical, environmental, and safety concerns exist, urging comprehensive research into long-term effects. Nanotechnology promises transformative innovations across industries, necessitating responsible development to harness its potential for a sustainable future.

2. SYNTHESIS OF NANOMATERIALS

Advancements in technology have led to the effective implementation of diverse nanotechnology manufacturing processes. These methodologies have been influenced by the cumulative expertise of researchers and the available technological resources. The spectrum of these procedures encompasses the creation of colloids, clusters, powders, tubes, rods, wires, thin films, and various other forms of nanomaterials.

Two primary techniques, known as the 'top-down' and 'bottom-up' approaches, characterize the synthesis of nanomaterials and the construction of nanostructures. The 'top-down' method involves mechanical, chemical, and biological processes that reduce largersized bulk materials into nanoscale entities. Conversely, the 'bottom-up' technique relies on assembling atomic or molecular components through chemical reactions or physical processes to fabricate nanoscale structures. In industrial nanomaterial manufacturing, the bottom-up method stands as the most prevalent synthesis strategy. The 'top-down' approach encompasses various subclasses distinguished by specific operations, reaction conditions, and adopted protocols, further refining the manufacturing processes and enhancing their precision.

2.1 BOTTOM – UP APPROACH

Nanoparticles, constructed from fundamental components, utilize the 'building up' or 'constructive' method, starting from atoms to clusters and culminating in nanoparticles. Chemical and biological techniques largely encompass this bottom-up approach. It fabricates nanoparticles such as titanium dioxide or iron oxide found in cosmetics, experimental devices, crystal films, and display tubes. Molecular self-assembly under near-thermodynamic equilibrium forms the basis of this technique, offering spatial control over attributes and composition. Notably cost-effective and reliable, it enables precise control over nanoparticle size and shape. Functionalization with diverse ligands mitigates particle agglomeration, making it a superior alternative with heightened control and minimized agglomeration.

2.2 TOP – DOWN APPROACH

The reduction of bulk substances into nanoscale particles characterizes the 'top-down' or destructive approach, employing methods like mechanical milling, nanolithography, laser ablation, sputtering, and thermal breakdown. These techniques rely on material removal or downsizing of bulk manufacturing processes to achieve desired structures. However, the primary drawback lies in imprecise surface structures. For instance, nanowires produced through lithography often possess surface irregularities and structural flaws. High-energy wet ball milling, electron beam lithography, and gas-phase condensation are among the varied processes employed in 'top-down' methodologies.

2.3 NANOFABRICATION OF NANOMATERIALS

2.3.1 PHYSICAL METHOD

The physical technique is a two-step top-down strategy to synthesizing nanostructure materials. The material is evaporated in the first phase, followed by a quick controlled condensation to achieve the desired particle size. Below are some of the most significant physical approaches.

2.3.1.1 LASER ABLATION

Laser ablation (LA) involves a laser's penetration into the sample surface, influenced by the laser wavelength and target material's refractive index as quoted in Fig 2.1. The laser generates an intense electric field, extracting electrons from the material and transferring energy upon collision with bulk sample atoms, leading to surface heating and vaporization. This transition, under strong laser flux, transforms material into plasma—comprising atoms, molecules, ions, and clusters. The subsequent rapid expansion and cooling of the plasma occur due to pressure variations. LA occurs in gaseous or vacuum environments, while pulsed LA can integrate with a tube furnace.



2.3.1.2 SPUTTERING

In 1852, Grove observed energetic ions sputtering the cathode surface within a discharge tube, depositing material within it as indicated in Fig 2.2. This process of bombarding a surface with powerful gaseous ions, ejecting surface atoms or clusters, remains in use today. Sputtering techniques include DC-diode, RF-diode, and mag-neutron sputtering. Notably, when employing Argon plasma for sputtering, unlike other vapor phase methods, there is no melting of the samples involved.



Fig.2.2 Sputter Deposition

2.3.2 CHEMICAL METHOD

The chemical synthesis of metal nanoparticles often involves several liquid-phase steps. Initially, metal atoms are generated by reducing metal precursors in a solution using chemical reductants. These newly formed metal atoms subsequently undergo elementary nucleation and gradual growth processes, ultimately leading to the production of nanoparticles.

2.3.2.1 SOL – GEL METHOD

The sol-gel method involves a multi-step, wet chemical process integrating both chemical and physical procedures like hydrolysis, condensation, gelation, drying, dehydration, and densification. Initially, precursors combine to form a stable solution referred to as the sol. As gel formation begins, the distinctive viscosity increase characterizes the 'sol-gel' process. The resulting gel encapsulates the solvent, forming a diphasic material. Subsequent drying, achieved through natural evaporation or supercritical drying, yields solid products known as aerogels as sited in Fig 2.3 sol – gel method.

Utilized for crafting metal oxide nanostructures, sol-gel synthesis is a solution-based approach for advanced material production, spanning ceramics and organic-inorganic hybrids. Water, alcohol, acid, or base control reaction kinetics, while varying precursor concentration, temperature, and pH allows particle size tuning. However, a notable drawback is the wet gel's tendency to shrink during drying, leading to potential fracture due to significant capillary stresses.



2.3.2.2 LANGMUIR-BOLDGETT (LB) TECHNIQUE

The LB technique involves transferring a monolayer of amphiphilic compounds from the air/water interface to a solid substrate (Langmuir films). Developed by Irving Langmuir and Katharina Blodgett in the 1930s, the Langmuir-Blodgett method creates monoand multimolecular films, widely applied in electronic equipment manufacturing. The method initiates with forming a monomolecular amphiphilic layer on water and transferring it to a solid substrate by compressing the surface with a specialized barrier according to the Fig.2.4. A flat substrate is dipped into the solution and withdrawn, carrying the adsorbed surface film onto the solid support. Repeating this monomolecular film transfer process enables the creation of diverse multimolecular layers.



Fig.2.4 Schematic of LB technique

3. PREPARATION OF PURE ZnO

3.1 ZINC OXIDE (ZnO): INTRODUCTION

Zinc oxide (ZnO) is a widely used n-type semiconductor metal oxide with a distinct band gap of 3.3 eV, valued for its optical properties in optoelectronic devices like photodetectors and innovative flexible devices made from human hair. However, conventional synthesis methods involving toxic reagents limit its usability by affecting ZnO's surface. Green synthesis methods offer a solution by enabling controlled particle size and finding applications across various fields like biomedicine, pharmaceuticals, cosmetics, environment, energy, and electronics. These methods utilize fungi, bacteria, and plants as alternatives to traditional chemical approaches, aiming for ecological and economic benefits.

Zinc oxide plays a significant role among semiconductor metal oxides, finding versatile applications in optoelectronics, field effect transistors, solar cells, photoluminescence devices, and diluted magnetic semiconductors. Advances in optoelectronics include eco-friendly laser diodes and white light technologies. Researchers manipulate ZnO's characteristics by doping or inserting impurities, altering its electronic structure to enhance optical, structural, electrical, and magnetic properties. These advancements improve electronic, spintronic, optoelectronic, photocatalytic, and antimicrobial applications. ZnO's exceptional properties, such as direct band gap energy, luminescence, high electron mobility, and potential room-temperature ferromagnetism in various structures, drive innovation across diverse fields.

3.2 PROPERTIES OF ZnO

Zinc oxide has the formula ZnO and is an inorganic substance. It is a white powder that is water insoluble. Cosmetics, food supplements, rubbers, plastics, ceramics, glass, cement, lubricants, paints, ointments, adhesives, sealants, pigments, edibles, batteries, ferrites, fire retardants, and first-aid tapes are just a few of the materials and products that contain ZnO. Although zinc oxide is found naturally in the mineral zincate, the majority of zinc oxide is manufactured synthetically.

3.3 CHEMICAL PROPERTIES

Pure ZnO is a white powder, but it is found in nature as the rare mineral zincate, which is yellow to red in color due to manganese and other impurities. When heated in air, crystalline zinc oxide changes color from white to yellow, then returns to white when cooled. At high temperatures, a little amount of oxygen is lost to the environment, forming the non-stoichiometric Zn1+xO, where x = 0.00007 at 800 °C.

An amphoteric oxide is zinc oxide. It is nearly insoluble in water, however most acids, including hydrochloric acid, will dissolve it:

$$2 \text{ HCl} + \text{ZnO} = \text{ZnCl}_2 + \text{H}_2\text{O}$$

In alkalis, solid zinc oxide dissolves to form soluble zincates:

$$Na_2 [Zn (OH)_4] = ZnO + 2 NaOH + H_2O$$

Slowly reacting zinc oxide with fatty acids in oils produces carboxylates like oleate and stearate. ZnO generates cement-like compounds called zinc hydroxyl chlorides when combined with a strong aqueous solution of zinc chloride. [16] This cement was utilized in the dental field.

When ZnO is treated with phosphoric acid, it forms cement-like substance, which is utilized in dentistry. Hoplite, Zn_3 (PO₄)24H₂O, is a key component of zinc phosphate cement formed by this process.

With a typical oxygen pressure, ZnO decomposes into zinc vapor and oxygen at around 1975 °C. Heating with carbon transforms zinc oxide to zinc vapor at a significantly lower temperature (about 950 °C) in a carbothermic reaction.

Zn (Vapor) + CO = ZnO + C

3.4 PHYSICAL PROPERTIES

Zinc oxide (ZnO) crystallizes mainly in hexagonal quartzite and cubic zinc blende structures, with quartzite being the most stable at room temperature. Substrates with a cubic lattice stabilize the zinc blende form. At high pressures (~10 GPa), ZnO transitions to the rock salt motif. Both structures feature tetrahedral zinc and oxide centers, common for Zn (II).

Hexagonal and zinc blende polymorphs lack inversion symmetry, leading to their piezoelectric and pyroelectric properties. Hexagonal ZnO has a point group of 6mm or C6v, space group P63mc or C6v4, with lattice constants a = 3.25, c = 5.2, and a c/a ratio close to the ideal 1.633. ZnO's bonding primarily involves ionic bonds (Zn2+O2-) due to Zn2+ (0.074 nm) and O2- (0.140 nm) radii. Unlike most materials, ZnO surfaces remain flat and stable without reconstruction, attributed to their charge production and specific quartzite structure characteristics.

3.5 APPLICATION OF ZnO

3.5.1. Rubber Manufacturing: Constituting 50%-60% of its use, zinc oxide, in conjunction with stearic acid, fortifies rubber in vulcanization and shields it from UV light and fungi.

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3.5.2. Ceramic Industry: Highly valued in ceramics for its heat capacity, thermal conductivity, and stability, zinc oxide influences glaze, enamel, and ceramic properties, improving flexibility and surface appearance.

3.5.3. Medicine: In calamine lotions, zinc oxide aids skin conditions; it's also used in dentistry. Nano ZnO exhibits antibacterial traits, benefiting fabric, oral care, and food packaging. Zinc oxide is found in mouthwashes, toothpaste, and sunscreen, combating plaque, bad breath, and skin ailments.

3.5.4. Cigarette Filters: Incorporated in cigarette filters, zinc oxide, along with charcoal and iron oxide, effectively diminishes harmful components in tobacco smoke without altering taste.

3.5.5. Food Additive: Added to various food products as a zinc source, zinc oxide was under scrutiny in the 2008 Chilean pork crisis due to dioxin contamination from pig feed.

3.5.6. Pigment: As zinc white, it acts as a pigment in paints, paper coatings, and artist materials, replacing toxic alternatives like lead white in painting.

7. UV Absorber: Micronized and Nano-scale zinc oxide offer robust protection against UVA and UVB rays, widely used in sunscreens, UV-blocking sunglasses, and protective gear for space activities and welding.

Zinc oxide plays a pivotal role across diverse industries, contributing significantly to their functionalities and applications.

4. RESULTS AND DISCUSSION 4.1 POWDER X-RAY DIFFRACTION ANALYSIS (XRD)

X-ray diffraction was used to characterize the phase purity and crystallinity of ZnO nanoparticles synthesized by hydrothermal method





The pure zinc oxide (ZnO) nanoparticles generated by a simple hydrothermal method's X-ray Diffraction (XRD) patterns the peaks of diffraction are indexed to hexagonal structure (JCPDS NO 36-1451).

The diffraction peaks are crisp and strong, indicating that the products are well crystalline. The diffraction peaks at 31.7° , 34.4° , 36.1° , 47.5° , 56.6° , 62.8° , 66.4° , 67.9° , and 69.1° can be indexed to ZnO planes (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 0 3), (2 0 0), (1 0 3), (2 0 0), (1 0 3), (2 0 0), (1 0 3), (2 0 0), (1 0 3), (2 0 0), (1 2 2), (2 0 1).

The Debye Scherer formula, $D=0.94/\cos \longrightarrow (4.1)$

Can be used to calculate the average particle size (D) of zinc oxide (ZnO) nanocrystals, where D is the mean particle size, is the full width at half maximum of the diffraction line, is the Bragg angle of diffraction, and is the wavelength of the X-ray radiation. From the full width of the screen to the half-maximum width of the screen (1 0 0) diffraction peak the particle size can be calculated. In this case, the average particles size is found to be 40.642 nm. The Lattice parameters calculated from the (100) diffraction peak are a = 3.23241 Å, c = 5.98162 Å, Volume of the hexagonal unit cell= $V = a^2 * c^* 0.866$, $V = 54.1240 Å^3$

4.2 UV DIFFUSED REFLECTANCE SPECTRAL ANALYSIS

In the reference to the graph Fig 4.2 the optical band gap energy is calculated using the Kubelka- Munk function

 $(hvF(R\infty))^2 = A(hv - E_g) \longrightarrow (4.2)$

Where h is Planck's constant, v is vibration frequency, E_g is energy gap, A is proportionality constant, and F (R) is reflectance function, and can be written as,

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 $(h\nu F(R\infty))n = (h\nu - E_g)$ (4.3)



Fig 4.3 shows the reflectance spectra of pure ZnO nanoparticles. Between (hv F(R)) 2 and hv F(R) 2 is plotted the Kubelka – Munk plot (eV). Extrapolating the linear component of the energy axis at zero reflectance yields the optical band gap of ZnO. In Fig 4.2, the Kubelka – Munk plot for pure ZnO nanoparticles is displayed. The optical band gap of pure ZnO nanoparticles was found to be 3.1 eV.



Fig 4.3 UV Reflectance spectra of ZnO

4.3 FOURIER TRANSFORM INFRARED SPECTROSCOPY

The chemical composition of the synthesized ZnO nanoparticles was examined by (FTIR) Spectroscopy. FTIR spectra are generally used to characterize compound.



Fig 4.4 Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR spectrum gives information about the functional groups, molecular geometry and inter/intro molecular interactions present in compounds. In the FTIR spectrum in Fig 4.4, the peaks at 3457 cm-1 and 1637 cm-1 arise due to the stretching and bending vibrations of absorbed water. The peak at 1377 cm-1 corresponds to the asymmetric stretching of C=O bond. The peak at 874 cm-1 can be attributed to the formation of tetrahedral co-ordination of ZnO. The stretching vibrations of Zn-O create strong peaks at 540 cm-1 and 442 cm-1. Thus the formation and purity of ZnO can be affirmed.

4.4 SCANNING ELECTRON MICROSCOPY (SEM)

A high-resolution scanning electron microscope is used to investigate the morphology of the generated zinc oxide (ZnO) Nano powders (HR-SEM). The ZnO nanoparticles exhibit nanocluster morphology as shown in Fig 4.5. The particles also seem to be highly agglomerated in nature.



Fig 4.5 HR-SEM analysis of ZnO

The EDX spectrum of pure ZnO nanoparticles Fig. 4.5 confirms the presence and formation of pure ZnO nanoparticles. The Nano rods contain only elements of Zn and O without any other impurities was indicated by the pattern.





Fig 4.6 EDAX analysis of ZnO

Element	Wt%	At%	
OK	13.77	39.48	
ZnK	86.23	60.52	
Matrix	Correction	ZAF	



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4.6 ANTIBACTERIAL ACTIVITIES

Stock Solution Preparation: (50 mg in 500µL)

50 mg of Nano particle was added to 500μ L Muller Hinton broth, mixed thoroughly. (100mg/mL concentration) So 100μ L contains 10mg (10000μ g); 10μ L contains 1mg (1000μ g) and 1μ L contains 0.1mg (100μ g) of the test compound.

For Well Diffusion Assay (High Concentration = 5000 μg; Low Concentration = 125 μg)

For 5000 μ g / well - add 50 μ L from stock; for 1000 μ g / Well – Add 10 μ L (Final Volume 50 μ L or desired volume) and similarly for the other tested concentrations the working solution was prepared and final volume was made up to 50 μ L

Test Bacteria: 2 Gram Positive bacteria *S aureu*, *B. subtilis* and 1 Gram Negative bacteria *E.Coli*, and yeast *C*. albicans will be tested by well diffusion method at 125µg to 5000µg concentrations.

Antibacterial activity by well Diffusion Method:

The antibacterial activity was evaluated using Gram-positive bacteria (S. aureus, B. subtilis), Gram-negative bacteria (E. coli), and yeast (C. albicans) via the well diffusion method. Cultures were grown on Muller Hinton Agar plates and then wells were created for testing. Nanoparticle suspensions at different concentrations were added to the wells, and after overnight incubation at 37°C, the zones of inhibition were measured to assess antimicrobial activity.

Results:

The antibacterial activity results indicate potent action of green-synthesized ZnO nanoparticles against both Gram-positive and Gram-negative bacteria in comparison to the standard antibiotic Streptomycin (10 μ g). However, these nanoparticles did not exhibit any activity against the yeast Candida albicans, unlike the standard antibiotic Ketoconazole (40 μ g). Table 4.2 demonstrate the zones of inhibition by green-synthesized ZnO nanoparticles against various bacteria and yeast.

Specifically, these nanoparticles displayed maximum bacterial growth inhibition (22mm) against B. subtilis at tested concentrations, effectively impeding growth on Muller Hinton agar plates. However, for the yeast C. albicans, no noticeable activity was observed across the tested concentrations.

Notably, the concentrations of 5000 μ g and 1000 μ g exhibited substantial zones of inhibition (22mm and 16mm, respectively) against S. aureus and E. coli. For E. coli, varying degrees of growth inhibition were observed: 22mm, 16mm, 12mm, 10mm, and no activity at 125 μ g concentration. Similarly, for S. aureus, the zones of inhibition were 22mm, 16mm, 12mm, 10mm, and 8mm at 125 μ g concentration.





Fig 4.7 Antibacterial activity of ZnO np against Fig 4.8 Antibacterial activity of ZnO np against *Bacillus* subtil Staphylococcus *aureus*

S.No	Test Sample	Conc (µg)	Zone of Inhibition (in mm)			
			S. aureus	B. subtilis	E. coli	C. albicans
1 LPE- ZnO n	LPE- ZnO np	5000 μg	20	22	22	-
		1000 µg	16	14	16	-
		500 µg	12	10	12	-
		250 µg	10	8	10	-
		125 µg	8	-	-	-
2	Streptomycin	10 µg	15	12	18	-
3	Ketoconazole	40 µg	-	-	-	30

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 Table 4.2 Zone of inhibition



Fig 4.9 Antimicrobial activity of LPE – ZnO np's by well diffusion method

Observations on the Fig 4.9 antimicrobial activity of green-synthesized Zinc Oxide nanoparticles using Lemon Peel extract revealed equal inhibition zones in Gram-negative (E. coli), Gram-positive (B. subtilis, S. aureus) bacterial strains, as observed in the yeast C. albicans at tested concentrations. Previous studies indicate that smaller ZnO particle sizes tend to exhibit enhanced efficacy in inhibiting bacterial growth by producing reactive oxygen species and nanoparticle accumulation.

Moreover, there's a dichotomy in ZnO nanoparticle behavior as they've been reported both as bactericidal agents and bacteriostatic agents in prior studies. This duality in function may potentially restrict their application in biomedical contexts.

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The synthesized ZnO nanoparticles via hydrothermal methods displayed compelling characteristics across various analyses: X-ray Diffraction revealed a crystallite size of 40.642 nm, FTIR identified functional groups, UV-Visible spectroscopy determined an optical bandgap of 3.0 eV, and antibacterial studies exhibited potent action against Gram-positive and Gram-negative bacteria, outperforming standard antibiotics. This green synthesis method utilizing citrus reticulate extract demonstrated promising results, showcasing superior structure, morphology, and optoelectronic properties compared to commercial ZnO. This opens avenues for optoelectronic sensors comprising ZnO rods.

Future research suggestions encompass photoluminescence analysis for electronic structure probing and semiconductor purity, exploring photocatalytic potential for environmental and healthcare applications, VSM studies for magnetic properties, and further antibacterial investigations for air and water purification applications. Additionally, experiments with metal-doped and non-metal-doped ZnO aim to enhance photocatalytic efficiency under UV and visible light, respectively.

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