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ORIGINAL ARTICLE

# Green Synthesis and Characterization of Magnesium Oxide (MgO) Nanoparticles as Biological Agent

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**ABSTRACT**

The synthesis of MgO nanoparticles is divided into various steps, such as mixing, stirring, filtering, drying, and grinding. Initially ($MgNO_3.6H_2O$) of weight 2 g taken into beaker to form 0.2 M solution in distilled water. Then 0.8 g (0.5 M) of NaOH dissolved in 100 ml distilled water. Magnesium nitrate solution stirred for half an hour using magnetic stirrer for constant stirring. Then 0.5 M sodium hydroxide solution was added drop wise by dropper to the prepared Magnesium nitrate ($MgNO_3.6H_2O$) Solution while Stirring it continuously. After 30 minutes, milky white color precipitate of Magnesium Hydroxide appeared in beaker. The PH of the solution was 12.5 as measured by the pH paper. Precipitate was filtered by filter paper and washed with methanol three to four times to remove ionic impurities and then dried in air, then after drying white powder sample were annealed in air for two hours at 300 ◦C and 500 ◦C. The dried powder is then crushed and made into very fine powder by using mortal pestle.

**Keywords:** Magnesium Oxide Nanoparticles, Green synthesis, Co-precipitation method, Tannic acid, UV–Visible and FTIR spectroscopy, X-ray diffraction, Antioxidant and Anti-inflammatory activity, Drug delivery, Biomedical and Pharmaceutical applications

## INTRODUTION

The properties of materials at the nanoscale are distinct from those of bulk materials. Nanoparticles, which range in size from 1 to 100 nanometers, have recently attracted a lot of attention due to their unique electrical, physical, magnetic, chemical, and optical features as compared to bulk materials[1]. The majority of researchers employ metal oxide nanoparticles because of their special qualities, which include stability, hydrophobicity, and photocatalysis. Coatings, catalysts, antibacterials, medical sciences, sensors, semiconductors, capacitors, and batteries are just a few of the various uses for them[2]. The special qualities of nanomaterials have piqued academics' interest over the past ten years in creating more straightforward and affordable methods for creating nanostructures with significant technological applications. Due to their potential application as operational components for nano electronics, optoelectronics, and sensing devices, metal oxide nanoparticles with large surface area and porosity have garnered a lot of interest for scientific research. Magnesium oxide is an inorganic element with incredible heat resistance, high chemical and alkali resistance, thermal stability, and high surface reactivity. The magnesium is 2$^{nd}$. Oxygen is a group element with atomic number 8 and a group element with atomic number 12. The chemical magnesium oxide has

melting and boiling temperatures of 2852ºC and 3600ºC, respectively[3]. Magnesium oxide is a white, hygroscopic solid mineral that is commonly referred to as periclase (from the Greek term periklao, peri "around," klao "to cut"). Its empirical formula is MgO, and its lattice is made up of $Mg^{2+}$ and $O_2^-$ ions joined by an ionic link. Usually, magnesium hydroxide ($Mg(OH)_2$) or magnesium carbonate ($MgCO_3$) are calcined to generate magnesium oxide. Thermal treatment, which is applied during the calcination process, has an impact on the final reactivity of the magnesium oxide as well as its surface area and pore size. Caustic calcined magnesium oxide is formed between 700 and 1000 degrees Celsius; lower chemical activity magnesium oxide is formed between 1000 and 1500 degrees Celsius; and reduced chemical activity refractory magnesium oxid, which is mainly used for electrical and refractory applications, is formed above 1500 degrees Celsius[4].

Magnesium oxide is used in many different industry sectors. Its refractory qualities make it an important component of building materials for fireproofing. Additionally, in industries like nuclear, chemical, and superalloy where corrosion is unacceptable. It is employed in medicine [5], where MgO is used as an antacid, a magnesium supplement, a temporary laxative, and to relieve sour stomach and heartburn. Additional uses include protective coatings, water treatment, insulators, fertilizers[6], and more. The usage of nanoscale fillers is currently popular.The creation of functional structures between 0.1 and 100 nm using a variety of physical or chemical techniques is generally referred to as nanotechnology[7]. This also holds true for magnesium oxide. Magnesium oxide at the nanoscale could be produced using the hydrothermal method or the sol-gel method. MgO has the potential to be used as a filler in electrical applications, such high-voltage insulation. Especially because of the high volume resistivity (1017 Wm) and large band gap (7.8 eV). Among commonly used nanoscale oxides, it has the highest volume resistivity value. It has also been utilized as a transition layer for the creation of many thin film materials due to its very large band gap, superior thermal stability, low dielectric constant, and refractive index[8]. MgO nanostructures were frequently produced by coprecipitation and thermal evaporation of various magnesium salts, or by dehydrating $Mg(OH)_2$.

While there are several metal oxides, including ZnO, CuO, MgO, TiO, CdO, etc., magnesium oxide has special qualities in comparison to other metal oxides, including optical, electrical, thermal, mechanical, and chemical properties (Diachenko *et al*., 2016). According to Abdoul *et al*. (2020) and Fatiqin *et al*. (2021), it has a high melting point, a wide energy bandgap, strong reactivity, and a low heat capacity, making it a very stable and safe material for usage in various industries[9].

These oxide materials can be made using a variety of synthesis methods, including solution combustion, coprecipitation, natural reducing and stabilizing agents, Sol-Gel, hydrothermal, Solvothermal, microwave assisted Sol-Gel, and green synthesis. One of the best methods for producing nanoparticles without agglomeration in the yield is co-precipitation with a natural reducing and stabilizing agent; size may be readily regulated. UV-visible spectroscopy and X-ray diffraction (XRD) are used to characterize the produced nanoparticles. FTIR spectroscopy and SEMEDX. This chapter discusses the interpretation or analysis of the data and confirms that the particles are made of MgO nanoparticles using a natural reducing and stabilizing agent technique and co-precipitation[4].

## REVIEW OF LITERATURE

1. Mirza and Makwana (2021) synthesized MgO nanoparticles using the co-precipitation method with magnesium nitrate and sodium hydroxide. Their study confirmed that the technique is simple, economical, and capable of producing highly pure, crystalline MgO NPs with uniform morphology, as validated by XRD and SEM analyses.

2. Rotti *et al*. (2023) adopted a green synthesis approach utilizing plant-based phytochemicals as natural reducing and stabilizing agents. The resulting MgO nanoparticles exhibited significant antibacterial activity, highlighting the role of bioactive components in enhancing biological performance. Their findings demonstrated the environmental and biomedical advantages of green synthesis.

3. Gatou *et al*. (2024) reviewed the biomedical potential of MgO nanoparticles, documenting their antimicrobial, antioxidant, anticancer, and anti-inflammatory properties. The authors further emphasized their suitability for drug delivery, tissue engineering, and diagnostic applications due to high biocompatibility and low toxicity.

4. Hijau *et al*. (2023) synthesized MgO nanoparticles using banana peel extract, demonstrating the effectiveness of fruit-peel phytochemicals in nanoparticle formation. Characterization confirmed good crystallinity, nanoscale dimensions, and the involvement of phenolic compounds. The study highlighted agricultural waste as a sustainable resource for green synthesis.

5. A 2023 study on green-synthesized MgO nanoparticles and MgO-based nanocomposites reported strong antimicrobial, antibiofilm, antifungal, and photocatalytic activities. Their multifunctional properties suggested applications in pharmaceutical formulations, environmental purification, and wastewater treatment.

**Aim:** Green Synthesis and Characterization of Magnesium Oxide (MgO) Nanoparticles as Biological Agent.

**Objective:**

1. To create magnesium oxide (MgO) nanoparticles using natural reducing and stabilizing agents (such as tannic acid) in an environmentally safe and green manner.
2. To use physicochemical methods as FTIR, XRD, SEM, UV-visible spectroscopy, and particle size analysis to analyze the produced MgO nanoparticles.
3. To examine the produced MgO nanoparticles' functional groups, surface morphology, particle size, crystalline nature, and purity.
4. To determine MgO nanoparticles' antioxidant capacity utilizing common in vitro tests as DPPH, ABTS, and FRAP.
5. To investigate how green synthesis (biomolecules) affects MgO nanoparticle biological activity.
6. To evaluate whether green-synthesised magnesium oxide nanoparticles are suitable as biological agents for use in pharmaceutical and biomedical applications.

## EXPERIMENTAL WORK

**Co-precipitation method[10]:** The block diagram of the entire process is displayed below, along with the chemical reaction that took place during the synthesis-

$$Mg(NO_3)_2 .6H_2O + 2\ NaOH \rightarrow Mg(OH)_2 + 2NaNO_3$$

$\Delta$

$$Mg(OH)_2 \rightarrow 2\ MgO + H_2O$$

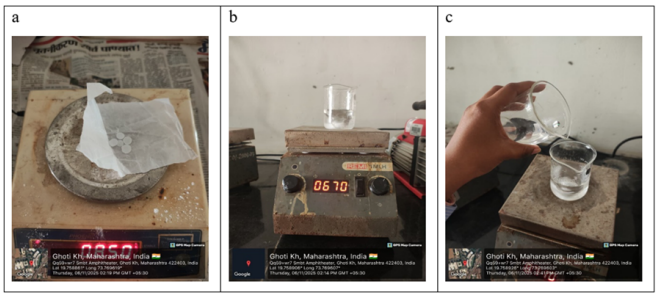


**Fig. 1: (a) Weighing, (b) Stirring, (c) Dropping, (d) White Precipitate, (e) Filtering, (f) drying, (g) Annealed at 300˚C, (h) Annealed at 500˚C, (i) MgO Nanoparticle are all shown in Experimental image of the synthesis process**

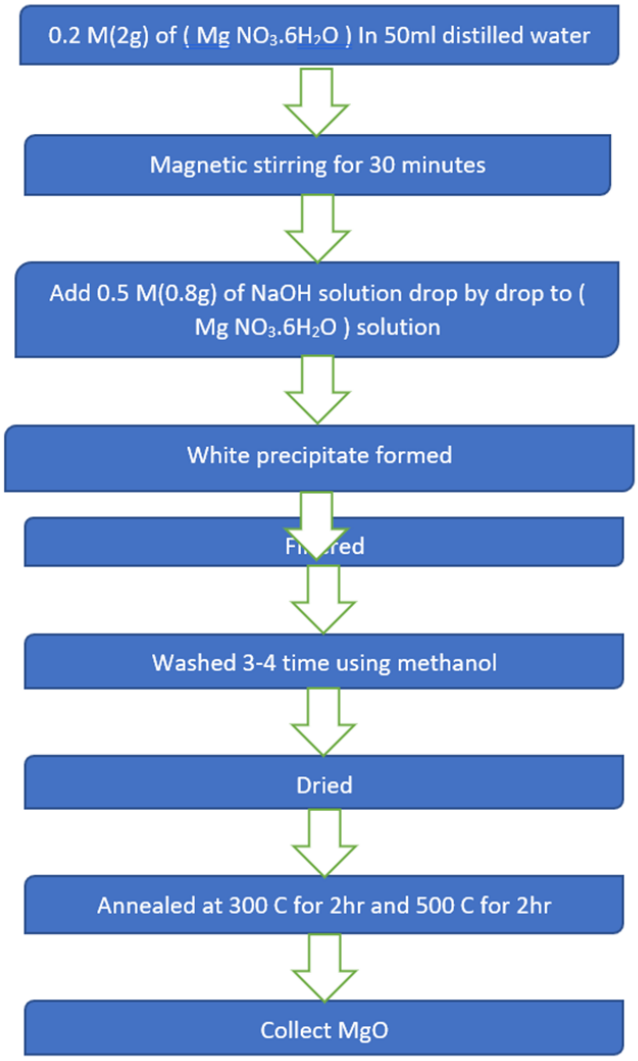


**Fig. 2: MgO nanoparticle synthesis by Co-precipitation method[10]**

There are several steps involved in the manufacture of MgO nanoparticles, including mixing, stirring, filtering, drying, and grinding. First, two grams of magnesium $NO_3.6H_2O$ were added to a beaker to create a 0.2 M solution in distilled water. Next, 100 milliliters of distilled water were used to dissolve 0.8 grams (0.5 M) of NaOH. A magnetic stirrer was used to continuously mix the magnesium nitrate solution for 30 minutes. Then, while continuously stirring the produced magnesium nitrate (Mg $NO_3.6H_2O$) solution, 0.5 M NaOH solution was added drop by drop using a dropper. A milky white precipitate of magnesium hydroxide formed in the beaker after 30 minutes. The pH paper indicated that the solution's pH was 12.5. After filtering the precipitate through filter paper and washing it with methanol three or four times to get rid of ionic contaminants, the white powder sample was dried in the air and then annealed for two hours at 300 and 500 degrees Celsius. A deadly pestle is then used to break the dried powder into an extremely fine powder[10].

**Objective:**

1. To develop a simple, low-cost and reproducible green method for MgO nanoparticle synthesis.
2. To avoid hazardous organic solvents and use only environmentally safe reagents.
3. To optimize reaction parameters (temperature, pH, concentration, stirring time) for maximum nanoparticle yield.
4. To obtain MgO nanoparticles of controlled size and morphology by adjusting calcination temperature.
5. To convert $Mg(OH)_2$ precipitate into MgO nanoparticles through calcination and confirm purity.
6. To identify functional groups involved in synthesis using FTIR spectra.
7. To measure particle size distribution using UV Spectroscopy
8. To evaluate potential biological properties, especially antioxidant activity, using standard methods (DPPH, Ferric reducing assay, $H_2O_2$ scavenging, etc.).
9. To compare the antioxidant potential of MgO nanoparticles with standard (ascorbic acid).
10. To study the stability of MgO nanoparticles under different storage conditions.
11. To analyze the color change, formation, and stability as visual confirmation of nanoparticle synthesis.
12. To demonstrate that the co-precipitation method can be completed at room temperature with simple lab equipment.
13. To minimize chemical waste and promote a green synthesis approach for student-level research.
14. To compare green co-precipitation with chemical co-precipitation in terms of safety and environmental impact.

**Application:**

1. Nanoparticle Synthesis
2. Pharmaceutical Formulation
3. Magnetic Nanoparticles for Drug Targeting
4. Catalysts Preparation
5. Water Treatment and Purification
6. Biosensing and Diagnostic Devices
7. Ceramic and Composite Material Preparation
8. Food and Cosmetic Industries
9. Tannic Acid as a Natural Reducing and Stabilizing Agent:

Magnesium oxide nanoparticles were synthesized using a tannic-acid. Firstly, 1 g of tannic acid was dissolved in 50 mL of distilled water to obtain a homogeneous phenolic solution. In a separate beaker, 2 g of magnesium nitrate hexahydrate ($Mg(NO_3)_2 \cdot 6H_2O$) was dissolved in 50 mL of distilled water. The two solutions were mixed and stirred continuously for 2–3 hours to promote chelation between $Mg^{2+}$ ions and tannic acid, leading to the formation of a stable tannic acid–magnesium complex[11].

The reaction mixture was then heated on a water bath to evaporate the solvent until a solid Mg–tannic acid precursor was obtained. This precursor was carefully collected, dried, and subsequently calcined in a muffle furnace at 400–500 °C for 2 hours. During calcination, the organic matrix decomposed, leaving behind crystalline magnesium oxide nanoparticles. The final product appeared as a fine white powder, confirming successful formation of MgO nanoparticles[11].

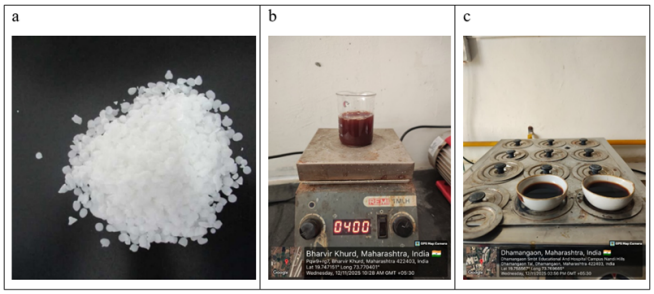


**Fig. 3: (a) Weighing, (b) Stirring, (c) Evaporation, (d) Dried, (e) Calcination, (f) MgO Nanoparticle are all shown in Experimental image of the synthesis process**

**Objective:**

1. To synthesize Magnesium Oxide nanoparticles using tannic acid as a natural, eco-friendly reducing and stabilizing agent.
2. To avoid toxic chemicals and promote a green, sustainable nanoparticle preparation method.
3. To utilize the antioxidant and polyphenolic nature of tannic acid to control nanoparticle size and stability.
4. To prepare $Mg(OH)_2$ precipitate through co-precipitation and convert it into MgO nanoparticles

by calcination.

5. To optimize reaction conditions (pH, concentration, temperature, stirring time) for efficient nanoparticle formation.
6. To characterize the synthesized MgO nanoparticles using UV–Vis, FTIR, or simple available techniques.
7. To evaluate the antioxidant activity of tannic-acid-mediated MgO nanoparticles using DPPH, , $H_2O_2$ scavenging, etc.
8. To compare the antioxidant activity of MgO nanoparticles with standard antioxidants (ascorbic acid).
9. To study the role of tannic acid in capping and stabilizing the nanoparticles.
10. To demonstrate an inexpensive, student-friendly synthesis method suitable for B. Pharmacy laboratories.

**Application:**

1. Natural stabilizing and lowering agent for green synthesis
2. Antioxidant agent
3. Antibacterial and antiviral properties
4. Anti-inflammatory agent
5. Astringent properties
6. Used in pharmaceutical formulations
7. Stabilizer in polymer and biomaterial preparation
8. Water purification
9. Food industry applications
10. Used in nanoparticle-based drug delivery

## EXPERIMENTAL RESULTS

### Physical and Visual Outcomes of Co-precipitation Synthesis

i. Formation of Precipitate: A milky white precipitate of magnesium hydroxide ($Mg(OH)_2$) appeared instantly when 0.5 M NaOH was dropwise added to the 0.2 M magnesium nitrate solution. The pH of the solution rose to 12.5, indicating that all of the $Mg^{2+}$ ions had precipitated.

ii. Filtration and Washing: Filtration was used for obtaining the white precipitate. Ionic contaminants were eliminated by washing with methanol three to four times, producing a clearer, brighter white solid.

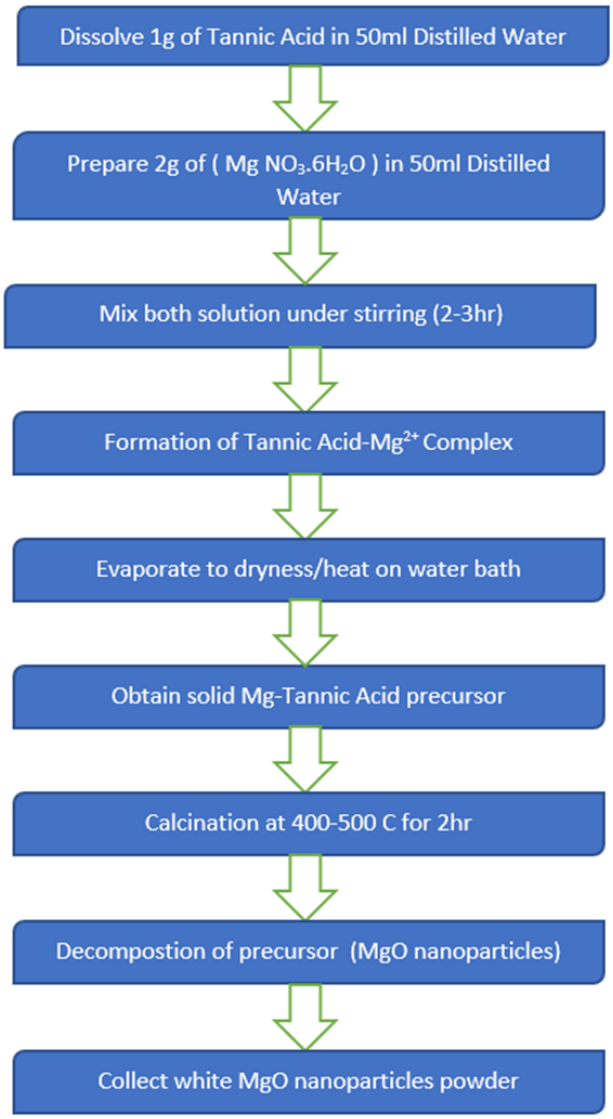


**Fig. 4: MgO nanoparticle synthesis by Co-precipitation method**

iii. Drying and Annealing: The precipitate turned into a dry solid mass that was easily ground into powder after air drying. Annealing at: 300 °C → Light white powder, 500 °C → finer, more brilliant white MgO powder.

iv. Physical Appearance of Final Product: Colour- White; Texture: Fine, smooth powder; Flow property: Non-sticky, non-hygroscopic; Grinding: Easy to powder using mortar & pestle.

### Observations From Tannic Acid–Assisted Green Synthesis

i. Solution Behaviour

- Dissolution of tannic acid in water produced a clear to light-brown phenolic solution.
- Addition of $Mg(NO_3)_2 \cdot 6H_2O$ solution led to:
  - Gradual complex formation between $Mg^{2+}$ and tannic acid
  - Slight change in colour due to chelation.
  - Formation of a stable Mg–tannic acid matrix after 2–3 hours of stirring.

ii. Heating and Precursor Formation

- Slow water-bath heating yielded:
  - Thickening of the solution
  - Gradual evaporation
  - Formation of a solid Mg–tannic acid precursor.

iii. Drying and Calcination

- The precursor became dry and brittle after water evaporation.
- Calcination at 400–500 °C for 2 hours caused:
  - Complete decomposition of tannic acid
  - Release of gases (observed as slight smoke)
  - Formation of a pure white MgO nanopowder

iv. Physical Characteristics

- Colour: Bright white
- Nature: Fine powder, similar to pharmaceutical-grade light magnesia
- Consistency: More uniform and smoother than chemically synthesized MgO
- Yield: Good (no numerical value provided in document)

v. Final Experimental Outcome

- Both methods resulted in successful preparation of high-quality white magnesium oxide nanoparticles.
- The green synthesis using tannic acid produced more uniform and smoother powder, indicating better stabilization during formation.
- Co-precipitation required pH adjustment and washing, while tannic acid method required evaporation and calcination.

## Characterization

Characterization of nanoparticles is an important step for determining their physicochemical properties such as structure, crystallinity, purity, functional groups, and optical behavior. Various analytical techniques are commonly employed to confirm the successful synthesis of magnesium oxide (MgO) nanoparticles and to evaluate their structural and chemical properties.

The crystalline structure and phase purity of nanoparticles are frequently ascertained by X-ray diffraction (XRD) investigation. The diffraction pattern helps in identifying the crystal planes and confirms the formation of crystalline MgO nanoparticles.

Fourier Transform Infrared Spectroscopy (FTIR) is used to identify the functional groups and biomolecules present on the surface of nanoparticles. FTIR analysis also helps in understanding the role of phytochemicals involved in the reduction and stabilization of MgO nanoparticles during green synthesis[12].

UV–Visible spectroscopy is an important analytical technique used to study the optical properties of nanoparticles. The absorption spectrum obtained from UV analysis confirms the formation of MgO nanoparticles and provides information regarding their optical behavior and nanoscale characteristics[13].

These characterization techniques collectively help in confirming the successful synthesis and stability of MgO nanoparticles for further biological and pharmaceutical applications.

- **Assessing the effectiveness of Antioxidant:**

**Assessing the effectiveness of Antioxidant by the Hydrogen Peroxide ($H_2O_2$):** As a standard, 100, 200, and 300 µg/ml solutions of magnesium oxide (MgO) nanoparticles and ascorbic acid were prepared using distilled water. A 40 mM hydrogen peroxide ($H_2O_2$) solution was prepared in phosphate buffer (pH 7.4). To the aforementioned solutions, 1 ml of $H_2O_2$ solution was added to 3 ml of each sample solution. After mixing, the reaction mixture was allowed to stand for 10 minutes at room temperature (≈25–26°C).

Using phosphate buffer as a blank, the absorbance was measured at 230 nm using a UV–Visible spectrophotometer. As a negative control, a mixture of 3 ml phosphate buffer and 1 ml $H_2O_2$ solution without sample or standard was used. The mean value was calculated after performing the experiment in triplicate.

The following formula was used to determine the percentage scavenging of the samples and the standard:

$$\% \text{ Scavenging} = [A_{control} - A_{sample}/A_{control} \times 100]$$

where

$A_{sample}$ = absorbance of the test sample

$A_{control}$ = absorbance of the control

**Antioxidant Activity by the Scavenging Method:** Distilled water was used to create solutions containing 100, 200, and 300 µg/ml of magnesium oxide (MgO) nanoparticles and the

standard (ascorbic acid). Phosphate buffer (pH 7.4) was used to create a 40 mM hydrogen peroxide ($H2O_2$) solution.

To each sample solution (3 ml), 1 ml of $H_2O_2$ solution was added. The reaction mixtures were mixed thoroughly and incubated at room temperature for 10 minutes. The absorbance of the resulting solution was measured at 230 nm using a UV–Visible spectrophotometer, with phosphate buffer used as a blank.

A control was prepared by mixing 3 ml of phosphate buffer with 1 ml of $H_2O_2$ solution without the addition of sample or standard. All experiments were performed in triplicate, and the mean values were calculated[14].

The following formula was used to determine the % scavenging activity of both the MgO nanoparticles and the standard (ascorbic acid):

$$\% \text{ Scavenging} = [A_{control} - A_{sample}/A_{control} \times 100]$$

where

$A_{sample}$ = absorbance of the test sample

$A_{control}$ = absorbance of the control

- **Anti-inflammatory activity:**

**Sample Preparation:** Distilled water was used to create solutions containing 100, 200, and 300 µg/ml of magnesium oxide (MgO) nanoparticles and standard (ascorbic acid or diclofenac sodium). Two milliliters of phosphate-buffered saline (PBS, pH 6.4) and 0.3 milliliters of egg albumin were added to each of the produced solutions.

After 20 minutes of incubation at 37°C, the reaction mixtures were heated for six minutes at 70°C. A UV-visible spectrophotometer was used to measure the absorbance at 660 nm after it had cooled to room temperature, using DMSO as a blank.

A control was prepared by replacing the sample with distilled water while maintaining all other components under identical conditions. All experiments were carried out in triplicate, and the mean values were recorded[14, 15].

The percentage inhibition of protein denaturation was calculated using the following equation:

$$\% \text{ Inhibition} = 100[V_{sample}/V_{control} - 1]$$

Where,

$V_{sample}$ = absorbance of the test sample and

$V_{contro\ l}$= absorbance of the control.

**Standard Sample Preparation:** Using distilled water, diclofenac sodium solutions were made at 100, 200, and 300 µg/ml. 0.3 ml of egg albumin and 2.9 ml of phosphate-buffered saline (PBS, pH 6.4) were added to each of the produced solutions.

The reaction mixtures were incubated at 37°C for 20 minutes, followed by heating at 70°C for 6 minutes. After cooling to room temperature, the absorbance was measured at 660 nm using a UV–Visible spectrophotometer, with DMSO used as a blank.

A control was prepared by replacing diclofenac sodium with distilled water while keeping all other components constant. All experiments were performed in triplicate, and the mean values were calculated.

The percentage inhibition of protein denaturation was determined using the following equation:

$$\% \text{ Inhibition} = 100[V_{sample}/V_{control} - 1]$$

Where,

$V_{sample}$ = absorbance of the test sample and

$V_{control}$ = absorbance of the control.

## FUTURE WORK

1. Targeted and controlled drug delivery systems.
2. Antioxidant formulations for oxidative stress-related disorders.
3. Anti-inflammatory therapeutic applications.
4. Antibacterial and antifungal pharmaceutical products.
5. Anticancer drug development and cancer therapy.
6. Wound healing materials and antimicrobial dressings.
7. Biomedical coatings for implants and medical devices.
8. Tissue engineering and bone regeneration applications.
9. Nano-carriers for sustained and controlled drug release.
10. Biosensors and diagnostic devices development.

## RESULT AND DISCUSSION

- **Characterization:**

### i. UV- Visible Absorption Sperctroscopy

The most popular analytical method for describing the electronic structure of the optical band gap of the nanomaterial is UV-Vis absorption spectroscopy. To characterize the size of nanoparticles in the range of 5–100 nm, the UV-Vis spectra of metal nanoparticles were recorded in the wavelength range of 200 nm to 800 nm. In the absorption spectra of MgO nanoparticles, distilled water was employed as a solvent. The charge transfer transition between energy levels causes a prominent band in the UV portion of the spectrum Fig. 5. First, as the wavelength increases close to the band edge (250 nm), the absorbance rapidly drops, indicating the size of MgO nanoparticles[16].

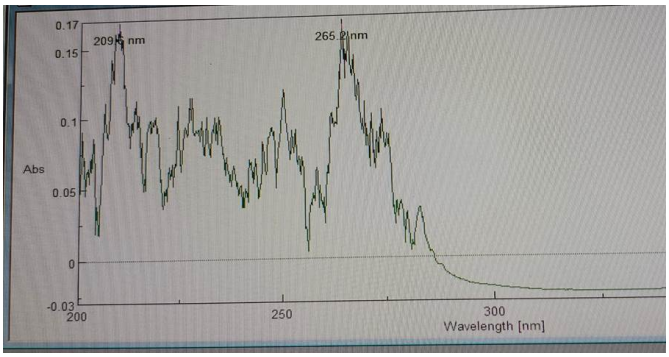


**Fig. 5**

The creation of nanosized MgO particles was confirmed by the UV-Vis spectrum, which showed a strong peak at 265.2 nm. The band gap energy of the synthesized MgO Nps was determined from the UV Vis spectrum using the formula Eg = h², where Eg, h, and Ψ stand for optical band gap energy, plank's constant, and frequency, respectively. The calculated band gap energy of MgO Nps was found to be 4.2 eV, in contrast to bulk MgO, which had a band gap energy of 7.8 eV. While the bulk materials include 6-coordinated surface anions, the presence of 4-coordinated surface anions at the margins of the MgO Nps is unquestionably responsible for the decreased band gap energy. This finding is consistent with the earlier research conducted by Berger *et al.*[17]. At addition to bulk materials, the band gap energy will also be influenced by MgO with varying particle sizes at the nanoscale. According to Moon and his colleagues, MgO Nps with an average size of 20 nm have a band gap of 5.6 eV. It is evident that the band gap energy increases as the particle size decreases because the band gap of our MgO Nps (average particle size ~28 nm, which will be covered in the XRD section) is 4.2 eV. Because the energy levels in bulk materials are tightly separated, they create quasi-continuous bands. Discrete energy levels are seen as the energy level separation rises in the nano-regime[18].

### ii. Fourier Transform Infrared (FTIR) Spectroscopy:

FTIR spectroscopy was used to determine the vibrational frequency of the molecules' stretching and bending modes as well as possible biomolecules responsible for the reduction and capping of MgO NPs. The MgO nanoparticle spectra and the analysis carried out in the 400-4000 $cm^{-1}$ band are shown in the Fig. 6. The stretching vibration of the O-H group is shown by the peaks at 3415 cm-1 and 2925 $cm^{-1}$. The stretching vibration of an aromatic C=C bond is shown by the peak at 1633 cm-1. MgO-NP formation is shown by the peak seen at 434 $cm^{-1}$[16].

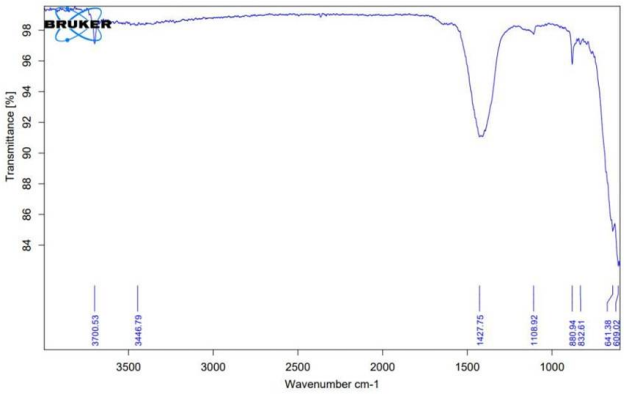


**Fig. 6**

### iii. X-ray diffraction

The X-ray diffraction (XRD) analysis of magnesium oxide (MgO) nanoparticles was carried out to determine their crystalline structure and phase purity. The diffractogram shows several sharp and intense peaks, indicating the highly crystalline nature of the synthesized MgO nanoparticles[14, 19].

The prominent diffraction peaks were observed in the mid-to-high 2θ range, particularly around 30°, 43°, and 62°, which are characteristic of MgO. These peaks correspond well with the standard diffraction pattern of cubic MgO, confirming successful formation of magnesium oxide nanoparticles[14].

Among these, the most intense peak at approximately 43° corresponds to the (200) plane, while the peak near 62° is attributed to the (220) plane of MgO. The presence of these well-defined peaks confirms the crystalline cubic phase of MgO nanoparticles.

The absence of additional impurity peaks in the diffractogram suggests that the synthesized nanoparticles are pure and free from secondary phases[19]. The sharpness and intensity of the peaks further indicate good crystallinity and nanoscale particle formation.

Overall, the XRD examination verifies that the produced MgO nanoparticles have a highly pure crystalline cubic structure, which qualifies them for additional biological and medicinal uses[14].

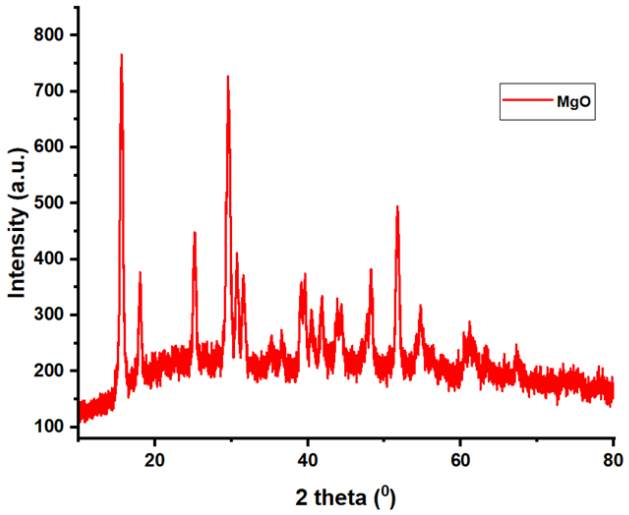


**Fig. 7**

### iv. Antioxidant activity

The antioxidant potential of magnesium oxide (MgO) nanoparticles was evaluated using the hydrogen peroxide scavenging assay and compared with the standard ascorbic acid. The results revealed that MgO nanoparticles exhibited a concentration-dependent increase in free radical scavenging activity.

**Ascorbic acid**

| Concentration ug/ml | Absorbance of Control | Absorbance of standard | % Scavenging |
|---|---|---|---|
| 100 ug/ml | 0.5963 | 0.238 | 60.08 |
| 200 ug/ml | 0.5963 | 0.149 | 75.01 |
| 300 ug/ml | 0.5963 | 0.089 | 85.07 |

**MgO Nanoparticles**

| Concentration ug/ml | Absorbance of Control | Absorbance of standard | % Scavenging |
|---|---|---|---|
| 100 ug/ml | 0.5963 | 0.3663 | 38.57 |
| 200 ug/ml | 0.5963 | 0.3226 | 45.90 |
| 300 ug/ml | 0.5963 | 0.2825 | 52.62 |

The percentage scavenging activity of MgO nanoparticles gradually increased at concentrations of 100, 200, and 300 µg/ml. This suggests that the nanoparticles' ability to neutralize hydrogen peroxide improves with concentration.

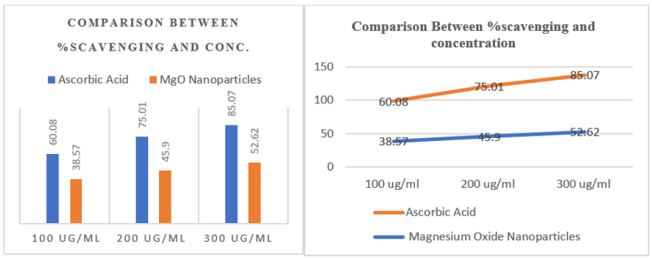


**Fig. 8**

The observed antioxidant activity of MgO nanoparticles can be attributed to their high surface area and active surface sites, which facilitate interaction with reactive oxygen species (ROS). These nanoparticles donate electrons to hydrogen peroxide, thereby converting it into harmless products such as water and oxygen.

Although the scavenging activity of MgO nanoparticles is lower than that of ascorbic acid, they still demonstrate moderate antioxidant potential, suggesting their possible application in reducing oxidative stress-related damage.

### v. Anti-inflammatory activity:

The anti-inflammatory activity of MgO nanoparticles was assessed using the protein denaturation method and compared with the standard drug diclofenac sodium. The anti-inflammatory activity of MgO nanoparticles was assessed using the protein denaturation method and compared with the standard drug diclofenac sodium.

**Diclofenac Sodium**

| Concentration ug/ml | Absorbance of Control | Absorbance of standard | % Inhibition |
|---|---|---|---|
| 100 ug/ml | 0.415 | 0.460 | 10.84 |
| 200 ug/ml | 0.415 | 0.486 | 17.11 |
| 300 ug/ml | 0.415 | 0.511 | 23.13 |

**MgO Nanoparticles**

| Concentration ug/ml | Absorbance of Control | Absorbance of standard | % Inhibition |
|---|---|---|---|
| 100 ug/ml | 0.415 | 0.537 | 29.40 |
| 200 ug/ml | 0.415 | 0.586 | 41.20 |
| 300 ug/ml | 0.415 | 0.636 | 53.25 |

The results demonstrated that MgO nanoparticles showed a significant inhibition of protein denaturation, which increased with concentration (100, 200, and 300 µg/ml). This confirms that MgO nanoparticles possess the ability to stabilize protein structures under stress conditions.

Protein denaturation is a well-known cause of inflammation, and substances that prevent denaturation can act as anti-inflammatory agents. MgO nanoparticles likely interact with protein molecules through surface binding and stabilization mechanisms, thereby preventing structural alteration.

When compared with diclofenac sodium, MgO nanoparticles exhibited comparable inhibitory activity, indicating their potential as an alternative or supportive anti-inflammatory agent.

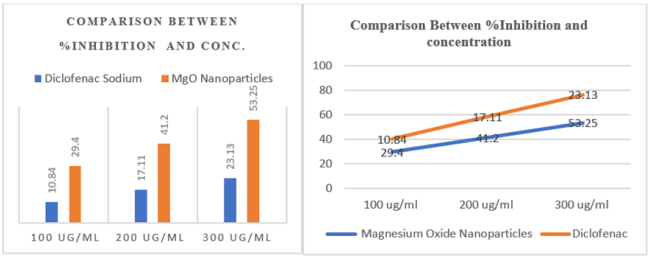


**Fig. 9**

## SUMMARY

Tannic acid was used as a natural reducing and stabilizing agent in the effective production of magnesium oxide (MgO) nanoparticles using both chemical co-precipitation and green synthesis techniques. The synthesized nanoparticles were characterized using UV–Visible spectroscopy, FTIR, and XRD analysis, which confirmed the successful formation, crystalline nature, purity, and nanoscale properties of MgO nanoparticles. UV analysis showed a characteristic absorption peak around 265.2 nm, while FTIR confirmed the presence of Mg–O functional groups and biomolecules involved in stabilization. The nanoparticles exhibited significant antioxidant and anti-inflammatory activities in a concentration-dependent manner when compared with standard drugs such as ascorbic acid and diclofenac sodium. The green synthesis method produced smoother, more uniform, and environmentally friendly nanoparticles with better stability. The study demonstrates that MgO nanoparticles possess promising biological and pharmaceutical potential for future biomedical, antioxidant, antimicrobial, and drug delivery applications.

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