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Article

Influence of Aging Hours on the Size, Dispersity and Stability of Phytosynthesized Magnesium Oxide Nanoparticles

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ABSTRACT

As a reliable, sustainable, and environmentally friendly method for producing a variety of nanomaterials, green synthesis has attracted a lot of interest. Magnesium oxide nanoparticles (MgO-NPs), which have a distinctive biocompatible nature, have been exploited as a superior nanocarrier. Due to their high degree of ionization, photocatalytic properties, and effective resistance to high temperatures, they have many advantages. They have recently been used as a substrate in the biomedical industry and as a novel utilization in refractory materials. The present study involved the 1st-time biogenic synthesis of MgO-NPs successfully by using the leaf extract of *Tinospora cordifolia*. *T. cordifolia* is commonly known as "Guduchi", which was used not only as a reducing agent but also as a capping agent too, resulting in increased stability. The effect of aging was observed for the 1st time on the properties (i.e., stability, dispersity, and size of MgO-NPs) of biogenic synthesized MgONPs. Here, the aging factor was studied for different hours (named as samples 1 and 2) along with the constant temperature during the green production of MgO-NPs. Sample 1 was stirred for 12 h, while correlated sample 2 was stirred for 24 h in the continuation form at constant temperature (i.e., 40°C). It was observed that as the time duration of stirring with temperature increased, the stability, homogeneity, and decrease in size of the nanoparticles increased.

Key words: Aging effect, Green method, Magnesium oxide nanoparticles, *Tinospora cordifolia.*

1. INTRODUCTION

Nanomaterials with diameters of <100 nm are being used in a number of applications across multiple domains, such as biology, physics, chemistry, cosmetics, optical components, polymer science, pharmaceutical drug manufacture, toxicology, and mechanical engineering. Magnesium oxide nanoparticles (MgO-NPs) are not toxic by nature. They are odorless. They also show high purity, a high melting point, and having high hardness. Application of MgO nanoparticles can be used in catalysis, ceramics, electronics, petrochemical products, etc. These are also used in wooden chips, and they are also applied in shavings for making sound-proof materials, heat insulation, light weight, and also in metallic ceramics [1].

Some methods, like the top-down method and the bottom-up method, are used for preparing the nanoparticles [Figure 1]. In recent years, researchers have moved toward biological systems for the synthesis of nanomaterials because these biological systems are eco-friendly in nature, reliable, non-toxic, and simple. According to Panchgavya, it is recommended that nanoparticles should be synthesized through biological methods by using extracts of plants, enzymes and microorganisms, and this is a good choice over physical and chemical methods [2]. In general, plant extracts act as capping and reducing agents for the synthesis of nanoparticles. Moreover, these show more value than other biological processes. The reason behind is like that they remove the inaccurate processes of maintaining and culturing the cells and can also be used for the synthesis of nanoparticles at large scales. Plant parts like bark, peels of fruits, callus, roots, stems, leaves, and extracts of these parts have been used for the synthesis of platinum, gold, titanium, magnesium oxide, and silver nanoparticles with different shapes and sizes [3]. Here, the process of biogenic synthesis of MgO-NPs was done by using *Tinospora cordifolia* leaf extract for the 1st time. The common name of *T. cordifolia* is "Guduchi" in Sanskrit. And it belongs to the family *Menispermaceae*. It possesses diterpenoid lactones, glycosides, alkaloids, sesquiterpenoids, steroids, phenolics, polysaccharides, and aliphatic compounds. It behaves as an appropriate fuel for the synthesis of nanoparticles. So, it can be said that *T. cordifolia* extract can be used as fuel in the preparation of MgO-NPs. It involves a reaction in a consistent solution of $MgCl_2$ salt and *T. cordifolia* leaf extract [4].

The present proposal involves the biogenic synthesis of MgO-NPs by the use of *T. cordifolia* leaf extract; for this, the precursor salt $MgCl_2.6H_2O$ was used. The phase change of MgO nanoparticles is some time in the synthesis of nanoparticles; aging is a kind of operation that is usually used. Generally, aging causes microscopic and macroscopic changes. The aging effect during synthesis processes caused changes in nanoparticles' dispersity, size, and stability. Usually, it is a slow process.

2. MATERIALS AND METHODS

2.1. Materials

Magnesium chloride hexahydrate ($MgCl_2.6H_2O$) purchased from Sigma Aldrich, sodium hydroxide (NaOH), ethanol (99%), whatman

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filter paper, *T. cordifolia* leaves collected from nearby places, and double distilled water (DDW) were used throughout the experimental work.

2.2. Equipment

Fourier transformed infrared (FTIR) spectroscopy, Model RZX (Perkin Elmer), and ultraviolet-visible (UV-Vis) spectroscopy (Make and Model: SHIMADZU UV 3600 Plus) Zeta potentiometer/sizer (Malvern, Nano-ZS), transmission electron microscope (TEM, Talos S), lyophilizer (Lyophilizer-FD-3, Allied Frost), laboratory hot-plate stirrer, and muffle furnaces (Microprocessor Programmable Furnace, Metrex, MT-14P).

2.3. T. cordifolia Leaves Collection and Extract Preparation

T. cordifolia leaves were collected from the MDU campus in Rohtak, Haryana (India). The collected leaves were properly washed and cleaned. Then it is dried at room temperature so that we can store the extract for long lasting. After 4–5 days of dryness, weigh 14 g of dried leaves in 120 mL of DDW. Boiled the leaves at 60–70°C for 60 min, and then removed the extract from the hot plate and placed it for cooling. Then the extract was filtered through the Whatman filter paper to get a clear solution of the extract. The filtered extract was stored at 4–8°C for further use [Figure 2].

2.4. Phytochemical Analysis of T. cordifolia Leaves Extract

Leaf extracts from *T. cordifolia* were subjected to phytochemical screening using the conventional approach. A preliminary phytochemical screening of a number of secondary metabolites in *T. cordifolia* leaf extract was carried out using the methods listed below. In the small amount of extracted material, alkaloids, phenols, steroids, flavonoids, saponins, tannins, anthraquinones, and cardiac glycosides were qualitatively screened for [5].

2.4.1. Test for determining phenols

2.4.1.1. Ferric chloride test

One milliliter of each solvent extract was treated with two drops of the $FeCl_3$ solution. The occurrence of phenols is indicated by the formation of a bluish-black color.

2.4.1.2. Tannins analysis

With 0.5 mL of each solvent extract, two drops of 1% lead acetate were added. With 0.5 mL of each solvent extract, two drops of 1% lead acetate were added. A yellow precipitate revealed the tannins' existence.

2.4.2. Assessment of flavonoids (NaOH)

2.4.2.1. Alkaline test

Two drops of the NaOH solution were added, one by one, to each solvent extract (0.5 mL). The intense yellow color that was produced became colorless when diluted HCl was introduced, showing the presence of flavonoids.

2.4.2.2. Steroids test

Separately dissolving 0.5 mL of each solvent extract in 6 mL of chloroform, an equal volume of concentrated sulfuric acid was then added by the test tube's sides. The sulfuric acid layer changed color from red to yellow with green fluorescence as the top layer got red. This implies the presence of steroids.

2.4.3. Glycosides analysis

2.4.3.1. Salkowski's test

0.5 mL of chloroform was added to 1 mL of each solvent extract. Each was then gently shaken after being carefully filled with 0.5 mL of concentrated H_2SO_4. The color was reddish-brown, indicating the presence of glycosides.

2.4.4. Test for saponins

0.5 mL of the extract was separately combined with 10 mL of distilled water and then vigorously shaken (agitated) in the graduated cylinder for 15 min. The formation of foam reveals the presence of saponins.

2.5. Synthesis of MgO-NPs by Using T. cordifolia Leaves Extract with Different Aging Hours

Two beakers (labeled as samples 1 and 2) of 200 mL were taken, each containing 60 mL of water. 1 mM $MgCl_2$ was added to these samples. Then, 60 mL of filtered *T. cordifolia* leaf extract was added to both beakers. Stirred the solutions at 600 rpm until they reached 90°C temperature. After reaching the temperature of 90°C, 2M NaOH solution was added in a dropwise manner to both mixtures, and kept the sample 1 for 12 h of aging and sample 2 for 24 h aging at 40°C. After

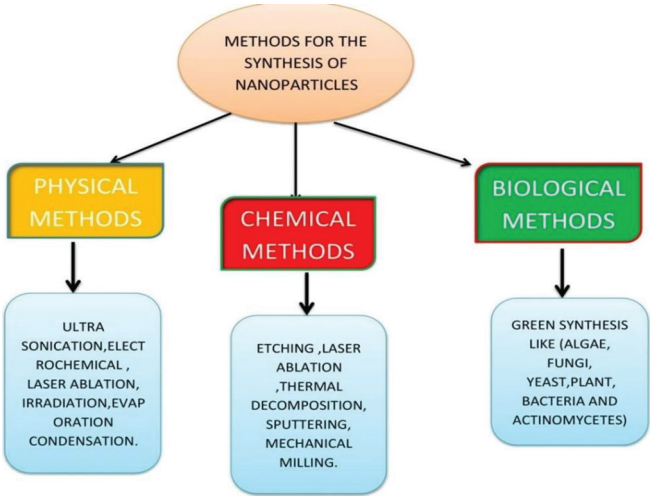

Figure 1: Synthesizing methods for preparation of nanoparticles.

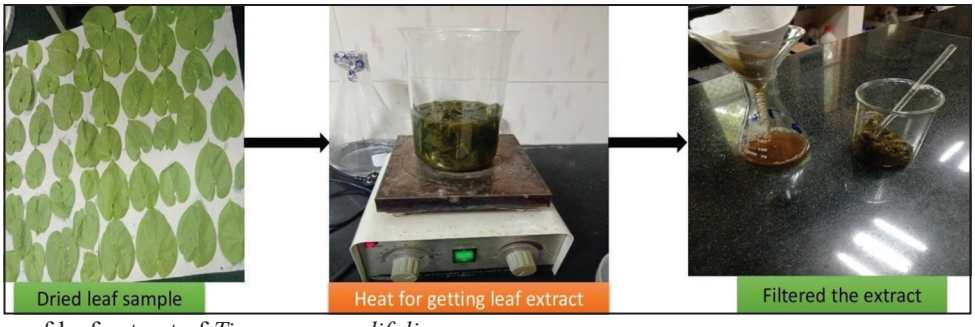

Figure 2: Preparation of leaf extract of *Tinospora cordifolia.*

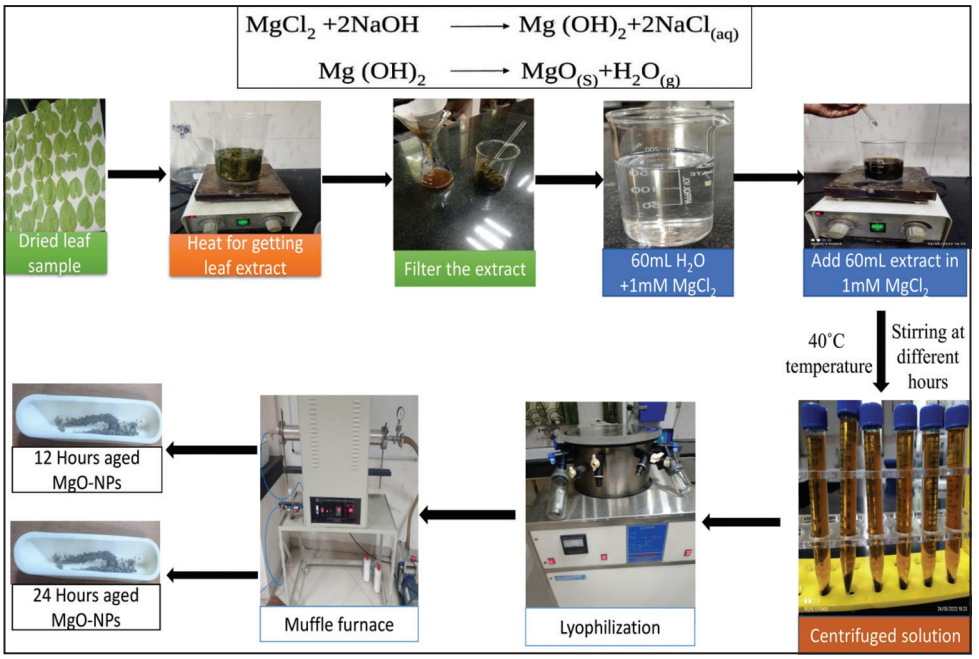

Figure 3: Schematic representation of green synthesis of magnesium oxide nanoparticles by using *Tinospora cordifolia* at different aging hours (12 h and 24 h) at constant temperature (40°C).

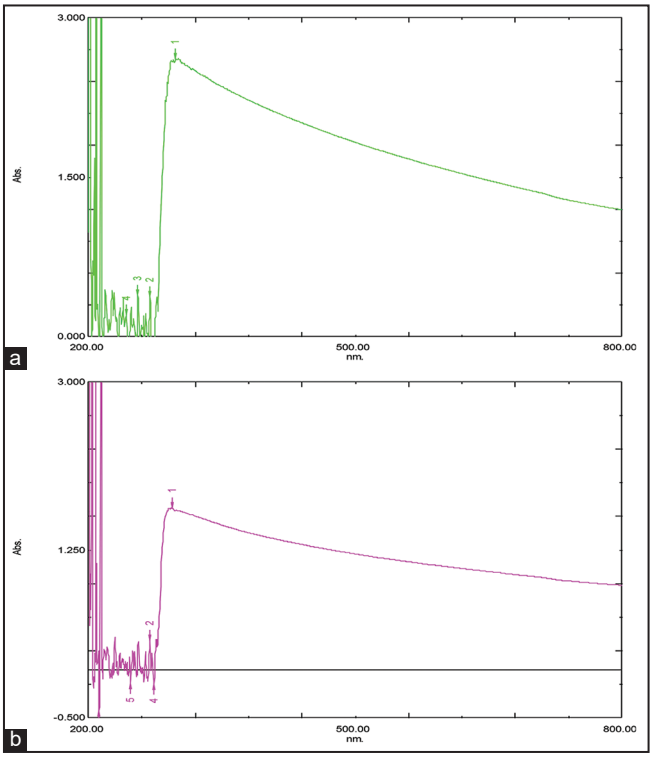

Figure 4: Ultraviolet-visible spectra of (a) Sample 1 (12 h aged Magnesium oxide nanoparticles [MgO-NPs]) showing absorption peak between 280 and 290 nm and (b) Sample 2 (24 h aged MgO-NPs) showing absorption peak between 260 and 280 nm.

completion of 12 h of aging of sample 1, the precipitate was separated from the mixture through the process of centrifugation with a rotation of 5200 rpm (round per minute) for 20–25 min, and removed the supernatant without disturbing the pallet portion. Separated precipitates were washed with a mixture of water and 99% ethanol. The sample was dried using a lyophilizer, as it helped in the removal of extra or unwanted water and ethanol. At last, the precipitate was calcined for 5 h at 600°C in muffle furnaces to remove the moisture content completely. Thus, the synthesis of MgO-NPs was done by following Tai *et al.* 2007 [Figure 3], and to confirm the synthesis of MgO-NPs, characterization was done by TEM, FTIR, UV-Vis spectra, XRD, and Zeta potential/sizer just after their preparation. The same steps were followed with sample 2 after the completion of 24-h aging. Thus, the synthesis of MgO-NPs was done by following different aging hours [5].

The chemical reaction of the biosynthesis synthesis of MgO-NPs:

3. RESULTS AND DISCUSSION

3.1. Evaluation of Phytochemical Presence

The results showed that *T. cordifolia* extract included steroids, flavonoids, glycosides, phenols, and saponins [Table 1]. Actually, the phytochemical screening test assists in separating and identifying the chemical components found in plant extracts. The precursor (magnesium chloride hexahydrate) was reduced with the help of these secondary plant metabolites, which increased the reducing ability.

3.2. Characterization of Green Synthesized MgO-NPs

The MgO-NPs of different aging hours were synthesized through *T. cordifolia* and were characterized by UV-Vis spectroscopy, Zeta potential/sizer/dispersity (Aryabhata, CIL, M.D. University Rohtak), TEM (SAIF-AIIMS, New Delhi), and FTIR (SAIF-CIL, Punjab University, Chandigarh). The results of samples 1 and 2, with aging hours and temperature factors, were compared in terms of size, dispersity, and stability.

3.3. UV-Vis Spectroscopy

The green synthesis of MgO nanoparticles was done by using *T. cordifolia* leaf extract, and the change in color was observed in the process of synthesis. When leaf extract was added to the $MgCl_2.6H_2O$ solution, the solution became dark brown. Then, after the addition of the NaOH solution, it turns a brighter color.

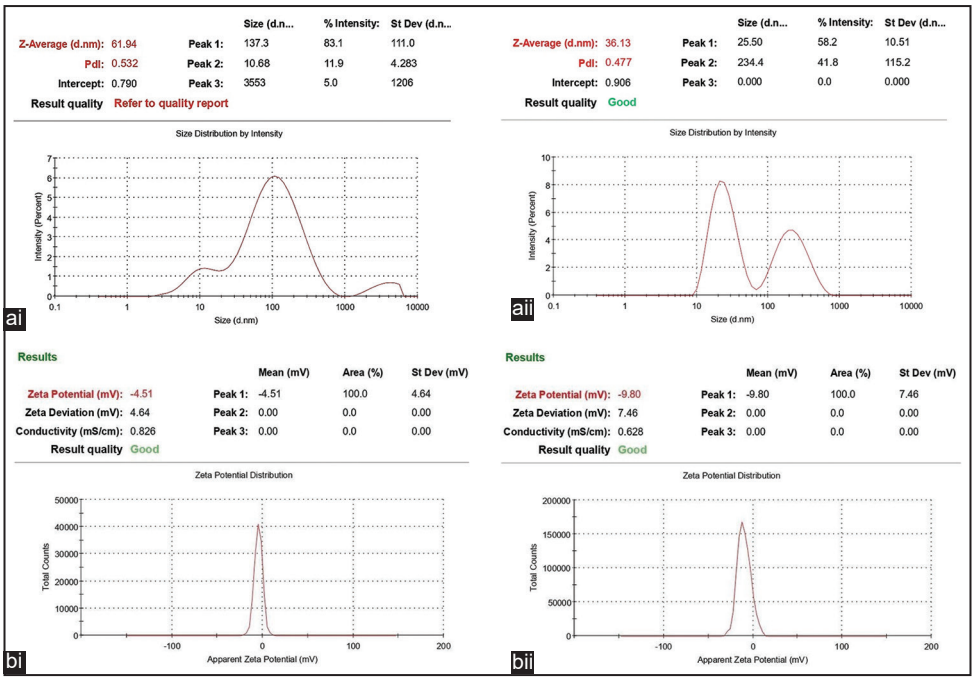

Figure 5: Zeta images of (a) size distribution of (i) Sample 1 (61.94 d.nm, 12 h aged Magnesium oxide nanoparticles [MgO-NPs]) (ii) Sample 2 (36.13 d.nm, 24 h aged MgO-NPs) (b) Zeta potential of (i) Sample 1 (−4.51 mV, 12 h aged MgO-NPs) (ii) Sample 2 (−9.80 mV, 24 h aged MgO-NPs).

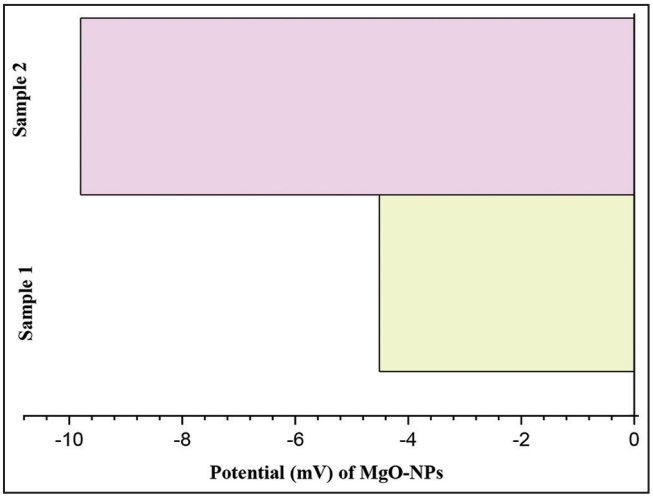

Figure 6: Effect of various aging hours on the stability of the phytosynthesized Magnesium oxide nanoparticles (MgO-NPs) depicting that longer the aging period results in increasing the stability of the MgO-NPs.

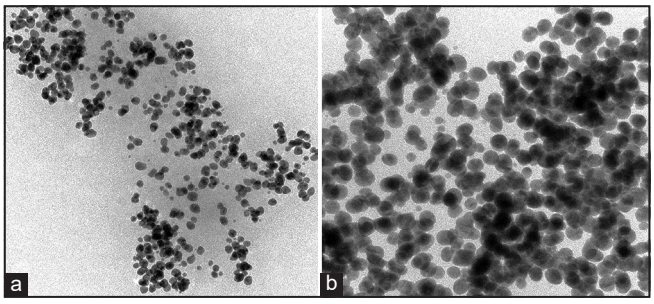

Figure 7: TEM images of (a) Sample 1 (100 nm, 12 h aged Magnesium oxide nanoparticles [MgO-NPs]) and (b) Sample 2 (50 nm, 24 h aged MgO-NPs).

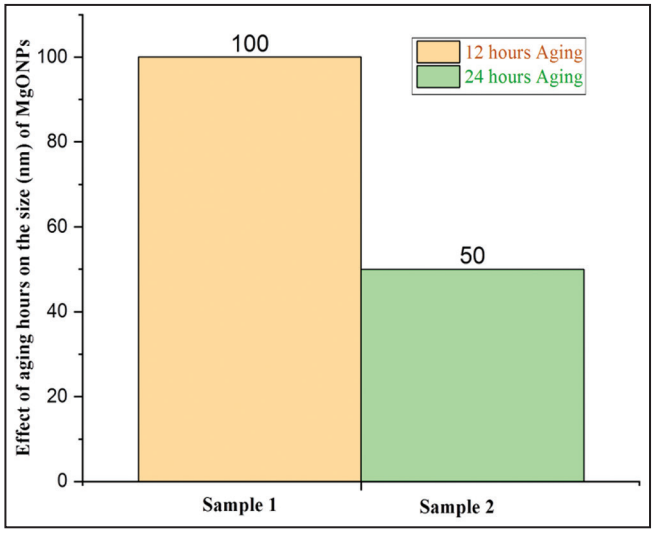

Figure 8: Effect of various aging hours on the size parameter of the phytosynthesized Magnesium oxide nanoparticles depicting that longer the aging period results in decreasing the size of the Magnesium oxide nanoparticles.

The change in color from dark brown to bright brown was due to the addition of NaOH solution. This change in color observance represented that the MgO nanoparticles had been formed. Moreover, the absorbance range for scanning the aqueous MgO-NPs suspension was observed between 200 and 800 nm. The absorption peak of sample 1 (12 h aging) [Figure 4a] and sample 2 (24 h aging) [Figure 4b] was found between 280–290 nm and 260–280 nm, respectively. The size of nanoparticles affects their color. Literature claims that as nanoparticles grow larger, their hue progressively shifts from bright brown to light brownish. Therefore, there was a decrease in the absorbance value of sample 2 rather than sample 1 [6].

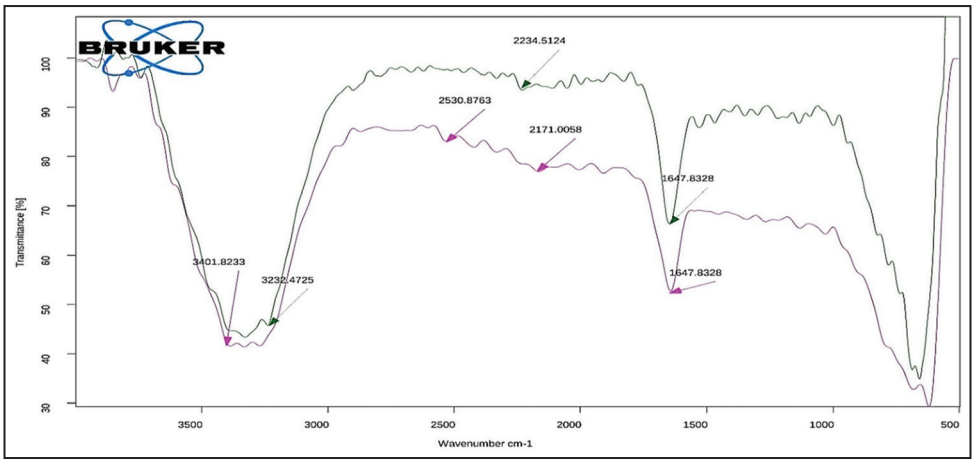

Figure 9: Fourier transform infrared spectroscopy Spectra of sample 1 (in Green) and sample 2 (in Pink).

Table 1: Qualitative phytochemistry of *T. cordifolia* leaf extracts

No.	Phytochemicals	Plants extract
		T. cordifolia
1.	Anthraquinone	−
2.	Phenols	+
3.	Tannins	−
4.	Saponins	+
5.	Glycosides	+
6.	Alkaline	+
7.	Steroids	+

(+): Present, (−): Absent. *T. cordifolia: Tinospora cordifolia*

3.4. Zetasizer/Polydispersive Index (PDI)/Zeta Potentiometer

Zetasizer defined the stable size range of MgO-NPs in different age groups. The Z-average size of sample 1 was 61.94 d.nm with 100% intensity ranges and the PDI value was 0.532 [Figure 5a.i], while the Z-average of sample 236.12 d.nm with 100% intensity ranges and the PDI value was 0.477 [Figure 5a.ii], which indicated that sample 2 was more homogeneity in nature than sample 1. Information about the stability of nanoparticles is provided by the magnitude of Zeta potential because there is an increase in the magnitude of potential, which represents the increase in the stability of nanoparticles. The effective electrical charge present on the surface of synthesized nanoparticles was measured through the Zeta potential [Figure 5b]. The stability of MgO-NPs can be determined by their Zeta potential. The value of Zeta potential helps to analyze that sample 1 possesses a negative potential value of −4.51 mV [Figure 5b.i], while for sample 2, the Zeta potential value of MgO-NPs was −9.80 mV [Figure 5b.ii]. The increase in negative potential value from −4.51 mV to −9.80 mV was due to an increase in aging hours. Thus, the 24-h aged (sample 2) MgO-NPs synthesized are more stable than the 12-h aged (sample 1) synthesized MgO-NPs due to the high negative value of Zeta potential [Figure 6].

3.5. TEM

The surface morphological features, such as size and shape, of samples 1 and 2 were monitored by a transmission electron microscope (TEM, SAIF-AIIMS, New Delhi). Tests for TEM research were created by dropping a tiny amount of dispersed nanoparticles on a copper network covered in carbon and allowing water to evaporate into a vacuum dryer. On the MgO-NPs-filled grid, TEM pictures were scanned. The green synthesized nanoparticles were oval. It was observed that the size of nanoparticles in samples 1 and 2 was 100 nm [Figure 7a] and 50 nm [Figure 7b], respectively. This decrease in size from 100 nm to 50 nm defines that increase in the aging hours' period, there is a decrease in the size of nanoparticles [Figure 8].

3.6. FTIR Spectroscopy

FTIR spectroscopy analysis was performed to find absorbed functional groups and molecules on the surface of MgO-NPS, which was synthesized using *T. cordifolia* leaf extract (aqueous) at different aging periods. FTIR spectra of MgO-NPS at different aging hours are shown in Figure 9. FTIR confirmed the presence of functional groups in the green-synthesized MgO-NPS. A small band in the green line (2234.5124 cm^{-1}) and a small band in the pink line (2171.0058 cm^{-1}) represent stretches of C-H and the rest of organic compounds. A band of green line on 1647.8328 cm^{-1} and a band of pink line lie on 1647.8328 cm^{-1} peaks, which represent the MgO interaction. Moreover, 594 cm^{-1} represents the Mg-O stretching. Hence, by the study of the spectrum, the MgO-NPs synthesis was confirmed [7].

4. CONCLUSION

The results acquired in this experimental observation represented that MgO-NPs were synthesized from leaf extracts of *T. cordifolia*, with different aging hours (the time period of stirring and temperature) affecting nanoparticle stability, dispersity, and size. The structural and optical properties of synthesized MgO-NPs were observed through UV-Vis spectroscopy. The FTIR indicates the stretching of Mg-O at 594 cm^{-1}. Zeta potential informed about the stability of MgO-NPs. The value of the Zeta potential of sample 2 is more negative (−9.80 mV) as compared to sample 1 (−4.51 mV), which represented that the increase in aging hours causes an increment in the stability of MgO-NPs. Zetasizer confirmed the size range of MgO-NPs. The decrease in size range of sample 2 (36.12 d.nm) than sample 1 (61.94 d.nm) showed the effect of different aging hours. Surface morphological studies of samples 1 and 2 were done by TEM. It was observed that the size of nanoparticles in samples 1 and 2 was 100 nm and 50 nm, respectively. This decrease in size from 100 nm to 50 nm defines the increase in the aging hours' period and there is the decrease in the size of nanoparticles. The PDI value of sample 2 was 0.477, while that of sample 1 was 0.532, which indicated that sample 2 was more homogeneity in nature than sample 1. Thus, it can be concluded that the effect of aging for different hours affects the size, dispersity, and stability of MgO-NPs. As there is an increase in the aging duration,

there is an increase in stability and homogeneity while decreasing the size of MgO-NPs.

5. FUTURE PROSPECT

There aren't many reports on the use of MgO-NPs in agriculture, the environment, and biosensing; more research is still needed to determine how these nanostructures work and how they might be applied to improve plant growth, stress tolerance, and other aspects of agriculture, the environment, and biosensing. The commercialization of this metal oxide nanoparticle will bring about in a time of cost- and environmentally-conscious applications. Magnesium oxides with nanostructures have the potential to be used as an electron mediator in the creation of more sensitive biosensors for the detection of environmental pollutants, biological diagnosis, etc. As it has been determined that different aging periods affect the stability, dispersiveness, and size of the MgO-NPs in the same way, more research can be done on a variety of other factors, such as different pH ranges, temperatures, salt concentrations, and different concentrations of green reducing agent, which can affect the size, shape, stability, dispersity, and toxicity level, among other things.

6. DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

7. RESEARCH INVOLVING HUMAN PARTICIPANTS AND/OR ANIMALS

The authors of the research confirm that the research did not involve the participation of any human or animal subjects.

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**Bibliographical Sketch*

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