GREEN SYNTHESIS AND CHARACTERIZATION OF MAGNESIUM OXIDE NANOPARTICLES FROM LEAF EXTRACTS OF *AMARANTHUS RETROFLEXUS* AND *AZADIRACHTA INDICA*

Abstract

Among plant extracts, neem leaf extract was found effective for successful synthesis of MgO nanoparticles. The main phytochemicals present in neem leaf extract acts as reducing and capping agents for synthesis of MgO nanoparticles. The bio reduced MgO nanoparticles were characterized by using surface plasmon resonance analysis (UV–Vis spectroscopy), particle size and distribution analysis (PSA), surface morphology analysis (SEM) and elemental distribution analysis (EDX). The UV–Vis spectral analysis confirmed that the maximum absorption at 279 nm range corresponds to the intrinsic band gap of MgO nanoparticles. Particle size analyzer confirmed synthesized particles are of nanoscale *i.e.*, 48.1 nm. SEM images confirmed synthesized nanoparticles were found to be relatively oval shaped and EDX confirmed peak is of MgO nanoparticles. This is a simple and effective method which could be an alternative for chemical and physical methods for the large-scale production of MgO nanoparticles.

Keywords: Green synthesis, MgO Nanoparticles, Phytochemicals, Plasmon resonance analysis

Introduction

Nanotechnology is a promising field of interdisciplinary research which opens up wide array of opportunities in various agricultural fields and raise hopes for improving agricultural productivity through encountering problems unsolved conventionally (Manjunath *et al.*, 2016). Metal-based nanoparticles such as nano magnesium can be synthesized using physical, chemical, and biological/green synthesis methods. Both physical and chemical methods result in the release of toxic byproducts that are not only health and environmental hazardous, but also expensive. On the other hand, green synthesis of metal nanoparticles is inexpensive, eco-friendly, with controlled size and shape (Khan *et al.,* 2018). Green synthesis of nanoparticles using microbes and plant extracts has been well documented in the literature (Hulkoti and Taranath, 2014, Ahmed and Ikram, 2015). However, using plants for nanoparticle synthesis can be beneficial over other biological methods as it eliminates the elaborate process of maintaining cell/microbial cultures and can also be easily scaled up for large-scale nanoparticle synthesis (Shankar *et al.*, 2004). Plant extracts are used for bio reduction of metal ions to form nanoparticles. It has been demonstrated that plant metabolites like sugars, terpenoids, polyphenols, alkaloids, phenolic acids and proteins play an important role in reduction of metal ions into nanoparticles and support their subsequent stability (Makarov *et al*., 2014). Synthesis of nanoparticles from plant extracts is safe to the environment and the user. Further, they are easily available and possess a broad range of metabolites. The present work aims to use the leaf extract of *Azadirachta indica* (Neem) and *Amaranthus retroflexus* (Amaranthus) for the green synthesis of magnesium nanoparticles. The eco-friendly method with the large scale production and without unwanted impurities is desirable for the cost-effective preparation of MgO nanoparticles. As a consequence, the low cost precursors such as magnesium sulphate heptahydrate and natural product such as neem and amaranthus leaves are used to synthesize the MgO nanoparticles through a simple green route.

Materials and methods

Preparation of leaf aqueous extracts

Fresh leaves of *Azadirachta indica* were collected from UAS, Dharwad campus and *Amaranthus retroflexus* were collected from local vegetable market in Dharwad. The collected leaves were thoroughly washed several times using deionized distilled water, air-dried and chopped finely into small pieces. Twenty grams of chopped leaves were taken in pestle and mortar and finely ground by adding 100 ml of deionized distilled water. The extract mixture was then filtered using Whatman filter paper No. 1 and the filtered mixture was stored in refrigerator at 4 °C for further utilization in the experiments.

Preparation of 1000 ml aqueous $MgSO_4$ (2000 ppm) stock solution

Magnesium sulphate ($MgSO_4.7H_2O$) procured from the firm Himedia was used as a precursor in the synthesis of magnesium nanoparticles. 2.0 g of $MgSO_4.7H_2O$ was dissolved in 1000 ml of deionized water and stored in bottle for use in further experiments.

Standardization of the protocol for green synthesis of magnesium oxide nanoparticles

The bio-active molecules present in the plant extracts of *Amaranthus retroflexus* and *Azadirachta indica* were being tried for green synthesis of nanoparticles with different methods *viz.*, hot plate heating method, heating with stirring and autoclave method. Though several methods were tried, based on the size (1-100 nm) of the MgO nanoparticles, one method (lowest size) was selected for further synthesis of MgO nanoparticles.

Hot plate heating method

The different combinations of precursor ($MgSO_4$) and plant extract (neem) were kept on the hot plate @ 60^0C for 30 and 60 minutes and particle size analyzer observations were recorded.

Heating with stirring method

The different combinations of precursor ($MgSO_4$) and plant extract (neem) were kept on the hot plate @ 60^0C along with stirring for 500 rpm for 30 and 60 minutes and particle size analyzer observations were recorded.

Autoclave method

The different combinations of precursor ($MgSO_4$) and plant extract (neem) were kept in autoclave (121 ^{0}C temperature and pressure of 15 pounds per square inch) for 15 and 30 minutes and particle size analyzer observations were recorded.

Characterization of synthesized magnesium oxide nanoparticles

The biosynthesized nanoparticles were characterized in order to know the shape, size and other parameters by using UV-Visible spectrophotometer (UV-Vis) and Particle Size Analyzer (PSA).

Results and discussion

Green synthesis of magnesium oxide nanoparticles from leaf extracts of amaranthus and neem

In the present study, we choose *Amaranthus retroflexus* and *Azadirachta indica* leaf extract for synthesis of magnesium nanoparticles. The amaranthus leaf extract used for the synthesis of nanoparticles by different methods *viz.*, hot plate heating method, heating with stirring method and autoclave method. These different methods were unable to reduce the particles below 100 nm and the obtained particle sizes were in the range of 211.6 to 390.1 nm (Table 1). Similarly, the neem leaf extract through hot plate heating method and heating with stirring method were also unable to reduce the particles below 100 nm and obtained particles were in the range of 272.8 to 329.2 nm (Table 1). However, neem leaf extract through autoclave method successfully synthesized particles in the nano range *i.e.*, 48.1 nm (Table 1). The magnesium oxide nanoparticles were successfully synthesized by autoclaving 10 ml of magnesium sulphate solution and 1.5 ml of neem leaf aqueous extract. After 30 minutes of autoclave, maximum number of Mg^{+2} ions to magnesium nanoparticles which was confirmed by change in colour from initial yellowish to light brown, indicating the reduction of Mg^{+2} to MgO in the $MgSO_4$ solution. Metal salts comprising of metal ion is first reduced to atoms by means of a reducing agent from the neem leaf extract. The obtained atoms then nucleate in small clusters that grew into nanoparticles. The presence of water-soluble phenolic acid and flavonoid compounds are believed to play a major role in bioreduction reaction and proteins seem to exhibit little importance in biosynthesis of nanoparticles as reported earlier (Raphael, 2012). A broad variability of metabolites *viz.* several terpenoids, reducing sugars and phenolic groups present in the neem leaf extract possesses reducing properties that aids in the immediate reduction of the magnesium ions into nanostructured MgO. These phytochemicals present in neem leaf extract acts as bio-reductant and are responsible for direct reduction of magnesium ions into their respective nanostructures (Prathna *et al*., 2010). In Addition, presence of amide groups of protein functions as capping agent to prevent agglomeration and hence helps in MgO nanoparticle stabilization. The results are in conformity with the findings of Moorthy *et al*. (2015), they synthesized MgO nanoparticles using neem leaf extract through hot plate heating method and obtained particles were of size 43 nm.

Characterization of synthesized MgO nanoparticles

Surface plasmon resonance analysis

UV-Visible spectroscopy was used to study surface plasmon resonance of metal nanoparticles. It is sufficiently effective as a preliminary analysis for the confirmation of nanoparticle formation. According to Mie theory, light absorbance is directly proportional to the particulate size of metal nanoparticles, as metal nanoparticles are conductors and possess surface plasmon resonance (Kelly *et al*., 2003). In this study, a strong and broad surface plasmon resonance peak at 279 for MgO was obtained confirming the successful synthesis of nanoparticles (Plate 1). Previous studies reported that a surface plasmon resonance peak located between 250 to 280 nm for MgO nanoparticles synthesized through neem leaf extracts as reported by Amina *et al*. (2020), Vergheese and Vishal (2018), Sushma *et al*. (2016) and Moorthy *et al*. (2015).

Particle size and distribution analysis

It was carried out by particle size analyzer (PSA). The sizes of the MgO nanoparticles synthesized were confirmed by dynamic light scattering. This analysis yielded a diameter of 48.1 nm for MgO nanoparticles (Plate 2). The Gaussian distribution confirmed by particle size distribution analysis at 100 per cent intensity single peaks. The comparable magnesium nanoparticles size was observed in various other studies also, for instance, neem leaf extract mediated MgO nanoparticle size was reported to be 43 nm (Moorthy *et al*. 2015), 18 nm with orange peel extract (Ganapathi *et al*., 2015), 8 nm with aloe vera extract (Anantharaman *et al*., 2016) and 50 to 90 nm with citrus extract (Umaralikhan and Jaffar, 2018).

Conclusion

Azadirachta indica (neem) leaves aqueous extract can be used as an efficient reducing and capping agent for green synthesis of MgO nanoparticles compared to *Amaranthus retroflexus*. This is a simple and effective method which could be an alternative for chemical and physical methods for the large-scale production of MgO-NPs.

Table 1: Standardization of the protocol for green synthesis of magnesium oxide nanoparticles

$MgSO_4$ 2000 ppm (ml)	Time (min)	Amaranthus leaf extract (ml)	PSA (nm)	Neem leaf extract (ml)	PSA (nm)
	Method: Heating @ 80 □				
10	30	0.5	390.1	0.5	329.2
10	30	1.0	370.6	1.0	301.9
10	30	1.5	331.5	1.5	296.9
10	30	2.0	309.9	2.0	294.3
10	30	2.5	299.7	2.5	277.2
10	60	0.5	386.4	0.5	318.5
10	60	1.0	350.7	1.0	307.1
10	60	1.5	314.6	1.5	303.1
10	60	2.0	297.2	2.0	289.6
10	60	2.5	258.9	2.5	272.8
	Method: Heating @ 80 □ with stirring @ 500 rpm				
10	30	0.5	351.3	0.5	295.9
10	30	1.0	330.1	1.0	272.6
10	30	1.5	314.7	1.5	262.8
10	30	2.0	312.1	2.0	319.9
10	30	2.5	280.7	2.5	326.8
10	60	0.5	380.2	0.5	285.4
10	60	1.0	332.2	1.0	297.2
10	60	1.5	320.2	1.5	311.8
10	60	2.0	317.4	2.0	310.8
10	60	2.5	275.1	2.5	317.9
	Method: Autoclave method				
10	15	0.5	223.2	0.5	184.5
10	15	1.0	236.7	1.0	182.3
10	15	1.5	271.3	1.5	156.8
10	15	2.0	268.4	2.0	178.4
10	15	2.5	212.4	2.5	167.3
10	30	0.5	211.6	0.5	105.7
10	30	1.0	221.7	1.0	102.3
10	30	1.5	260.9	1.5	48.1
10	30	2.0	234.8	2.0	120.6
10	30	2.5	214.1	2.5	127.1

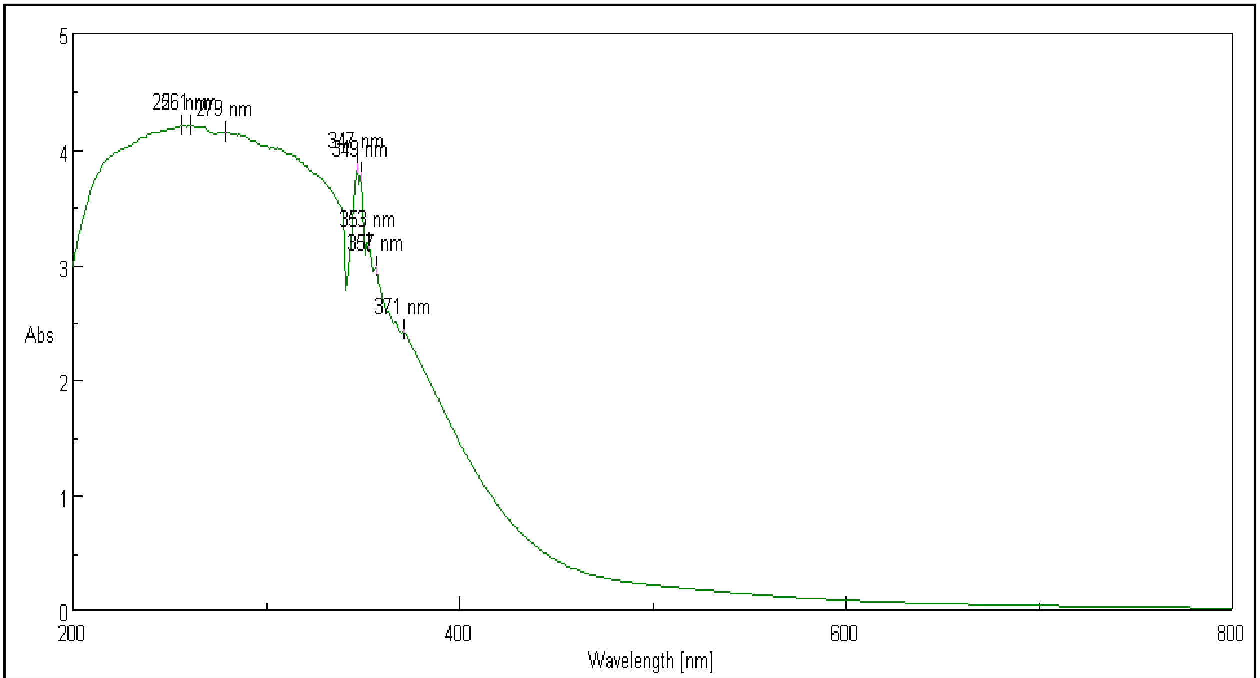

Plate 1: Characterization of Synthesized MgO nanoparticles from neem leaf extract by UV-Visible spectrophotometer

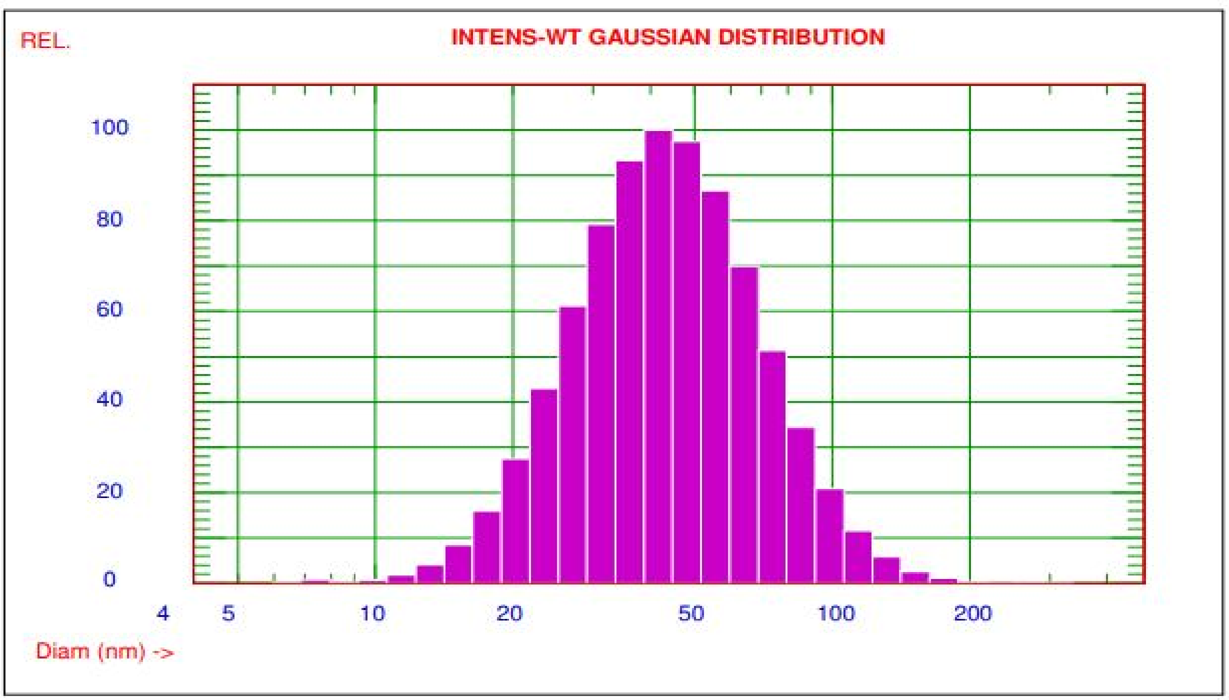

Plate 2: Characterization of Synthesized MgO nanoparticles from neem leaf extract by particle size analyzer (Nicomp)

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