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# Green Synthesis and Characterization of Fe Nanoparticles Using Neem (*Azadirachta indica* L.) Leaves

**Pushpa S. Parmar [a++*], K. C. Patel [b#] and G. B. Patil [c†]**

*[a] Department of Soil Science and Agricultural Chemistry, College of Agriculture, Anand Agricultural University, Vaso, India.*
*[b] Micronutrient Research Center (ICAR), Anand Agricultural University, Anand, India.*
*[c] Department of Nanotechnology and Centre for advanced Research in Plant Tissue Culture, Anand Agricultural University, Anand, India.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

A laboratory study for synthesis and characterization of iron nanoparticles was conducted at laboratory of Department of Nanotechnology and Centre for advanced Research in Plant Tissue Culture, Anand Agricultural University, Anand. The Fe nanoparticle synthesized by green method and subsequently characterized for size (Dynamic Light Scattering), functional group (FTIR) and absorbance spectra (UV-VIS Spectrophotometer). The hydrodynamic average diameter was 22.02 nm, Polydispersity index (Pdi) of 0.248 and Kilo count per second (Kcps) of 228.6 for Fe

*[++] Assistant Professor;*
*[#] Research Scientist;*
*[†] Associate Research Scientist;*
**Corresponding author: Email: Pushpa78767@aau.in;*

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nanoparticles. The FTIR showed 3219.06 $cm^{-1}$, 1637.15 $cm^{-1}$ confirmed presence of Fe nanoparticles. The UV-Visible spectra of the green synthesized Fe nanoparticles found an absorption peak at 256 nm. The $FeCl_3.6H_2O$ and the neem leaf extract reacted, the color of the reaction mixture quickly changed from yellow to a dark brown color.

*Keywords: Fe nanoparticles; neem leaf extract; DLS; FTIR; UV-VIS Spectroscopy.*

## 1. INTRODUCTION

Iron is an essential element for plant growth and development. Iron is involved in chlorophyll formation its deficiency will cause plant disorder known as chlorosis. Iron is a micronutrient which has a vital role in improving crops in terms of quality and quantity of rice. Though plants need the Fe in small quantities for their growth, its insufficiency or excess has detrimental effects on the physiological and metabolic functions of plants, thereby decreasing their yield.

Nano fertilizers are the most important function of nanotechnology in the production phase of agriculture. Application of nano fertilizers instead of common fertilizers, nutrients are provided to plants gradually and in a controlled manner. The nanotechnology increases the application efficiency of fertilizers, reduces soil pollution and environmental risks of chemical fertilizers (Naderi et al., 2011). Because nano materials are much smaller and lighter, they interact better in the environment and may be a solution for the problem of iron nutrition in saline and lime soils. Iron Nano-oxide is smaller than the common iron oxides and forms more complexes and makes the Fe more available to plants (Mazaherinia et al., 2010).

Smaller particles allow better coverage of surface area. Nano sized particles can even pass through the plant cell wall. Nanotechnologists exploit this process to deliver at cellular level and atomic levels which is more effective than the conventional method (Majid et al., 2021; (Yilleng et al., 2020)). Nanogenetic manipulation of agricultural crops, agricultural diagnostics, drug delivery and nanobiotechnology, nano biosensors, nano insecticides, nano herbicides and nano fertilizers are some of the key focus areas for agricultural nanotechnology research (Nagula & Usha, 2016; Kumari & Shukla, 2021; Moorthy et al., 2015; Akhtar et al., 2024). Nano-iron has a high degree of bioavailability, which indicates its alternative use in living systems. Iron forms part of the catalytic centres of many redox enzymes, and also contributes to the formation of chloroplast proteins, which stimulate the development of the root and shoot systems (Kovalenko & Folmanis, 2006; Carvalho et al., 2024).

## 2. MATERIALS AND METHODS

A laboratory study was carried out at Department of Nanotechnology and Centre for advanced Research in Plant Tissue Culture, Anand Agricultural University, Anand.

### 2.1 Synthesis of Fe Nanoparticle *Via* Green Synthesis Method

The synthesis of iron nano particles was carried out as describe below (Plate 1).

#### 2.1.1 Materials

Analytical reagent-grade iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) and dry neem leaf powder and double distilled water was used for the synthesis of iron nano particles.

#### 2.1.2 Protocol

The iron nanoparticles (Fe nanoparticle) were synthesized via green synthesis method. The 5 gm dry neem leaves were taken and boiled them in 100 ml of double distilled water in microwave oven at 70˚C for 3-5 minutes to prepare 5 % (w/v) of the neem leaf extract. Boiled extract was cooled and filtered by sieve (1.00 mm) to get clear supernatant. After filtering the neem leaf extract, it was used for preparing the Fe nanoparticle. The Ferric chloride hexahydrate (0.1 M) yellow colour solution was prepared under continues stirring condition in glass beaker. For making 100 ml of Fe nanoparticle, 96 ml of 0.1 M iron (III) chloride hexahydrate had been taken and then 4 ml of 5 % (w/v) neem extract was added in drop wise manner and mixed it by using a magnetic stirrer at room temperature for 10-15 minutes. The dark brown colour solution was obtained after kept at water bath at 90 ˚C for 80 minutes.

### 2.2 Characterization of Green Synthesized Fe nanoparticle

After the preparation of Fe nanoparticle, different characterization techniques were used to investigate their particle size (nm), poly dispersity index (PDI) and count rate (KCPS).

### 2.2.1 Dynamic light scattering (DLS)

The zeta sizer, also known as dynamic light scattering (DLS) instrument, is an analytical tool used to measure the size, distribution and surface charge of particles in a solution. The zeta sizer works by passing a laser beam through a sample solution and measuring the scattered light at different angles. The intensity and rate of the scattered light are analysed to determine the size of the particles, which is a measure of their surface charge.

### 2.2.2 Fourier transform infrared spectroscopy (FTIR)

FTIR is widely used in various fields, including chemistry, biology and materials science, for the identification and quantification of compounds in complex mixtures.

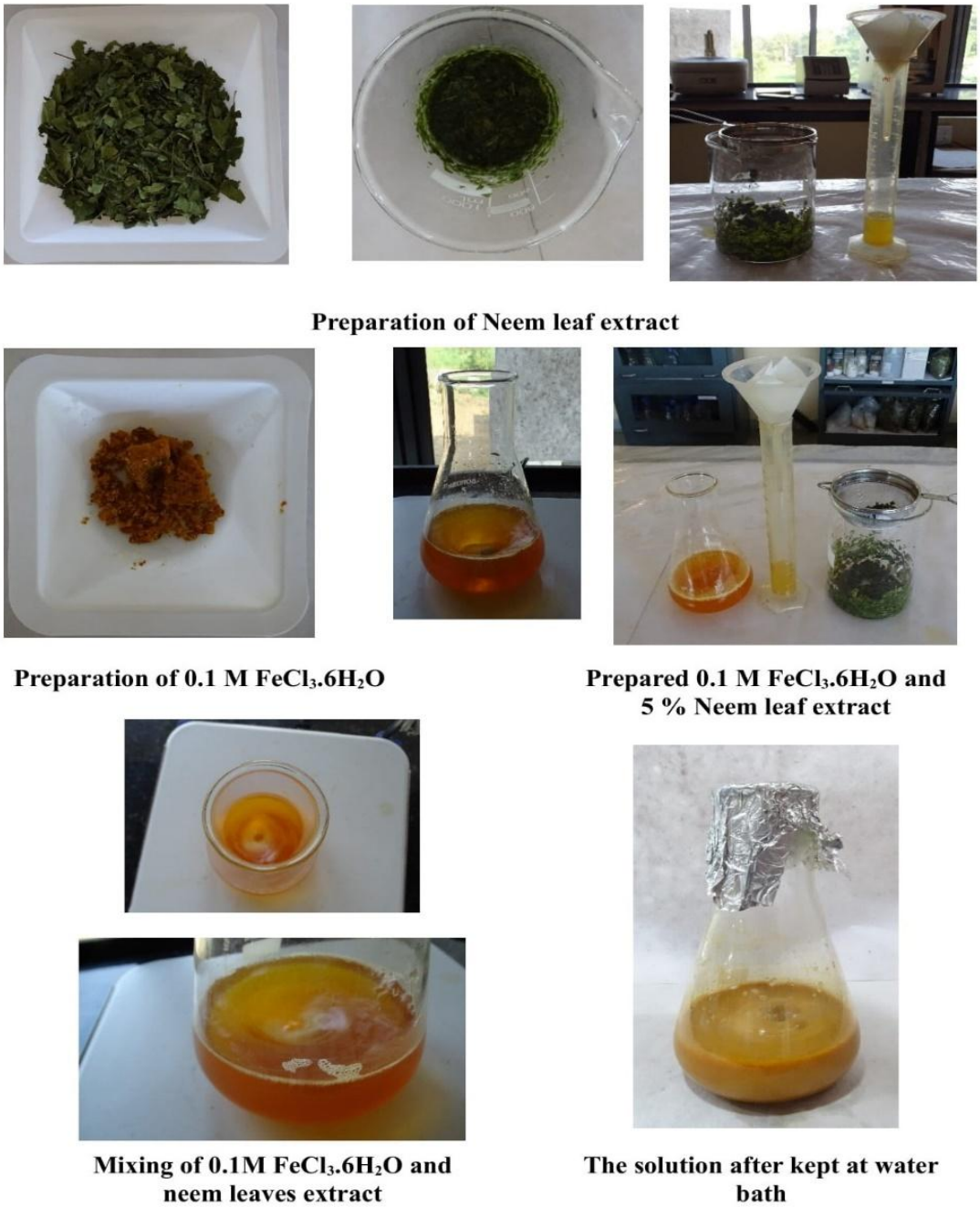
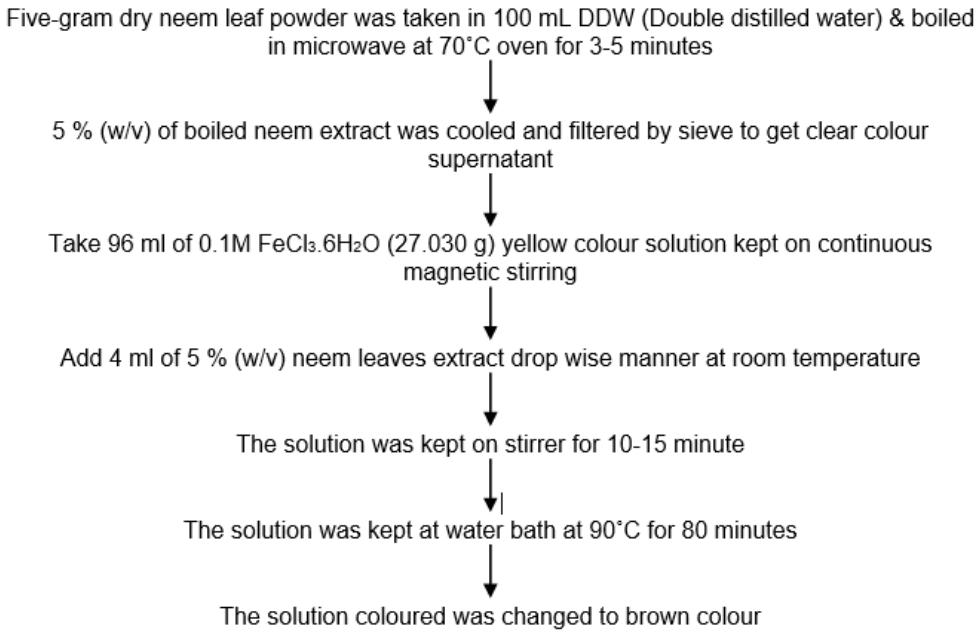



**Plate 1. Protocol of the green synthesis of iron nano particles**

**The step-wise method of synthesis of Fe nanoparticle is presented below**




The synthesized Fe nanoparticle was utilized for further studies. Its total Fe content was 4000 mg $L^{-1}$.

### 2.2.3 UV-spectrophotometer

The UV spectrophotometry is a versatile and valuable tool in nanoparticle research, allowing for the characterization, quantification, and assessment of nanoparticles and their interactions. It provides important insights into the optical properties, stability and behavior of nanoparticles, contributing to the understanding and advancement of nanotechnology applications.

## 3. RESULTS AND DISCUSSION

## 3.1 Characterization of Fe Nanoparticles

The green synthesis method was selected for the synthesis of Fe nano particle, which was characterized by Dynamic Light Scattering (DLS), Fourier-Transform Infrared Spectroscope (FT-IR) and UV visible spectrophotometer and the results of the particle size, particle size distribution, poly-dispersity index, functional group and absorption spectrum are given below:

### 3.1.1 Dynamic light scanning (DLS)

The hydrodynamic diameter of Fe nanoparticles was measured using Malvern, USA, Model Name: Zeta Sizer ZS-90. Dynamic Light Scattering (DLS) analyzes the velocity, caused by the random motion of the particle. The hydrodynamic average diameter of Fe nanoparticles determined by DLS was 22.02 nm (Table 1 and Fig. 1). The perfect correlogram of the metallic nanoparticles clearly suggests monodisperse particle solution with poly dispersity index (Pdi) of 0.248 and Kilo count per second (Kcps) of 228.6 for iron oxide nanoparticles.

### 3.1.2 Chemical bonds in synthesized nanoparticles: FTIR spectra analysis

Fourier Transform Infrared Spectroscopy is a characterization technique used for the determination of functional groups in compounds. FTIR analysis shown in Fig. 2 was used to identify the iron oxide forms. The FT-IR spectrum of synthesized nanoparticles in the

**Table 1. Characterization of Fe nanoparticles for particle size (nm), PDI and KCPS**

| Sr. No. | Z-Average size (nm) | Polydispersity Index (PDI) | Kilo count per second (KCPS) |
|---|---|---|---|
| 1. | 22.02 | 0.248 | 228.6 |

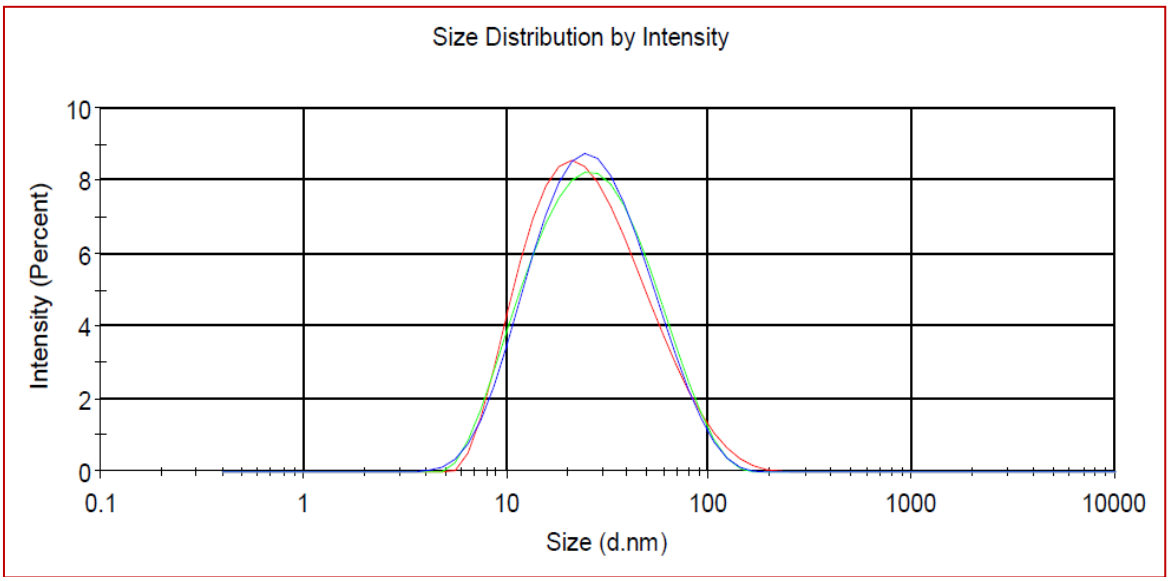
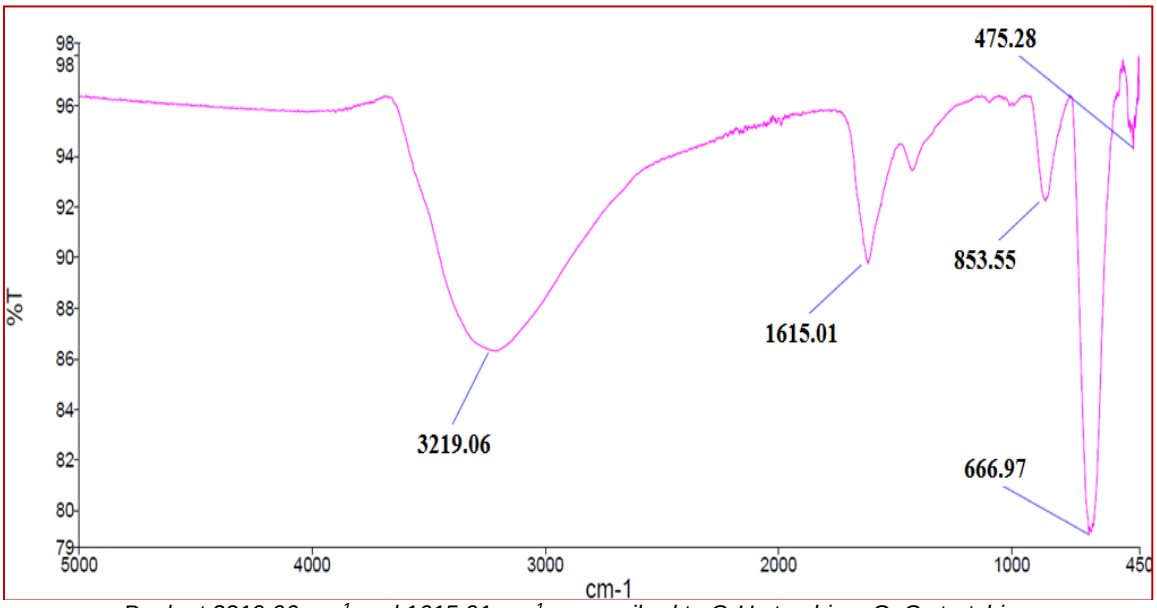



**Fig. 1. Particle size distribution of green synthesized Fe nanoparticles**




*Peak at 3219.06 $cm^{-1}$ and 1615.01 $cm^{-1}$are ascribed to O-H streching, C=C stretching*

**Fig. 2. FTIR spectrum of green synthesized Fe nanoparticles**

wave number range from 450 to 4000 $cm^{-1}$ and the graph revealed the presence of five peaks in given ranges as shown in Fig. 2. IR band at the 3219.06 belongs to the hydroxyl (-OH) group between 2500 and 3800 $cm^{-1}$. The finding showed fundamental mode of vibration at 3219.06 $cm^{-1}$ O-H stretching due to present of carboxylic acids group, 1637.15 $cm^{-1}$ confirmed the C=C (C double bond C) due to the alkene functional group. FeO vibrations at the lower wavenumber bands belong to the iron oxide nanoparticles.

### 3.1.3 Characterization by UV–visible spectroscopy

UV–Visible Spectroscopy analysis the UV-Visible spectra of the green synthesized Fe nanoparticles by the neem leaf extract are shown in Fig. 3. The green synthesized Fe nanoparticles showed an absorption peak at 256 and 330 nm. When $FeCl_3.6H_2O$ and the neem leaf extract reacted, the color of the reaction mixture quickly changed from yellow to a dark brown color of solution.

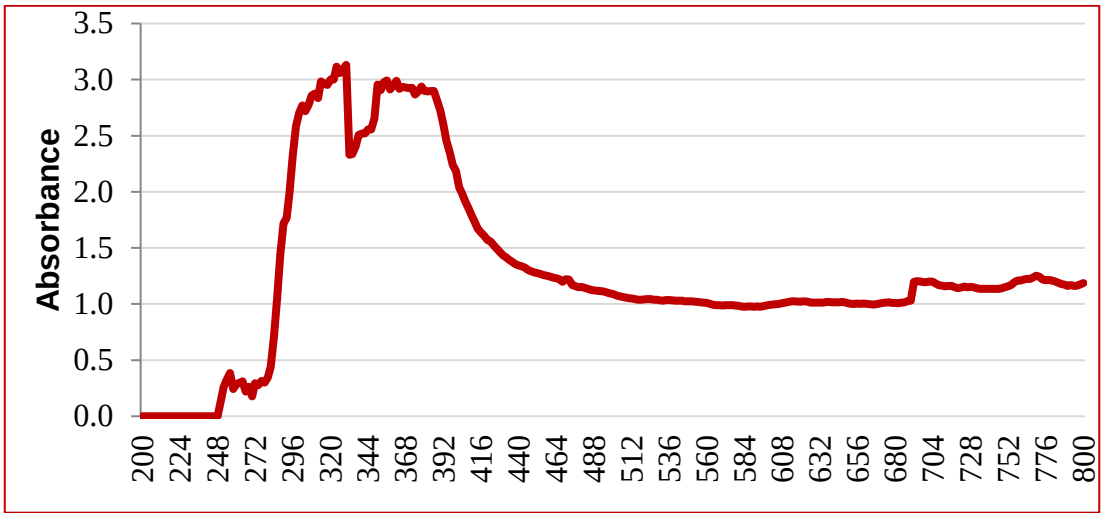



**Fig. 3. UV-Vis absorption spectrum of green synthesized Fe nanoparticles**

## 4. CONCLUSION

The synthesis of Fe nano particle by green method using neem leaf extract gave less than 100 nm particle size, carboxylic acids and alkene functional groups, absorption peak at 256 nm confirmed the Fe nano particles.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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