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Biosynthesis of Iron Oxide Nanoparticles Using Fenugreek

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Abstract

Due to the environmental and economic advantages of synthesis of nanoparticles (NPs) from plantextract has gained wide popularity in the recent time. The synthesis of NPS by plantextract is a 'green' technology as it avoids use of harmful chemicals and formation of toxicbyproducts. In this study, iron oxide NPsare synthesized by reducing ferrous sulfate in a aqueous solution by plant extract of Fenugreek *(Trigonella Foenum-Graceum)*plant extract (PE). Various conditions of NPs formation were tested to see maximum synthesisby varying parameters like Ferrous sulfate concentration, PE concentration, PE: ferrous sulfate ratio, and incubation temperature. The reducing and capping potential of Fenugreek was confirmed using UV-visible spectrophotometer analysis of reaction mixtures. Peak around 310nm is characteristic of NPs having iron oxide chemical composition. The size of iron oxide NPsas investigated using DLS was found to beapproximately 500 nm. FE-SEM analysis revealed that the NPs wereapproximately 50 nm in size and spherical in shape. XRD analysis confirmed amorphous coating of PE biomolecules on the surface of iron oxide NPs. FTIR analysis revealed role of phenolic and protein moieties in iron oxide NPs synthesis. Being synthesized through greener route, NPs can be used for environmental, agricultural and various in vivo human applications.

1. Introduction

Nanotechnology deals with synthesis, characterization and modification of particles having atleast onedimension preferably between 1 and 100 nanometers. The ultra small size as well as largesurface to volume ratio dictates unique physical and chemical properties of NPs ascompare to bulk molecule of the same chemical composition. Due to their uniquechemical, optical, thermal, physical and electrical properties, NPs have hugeapplications [1-3].There are large numbers of methods of NPs synthesis are broadly categorized into physical, chemical and biological methods. Every method has some advantages and disadvantages. Each method provides some specific properties to NPs synthesized. Physical and chemical methods are commonly used for NPs synthesis but have some disadvantages like low yield, costly manufacturing, huge energy requirementand environment toxicity due to use of toxic precursor chemicals. Further generation of toxic byproducts is also a major challenge. So, in comparison to other methods, biological green synthesis of NPsuses low amount of energy, environment friendly, low cost and simple method. This method is also suited for industrial scale production of NPs. Green nanotechnology is emerging field that involve range of techniques that can minimize or remove toxic substances to regain better environmental conditions [4]. Plant extract based green synthesis

methods specifically use green reagents that are non-toxic, inexpensive, renewable and easily available. In addition the synthesis procedure uses environmentally favorable synthesis conditions like low temperature and low waste generationHowever, extract preparation and optimization of reaction conditions for NPs synthesis is a tedious task and require thorough investigation of phytochemicals[5-10]. This become more challenging as photochemical varies from one plant to another as well as even within different parts of same plant. Plants have been use for iron oxide NPs but the photochemical varies the size as well as surface properties of NPs synthesized also varies [1,10-18]. This has advantage as we can tap plant biodiversityto obtain diverse iron oxide NPs for various applications [18-22].

Fenugreek (*Trigonella Foenum-Graecum*) is herb used as vegetable and spice all around the globe. It belongs to the plant family of fabaceae. Thisfamily is well known for chemicals that possess a distinctive smell. It is used in India,Turkey, Egypt, Persia, Ethiopia in various cuisines. Basically it is known to reduce blood sugar level in diabetics, improving digestion and metabolism. It also contains highlevel of antioxidants due to the presence of compounds like polyphenols and flavonoids that has good antioxidant potential. Due to its good medicinal value Fenugreek has been reported for the synthesis of goldNPs [11].Gold salt is costly that make synthesis of gold nanoparticle costly. The application of NPs are seen in different fields like chemical sensors, cancer therapy, optics, antimicrobial activity, computer transistors and wastewater treatment. The specificapplication of iron oxide nanoparticle is observed in biomedical and technological applicationslike drug delivery, magnetic resonance imaging (MRI), magnetic separations and magnetic resonance imaging enhancements [3]. It may also be useful in heatconductoras high strength meterial, bactericidal agents over biomedical devices and nanocatalyst[1,12,13,].In current study Fenugreek extract (FE) has been explored for the green synthesis of iron oxide nanoparticle.

2. Experimental

2.1 Material

Ferrous sulfate, NaOH and HCL use in this study were procured from Qualigens, HIMEDIA. Fenugreek leaves were procured from local market, Jalandhar.

2.2. Plant extract preparation

The plant extract is prepared by modification to our previously reported method [6]. 1 g of dried and finely grinded plant leaves powder was mixed with to 50 ml of distilled water. The mixture was heated to boil for 10 minutes. The sample was cooled and centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected stored at 4^0C for further use.

2.3. Synthesis of iron oxide Nps

Plant extract (PE) prepared from Fenugreek leaves was incubated with Ferrous Sulfate at appropriate conditions to obtain iron oxide NPs. Various factors like Ferrous Sulfate concentration, PE concentration, PE ratio, andincubation temperatures were varied to obtain standardize synthesis of iron oxideNPs.Ferrous Sulfate concentration was varied from 1, 10,50, 100, 500 mM. FLEconcentration was varied from 0.25, 0.5, 1, 1.5 and 2 ml keeping final reaction volumeconstant (4ml).PE:

Ferrous Sulfate ratio was varied, 0.1 : 1, 0.25 : 1, 0.5 : 1 and 1 : 1.Incubation temperature was varied, 25, 35, 45, 55, 65 °C. UV-visible spectroscopy was used to screen reaction mixture for the synthesis of iron oxide NPs.

2.4. Characterization of iron oxide NPs

Initial characterization of reaction mixture for the synthesis.The NPs were characterized for morphology using DLS (Malvern zetasizer) and field-emission scanning electron microscope, FESEM (Hitachi). FTIR (Perkin Elmer Spectrum) was used to confirm the type of molecules involved in NPs synthesis. The iron oxide NPs obtained at optimum conditions i.e. 100 mM Ferrous Sulfate concentration, 1 ml FLEconcentration, 1: 1 PE: Ferrous Sulfate ratio and room temperature incubation were centrifuged at 10,000 rpm for 10 minutes. The Pellet was washed with double distilled water twice. The purified iron oxide NPs were subjected to DLS and FESEM analysis. For DLS analysis the iron oxide NPs were diluted 10 times and filled in disposable cuvette. For FESEM analysis purified iron oxide nanoparticle samples were mounted on carbon tape and subjected to drying. The samples were analyzed using FESEM under appropriate magnification. The purified iron oxide NPs were dried completely. For X-ray diffraction (XRD) analysis NPs powder was analyzed using (Panalytical D/Max-2500).For FTIR characterization, dried NPs samples were mixed with potassium bromide to form pellet in pellet maker. The pellets were subjected to FTIR analysis.

3. Result and discussion

3.1. Nanoparticle synthesis and UV-visible screening ofNPs

UV-visible spectroscopic analysis was done to analyze the synthesis of ironoxide NPs in the reaction mixtures.

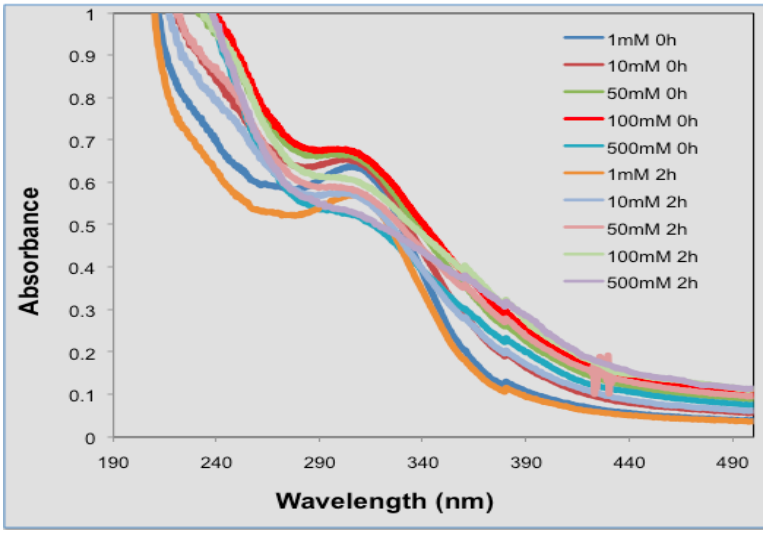

Figure 1: UV-visible spectra showing iron oxide NPs synthesized at differentFerrous Sulfate concentration ranging from 1-500 mM using FE.

All the tested Ferrous Sulfate concentrations, 1, 10, 50, 100 and 500mM resultedinto synthesis of iron oxide nanoparticle (Figure 1). Among these100 mM concentration was optimum concentration for iron oxide NPs synthesis asthe UV- visible peak intensity was maximum at this concentration. Further visibly no aggregationwas

observed at 100mM concentration during synthesis while higher concentration led tovisible aggregation of particles. So, iron oxide NPs obtained at 100mM ferrous sulfateconcentration were most stable and were selected for further study.

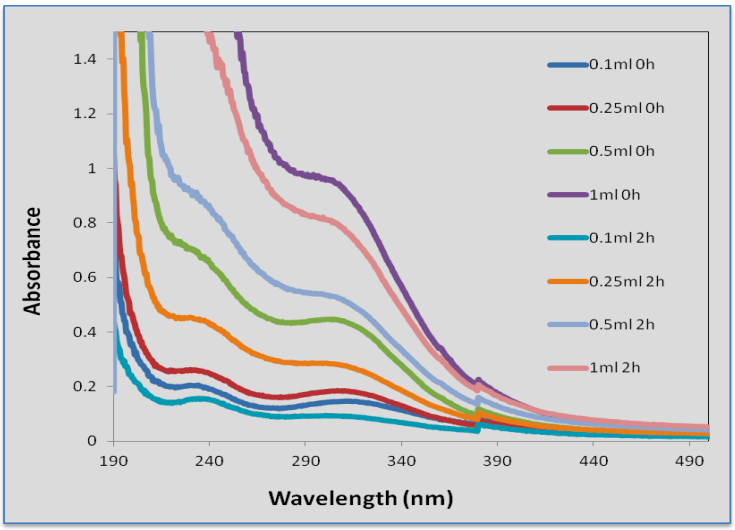

Figure 2: UV-visible spectra showing iron oxide NPs synthesized at different FLE: Ferrous Sulfate ratios, 0.1:1 to 1: 1. Other conditions were fix, 100 mM Ferrous Sulfate concentration and 25^{0}C incubation.

UV- visible spectroscopic analysis of reaction mixtures using different PE: ferrous sulfate ratio revealed that 1ml PE : 1ml ferrous sulfate was optimum condition for iron oxide NPs synthesis (Figure 2). So this 1:1 ratio was used in further studies.

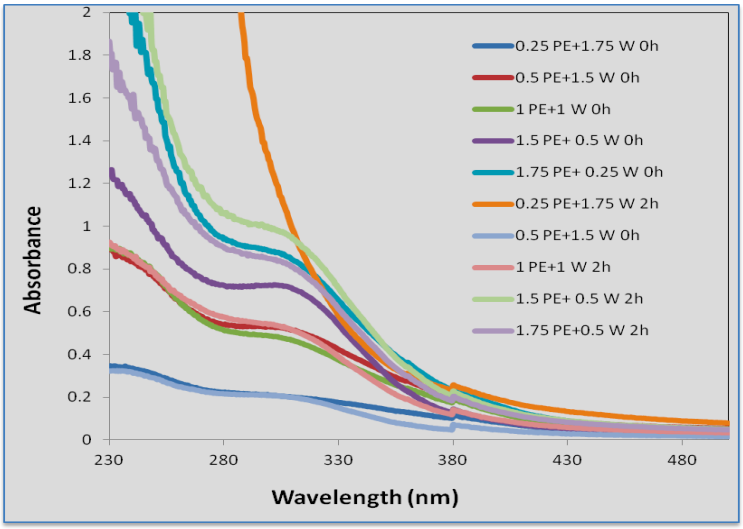

Figure 3: UV-visible spectra showing iron oxide NPs synthesized at different PE concentration, 0.25-1.75 ml keepingtotal PE volume 2ml fix. For synthesis 2ml PE of varying concentration was incubated with 2ml ferrous sulfate was used for synthesis (Overall FLE: metal ion ratio 1:1). Other conditions were fix, 100 mM Ferrous Sulfate concentration and 25^{0}C incubation temperature.

As clear from Figure 2 and Figure 3, the peak intensity was better at 2ml PE concentration. Further the iron oxide NPs tend to aggregate immediately in case of NPs obtained at 1.75+0.25 ml PE concentration. While iron oxide NPs obtained at

pure 2 ml PE were stable.UV-visible spectra obtained at different incubation temperature revealed that roomtemperature 25^0C incubation was optimum temperature for iron oxide nanoparticle synthesisusing PE as reducing and capping agent (Figure 4).

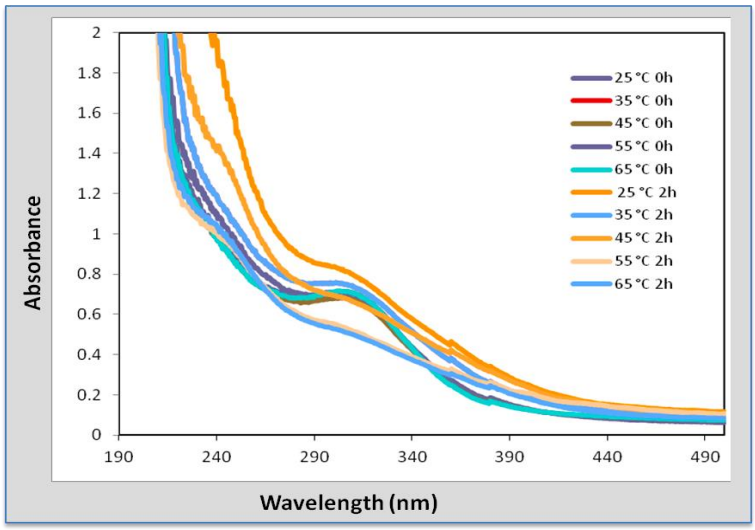

Figure 4: UV-visible spectra showing iron oxide NPs synthesized at differentincubation temperature, 25-65 0C. Other conditions were fix, 100 mM Ferrous sulfateconcentration, 1:1 PE :Ferrous sulfate ratio and 2 ml PE concentration.

As clear from above discussion, the optimum conditions for iron oxide NPs synthesiswas, 100 mM Ferrous Sulfate concentration, 1:1 PE : Ferrous sulfate ratio, 2 ml PE concentrationand room temperature incubation. So, NPssynthesis at these optimum conditions wasscreened at different time interval (Figure 5).

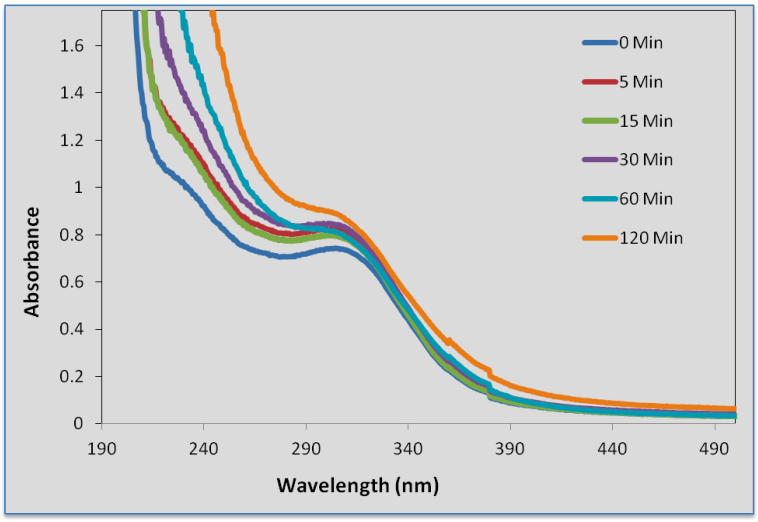

Figure 5: UV-visible spectra showing iron oxide NPS synthesized using optimumconditions at different time interval 0-120 min.

It is clear from careful analysis of figure 5 that the synthesis of iron oxide NPs using PE was over in 5 min.Further increase in incubation time has only very small change in NPs synthesis.

3.2. DLS characterization of NPs

DLS characterization revealed that the synthesis of iron oxideNPs using Fenugreek LE were 500 nm in size (Figure 6).As documented earlier, the DLS size is a hydrodynamic size and is not accurate but it gives initial rough idea about synthesis of NPs andsize of NPs [23].

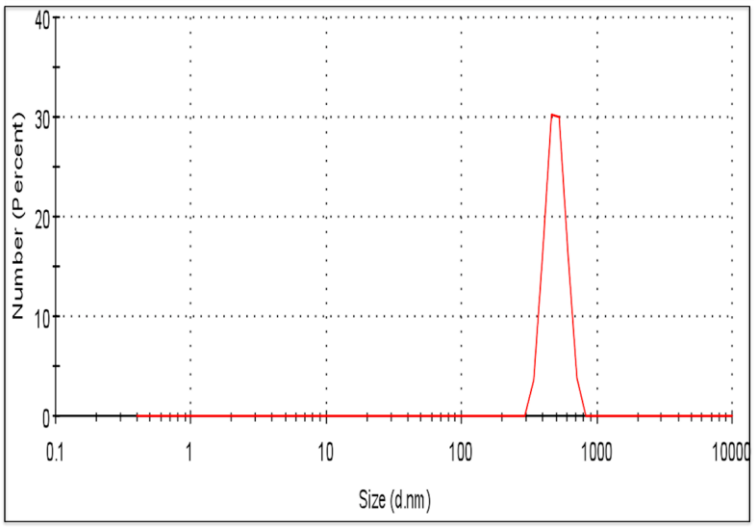

Figure 6. DLS graph showing synthesis and size of iron oxide NPs.

3.3. Iron oxide NPs characterization using XRD

XRD characterization of iron oxide NPs revealed broad peaks in the XRD spectrum(Figure 7). The sharp peaks appear in case of crystalline material, while the broad peaks appeardue to amorphous material. The broad peaks in the spectrum is due to surface covering ofiron oxide NPs with amorphous PE molecules that act as surface stabilizer over the surface ofNPs. Further the small size of the NPs may be another reason behind the broadpeaks in the XRD spectrum of iron oxide NPs.

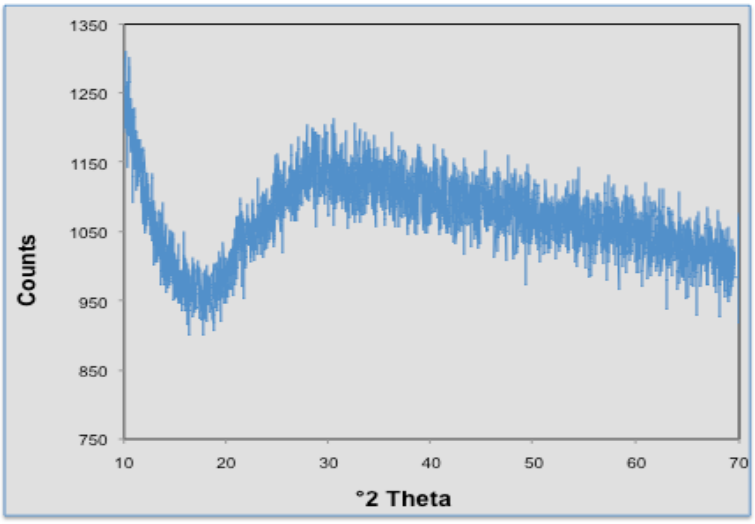

Figure 7: XRD spectrum of iron oxide NPs prepared using FLE.

3.4. FESEM characterization of iron oxide NPs

FESEM is a reliable technique for determining the size of NPs (Figure 8). Furtherit

gives information regarding the shape of the iron oxide NPs. The NPs synthesized using PE were ~50 nm in size. The shape of NPs wasspherical.

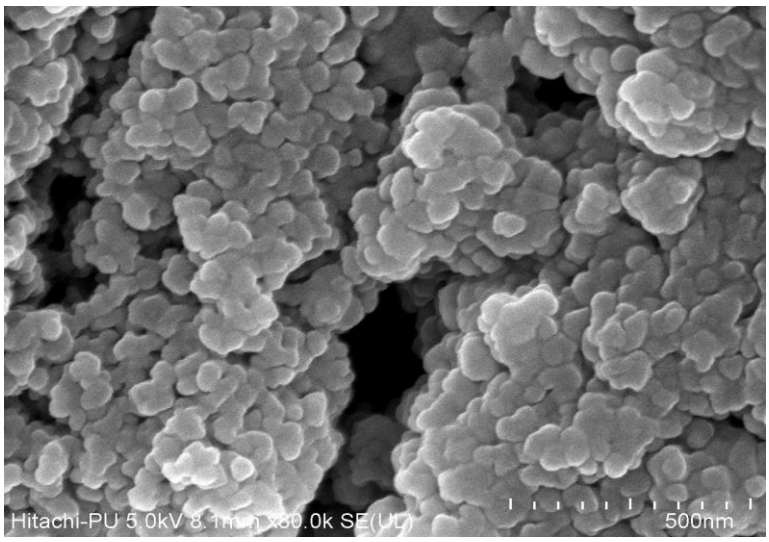

Figure 8: FESEM image of iron oxide NPs prepared using FLE.

The size shown in DLS is hydrodynamic diameter of NPs. So sometimes it is comparatively 10-20% more than size obtained through FESEM as reported earlier [23].

3.5. FTIR characterization of NPs

Peaks in the IR spectrum of iron oxide NPs at around 1010 (aliphatic C-N stretching)1270 (C-O-C bending of CH_3-O aromatic ring), 1405, 1482 (CH_2group bending, aromatic C=C),1621 (amide I band of proteins, aromatic C=C bond or C=O stretching), 2855, 2913 (sp_3C-Hstretch) and 3274 (polyphenols) cm^{-1}revealed polyphenolic and protein moieties. So,polyphenolic and protein moieties of PE were mainly responsible for the synthesis of iron oxideNPs [24,25].

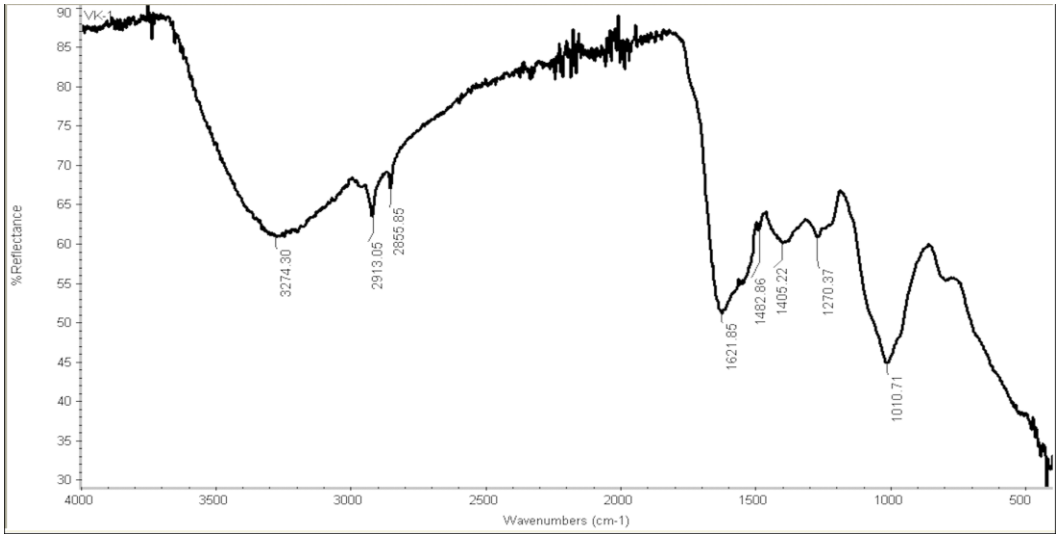

Figure 9: FTIR spectra of iron oxide NPs synthesized using PE as reducing andstabilizing agent.

So, PE has ability to act as reducing as well as surface stabilizing agent for the synthesis of iron oxideNPs from ferrous sulfate.

4. Conclusion

The disadvantages of physical and chemical reduction methods had developed a critical need todevelop an ecofriendly process to synthesize iron oxide NPs. NPswere synthesized by the bioreduction of ferrous sulfateby FLE. Current study is a simple, pollutant-free and a low costapproach for iron oxide NPs synthesis. The advantage of green synthesis method iseven NPs obtained by same plant extract and ferrous sulfate at different conditions willvary in size and shape. So, ~50 nm iron oxide NPs were obtained at optimum conditions using PE as reducing and capping agent. The greeniron oxideNPs may be vary useful for safe direct humanapplication.

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