

RESEARCH ARTICLE

Ecofriendly Green Synthesis of Iron Oxide Nanoparticles Using *citrus sinensis*

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Abstract: Green protocols being eco-friendly and cost effective approach are most widely used for the production of iron oxide nanoparticles using plant-mediated extract of *Citrus Sinensis*, moreover biosynthesized iron oxide (FeO) nanoparticles shows better antibacterial activity. Green synthesis of nanoparticles has been broadly studied from the past few years because of their different features and potential applications in various fields. The successful biosynthesis of iron oxide nanoparticles was confirmed and characterized using UV-Visible spectroscopy, Scanning Electron Microscope (SEM), Fourier Transform Infrared (FTIR) analysis and Zeta sizer. Antibacterial effect of biologically produced iron oxide nanoparticles was tested against Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Macrococcus*). These results exhibited that iron oxide nanoparticles have high antibacterial potential as these nanoparticles showed significant zone of inhibition against bacteria strains. The proposed green synthesis of iron oxide nanoparticles (NPs) from *Citrus Sinensis* can be strongly recommended as a potential method for industrial application.

Keywords: Green synthesis, Nanoparticles, Citrus Sinensis, Antibacterial activity

INTRODUCTION

Nanotechnology has induced the significant advancement in the field of scientific research. Nanoparticles have the unique properties that based on improved characteristics like morphology and size distribution. Metal Nps have gained considerable interest from few years ago owing to their specific properties and wide ranging convenient applications [1,2]. Conventionally, physical and chemical techniques were used to produce metallic nanoparticles while their synthesis involved the use of hazardous and toxic chemicals, but these methods are also very costly and frequently raise the environmental risk [3]. To avoid chemical

toxicity, bio-inspired methods were developed because green route has been proposed as the environment friendly and cost effective way to synthesize the metal nanoparticles. Bio-inspired ways are valuable as compared to some other synthetic protocols because these methods are efficient, inexpensive and restrict the use of harmful chemical reagents plus high pressure, energy and temperatures [4].

Plant mediated production is purely a green synthetic method that provides clean, eco-friendly, safe, cost effective and useful way to the synthesize the metal NPs at huge level. Various plants are investigated to facilitate the

development of iron oxide NPs as well as their promising applications [5-7]. The amount of synthesized NPs differs with reduction ability of ions. The occurrence of numerous polyphenols and heterocyclic compounds influences the reducing capacity of plants [8,9]. Plants have antioxidants that act as reducing agents and control the reduction of metal oxide and metal ions to their corresponding nanoparticles. Biosynthesis gives good results as compare to chemical and physical methods because it is low cost, environmentally favorable, easily established for industrial-scale synthesis. Moreover, in above method pressure, temperature and harmful chemicals are not necessary. Biosynthesis gives us better handling, control the growth and stability of crystals [10]. Among all other nanoparticles of metals and metal oxides, iron nanoparticles (FeNPs) take great advantage that they can fight with ecological pollution. The attention in nano-scale iron oxide nanoparticles for removal of toxic substances and pollutants from environment is increasing due to the reactivity of IONPs, as they have huge surface to volume ratio [11].

IONPs have been utilized like catalyst for the degradation of organic compounds [12]. A commercial technology utilized IONPs to reduce the chlorinated organic material in water [13,14]. Due to large surface area, catalytic, optical, high electrical and magnetic properties, IONPs have been exploited for a variety of applications. In recent works, IONPs have been employed as strong catalyst as well as magnets. Research work on different applications of NPs for environmental remediation has recently gained massive attention among the researchers, with most obvious application in the removal of natural and inorganic contaminations from environment [15]. Super-paramagnetic IONPs have suitable surface are combined with targeting ligands or proteins and employed in drug delivery applications, magnetic resonance

imaging (MRI) and in electrical components like the core of transformers and inductors [16].

In recent work biosynthesis of iron oxide nanoparticles using citrus sinensis peels has been reported. Peels of citrus species contains various important components including pectin, limonene, carotenoids, sugars, ascorbic acid and flavonoids, as these components are reliable to reduce the metal ions [17]. The citrus sinensis (orange peels) have excellent antioxidant activity [18]. Biosynthesized IONPs were characterized by Fourier transform infra-red spectroscopy (FT-IR), UV-Vis spectroscopy, Zeta sizer analysis and Scanning electron microscopy (SEM). Additionally, these plant-mediated synthesized IONPs exhibit potential antimicrobial activity and they are considered sensitive for different pathogenic bacterial strains.

EXPERIMENTAL METHOD

Fresh peels of Citrus sinensis(orange) and Ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), Ferrous chloride ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), Sodium hydroxide (NaOH), distilled and deionized water were used for all experimental work.

Preparation of Aqueous Extract:

Four grams of finely powdered citrus sinensis peels were weighed and thoroughly mixed with 100mL of deionized water and boiled for 20 minutes with constant stirring and allowed to cool. Then this extract is filtered with whatman filter paper and collected in dark colored glass bottle and stored in refrigerator at 40 °C for further experimental work.

Preparation of Solutions:

0.1M Ferrous chloride ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), 0.2M Ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and 1N sodium

hydroxide (NaOH) solutions were prepared in deionized water.

Biosynthesis of Iron Oxide Nanoparticles:

For green-synthesis of IONPs, 100ml of ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) solution was added drop-wise in ferrous chloride ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) under constant magnetic stirring at room temperature. After addition, the solution was kept for constant magnetic stirring for 20 minutes at 80 °C. Then 20 ml of citrus sinensis peel extract was added drop wise in above solution. kept this solution for 20 minutes under constant stirring at 80 °C temperature. Then 1N Sodium hydroxide solution was added drop wise in the reaction media. Black suspension was formed as the pH of the reaction mixture raised to 9.0.

The reaction media containing precipitates was centrifuged at 4000 rpm for 10 minutes. The pellets were collected in petri plates and pellets were washed by 70% ethanol solution. These petri plates were kept for evaporation. After complete evaporation, black colored nanoparticles were collected in eppendorff tubes for further characterization. Biosynthesis of IONPs was confirmed by UV-Visible spectroscopy. These biosynthesized iron oxide nanoparticles using Citrus Sinensis was named as IONPs-CS for further use.

Characterization of IONPs-CS

Biosynthesis of IONPs was conformed via UV-Visible spectrum and various analytical techniques such as UV-visible spectroscopic analysis, Fourier transform infra-red spectroscopy to determine functional groups, scanning electron microscope to study the morphology and zeta sizer to study size distribution by intensity was employed for the characterization of IONPs.

Antibacterial Activity of IONPs-CS:

The antibacterial effect of biosynthesized IONPs was assayed through agar well diffusion method [19]. Powdered NPs was dissolved in water for analysis. The assay was performed on strains Escherichia coli and Macroccusto calculate antimicrobial potential. The antimicrobials present in the nanoparticles were allowed to diffuse into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition were uniformly circular as there was a confluent lawn of growth.

RESULTS AND DISCUSSION

The IONPs were successfully bio-synthesized in eco-friendly, easy and simple way using peel extract of *citrus sinensis*. *Citrus sinensis* peels found highly effective for the bio-synthesis of FeO-NPs. Citrus sinensis was employed for the green-synthesis of IONPs because it is responsible for the reduction of iron ions to IONPs. Biologically synthesized black colored IONPs using Citrus Sinensis was designated as IONPs-CS for further discussion shown in figure 1. Biosynthesis of IONPs was observed with the change in color of the solution of ferrous chloride and ferric chloride after reduction of solution. Synthesis of IONPs was indicated by change in color of the solution from yellowish to brown then black as shown in figure 2. Biosynthesis of IONPs was further confirmed by UV-Visible spectrophotometer.

CHARACTERIZATION of IONPs-AV

UV-VIS Spectroscopic Analysis:

UV-Visible spectrum of IONPs-CS showed the characteristic peak at 370nm. This result is in accordance with the results of [20] who observed the absorption between 350-450nm wavelengths indicate the formation of iron

nanoparticles. It is the characteristic absorption peak for iron oxide nanoparticles. UV-VIS spectrum of IONPs-CS is shown in figure 3.

Scanning Electron Microscope (SEM) Analysis:

SEM images of green synthesized IONPs-CS were recorded using FEI Quanta 250 scanning-electron microscope while 15.00 kV accelerating voltage was used for analysis. The size and morphology of IONPs-CS is shown in figure 4 and 5. Different magnification powers were used for the SEM image of iron nanoparticles were 1500x, 3000x and 6000x. Because of different magnifications average size ranges between 5 μm to 30 μm . SEM images reveals that IONPs-CS was also spherical in shape and in the form of clusters. It is stated that small sized particles forms aggregations because of their definite surface energy and due to this reason unclear images obtained under SEM [21].

Our results are in accordance with [22] that synthesized iron nanoparticles from sorghum bran and observed spherical shaped nanoparticles. Moreover, the current results also relates with Devatha et al., 2018 findings, they biosynthesized iron nanoparticles from *Azadirachta Indica* and observed spherical shaped nanoparticles in SEM images. Similarly, spherical cluster in SEM images of biosynthesized FeO nanoparticles from Shirazi thyme and pistachio green hulls are reported [23].

Zeta-Sizer /DLS Dynamic Light Scattering Analysis:

Zeta sizer analysis of iron oxide nanoparticles is performed using Malvern zeta sizer (Malvern ZS). IONPs-CS was also analyzed by zeta sizer analyzer. Figure No.6 shows the zeta sizer analysis in this that z-average size is 562.6 and poly dispersity index (PDI) is 0.404.

Fourier-Transform Infrared Spectroscopy (FTIR) Analysis:

The FTIR analysis of IONPs-CS was carried out using Bruker spectrophotometer in the wave number range from 4000 cm^{-1} to 500 cm^{-1} . FTIR spectra of IONPs-CS are shown in figure 7. Number of peaks such as 3852.63 cm^{-1} , 3749.56 cm^{-1} , 3648.66 cm^{-1} , 3355.62 cm^{-1} , 1541.40 cm^{-1} , 1338.85 cm^{-1} , 1074.86 cm^{-1} and 541.66 cm^{-1} was observed.

The FTIR peak at 3852.63 cm^{-1} is due to stretching vibrations of C-H bonds in CH_2 groups. The absorption band at 3749.56 cm^{-1} is due to stretching vibrations of C=C bonds. Markova et al., also observed similar peak at 2890 cm^{-1} and 2828 cm^{-1} due to stretching mode of vibrations of C-H bonds in CH_2 groups and also observe band at 3735 cm^{-1} due to stretching vibrations of C=C bonds [24]. A major peak was identified at 3648.66 cm^{-1} due to the O-H stretching vibrations of polyphenolic groups. This peak is related to Venkateswarlu et al., who observed O-H stretching vibrations of polyphenolic groups [26]. In addition, absorption band at 3355.62 cm^{-1} is due to O-H stretching vibrations, this result is according to Mahdavi et al., who observed peak around 3355 cm^{-1} [24]. The broad peak at 1541.40 cm^{-1} due to the CN stretching vibrations modes of amide bond, current peak is related to Naseem and Farrukh., who observed the peak at 1521.71 cm^{-1} [27].

The absorption peak at 1338.85 cm^{-1} is due to C-N stretching of aromatic amines which was observed by Wang et al., around 1367 and 1361 cm^{-1} [28]. Band at 1074.86 cm^{-1} is because of stretching vibrations of C-O-C bond. According to Solimanzadeh et al., bands in between 1000 and 1616 cm^{-1} indicate the occurrence of phenolic compounds [23]. The presence of iron nanoparticles was confirmed by peak found at

541.66 cm^{-1} which is due to Fe–O bond stretching vibrations. Wang et al., also observed Fe–O bond stretching vibrations approximately at 540 cm^{-1} . In our present findings, FTIR spectra confirm the synthesis of FeO NPs and the presence of polyphenols in biosynthesized IONPs-CS.

ANTIBACTERIAL ACTIVITY

The antibacterial activity of chemically synthesized nanoparticles was studied through agar well diffusion method [19]. Iron oxide nanoparticles displayed significant antibacterial potential against both (gram positive) and (gram negative) bacteria *Macrococus* and *E. coli* respectively. Ciproflaxin, an antibiotic was also used as standard for investigating antibacterial potential of *macrococus* and its zone of inhibition was calculated 30.3mm. IONPs-CS exhibited 12.6mm and 11.3mm area of inhibition against *E. coli* and *Macrococus* respectively. Inhibition zone size was different according to the type of bacterium. IONPs-CS was potent against *Macrocococcus* and *E. coli* bacterial strains

[25]. Similarly, Devathaet al., found that IONPs synthesized by plant extract exhibited excellent antibacterial potential against gram negative and gram positive bacterial strains [29]. Similar results observed Daniel et al., who produced IONPs and their potent antimicrobial effect was estimated against human pathogens (gram negative) bacteria *Escherichia-coli*, (gram positive) bacteria [30].

CONCLUSION

The present results showed eco-friendly as well as non-toxic method for the synthesis of IONPs using citrus sinensis peels. Current study proposed an efficient and simple way for the production of IONPs with high antibacterial property. These iron oxide nanoparticles are stable, safe to use, easy to handle and are cost effective. From these findings it concluded that, the biosynthesized IONPs could be effectively used in the field of medicine as well as in food industry due to their potential antibacterial activity. Consequently, our present study shows an imperative role in this direction.



Figure 1. IONPs-CS and attachment with magnet

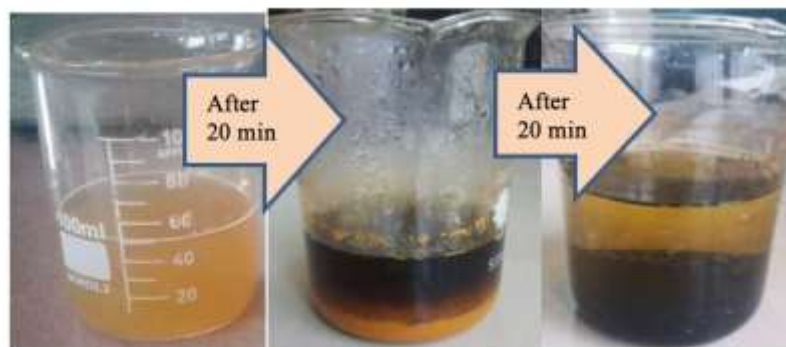


Figure 2. Change in color during formation of iron nanoparticles.

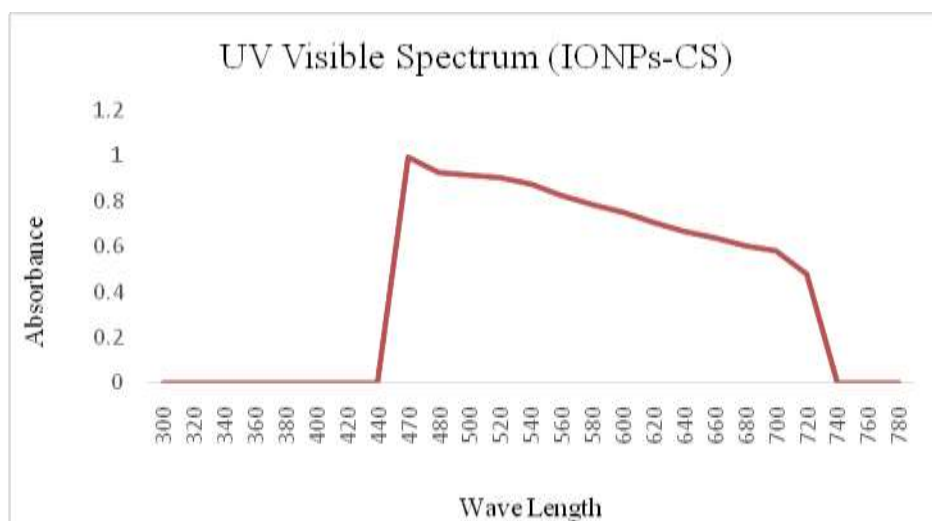


Figure 3. UV-VIS spectrum of IONPs-CS

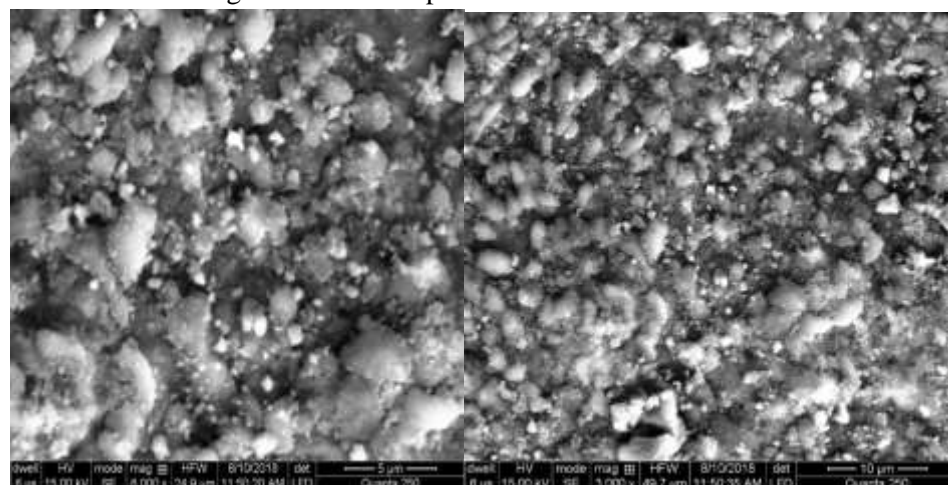


Figure 4. SEM images of IONPs-CS under different magnifications

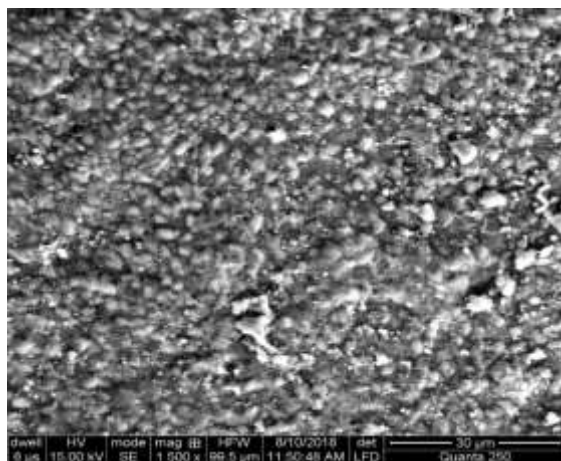


Figure 5. SEM images of IONPs-CS under different magnifications
Size Distribution by Intensity

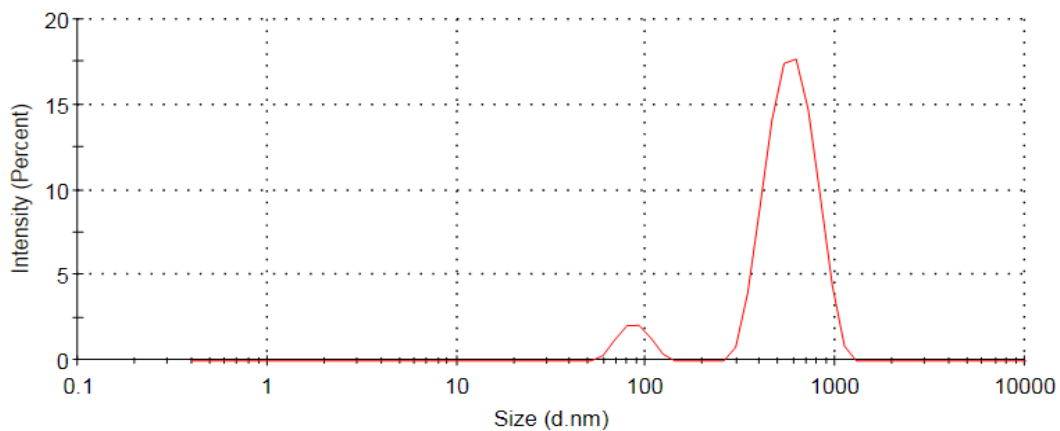


Figure 6. Size distribution by intensity of IONPs-CS

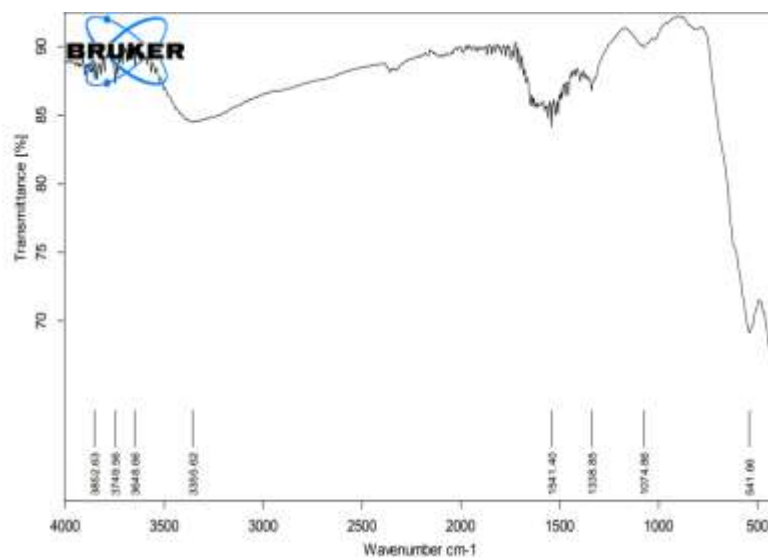


Figure 7. FT-IR spectra of IONPs-CS

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