

JOURNAL OF BIOTECHNOLOGY AND FOOD ENGINEERING

Volume: 2 Issue: 1
January-March, 2024
ISSN: 2959-0116



DOI:

<https://doi.org/10.5281/zenodo.11060548>

Research Article



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Green Synthesis, Characterization and Antimicrobial Activity of Iron Nanoparticles Using Hibiscus Leaf Extract

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ABSTRACT

Green chemistry has a significant role in improving and protecting the global environment and they are even competent over other conventional methods. The present research work was focused on the synthesis of iron oxide nanoparticles using hibiscus leaf extract as a plant resource. This method has been proven cost-effective, simple, economical, eco-friendly, and non-hazardous. The formation and nature of synthesized iron oxide nanoparticles were characterized by UV-Vis spectroscopy, FTIR, XRD, EDAX, and SEM techniques. The synthesised iron nanoparticles exhibited higher antimicrobial activity than the known standard (Chloroamphenicol). The synthesised sample was used effectively in the reduction of 4NP to 4AP in the presence of NaBH₄. The time required for the reduction of the 4NP was found to be 25 min. The present work focused on providing an alternative approach for treating PRW in an eco-friendly, economical, and efficient mode with a considerable reduction in time requirement for treating wastewater.

ARTICLE HISTORY

Received 3 January 2024

Revised 23 February 2024

Accepted 23 March 2024

KEYWORDS

Iron nanoparticle, eco-friendly, characterization, hibiscus

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INTRODUCTION

Nanotechnology is the ability to measure, see, manipulate, and manufacture things on an atomic

or molecular scale, usually between one and 100 nanometres. Nanoparticles show various applications such as environmental, food, health care, optics, healthcare, chemical industries, etc.

Nanobiotechnology is a part of nanotechnology and multidisciplinary in nature which investigates the use of nanoparticles in biological systems. Nanobiotechnology provides a crucial technique for the development of a clean, nontoxic, and environment-friendly process for metal nanoparticle synthesis which can reduce metals by specific metabolic pathways. Nanoparticles show specific characteristics as compared to large particles such as their morphology, size, and distribution. Chemical and physical methods for the synthesis of nanoparticles are costly and release toxic byproducts in nature. Due to these problems, there is a requirement for an alternative for the synthesis of nanoparticles. It has also been seen that silver nanoparticles synthesized from chemical methods show less antibacterial activity as compared to the nanoparticles synthesized from a biological approach. This may be due to the presence of protein coating of nanoparticles obtained from plant extract. These tiny products also have a large surface area to volume ratio, which is their most important feature responsible for the widespread use of nanomaterials in mechanics, optics, electronics, biotechnology, microbiology, environmental remediation, medicine, numerous engineering fields and material science [1].

Accessibility of good-quality food on a sustainable basis has become a global life-threatening problem. Nanotechnology is an emerging strategy designed to eliminate the awful effects of food shortage, heavy metal contamination, and other abiotic stresses. Iron

oxide nanoparticles play a proficient role in increasing the growth, development, and enhancement of the stress tolerance of plants and the provision of nutrients. It has also been revealed that iron oxide nanoparticles have a high sorbent affinity toward hazardous contaminants such as arsenic. Because iron oxide nanoparticles seem to have revolutionized the world by providing proximal food, efficient ways to combat diseases, and proficient methods to resolve environmental concerns, it is necessary to study the behavior, response, and ultimate fate of these iron oxide nanoparticles.

Synthesis of metallic nanoparticles was performed by a variety of physical and chemical methods [2]. However, these methods may use toxic chemicals and are harmful to the environment [3]. Recently, biological or green synthesis of nanoparticles (NPs) received enormous attention over the physical and chemical synthesis, as it is a clean, non-toxic, and eco-friendly approach that includes the design and development of syntheses using renewable materials, benign reaction media, and non-hazardous as well as non-toxic solvents [4, 5].

Metal oxide NPs are finding increasing applications in a wide range of fields and represent about one-third of the consumer products nanotechnology market [6]. These materials are used as pigments in paints (TiO_2), as sunscreens and cosmetics (TiO_2 , ZnO), as antimicrobial agents (MgO , CuO), in industrial operations (Al_2O_3 , MnO_2), and for medical purposes (Al_2O_3 , Fe_3O_4 , Fe_2O_3). Aluminum

nanomaterials act as drug delivery systems, by encapsulating the drugs the drugs to increase solubility for evading clearance mechanisms and allowing the site-specific targeting of drugs to cells [7]. Previous toxicological studies on nanomaterials were conducted on TiO₂, CdO₂, C₆₀, and carbon nanotubes only [8, 9]. The toxicity of iron oxide nanoparticles (IONPs), although they are the only metal oxide nanoparticles approved for clinical use, has been investigated only in a small number of studies. Plant-mediated NP synthesis is currently the most efficient method to produce large-scale NPs in a short time. The bioactive components contained in the plant extract act as reducing and capping agents in the synthesis process and reduce the metal ions to NPs [10, 11]. The overall synthesis procedure is simple, cost-effective, reproducible, and sustainable [12-14]. Hence, biologically formed NPs have superior properties as compared to chemically synthesized NPs.

The present investigations focused on the synthesis of iron nanoparticles using leaf extract. It was characterized using different analytical and spectroscopic techniques. Further, it was subjected to antimicrobial study against different microbial species. Then, the photocatalytic degradation of methylene blue by solar irradiation technique was also performed. The petrochemical refinery wastewater treatment is also accessed.

EXPERIMENTAL METHOD

Collection of Plant Material and Preparation of Extract:

Hibiscus rosa-sinensis (HR) leaves were collected from, Courtralam, Thirunelveli District, Tamil Nadu, India. The plant was authenticated by Botanical Survey of India. The fresh leaves were used for all experimental procedures. The Hibiscus leaf shown in Fig. 1 was washed several times with deionized water. 20 g of hibiscus leaf was finely cut and stirred with 200 mL de-ionized water at 300 K for 1 min and filtered to get the extract. The filtrate is used as a reducing agent and stabilizer.



Figure 1. Hibiscus Leaf Used For The Synthesis of Nanoparticles

Chemicals:

The chemical Ferric chloride hexahydrate (FeCl₃·6H₂O, 98%) and solvent used in the study were of highest purity and analytical grade purchased from Sigma Aldrich.

Extraction Preparation:

The HR plant leaves were washed and stored at -4 °C. For the production of extract, ground, air-

dried HR samples about 5 g were boiled with double distilled water (100 ml) in an Erlenmeyer flask while being continuously stirred for 15 min. The extract was cooled to room temperature after that filtered, and stored at $-4\text{ }^{\circ}\text{C}$ for further use.

Preparation of Fe₃O₄-NPs:

Iron oxide nanoparticles were synthesised by adding 0.01 M FeCl₃·6H₂O solution to the HR extract in a 1:1 volume ratio. Fe₃O₄-NPs were immediately obtained with the reduction process. The mixture was stirred for 60 min and then allowed to stand at room temperature for another 30 min to obtain colloidal suspension. The mixture was centrifuged and washed several times with ethanol and then dried at 40 °C under vacuum to obtain the Fe₃O₄- NPs. HR leaves have the best reduction capability against ferric chloride when compared to other parts of the plants (seeds and fruit) as observed by the external color change. After the confirmation test, the Fe₃O₄-NPs were synthesised by using the above procedure for further characterisation.

Characterization of Nanoparticles:

Characterization techniques help us to understand the specific properties of the substance or nanocrystals to be studied in an accurate rapid manner which is reliable for understanding the measured values. The synthesised Fe₃O₄-NPs were subjected to various characterisation studies to understand the specific properties such as optical, structural, morphological, elemental composition, particle size, and functional groups studies which could be made precisely using sophisticated techniques such as UV-VIS

spectroscopy (SHIMADZU 3600 UV-Vis NIR model), Zeta sizer (Malvern make), XRD (PANalytical X'Pert Pro instrument with Cu K α 1 radiation of wavelength (λ) of 1.5406 ($^{\circ}\text{A}$)), SEM, FT – IR (SHIMADZU FTIR Affinity 1) instruments. These techniques helped verify our method is well optimised and meets the requirements.

RESULTS AND DISCUSSION

The leaf extract of *H. Rosa sinensis* was utilized as a reducing agent to arrive at the morphology of iron oxide nanoparticles because it contains a huge number of polyphenols and other organic scaffolds. It has been found that more than 50 species are present out of which 1/3rd of the total is polyphenols [15] that assist in the reduction of the salt precursors to nanoparticles. These phytochemicals are accountable for the formation of NPs.

The extract plays a double role in the process of NP synthesis; it reduces the metallic salts to NPs and act as stabilizing agent hindering the aggregation of the synthesized NPs. The light yellow color of reaction mixture changes to dark brown on incorporation of leaf extract and slowly to red brown, indicating the formation of iron(III) oxide particles. The extract of *H. Rosa sinensis* may not be able to reduce Fe³⁺ to Fe⁰; instead, the organic components of the leaf extract react with the iron ions to give iron oxide NPs, as the first-row transition metals are prone to oxidation. Confirmation for the formation of iron oxide NPs is given by XRD. From the XRD data, it can be

concluded that particles of iron oxide are crystalline in nature and free from impurities.

UV-Visible spectroscopy:

The phytochemicals include hydroxyl, carboxyl, and amino functional groups, which can serve both as effective metal-reducing agents and as capping agents to provide a robust coating on the metal nanoparticles in a single step and lead to the colour change of Yellow to brown. This colour change confirmed the synthesis of Fe₃O₄-NPs. The synthesised iron oxide nanoparticle was confirmed using the UV-visible spectrum (Fig.2).

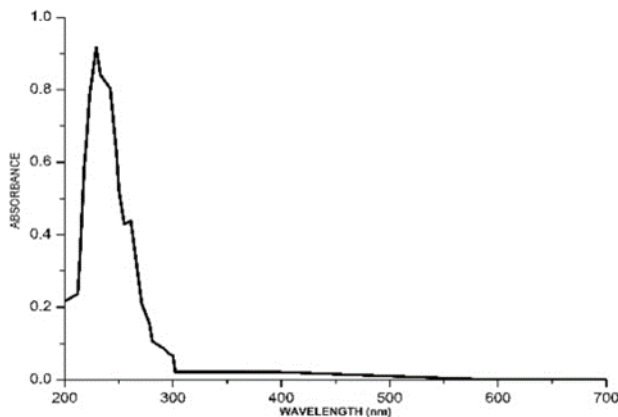


Fig.2. UV-Visible spectrum of iron oxide nanoparticles

FT IR Spectroscopy:

The FT-IR analysis gave the stretching vibrations at 3444 cm⁻¹, 1633 cm⁻¹, and 533 cm⁻¹ within the region of 400-4000 cm⁻¹ (Fig.3.). These peaks represent the following bonding in the sample confirms the reducing agent role in the formation of Fe₃O₄-NPs. The peak at 3444 cm⁻¹ corresponds to the -OH bond stretching denotes

the aqueous phase as well as the reduction of the Ferric chloride, 1633 cm⁻¹ corresponds to the C=O bond stretching denotes the phytochemicals present in the plant extract and amino acids which stabilise as well as act as a capping agent. The remaining unclear peaks represent a small amount of organic acids which is responsible for the low pH of the sample which helps to the synthesis of the Fe₃O₄-NPs. The strong peak at 533 cm⁻¹ corresponds to the inorganic stretching indicating the Fe₃O₄-NPs. Further, the strong vibration was observed at 466 cm⁻¹ corresponds to Fe-O vibration.

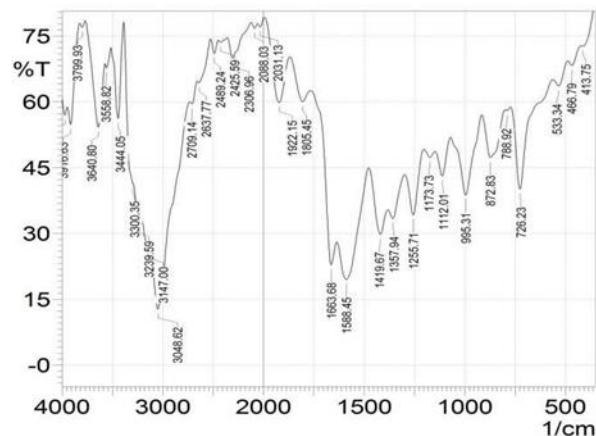


Figure 3. FT IR Spectrum of Iron NPs

SEM analysis of Iron Nanoparticles:

The formation of Fe₃O₄-NPs and its morphological dimensions were studied using the SEM. The observations demonstrated that the average size of the NPs was in the range of 30 nm – 100 nm similar phenomenon was reported in the previous studies. That also exhibits the formation of needle shape iron nanoparticles as shown in Fig. 4. The needle-shaped nanoparticle formation

induced by phytochemicals present in the extract influences the morphology of the nanoparticles. Due to the very narrow electron beam, SEM micrographs have a large depth of field yielding a characteristic three-dimensional appearance useful for understanding the surface structure of a sample.

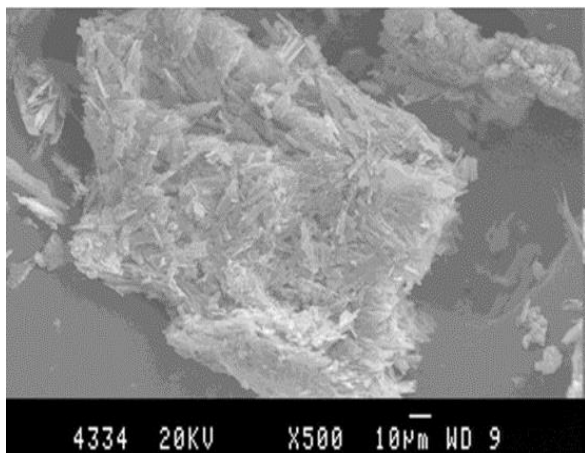


Figure 4. SEM image of FeNPs

XRD Pattern of Prepared Nanoparticles:

The X-ray diffraction patterns obtained for the Fe₃O₄-NPs synthesised using HR extract is shown in Fig.5. It is found that the strong diffraction peaks with 2θ values of 28.26°, 32.28° corresponding to the hkl value of 220, 222, that denotes the crystalline phase of Fe₃O₄-NPs matches with JCPDS card No. 39-1346 and JCPDS card No. 89-4319 for Fe₃O₄ - Nanoparticles, the grain size has calculated using Debye-Scherrer equation, which gives a relationship between peak broadening in XRD and particle size. Using the Scherrer equation the average crystallite sizes of the Fe₃O₄-NPs are

found to be in the range of 14 nm - 18 nm. The results indicated that all the nanoparticles were in spinel structure with face-centered cubic phase.

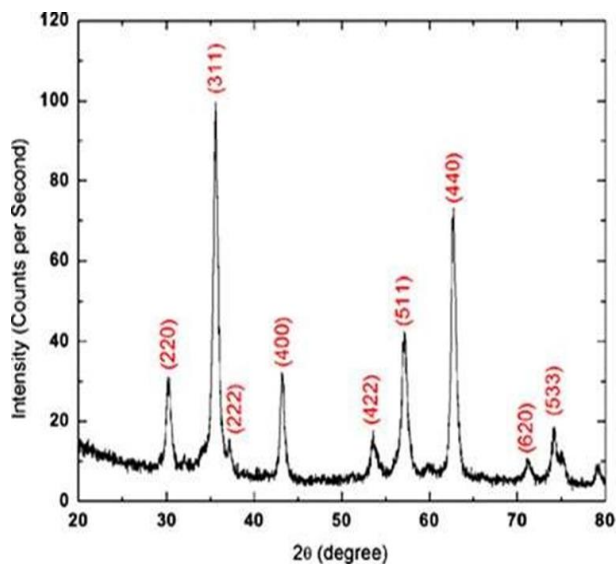


Figure 5. XRD spectrum of Fe₃O₄-NPs

Antibacterial activity:

The antibacterial activity of samples was determined using a well diffusion method. The antibacterial activities were performed by using *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, and *Pseudomonas aeruginosa*, respectively. The nutrient agar medium was boiled to dissolve completely and sterilized at 15 lbs pressure (120°C). After sterilization, 20 mL of media was poured into the sterilized petri plates. These plates were kept at room temperature and the medium solidified in the plates. Then, it was inoculated with microorganisms using sterile swabs. The stock solutions were prepared by dissolving the compounds in appropriate

solvents. The sample solutions were filled in the incubated plates using a micropipette and incubated for 24 hrs at 37 °C. During the incubation period, the sample solution was diffused into the gel and inhibited the growth of the microorganism. The zone of inhibition was developed on the plate and measured.

Antibacterial properties of phytogetic Fe3O4-NPs:

The antibacterial properties of phytogetic IronNPs have been tested under well diffusion method against different bacteria strains such as Escherichia coli, Staphylococcus aureus, and Staphylococcus typii. The above three bacteria strains showed a zone of inhibition at 20 mm, 17 mm, and 16 mm respectively (Table 1 and Fig.6). The antibacterial activity of prepared iron nanoparticles was greater because smaller particles have a bigger surface area, which can enhance the capability to go through the cell membrane and provide a more bactericidal effect.

The iron NPs and secondary metabolites present in HR leave extract showed synergistic effects and destroyed the bacterial cell walls by impairing membrane integrity and cell respiration due to the decline of the energy compounds. Different responses of bacteria strains against the phytogetic IronNPs in various reports may be due to various factors such as initial CFU used of the bacterial samples, different origins of bacterial strains, used precursor material for the synthesis of NPs, use of different reducing and capping agents and difference in the size and shapes of AgNPs. The iron ions from iron

nanoparticles are believed to become attached to the negatively charged bacterial cell wall and rupture it, which leads to the denaturation of protein and finally cell death. NPs have a large surface-to-volume ratio, so they can strongly adhere to the cell surface of the fungus. Also, due to its small size, it can directly penetrate the cell and damage the cell wall. Inactivation of fungus by iron oxide NPs involves the direct interaction between NPs and cell surfaces, which affects the permeability of membranes where NPs enter and induce oxidative stress in fungus cells, subsequently resulting in the inhibition of cell growth and eventually cell death [16]. Possibilities of membrane damage caused by direct or electrostatic interaction between iron oxide NPs and cell surfaces, cellular internalization of NPs, and the production of active oxygen species such as H2O2 in cells due to metal oxides have been reported in the literature [17,18].

Table 1. Antibacterial activity test of Fe3O4-NPs – Zone of inhibition

Name of copound/ standard	Zone of inhibition (mm)		
	S. aureus	E. coli	S.typii
FeNPs	23	18	19
Chloroamphenicol	27	29	24

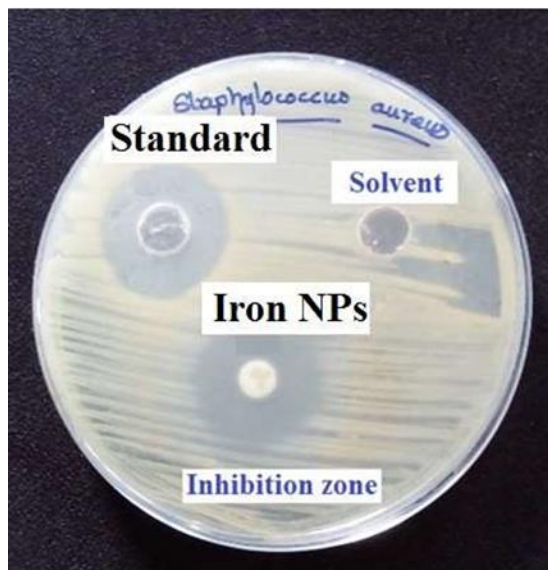


Figure 6. Zone of inhibition of FeNPs against *Staphylococcus aureus*

Treatment of petroleum refinery wastewater:

Petroleum Refinery Wastewater (PRW) was collected from the nearby refinery, which is situated in Chennai, Tamil Nadu, India. The composition of PRW depends on the complexity of the refining process but in general, compounds in PRW include dissolved and dispersed oil and dissolved formation minerals. The sample collected is subjected to analyze of the initial characteristics.

Industrialization is linked to major pollution sources of hazardous pollutants in water bodies, especially in developing countries where untreated or only partially treated industrial wastewater is discharged into the environment. This poses considerable environmental problems because most of the people's livelihood in the

pollution-affected communities depend on the water bodies for fishing, domestic use, and irrigation. There is an increasing awareness of the need for the discharge of well-treated wastewater into the environment, especially with the recent agitations for the demand for compensation by oil pollution affected communities in Nigeria based on human and environmental rights reported that their struggle for survival was more an ecological than a political one. Hence, there is a need for economically and ecologically friendly refinery wastewater treatment technologies.

Experiments were conducted to analyze (pH, COD, and Nitrates) of PRW and were found out to be 7.2, 1350 mg/l, and 6.0 mg/l. Experiments were conducted daily using Fe₃O₄-NPs for treating PRW and results were obtained for all the proportions and 5th day. On the 5th day, 75% removal of COD and nitrates was achieved for 1:5. The removal efficiency is showing a sharp increase of about 55% for COD and 75% for nitrates on the 5th day for 1:5. It is mainly due to the increase in active sites for adsorption to takes place as explained by other studies. An attempt is made to identify the effect of some selected polyphenols on the removal efficiency. It is significant to note that an increase in polyphenol content increases active sites for FeNPs adsorption to serve as a reducing/capping agent and hence it showed high removal efficiency without any pH adjustment.

Degradation of Methylene Blue by Fenton-like Catalyst:

The need for the purification of wastewater is of great concern to today's world. The water gets polluted by various ways. Pollution by the dye is one of these factors. The water containing organic pollutants such as dyes coming out from textile industries affects the biological cycle mainly photosynthesis in aquatic plants and also makes it unfit for human use and also for marine animals. Many studies have shown that some of the dyes can also be carcinogenic and mutagenic. In this study, we have synthesized an eco-friendly method of treating these pollutants on a large scale and making them free from these pollutants for different uses. The sample that was collected at regular intervals was characterized by UV-vis spectroscopy. The peak of the MB dye is found at 668 nm. With the increase in the reaction time, the intensity of the peak gradually decreases and at 80 min of the reaction, the peak at 668 nm nearly vanishes. It indicates that the total MB present in the solution has been degraded as shown in figure 7.

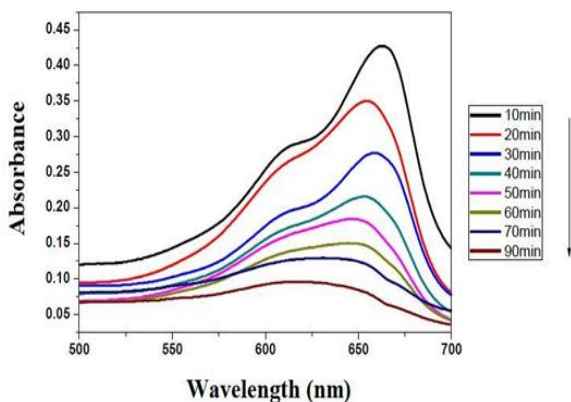


Figure 7. UV-Vis., spectrum for methylene blue degradation

Reduction of 4-Nitrophenol: 4-Nitrophenol is a common organic pollutant in wastewater. 4-NP has many ill effects in the body of the human body such as reacting with blood and forming methemoglobin that is responsible for causing unconsciousness. Different methods are being carried out for purification purposes such as adsorption, photocatalytic degradation, electrochemical process, etc. 4-NP is also a starting material for the production of the 4-AP that has many applications such as an anti-corrosion lubricant, hair dyeing agent, antipyretic drugs, and many more. In this study, we have synthesized a catalyst and reduced 4-NP to 4-AP using Au as the primary catalyst and iron oxide as the catalyst carrier. The confirmation of the reduction of 4-NP was done by UV-vis spectroscopy as shown in Figure 8. It has been analyzed from the graph that the peak for the reactant sample showed a peak at 400nm. After the completion of the reaction (Nitrophenol in the presence of NaBH₄ with Fe₃O₄-NPs), the peak vanished confirming the reduction of 4-NP.

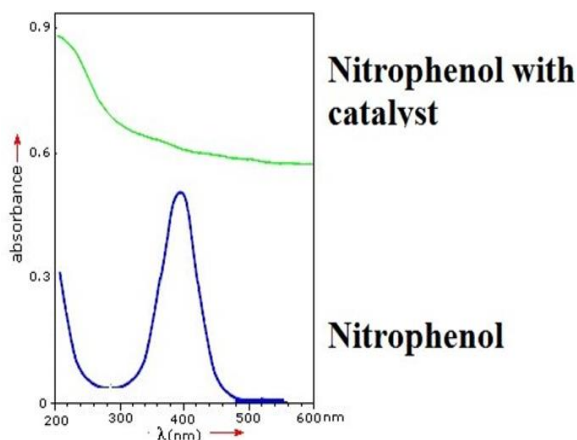


Figure 8. UV-Visible spectrum for reduction of 4-nitrophenol using FeNPs

CONCLUSION

Iron oxide nanoparticles are continuously drawing researchers' attention because of their unique electronic and physiochemical properties in the fields of catalysis, environmental remediation, bio-imaging, drug delivery, and so on. Iron oxide nanoparticles are mainly prepared by the chemical reduction of iron precursors, but the environmental toxicity and expensiveness of reducing agents limit their application. Recently many people reported that leaf extract as a reducing agent for the synthesis of a variety of NPs as an economical, environmentally friendly, and biocompatibility source. Synthesis of iron nanoparticles using plant extract is useful not only because of its reduced environmental but also because it can be used to produce large quantities of nanoparticles. Plant extracts-based synthesis of nanoparticles gained attention because of its eco-friendly nature. The ability of water extract from locally available plant

materials in the formation of iron oxide nanoparticles was screened in the present study. Plant extracts were mixed with a ferric chloride solution for the synthesis of nanoparticles.

In the present study, the synthesis of iron nanoparticles using Hibiscus Rosa sinensis leaf extract was performed. The synthesized iron NP was characterized using Uv-Vis., and FT IR techniques. The particle morphology and size were studied using SEM and XRD techniques. It is noticed that phenolic content in the reaction mixture decreases during the formation of nanoparticles. This indicates the putative role of the hydroxyl group present in the phenolics as a priming agent of iron oxide nanoparticle formation via the reduction of Fe^{3+} to Fe^{2+} during green synthesis. Then, the study is emphasized to present the effect of size of nanoparticle formed and concentration of polyphenols present in HR leaves, and its efficiency in treating PRW (COD and Nitrates). Further, Its antibacterial activity also had been studied considering three bacterial strains. It is evident that the augmented polyphenolic content along with FeNPs resulted in increased ROS production and accumulation of FeNPs within the cytoplasm region due to size variation showed increased antibacterial activity. The synthesized sample was used effectively in the reduction of 4NP to 4AP in the presence of NaBH_4 . The time required for the reduction of the 4NP was found to be 25 min. The present work focused on providing an alternative approach for treating PRW in an eco-friendly, economical, and

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