Experimental Investigation on Green Synthesis of FeNPs using Azadirachta indica leaves

Vijay Devra (v_devra1@rediffmail.com)
Janki Devi Bajaj Government Girls College https://orcid.org/0000-0002-7885-9179
Ajay Rathore
Janki Devi Bajaj Government Girls College

Research Article

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Experimental Investigation on Green Synthesis of FeNPs using *Azadirachta indica* leaves

Ajay Rathore, Vijay Devra*

V_devra1@rediffmail.com

Department of Chemistry, Janki Devi Bajaj Government Girls College, Kota, Raj.

Abstract

In the field of nanotechnology, developing an environmentally friendly method for the synthesis of iron nanoparticles (FeNPs) an important aspect. The use of secondary metabolites from plant leaf extract has recently emerged as a novel technology for the synthesis of various nanoparticles, according to recent studies. The leaf extract of *Azadirachta indica* was used to synthesize iron nanoparticles in this research. The effects of reactant concentrations, reaction temperature and pH of the solution on the synthesis process of iron nanoparticles were studied. The formation of iron nanoparticles in dispersion was monitored using a UV-Visible Spectrophotometer that analyzed absorbance spectra. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) characterized the morphology of iron nanoparticles and results reveal the particles are spherical in shape with an average size of 48 nm. The following are the ideal conditions for synthesis: leaf extract 15%, [$FeCl_3$] = 1.0 mM, pH 6.0 and temperature 60°C. The plant biomolecules induce the reduction of Fe^{3+} ions to FeNPs and act as a capping and stabilizing agent, which is confirmed by the FTIR technique. Therefore, they have good stability for various applications.

Keywords: Green Synthesis, *Azadirachta indica*, Iron nanoparticles, Experimental investigation

1 Introduction

Currently, nanotechnology has become a technology that has revolutionized every field of applied science. One of the nanotechnology networks associated with nanoscale materials with particle sizes ranging from 1 to 100 nm is the field of nanoparticles (NPs). Metal nanoparticles have distinct chemical, electrical, optical, magnetic, and mechanical properties over bulk

materials due to their high surface to volume ratio and size effects [1]. Researchers have paid a lot of attention to nanoparticle synthesis, which has controlled morphologies and important characteristics, making it a large field of study. One of the key priorities in chemistry that could be used for future applications has been a marked increase in the field of biosynthesis of metal nanoparticles (MNPs) in recent years, with control over particle size, shape, and crystalline nature [2]. Additionally, in comparison to their bulk counterparts, transition metal nanoparticles have a wide range of applications due to their high surface to volume ratio and multiple oxidation states. Transition metals are defined as *d*-block elements as their atomic structures have incomplete d-orbitals in their electron configuration and, therefore, give rise to physical and chemical properties that differ from the main group elements [3]. This provides a large number of configurations for their oxides, thus, widening the field of applications for transition metals.

The reactivity of nanoscale iron with a high surface area to volume ratio [4] has made iron nanoparticles (FeNPs) one of the most promising metallic nanoparticles for numerous applications. The use of a more convenient greener route to synthesize iron nanomaterials like metallic iron and iron oxide is a big step forward in nanomaterial production. Nanoparticle synthesis can be accomplished in two ways. One strategy is top-down, while the other is bottom-up. For the synthesis of nanoparticles, a top-down technique uses size reduction from a suitable starting material [5]. The physical technique includes the use of high energy, pressure, and temperature intake, while the chemical technique requires the use of dangerous and harmful chemicals that lead to environmental contamination [6]. Smaller entities are joined together to form nanoparticles in bottom-up synthesis. Smaller entities are created first, and then these entities are assembled to produce the final particles, which are in the nanometer scale, using chemical and biological methods. While biological methods take longer to synthesize nanoparticles than chemical methods, the time can be reduced in half by using the appropriate microorganism or organism. [7]. As a result, the advantages of biological methods over physical and chemical methods include a cost-effective, environmentally safe, single-step process for large-scale nanoparticle production.

Researchers, constant efforts to develop a green process for the synthesis of nanoparticles that is simple, efficient, and genuine. In order to synthesize stable and well-defined functionalized nanoparticles, several species serve as safe, eco-friendly, and green precursors [8]. For nanoparticle synthesis, a broad variety of biological tools such as microorganisms (bacteria, yeast,

fungi, algae, and viruses) and plants can be used [9]. Nanoparticles can be produced in a matter of minutes or hours, depending on the plant type and phytochemical concentration, while microorganism-based methods take a longer time [10]. Additionally, plants are favored biological tools over microbes because of their easy availability in nature [11]. In terms of eco-friendly alternatives, this method is beneficial for toxic chemicals that can be processed in comparatively less time, feasible, and can provide immense applications. Iron nanoparticles have emerged as a new class of significant nanoparticles due to their unique properties, such as superparamagnetism and high coercivity. The leaf extracts of *Eucalyptus tereticornis, Melaleuca nesophila, and Rosemarinus officinal*[12] , *Plantago major* [13], *Mangifera indica, Murraya Koenigii, Azadiracta indica, Magnolia champaca* [14] were chosen as reducing and capping agent to successfully synthesize iron nanoparticles in the past studies

Neem is a plant that belongs to the Meliaceae family and has the botanical name *Azadirachta Indica.* It has been used as a medicinal plant for many years and has a wide range of biological activity. Terpenoids, nimbaflavone, sugar, and other biomolecules are present in neem leaves extract and are responsible for metal ion reduction with capping agents [15]. Using leaf extract from the Indian medicinal plant *Azadirachta indica*, we established a quick, eco-friendly, and convenient green route for the synthesis of FeNPs from their salt solution. Different parameters, such as reactant concentrations, temperature, and pH, affected the reaction's outcome, and synthesized plant-mediated FeNPs were described using various instrumental techniques.

2 Material and Method

2.1 Chemical and Materials

Ferric chloride (E. Merck) and neem (*Azadirachta indica*) leaf extract were used in the synthesis of iron nanoparticles. Based on cost-effectiveness and ease of availability, the plant *Azadirachta indica* (neem) was chosen from Kota (Rajasthan) India. New, healthy leaves were collected, thoroughly rinsed, and allowed to dry at room temperature. For 20 minutes, ten gm of these finely incised leaves were stirred on a magnetic stirrer at 80^0C. The extract was then filtered twice through Whatman filter paper and stored in Erlenmeyer flasks at 4°C for further testing. Sterile conditions were maintained during the experiment to ensure the feasibility and precision of the findings.

2.2 Instrumentation

UV-Vis spectra were obtained as a function of the time of reaction on a double beam spectrophotometer (UV 3000+ LAB INDIA) with a resolution of 1 nm. To identify functional groups of biomolecules present in the leaves extract of *Azadirachta indica,* the spectrum was recorded using an FTIR model (ALPHA-T Bruker, Germany) transmittance mode operating at a resolution of 4 cm^{-1}. Morphological study of the Iron nanoparticles was carried out with scanning electron microscopy (SEM) (Model-Nova Nano FE-SEM 450 (FEI)) and Transmission electron microscope (TEM) (Model-FEI Techni G2S2 Twin) instrument. The presence of metal in the sample was investigated using the energy dispersive spectroscopy (EDS) technique. A pH meter (Model-MSW -552) was used to determine the pH of the reaction mixture.

2.3 Iron Nanoparticle Synthesis

Solution (aqueous) in a 250 ml Erlenmeyer flask, 1 mM ferric chloride ($Fecl_3$) was prepared, and 15% leaf broth was added to reduce Fe^{+3} ions. At 60^0C, the mixture was held on magnetic stirring. Periodic sampling and scanning with a UV-Visible (UV-Vis) spectrophotometer were used to record time and color change. The color change from light yellowish to colloidal brownish-black confirmed the complete reduction of Fe^{+3} ions. The colloidal solution was sealed and stored properly for further use. The formation of Iron nanoparticles was further confirmed by different spectrophotometric analyses. The effects of different concentrations of $Fecl_3$ solution, percentage of leaf extract, Temperature, and pH on the synthesis rate were also investigated.

3. Results and Discussion

3.1 Effect of Leaf Extract's Percentage

The color of the dispersion changed from yellow to brownish-black as a result of the biosynthesis of FeNPs, indicates the reduction of Fe^{+2} into Fe^0 particles can impart such color. Previous research has also observed similar color variations. [4,16]. The recent studies report that the optical properties of metal nanoparticles depend upon the size and shape, thus the optical response of nanoparticles can be controlled by geometry and size of nanoparticles [17,18]. During the synthesis, the process is an aqueous solution; optical spectroscopy can be used as a primary tool for confirmation for metal nanoparticles. The absorption peak was obtained at 258 nm, which can be attributed to the SPR of Fe^0 particles or FeNPs formation [19] Fig.1.

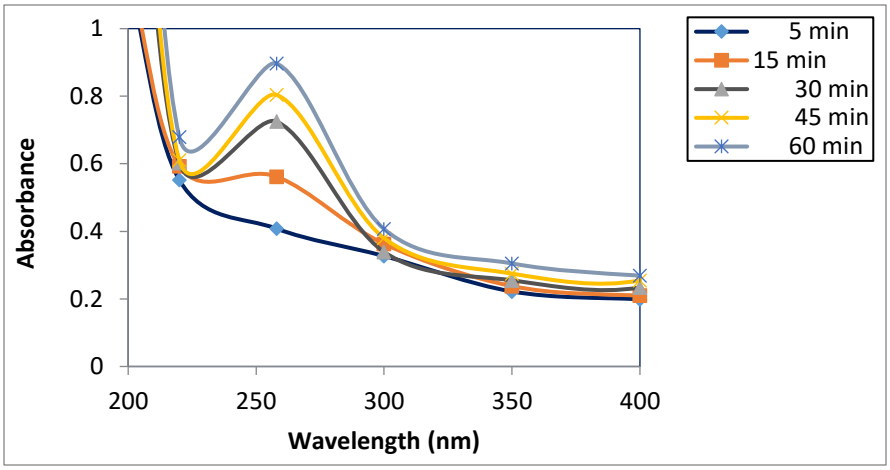

Fig.1 U.V. spectra recorded as a function of reaction at different wavelength versus absorbance during synthesis of iron nanoparticles at different time intervals.

The effect of different percentages of leaf extract on synthesis rate was investigated. When the leaf extract concentration was increased, a significant difference emerged between the intensity of UV–vis at 258 nm in varied extract percentage (5 to 15%) and 1 mM $FeCl_3$ at 60°C as shown in fig. 2. Weak absorption peak at 258 nm was observed when a low percentage (5%) of leaf broth was used, indicating that due to inadequate reduction, relatively low concentrations of iron nanoparticles were formed. The degree of dispersion of iron nanoparticles is considered to be determined by the UV-Vis absorption peak [20]. The intensity of the absorption peak at 258 nm increases as the percentage of leaf extract (5-15%) increases. However, the maximum absorption peak was found at 15% neem leaf extract, indicating that this is the optimal percentage of leaf extract for iron nanoparticles synthesis.

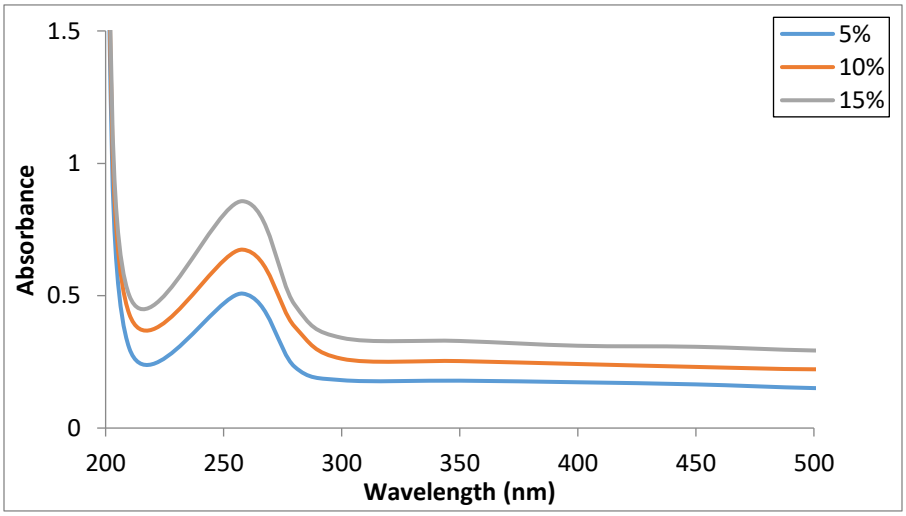

Fig. 2. U.V. spectra recorded as a function of reaction at different wavelength versus absorbance during synthesis of iron nanoparticles at different Neem leaf extract (5 %, 10 %,and 15%).

Biosynthesized Iron nanoparticles are documented that polyphenols from plant leave to be encased in a thin layer of organic material that acts as a cap [21, 22, 23]. It is also reported that the leaf polyphenols not only capped the ensuing nanoparticles[24] but also reduce the iron salts since the reduction potential of polyphenols was in the 0.3–0.8 V range, while the reduction potential of Fe was only 0.44 V [21], leading to Fe^{+3} to be reduced to FeNPs. TEM images (Fig. 3) show that the synthesized Iron nanoparticles are spherical in shape with an average size of 48 nm and encased in a thin layer of some capping material and remain stable in solution for four weeks. Energy-dispersive X-ray spectroscopy was used to calculate the elemental analysis of Iron (EDS) (Fig.4). The number of X-ray counts is shown on the vertical axis, while the energy is shown on the horizontal axis. Strong signals in the Iron region 7 Kev are revealed by EDS spectra, confirming the formation of nano iron in its elemental state [25]. Other than, these signals for C, O are observed which may originate from the biomolecules capped to the surface of the FeNPs, Mn, and Cl due to plant constituents and precursor salt respectively.

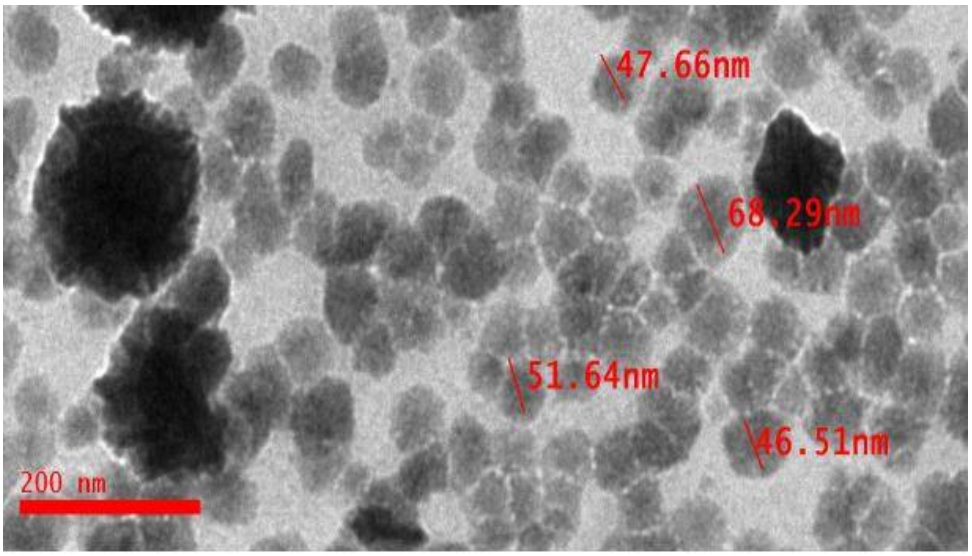

Fig. 3. TEM image of synthesized Iron nanoparticles

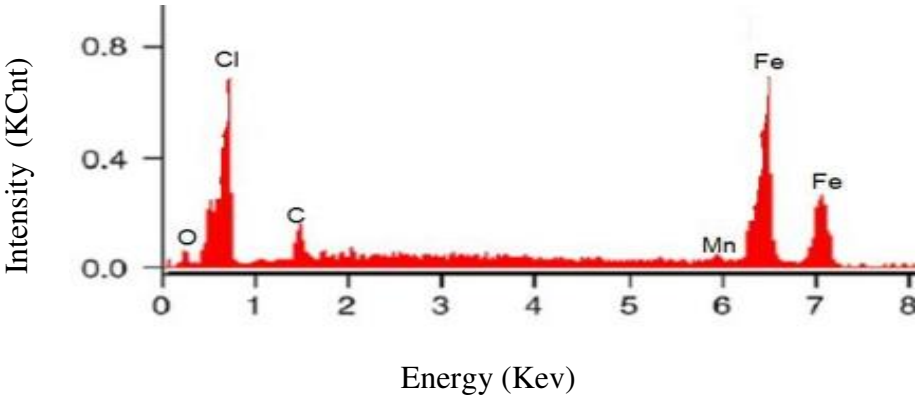

Fig. 4. Spot profile EDS spectra of synthesized Iron nanoparticles

3.2 Effect of Precursor salt Concentration

The previous study reported, when metal nanoparticles form in a solution, there are two stages. The first stage is to produce metal nuclei, and the second is to watch them expand [26]. As a result, it is critical to keep track of the preparation phase because iron nuclei must produce faster and develop slower, which necessitates better control of the Fe+3 concentration at the initial. In this research between 0.5 and 2 mM concentrations of iron chloride, the effect of initial precursor salt concentration on the formation of iron nanoparticles was investigated

The UV-Vis spectra observed as a function of reaction at various $Fecl_3$ concentrations versus absorbance during the synthesis of iron nanoparticles at various time intervals are shown in Fig. 5. It can be shown that as the concentration of Fe+3 increases, the reaction rate increases. As the reaction rate increases, the number of iron nuclei produced increases, resulting in smaller particle sizes. Figure 6 shows SEM images of the synthesized iron nanoparticles at various $Fecl_3$ initial concentrations. When the reactant concentration is too high, an excess number of nuclei is produced, according to the SEM results. This result is the agglomeration of the nuclei and growing particle size. This may be due to collision between small particles, which leads to particle growth [27]. Therefore, the optimal reaction condition is 1 mM $Fecl_3$ concentration, 15% leaf extract, 60 ^oC temperature, and pH-6.0 for the synthesis of average size 48 nm iron nanoparticles.

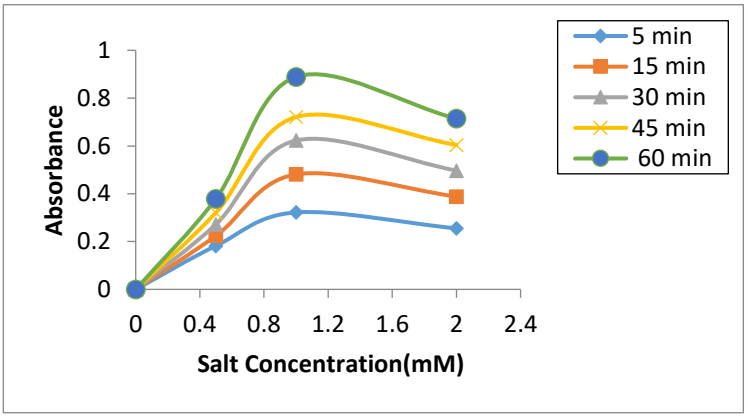

Fig.5. Time course of iron nanoparticles synthesis with different initial concentration of $Fecl_3$ (0.5 to 2 mM), leaf Extract =15%, temperature=60 ^oC, pH=6.0

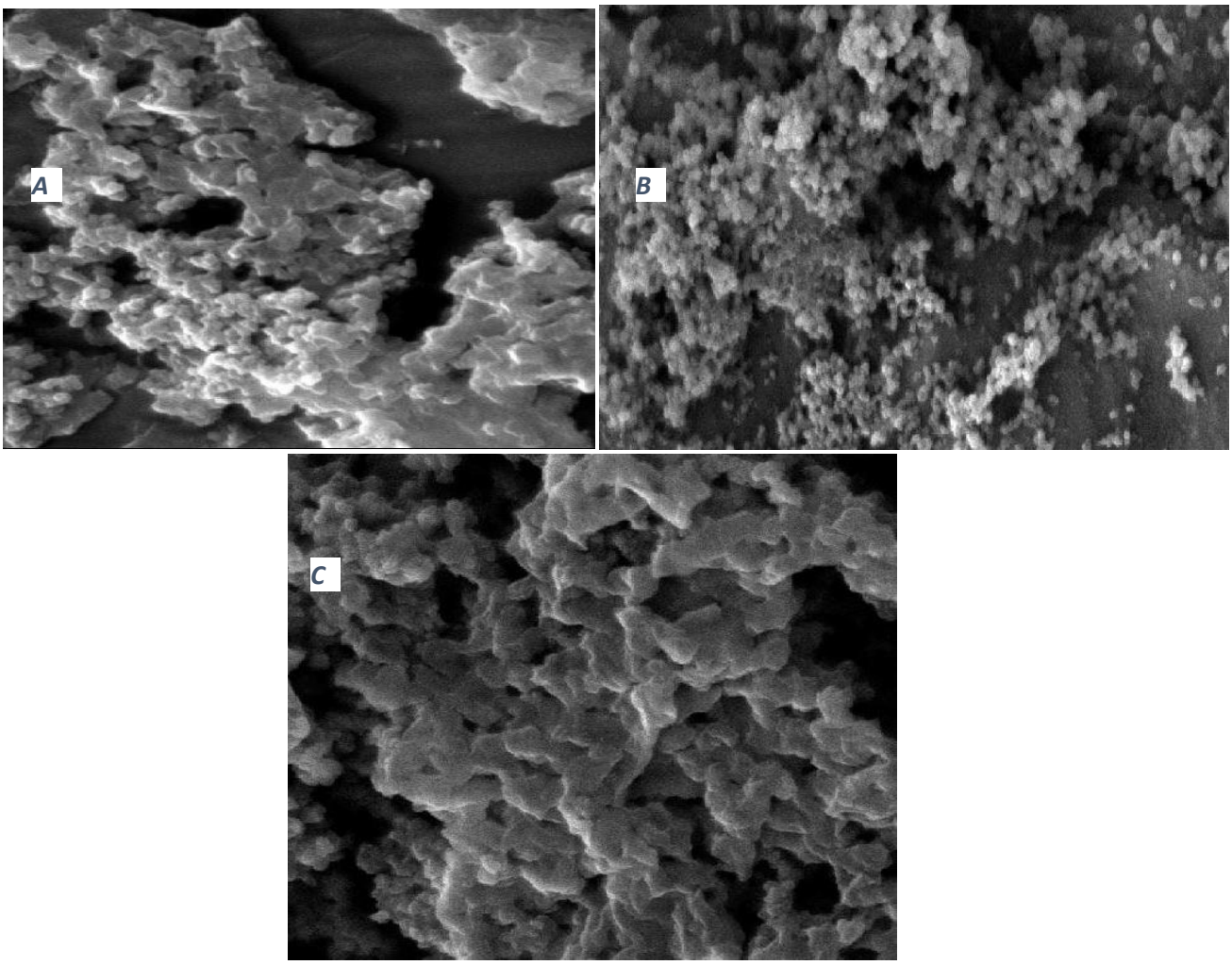

Fig. 6. SEM images of the synthesized iron nanoparticles at three concentration of the precursor salt ($FeCl_3 .6H_2 0$) (A) 0.5 mM, (B) 1.0 mM, (C) 2.0 mM

3.3 Effect of Reaction Temperature

The present study also investigated the effect of temperature on the synthesis of nanoparticles at three different temperatures viz 50^{o}. 60^{o}, 70^{o}c respectively. Fig. 7 shows that at higher temperature (70^{o}c), the nanoparticles were agglomerated, while at 60^{o}c are well dispersed with an average size at about 48 nm. The reduction of Fe+3 was increased by raising the reaction temperature. As a result, at high temperature, the synthesis rate is too high to regulate particle size. Harshiny et al. [28] found that raising the reaction temperature of iron nanoparticles synthesis with Amaranthus Dubius leaf extract increased the reduction rate. When a reducing agent was added to the precursor solution at 70°C, the rate of growth, agglomeration, and nucleation of iron nanoparticles all accelerated almost simultaneously, resulting in agglomeration of the formed iron nanoparticles. Also confirmed by SEM results Fig. 8. As a result, for the synthesis of iron nanoparticles with appropriate control on size, a moderate temperature (60°C) should be selected.

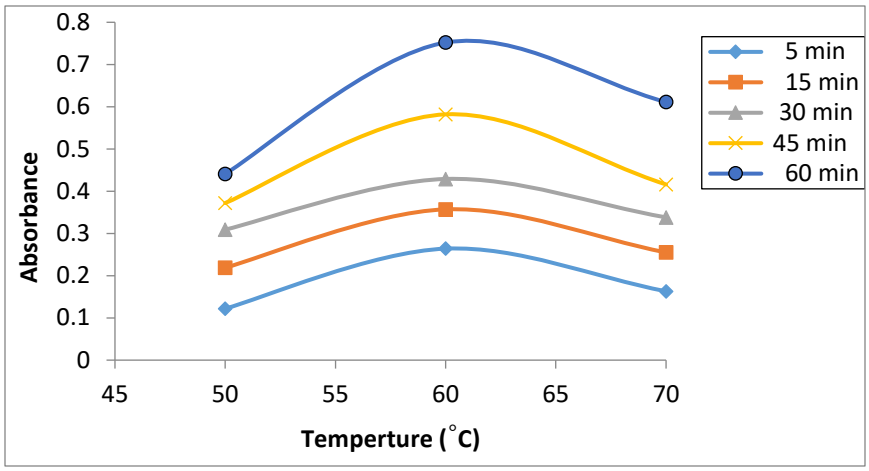

Fig. 7. Time course of iron nanoparticles synthesis with different reaction temperature (50-70 ºC), $Fecl_3$ (1 mM), leaf Extract= 15% pH=6.0

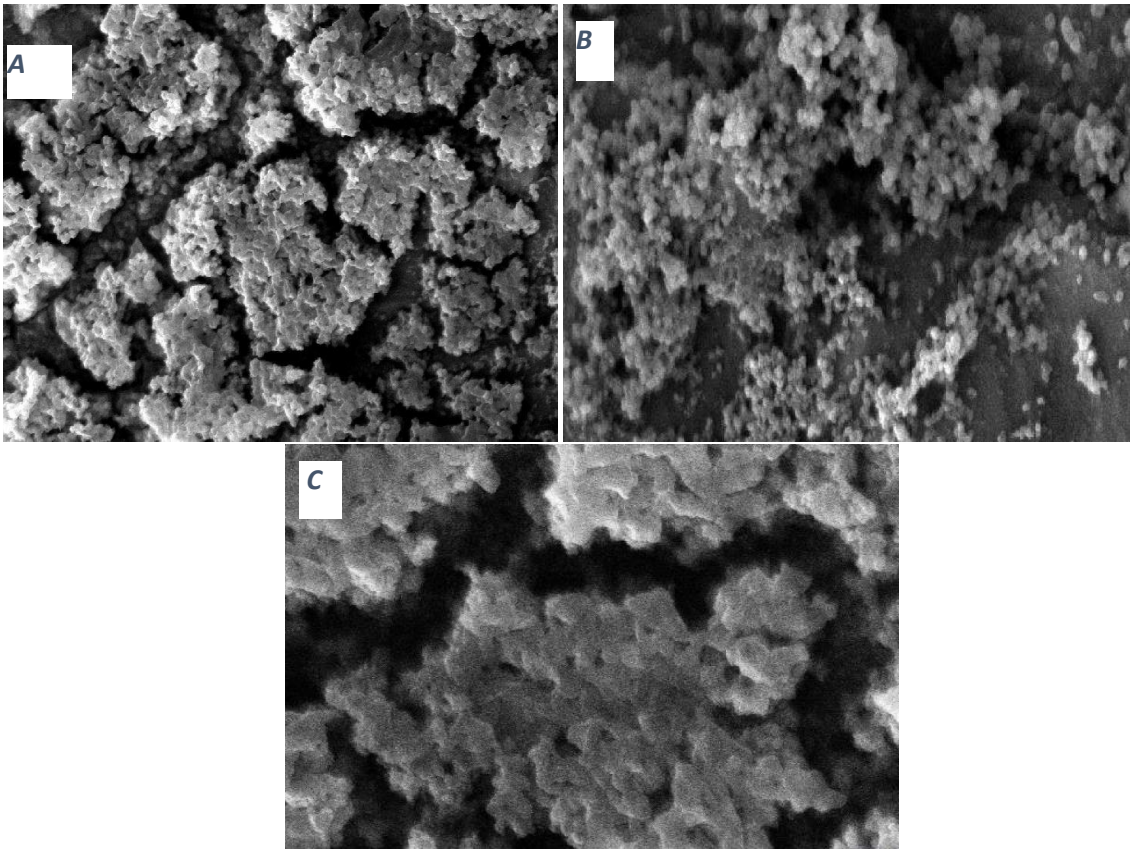

Fig.8. SEM images of synthesized iron nanoparticles at three different temperature (A) 50ºc (B) 60ºc (C) 70ºc

3.4 Effect of pH

The solution pH was one of the key factors for the synthesis of nanoparticles and its absorbance peak was shown in Fig. 9 at different pH of the solution. The rate of synthesis and morphology of NPs are affected by pH variations [29]. At acidic pH (3) conferred a less number of particles at spectrum peak at 258 nm, due to unreacted organic molecules present in the reaction solution. At almost neutral pH (6), high absorbance obtained was due to activation of phytochemicals present in the leaf extract. However, at higher pH (9) the absorbance peak was shifted, which may be an agglomeration of nanoparticles. As a result, the pH of 6.0 is favorable for FeNPs biosynthesis at 1 mM $Fecl_3$ concentration, 15% leaf extract, and 60 °C. During the synthesis process, the pH of the medium drops due to the release of H^+ ions by the species of leaf broth when they oxidize in the presence of Fe^{3+} ions. [28].

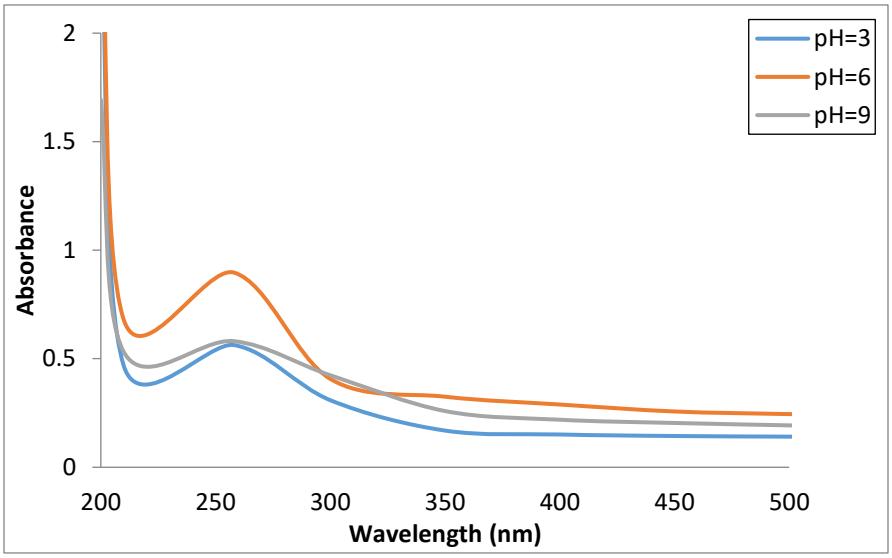

Fig. 9. U.V. spectra recorded as a function of reaction at different wavelength versus absorbance during synthesis of iron nanoparticles at different solution pH (3, 6, and 9).

ZTIR measurements were carried out to identify the potential functional groups of the bio-molecules in the leaf extract of Azadirachta indica (neem), which are responsible for the reduction of iron ions into iron nanoparticles. The synthesized Fe-NPs are stable for one month at 4°C by the bio capping of Neem leaf broth, which was confirmed by the FTIR Spectra of synthesized Fe-NPs given in Fig.10. The absorption bands observed at 3393cm^{-1} and 1627 cm^{-1} are attributed to the O-H and C=C stretching vibrations, respectively. The band that appears at 1076 cm^{-1} is related to C-O-C and absorption peaks [30]. In general, a band observed at 1384 cm^{-1} is assigned to the germinal methyl groups. From the analysis, it is clear that flavonoids could be adsorbed on the surface of metal nanoparticles by a possible interaction through electron or carbonyl groups. The presence of reducing sugar in the leaf extract may be responsible for the formation of bio-capped FeNPs.

The physisorbed leaf extract may cause steric/ electrostatic barriers around the surface of FeNPs and hence they show good stability.

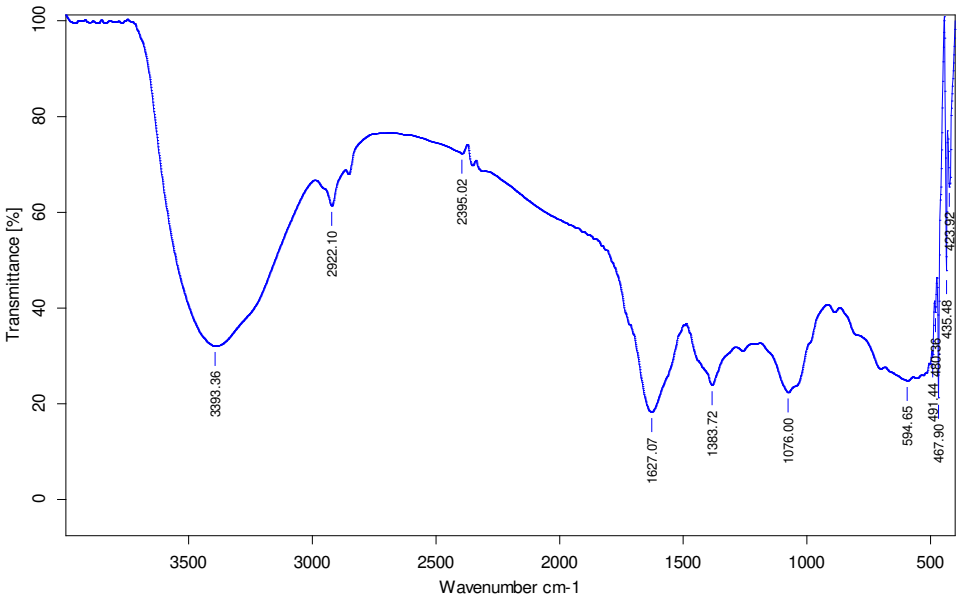

Fig. 10.FT-IR Spectra of synthesized Fe nanoparticles

4 Conclusion

The study suggests that the synthesis route is low cost, environmentally friendly, and can be prepared in simple laboratory equipment in ambient conditions. This biological reduction of iron ions would be a boon for the development of a clean, non-toxic, environmentally acceptable green approach to produce iron nanoparticles. The characterization results revels biosynthesized FeNPs is spherical with an average size of 48 nm. Moreover, it was clearly shown that the initial concentration of reactant, reaction temperature, and pH has a remarkable effect on particle size and agglomeration of the synthesized iron nanoparticles. The synthesized nanoparticles have good stability, thus have a potential for use in biomedical applications and will play an important role in the field of catalysis.

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Compliance with Ethical Standards

Conflict of interest: The authors have no conflicts of interest to declare.

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Figures

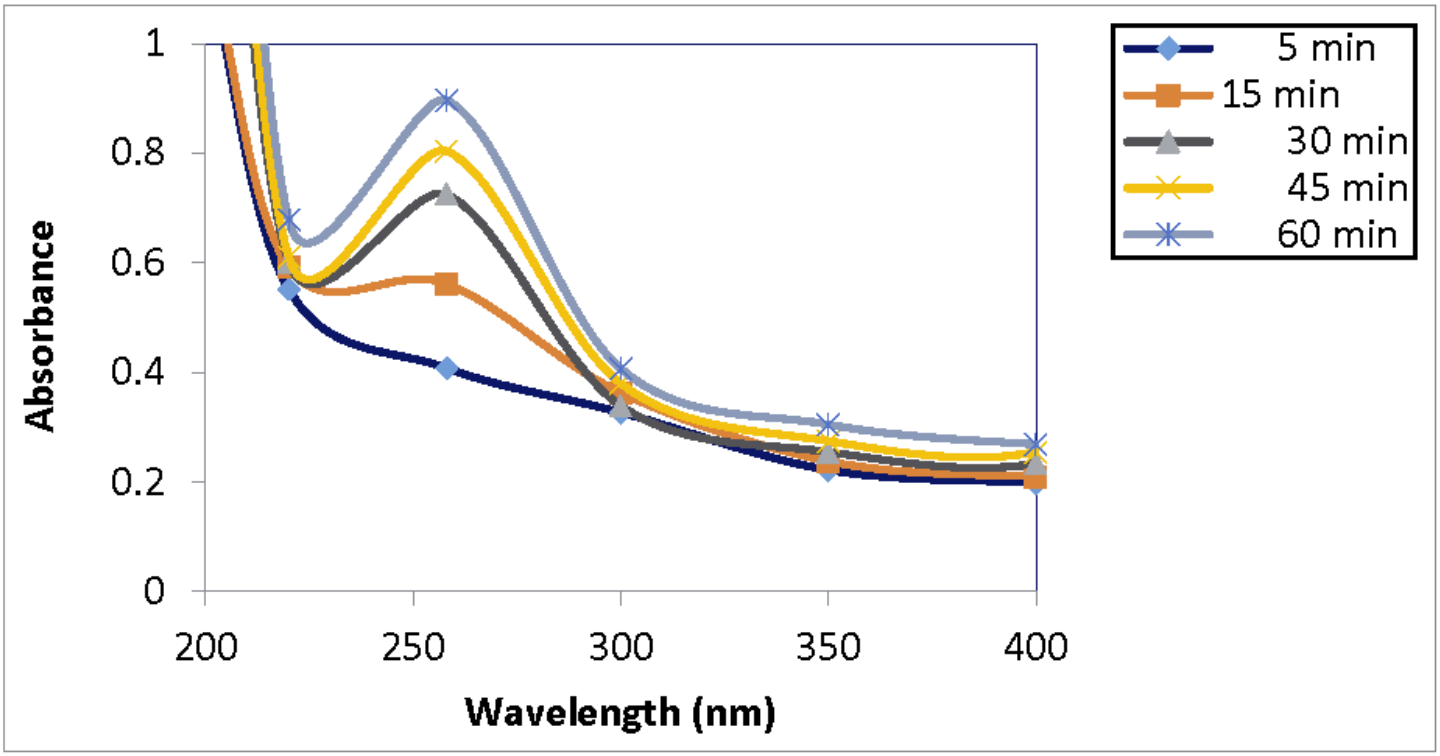

Figure 1

U.V. spectra recorded as a function of reaction at different wavelength versus absorbance during synthesis of iron nanoparticles at different time intervals.

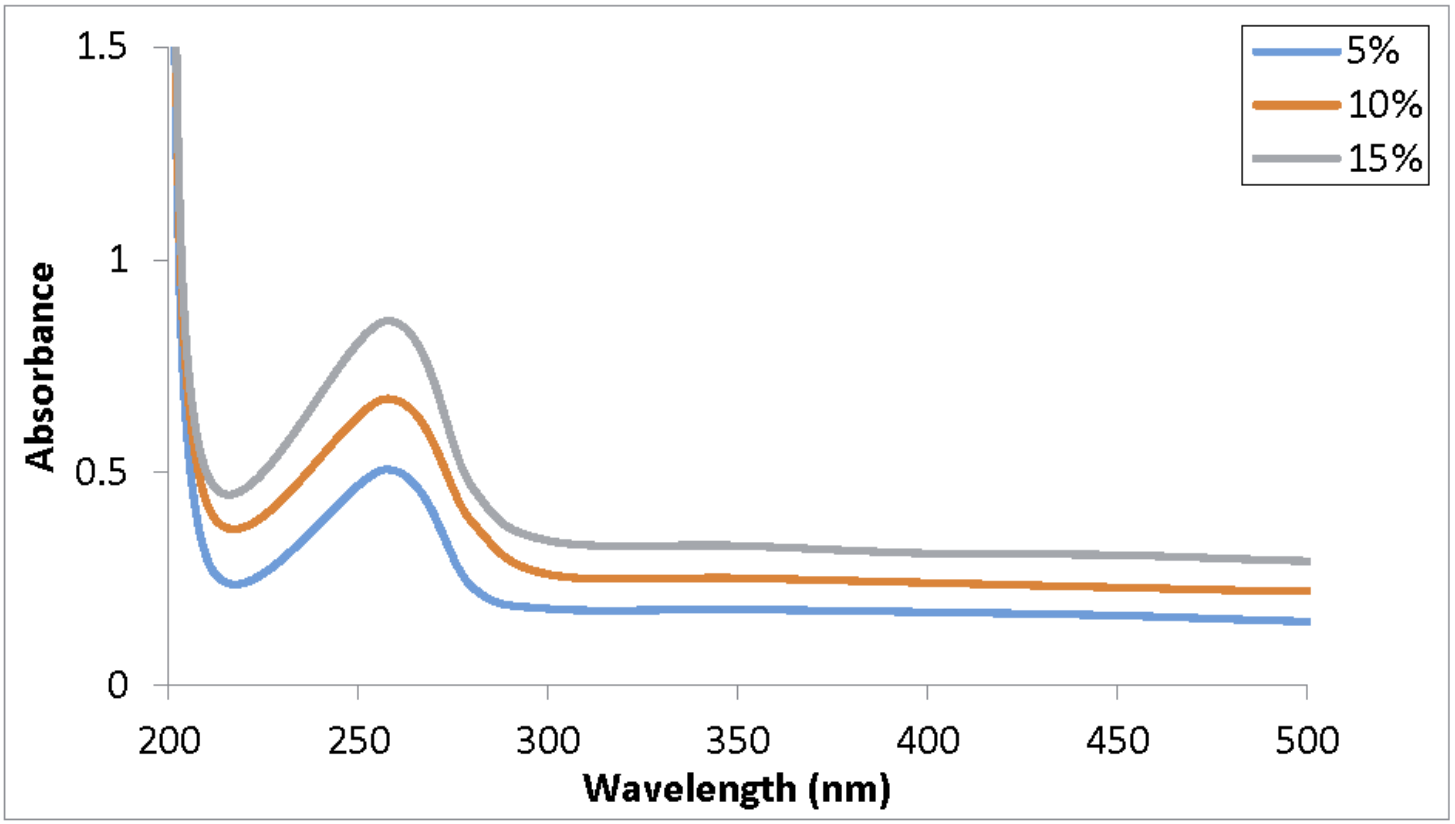

Figure 2

U.V. spectra recorded as a function of reaction at different wavelength versus absorbance during synthesis of iron nanoparticles at different Neem leaf extract (5 %, 10 %, and 15%).

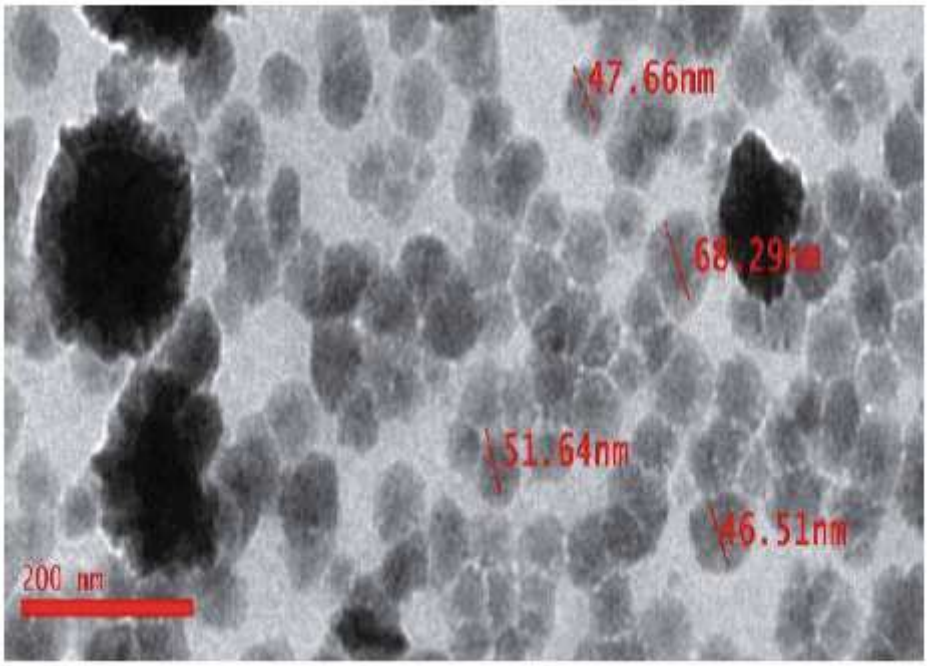

Figure 3

TEM image of synthesized Iron nanoparticles

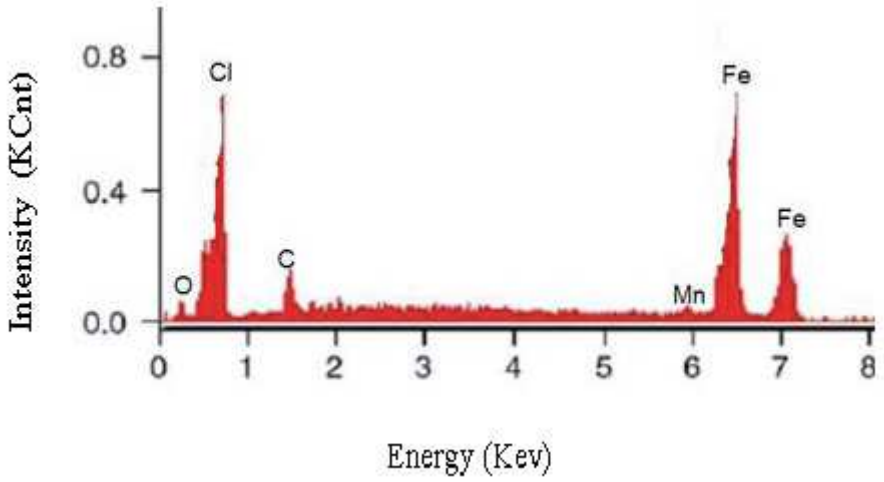

Figure 4

Spot profile EDS spectra of synthesized Iron nanoparticles

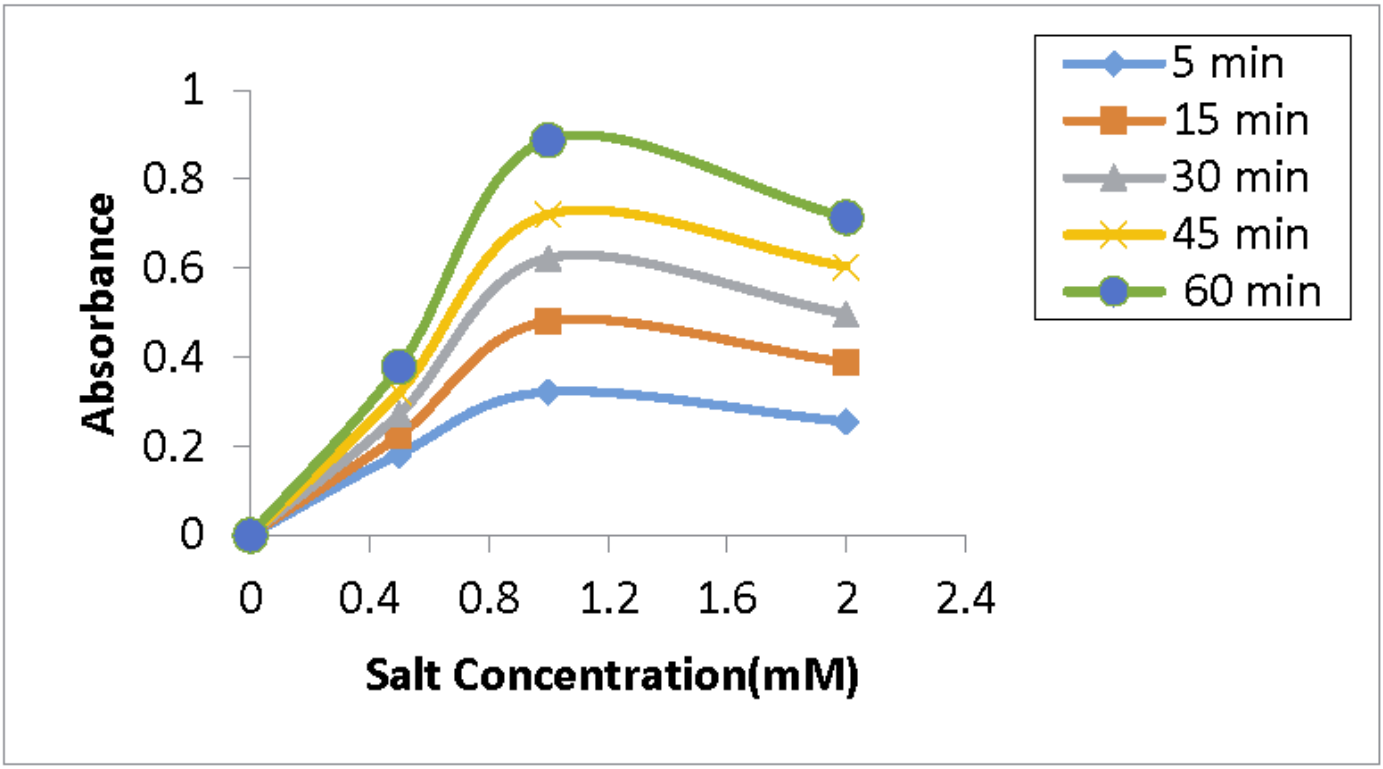

Figure 5

Time course of iron nanoparticles synthesis with different initial concentration of Fecl3 (0.5 to 2 mM), leaf Extract =15%, temperature=60 oC, pH=6.0

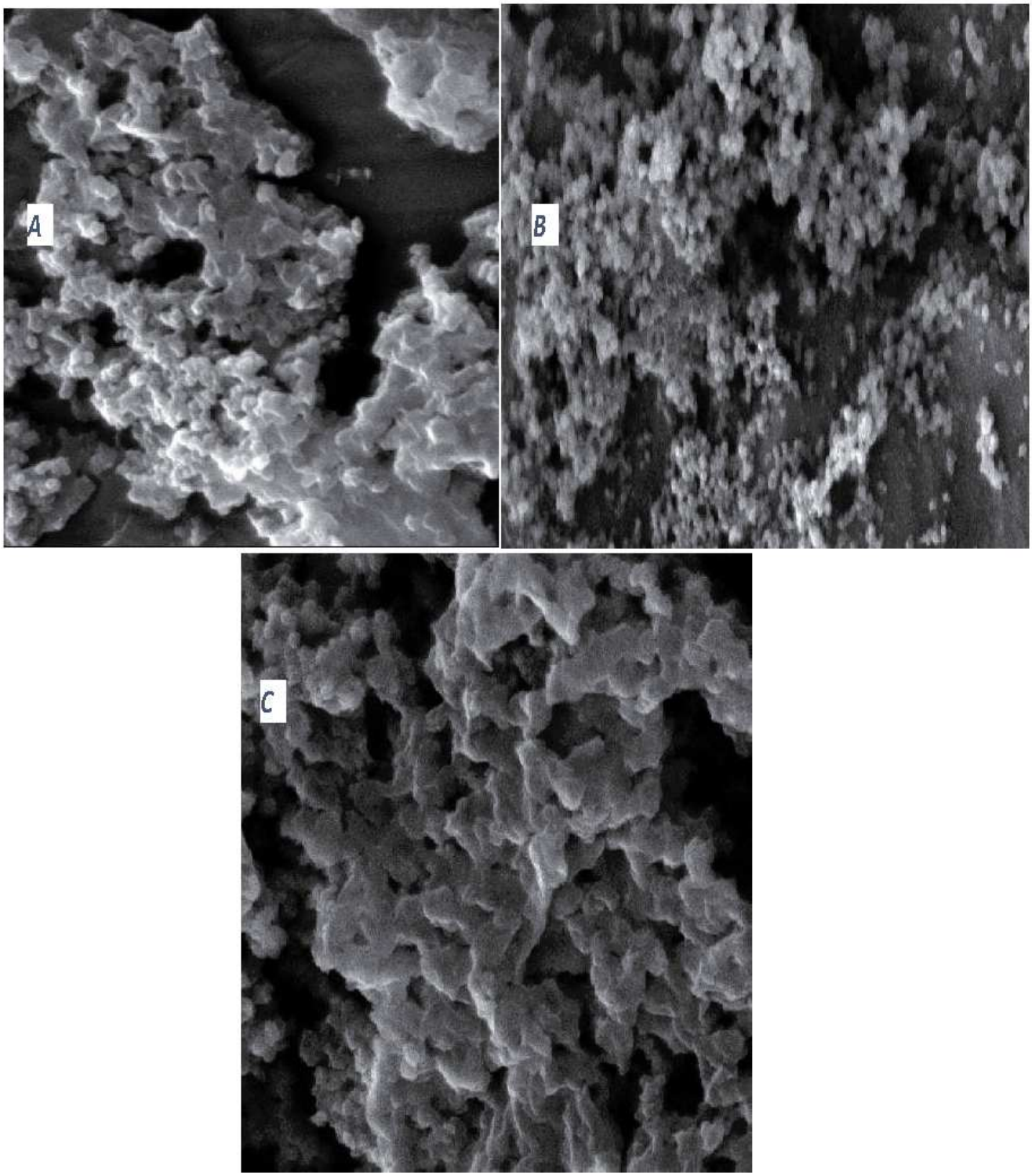

Figure 6

SEM images of the synthesized iron nanoparticles at three concentration of the precursor salt (FeCl3 .6H2 0) (A) 0.5 mM, (B) 1.0 mM, (C) 2.0 mM

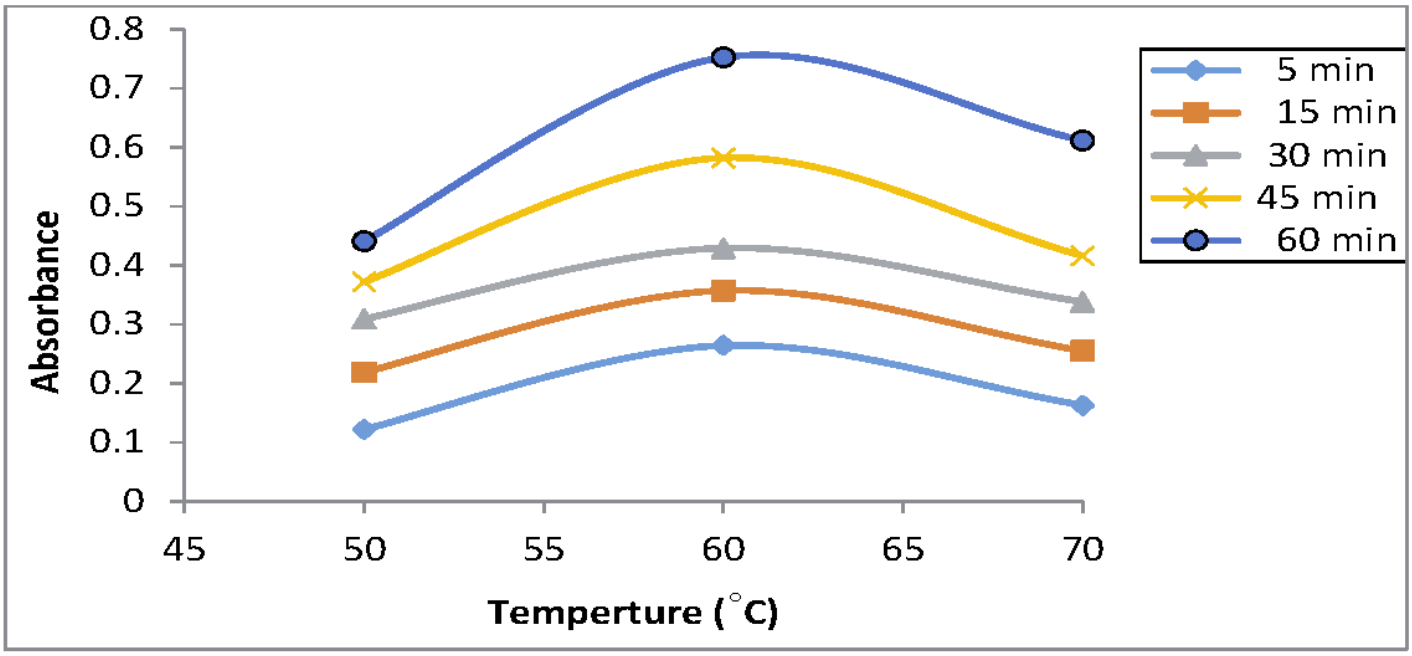

Figure 7

Time course of iron nanoparticles synthesis with different reaction temperature (50-70 oC), Fecl3 (1 mM), leaf Extract= 15% pH=6.0

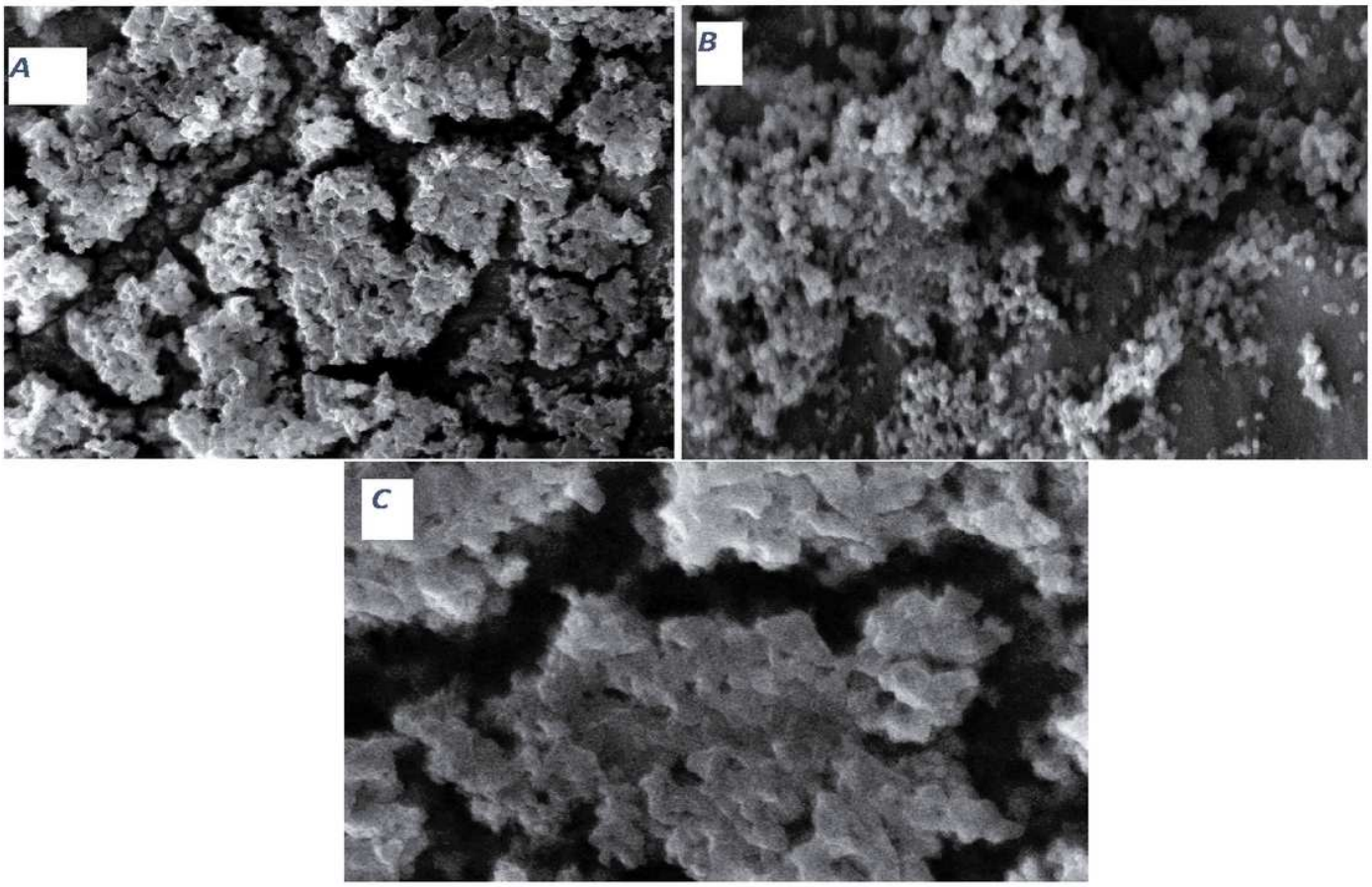

Figure 8

SEM images of synthesized iron nanoparticles at three different temperature (A) 50oc (B) 60oc (C) 70oc

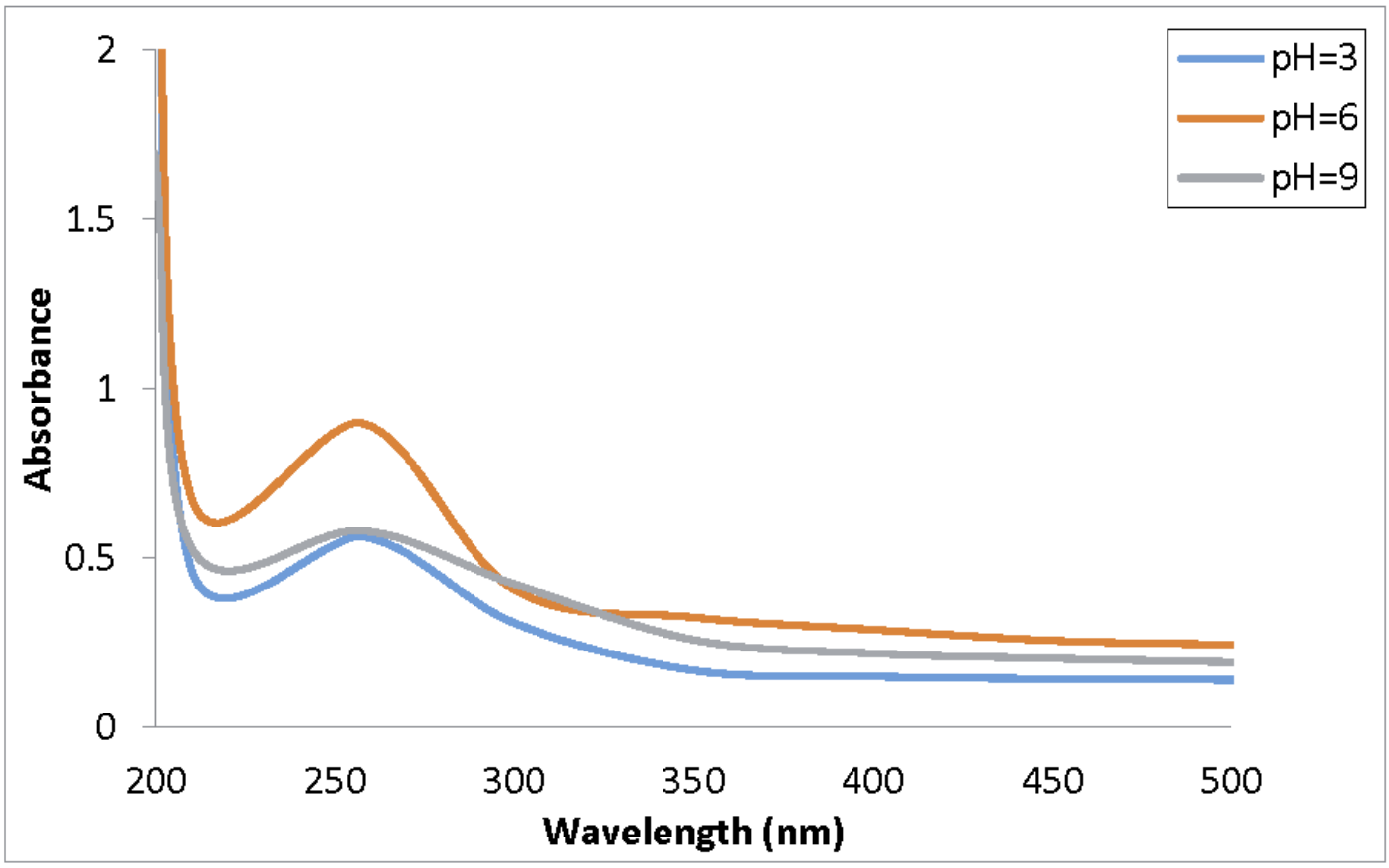

Figure 9

U.V. spectra recorded as a function of reaction at different wavelength versus absorbance during synthesis of iron nanoparticles at different solution pH (3, 6, and 9).

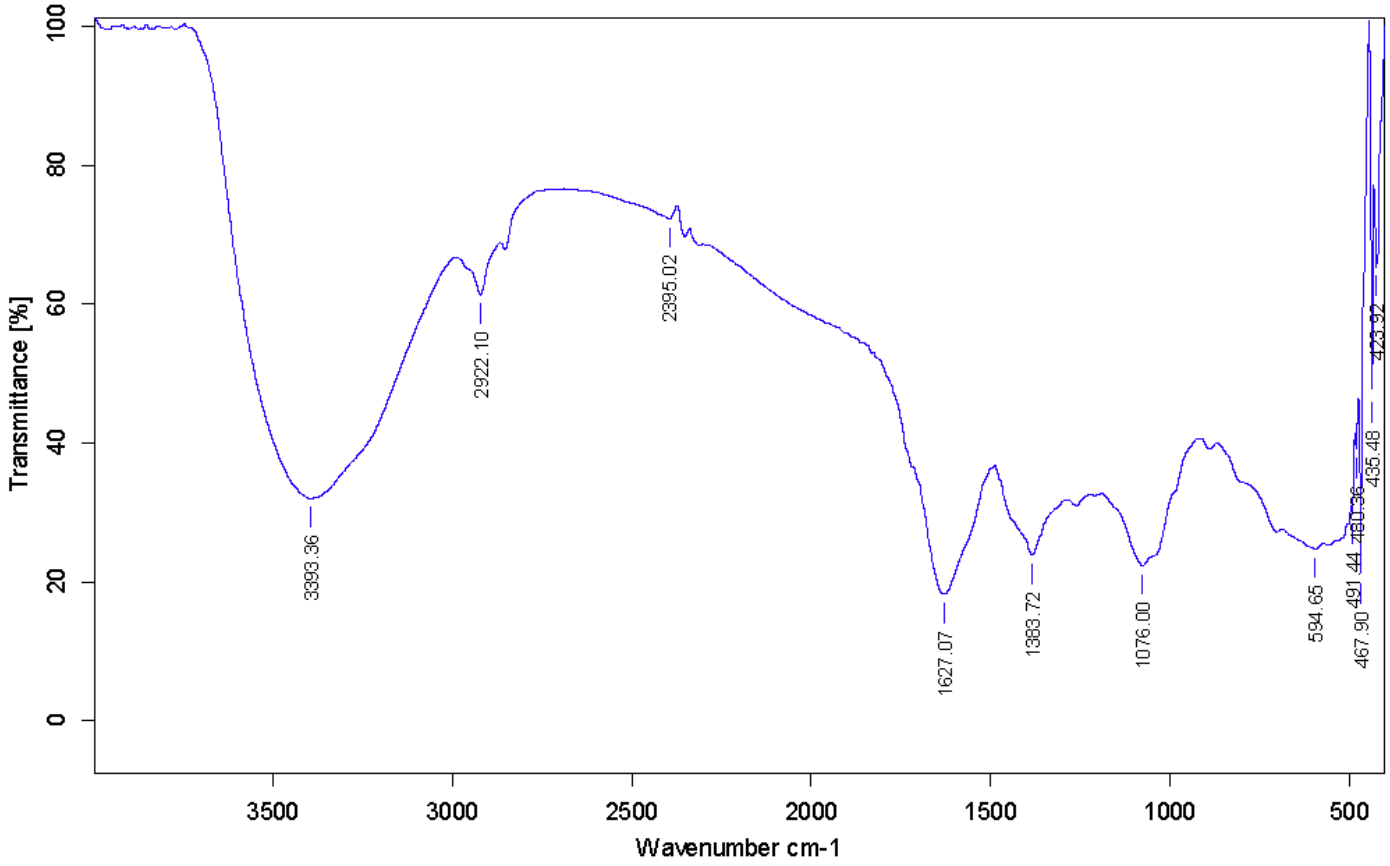

Figure 10

FT-IR Spectra of synthesized Fe nanoparticles