Green Synthesis of Iron Nanoparticles Using Spinacia oleracea and Their Role in Antioxidant Defence

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Abstract - The green synthesis of iron oxide nanoparticles (Fe_2O_3) using Spinacia oleracea leaf extract is explored in this study. Iron oxide nanoparticles are noted for their unique properties, such as enhanced chemical reactivity due to their high specific surface area to volume ratios. Unlike traditional physical and chemical methods, green synthesis presents an environmental friendly alternative, leveraging biological processes for nanoparticle production. This method is particularly significant due to its potential applications in various fields, including medicine, where iron nanoparticles demonstrate antibacterial activity. However, limited research exists on their antioxidant properties. In this research, Spinacia oleracea leaf extract is utilized to synthesize iron oxide nanoparticles, followed by characterization using UV-Visible spectroscopy, FTIR, XRD, and SEM techniques. The results underscore the necessity for further investigation into the impact of nanoparticles on biological processes and the long-term effects of high nanoparticle concentrations on ecological stability. Additionally, this study includes an evaluation of antioxidant properties of the nanoparticles to address gaps in current research.

Key Words *Green synthesis, iron oxide, ecological stability, antioxidant, biological, nanoparticles.*

1. INTRODUCTION

Nanoscience is a vital area of science which deals with the study of small dimensional particles, which brings nanotechnology in focus. Nanotechnology is the field of science which deals which the study of particles in the range of nano scale to bring into existence a variety of products which has its application to our society. Nano means "drawf" in Greek. Nanoscale materials shows how the molecule can be distinguish on the basis of size obtained from the study of nano particles. Recently all the research is mainly focused on the synthesis of nanoparticles by eco-friendly method[1].The nanoparticles synthesized from plants by green synthesis method are much stable as compared to all other nanoparticles synthesized by chemical process. The advantage of using plant extract is that it has its wide application and is beneficial to our society in many ways. The rate of synthesis is faster and the shape and size of these nanoparticles are unique in comparison with those produced by other method. In short Nanotechnology is the study of extremely small structures, having size of 0.1 to 100 nm[2]. Nanotechnology is the treatment of individual atom, molecule or any compound to enhance its special properties. Structure also changes with size however it is important that these materials display different properties such as electrical conductance, reactivity, melting point, surface area, and physical strength[3]. Nanotechnology is a highly attractive field having applications in the wide range of areas. Nanoparticles due to its flexible size help in the smooth passage of drug from the blood vessels and capillaries. It shows enhancement in the fields like biomedical, chemical, biological, physiological, nanomedicine, green nanotechnology, drug delivery, biotechnology, electronics, textile, cosmetics, optical engineering and communication, energy storage, metallurgy and materials, agriculture and food industry[4].

2. MATERIAL AND METHOLOGY

Spinacia Oleracea leaf extract, Ferric Nitrate are used as starting materials.

A. Preparation of *Spinacia Oleracea* leaf extract

The fresh spinach leaves were collected from the fam of Maulana Ahmad Khan of Warthoda Shukleshwar in the month of December 2018 and January 2019. These leaves were slowly washed with distilled water several times. Then the leaves were shade dried at room temperature and then grinded properly in mortal and pestle to make a proper paste of it. The paste was soaked in 100ml boiling deionised water for about 2 hour and the reaction mixture was kept on the magnetic stirrer and then it was filtered through Whatmann filter paper No.41.

B. Synthesis of Fe nanoparticles

In 250ml conical flask a mixture of *Spinacia Oleracea* leaf extract(150ml), ferric nitrate (0.2M, 75ml)and deionised water(9ml) was kept on magnetic stirrer for 2hours and the reaction mixture was kept in dark for 24hours. Cherry red solution was obtained. It was centrifuged at 1500rpm. Supernatant solution was removed by decantation. Nanoparticles were collected after calcinations. The obtained nanoparticles was amorphous in nature. Brown in color and the yield is about 5g.

Figure 1.1 Color change shows the formation of Iron Nanoparticle

3. RESULT AND DISCUSSION

3.1. UV Visible Spectroscopy

The reduction of metallic Fe^{3+} is taken by using a small aliquot from reaction mixture. The spectrum is recorded between the wavelength of 200-600nm. The UV was recorded at two different time, first after immediate addition of solution in the *spinacia oleracea* leaf extract and then after 24hr. The UV absorption spectra shows the absorption range at 0.615 at 334nm wavelength as soon as both the solutions are mixed. While after 24hr it shows absorption range at λ_{max} 3.875 at 403nm wavelength.

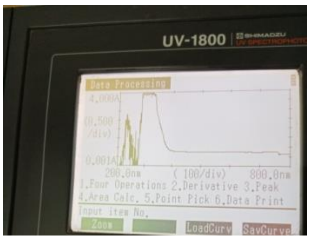

Figure 1.2 UV after immediate mixing of solution

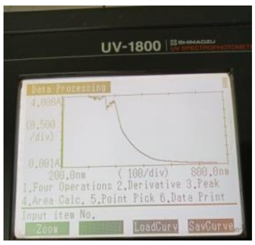

Figure 1.3 UV after 24 hours

3.2. Fourier transmission infrared spectroscopy (FTIR) spectroscopy

The characteristic FTIR bands Fe_3O_4 nanoparticles shown in (Figure1.4) The band located at 3248.27cm $^{-1}$ and 3402.58 cm $^{-1}$ is attributed to the stretching vibrations of -OH, which is assigned to OH^- absorbed by Fe_2O_3 nanoparticles. The bands at 29331.93 cm $^{-1}$ and 2858.63 cm $^{-1}$ were assigned to asymmetric and symmetric CH2 stretching respectively, the band at 1739.87 cm $^{-1}$ corresponds to a carbonyl stretching (C=O), the band at 1643.42 cm $^{-1}$ correcponds to the C=C stretching frequency. The located band at 1608.70 cm $^{-1}$ corresponds to O-H bending vibrations from adsorbed water on the iron surface, at 1427.39 cm $^{-1}$ were assigned to CH3 symmetric and asymmetric bending, the bands at 1280.79 cm $^{-1}$ and 1114.90 cm $^{-1}$ are related to C-C and C-O stretching from the crystalline and amorphous regions of the nanoparticle, the band at 1030.03 cm $^{-1}$ from C-O-C asymmetric stretching, and a band at 540.09 cm $^{-1}$ form the Fe-O surface vibration in the iron oxide core .

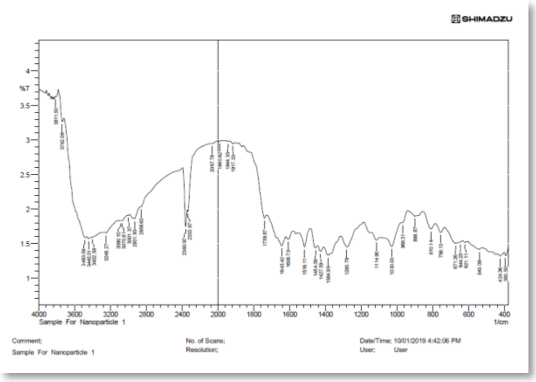

Figure 1.4 FTIR Spectra of Iron nanoparticles

3.3. X-Ray Diffraction (XRD) Analysis

X rays are used to establish the atomic arrangement or structure of the materials because the interplanar spacing (d) of the diffracting planes is of the order of X-ray wavelength. For a crystal with a given d-spacing and for a given wavelength λ, the various orders n of reflection occur only at the precise values of angle θ, which satisfy the Bragg equation,

2*d* sin□ = n□

where *n* is an integer, □is the wavelength of incident wave, *d* is the spacing between the planes in the atomic lattice and □□is the angle between the incident ray and the scattering planes. A typical powder X-ray diffractometer shown in figure 1.5, consists of a source of radiation, a monochromator to choose the wavelength, slits to adjust the shape of the beam, a sample and a detector. A goniometer is used for fine adjustment of the sample and the detector positions. The goniometer mechanism supports the sample and detector, allowing precise movement. The source X-rays contains several components; the most common being $K_{□}$ and $K_{□}$. Filters are used to absorb the unwanted emission with wavelength $K_{□}$, while allowing the desired wavelength, $K_{□}$ to pass through. The X-ray radiation most commonly used is that emitted by copper, whose characteristic wavelength for the $K_{□□}$radiation is equal to 1.5418 A°. When the incident beam strikes a powder sample, diffraction occurs in every possible orientation of 2□. The diffracted beam may be detected by using a moveable detector such as a Geiger counter, which is connected to a chart recorder. A detector records and processes this X-ray signal and converts the signal to a count rate which is then fed to a device such as a printer or computer monitor. The sample must be ground to fine powder before loading it in the glass sample holder. Sample should completely occupy the square glass well[5].

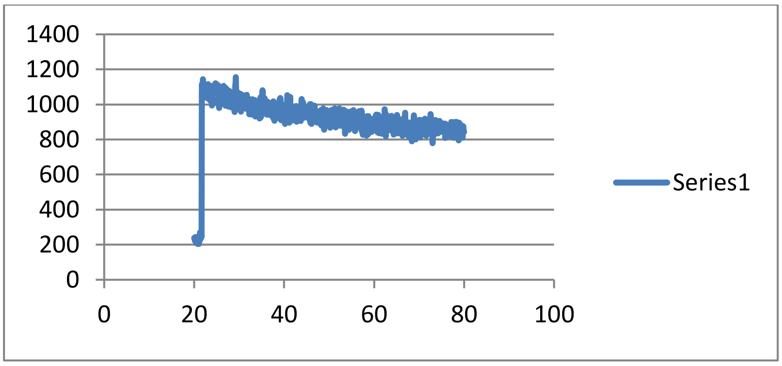

Figure- 1.5 Powder X-Ray Diffraction (XRD) pattern of Fe nanoparticles

The X-ray diffraction patterns obtained for the Fe_2O_3 nanoparticles synthesized using *Spinacia Oleracea* leaf extract is shown in Figure 1.5 The peaks with 2θ values of 21.95, 29.25, 34.75 and 35.2 are the different peaks of Fe_2O_3 respectively. The better crystallinity of an XRD pattern are due to the higher peak intensities. The corresponding X-ray diffraction pattern agrees with the JCPDS card no-36-1451. From above XRD pattern high intensity peak observed at 29.25 degree, hence we calculate particle size by using Scherrer formula and we get 16 nm particle size. The XRD pattern of the broad nature of the diffraction bands indicated that iron oxide has small particle sizes[6].

3.4. SCANNING ELECTRON MICROSCOPY

Scanning Electron Microscopy (SEM) photographs of spinach-Fe nanoparticles by using Bath deposition technique is shown in Figure1.6

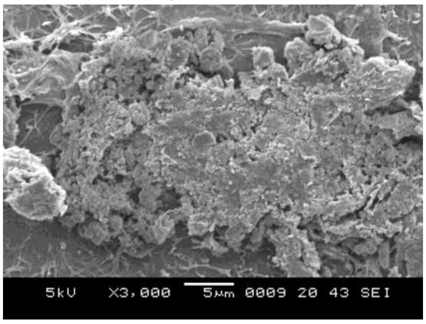

Figure 1.6 SEM image of spinach-Fe nanoparticles

SEM micrographs of spinach-Fe nanoparticles show polymeric solid structure with granular appearance. There is formation of aggregates of polymeric chains is observed in spinach-Fe nanoparticles. Fe image seems to be uniform microporous on the surface and the particles were in nanometer scale. Nanoparticles agglomerated grain structure observed. The grains are highly agglomerated shape but they are well interconnected each other.

3.5. DETERMINATION OF ANTI-OXIDANT ACTIVITY OF IRON NANOPARTICLES BY REDUCING POWER ASSAY METHOD

with various cellular When the oxidizable substrates is present at lower concentrations it prevents the oxidation. Anti-oxidants can be synthesized *in vivo* using reduced glutathione (GSH) and superoxide dismutase (SOD) or we can take it in the form of diet. These low molecular weight anti-oxidants works as redox buffers and interact components and plays an vital role in plant growth and development by modulating processes from mitosis and cell elongation to senescence and death.116 The reducing property is generally associated with the presence of reductants. The anti-oxidant action of reductants is based on the breaking of free radical chain by donation of a hydrogen atom. Reductants also react with certain precursors of peroxide, thus preventing peroxide formation. The presence of anti-oxidant molecules in sample act as reductants by donating the electrons and reacting with free radicals to convert them to more stable products and terminate radical chain reaction. Results confirms the reducing power (as indicated by absorbance at 700 nm) of samples which increased with increasing concentration. In the presence of various concentration of Fe nanoparticles the Fe^{3+} or ferricyanide complex is transformed to Fe^{2+}.
Reducing power was determined according to the method of Oyaizu. The concentration was taken in the range of 10 ug/ml to 50 ug/ml respectively. The Maximum reducing power of iron oxide nanoparticles was found when it was compared to the crude algal extract. The increased in the reducing power of Fe nanoparticles was attributed from the increased in absorbance of reaction mixture. The sample and standard exhibited maximum reducing power only at higher concentration. The reduction of Fe^{3+} or Ferricyanide complex to the ferrous form is due to the presence of reductants in the synthesized iron oxide nanoparticles[7].

Different concentrations of the drug (10-50µg/mL) was added to 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide $[K_3Fe(CN)_6]$ solution. The reaction mixture was vortexed well and then incubated at 50°C for 20 min using vortex shaker. At the end of the incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3,000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of deionised water and 0.5 mL of 0.1% ferric chloride. The coloured solution was read at 700 nm against the blank with reference to standard using UV Spectrophotometer. Here, ascorbic acid was used as a reference standard, the reducing power of the samples were comparable with the reference standard[117].

In the present study the antioxidant property of Fe nanoparticles was carried out in Pharmacy College, Amravati. It is carried out through reducing power assay method. The main aim was to find out the reducing power of Fe nanoparticles synthesized from *Spinacia oleracea* leaf extract. Ascorbic acid was used as the standard. The absorbance of the standard was taken at 720nm and the process was repeated in the triplet. The concentration was taken as 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50µg/ml. The wavelength that showa maxima=um absorbance was set.

Similarly, the Fe sample was also prepared in the concentration range of 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50µg/ml and the absorbance was measured. The highest absorbance was taken to calculate the reducing power of Fe nanoparticles synthesized from *Spinacia oleracea* leaf extract. Absorbance should increase with increase in concentration. Result confirms the reducing power of samples which increased with increasing concentration. Higher value absorbance of the reaction mixture indicated greater reducing power.

Concentration(ug/ml)	Absorbance 1	Absorbance 2	Absorbance 3
10µg/ml	0.002	0.006	0.061
20 µg/ml	0.395	0.251	0.029
30 µg/ml	0.941	0.775	0.499
40 µg/ml	1.451	1.350	1.036
50 µg/ml	2.000	2.000	2.000

Table 1.1 Ferric Reducing Anti-oxidant Power Assay

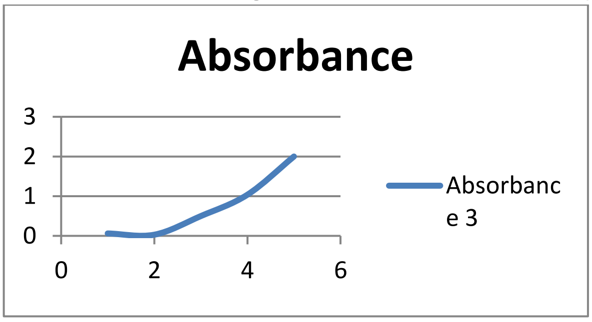

Figure 1.7 Graph of Standard Observation of Ascorbic acid

Sample name	Concentration	Absorbance
1) Fe	a) 10 µg/ml	0.029
	b) 20 µg/ml	0.038
	c) 30 µg/ml	0.048
	d) 40 µg/ml	0.122
	e) 50 µg/ml	0.144

Table 1.2 Sample Observation- (720nm)

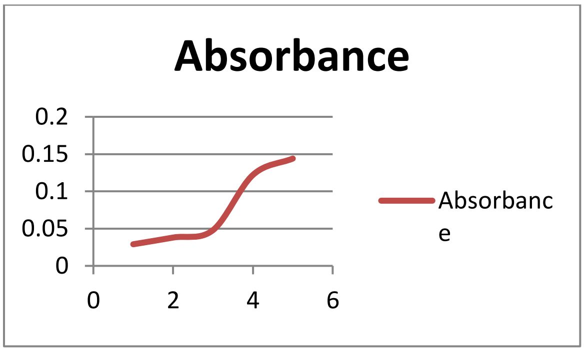

Figure 1.7 Graph of Fe sample against concentration

CONCLUSION

Green nanotechnology has attracted a lot of attention and includes a wide range of processes that reduce and eliminate toxic substances to restore the environment. Iron oxide nanoparticles with smooth surface chemistry exhibit many properties that can be used in a variety of biomedical applications such as magnetic resonance imaging contrast enhancement, tissue repair, hyperthermia, drug delivery and in cell separation. The phytochemicals present in the leaf has a main role as reducing agent that makes it eco friendly synthesis of Fe_3O_4 nanoparticles with enhanced antioxidant property.

In the present study, the small scale iron nanoparticles are synthesized from the fresh Spinacia oleracea leaves extract using the ferric nitrate salt by the non-toxic, non-hazardous green synthesis technique. The confirmation of the Fe nanoparticles was done by the help of UV-VIS, FTIR, XRD and SEM studies. UV conform the colour change and formation of nanoparticles. FTIR shows the Fe-O bonding and presence of functional groups. XRD shown the broad peak and particle size of 16nm and confirms that crystalline nature of the iron oxide nanoparticles. SEM studies are used to study the morphology of nanoparticles. These nanoparticles were further evaluated and it showed higher anti-oxidant properties as compared to the crude extract. Iron nanoparticles synthesized by this method have very useful applications in many versatile spaces.

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