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RESEARCH PAPER

Green synthesis and characterisation of iron oxide nanoparticles using hydroponically grown spinach plant extract

Smital Kulkarni[1] **and Mansee Thakur[2]**

[1]Department of Medical Biotechnology, MGM School of Biomedical Sciences, MGMIHS, Kamothe, Navi Mumbai, (M.S.) India
[2]Department of Medical Biotechnology and Central Research Laboratory, MGMCET, MGM School of Biomedical Sciences, MGM Medical College Building, MGM Institute of Health Sciences, Kamothe, Navi Mumbai (M.S.) India
Email : mansibiotech79@gmail.com

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Recent advances in Nanoscience and Nanotechnology radically changed the way we diagnose, treat, and prevent various diseases in all aspects of human life. Iron oxide nanoparticles (IONPs) are one of the most vital and fascinating nanomaterial among several metallic nanoparticles that are involved in biomedical applications. IONPs have been focused on budding applications in magnetic resonance imaging, drug delivery across biological barriers or in cancer treatment by magnetic field-induced hyperthermia. In this article, we discuss the green synthesis and characterization of IONPs using hydroponically grown spinach plants leaf extract. The use of plants in the green synthesis of nanoparticles emerges as a cost-effective and eco-friendly approach. Characterization of nanoparticles was done using different methods, which include; Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), Scanning Electron Microscope (SEM), Energy Dispersive Spectroscopy analysis (EDS) and Atomic Absorption Spectroscopy (AAS). Fourier transform infrared spectroscopy identifies the functional groups of active components presents on the surface of nanoparticles. The crystalline nature of the particles was validated from an X-ray diffractometer. The size and stability were detected using SEM-EDS analysis. Iron content was found to be 40.34% by AAS.

Key words : Green synthesis, IONPs, Spinach leaf extract, Hydroponic method

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INTRODUCTION

Iron deficiency anaemia (IDA), a most severe consequence of iron depletion is considered as one of the most common nutritional disorder which in adverse conditions may leads to diminished work or exercise capacity, impaired thermoregulation, immune dysfunction, GI disturbances, and neurocognitive impairment (Dupont, 2017). Oral iron is normally provided in IDA conditions. But oral iron is not well-tolerated by all patients (El-Din Taher *et al.*, 2008). As these iron supplements rely on soluble iron compounds, it may have adverse effects on the gastrointestinal tract leading to constipation and diarrhea. Another problem is iron absorption which is not always adequate. However the natural sources of iron are meat and leafy vegetables, present in the form of ferritin (Mason, 2014). As it is difficult to isolate ferritin, nanotechnology has emerged with wide applications involving nanoparticles in medicine and research. In the last decade biosynthesis of metal oxide nanoparticles has received increasing attention due to their potential properties such as optical, electronic, mechanical, magnetic, and chemical properties (Satishkumar *et al.*, 2009). Iron oxide nanoparticles (IONPs) show several advantageous properties including a non-toxic and biocompatible. Due to the magnetism of these iron oxide modifications, magnetic IONPs have gained high interest and a wide range of technical, biological and clinical application (Weinstein *et al.*, 2010; Winer *et al.*, 2011; Chertok *et al.*, 2008 and Kamau *et al.*, 2006). It is also used as catalytic materials, wastewater treatment adsorbents, pigments, flocculants, coatings, and medicine etc. (Tiwari *et al.*, 2008 and Ghobadian, 2016). Due to its vast application in research and medicine (for eg. as antianaemic preparation) its synthesis has become an important aspect.

Use of plants for the synthesis of metallic nanoparticles offers a cost-effective and regulated alteration in its size and shape (Iravani, 2011 and Mukunthan and Balaji, 2012). Inactivated plant tissues, exudates, plant extracts and other living plant parts serve as a substitute for the production and synthesis of metallic nanoparticles. The advantage of using plants in nanoparticles synthesis is their ease of availability, safe to handle and possess a broad spectrum of metabolites such as antioxidants, nucleotides,and vitamins. The green synthesis of Iron oxide nanoparticles (IONPs) using various plant extracts has been reported by many researchers (Hoag *et al.*, 2009; Shahwan *et al.*, 2011 and Markova *et al.*, 2014).

An extensive literature survey shows that there is no research report available for synthesis of iron oxide nanoparticle using hydroponically grown spinach leaf extract. Hydroponics, the technique of growing plants in water and inorganic nutrients without using soil. These plants will be free of soil pathogens (Hussain *et al.*, 2014).

So that the present study discusses the plant mediated green synthesis of iron oxide nanoparticles using Spinach leaf extract which is used as a reducing agent to convert the precursors into nanoparticles. Spinach, a green leafy vegetable with high iron content and vast medical applications has been chosen for the synthesis of Iron oxide nanoparticles (IONPs). It contains several essential nutrients phytonutrients, minerals, vitamins and therapeutic properties. It is recommended for variety of disorders like constipation, anemia, high blood pressure, obesity, tumors etc. In addition to that the synthesized nanoparticles are characterized with the aid of Fourier transform infrared spectroscopy (FTIR), X-ray diffractometer (XRD), Scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS) and Atomic absorption spectroscopy (AAS).

RESEARCH METHODOLOGY

Raw material and preparation of leaf extract :

Fig. A shows hydroponically fully grown plants (*Spinacia oleracea*) 24 days old. A number of measurements were made to ascertain the physiological state of plants grown. The study involves different parameters which include height of plants, estimation of iron and chlorophyll content. The iron content for hydroponics spinach was found to be 0.5 g/100 g and plants had vibrant green coloration for which total chlorophyll content of hydroponics plant leaves of spinach was found to be 11.5 ± 0.4 lg mg-DW^{-1}, about 20 - 25 g of fresh and healthy leaves of *Spinacia oleracea* were collected, washed thoroughly with double distilled water, cut into fine pieces and boiled with 100 mL double distilled water in water bath at 70°C for 15-20 min. After boiling, the color of the aqueous solution changes from watery to brown color. The extract was cooled at room temperature and filtered using Whatman filter paper number 42 and stored at 4°C for further experiments (Kulkarni *et al.*, 2016).

Fig. A : Hydroponically grown *Spinacia oleracea*

Biosynthesis of nanoparticles using plant extract:

0.1 M solutions of $FeCl_2.4H_2O$, $FeCl_3.6H_2O$ and NaOH were prepared. $FeCl_2$ and $FeCl_3$ were added in 1:2 ratio in a conical flask under constant stirring. 12 ml of spinach plant extract was added drop wise to the above solution and allowed to remain at 80°C for 15 min under constant stirring. After 15 min, 0.1 M NaOH was added to the solution which starts formation of black solid particles under constant stirring. Once NaOH was added, temperature was allowed to decrease to 20°C and stirred for further 45 min. The colour changes from light brown to dark brown with black colored particles in it. The solution was allowed to cool at room temperature. It was then centrifuged at 1500 rpm for 15 min, supernatant was removed and pellet was washed with ethanol (3 times) to remove the possible impurities. The ethanol was again added to the pellet and it was kept for sonication for 30 min. After sonication, the solution was poured in beaker and kept in oven for drying. After 48-72 hrs, black solid IONPs obtained. These particles were further characterized.

Characterization of nanoparticles :

The characterization of the synthesized nanoparticles from spinach leaf extracts was characterized using FTIR, XRD, SEM-EDS and AAS. Functional groups present in the synthesized nanoparticles were found by FTIR spectrometer 3000 Hyperion Microscope with Vertex 80 FTIR System, IIT Bombay. X-ray diffraction pattern was obtained from Seifert XRD 3003 diffractometer having the radiation source which was used to confirm the presence of green synthesized nanoparticles. The structural morphology of prepared nanoparticles was carried out by JSM-7600F scanning electron microscope with an accelerating voltage between 10 and 20 KV under the vacuum condition.

RESEARCH FINDINGS AND ANALYSIS

The morphology and size were verified by characterizing the sample by FTIR and SEM analysis for studying the functional groups and diameter of the nano particles.

Fourier transform infrared spectroscopy (FTIR) :

FTIR spectroscopy is a technique used for identification of functional groups of active components. The strong absorption peak at 3133 cm^{-1} for FTIR of IONPs was assigned to O-H stretching of alcohol and phenolic compounds simultaneously. The peak at 1631 cm^{-1} of IONPs was due to stretching vibration of CO groups in the ketones, aldehydes and carboxylic acids. The formation of Fe_2O_3 is characterized by the absorption bands at 691 cm^{-1} of IONPs correspond to the Fe–O band. These peaks were absent in their corresponding plant extracts which indicate the formation of iron oxide nanoparticles. FT-IR analysis confirmed that the bioreduction of ferric chloride into iron oxide nanoparticles are due to the reduction by capping material of spinach leaf extract (Matheswaran *et al.*, 2014, Omnidvari *et al.*, 2014 and Mahdavi *et al.*, 2013).

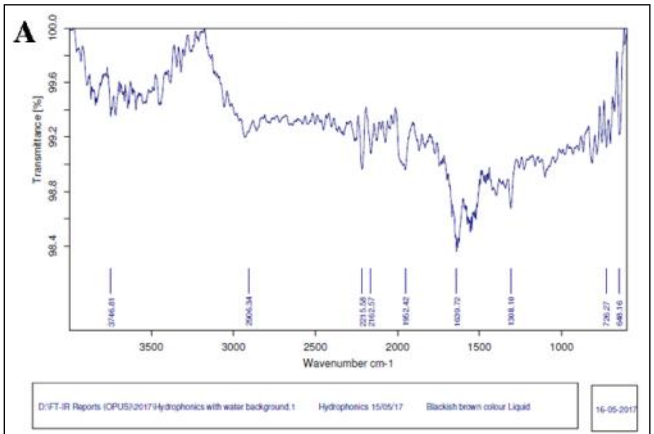

Fig. 1 A : FTIR of plant extracts

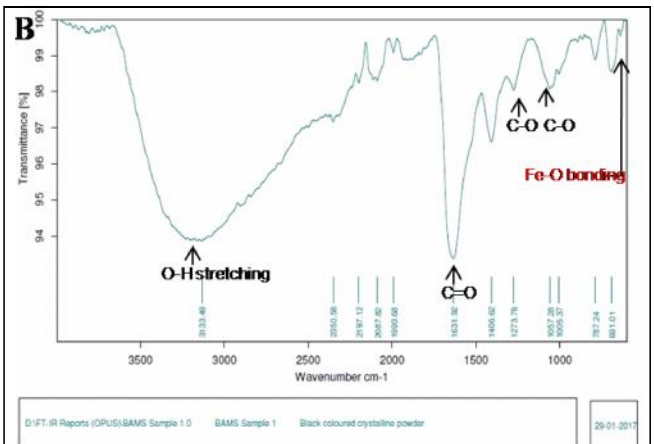

Fig. 1 B : FTIR of IONPs

X-ray studies :

X-ray diffraction of synthesized iron oxide nanoparticles is shown in Fig. 2. X-ray diffraction pattern of pure iron oxide nanoparticle has showed two peaks – 33.09°2θ and 35.56°2θ. All these peaks denote presence of hematite (Fe_2O_3) structure (Fig. 2). The X-ray diffraction plot shows peaks only due to Fe_2O_3 and no peak is detected due to any other material or phase indicating a high degree of purity of the as-synthesized sample. The broadening of the X-ray diffraction lines, as seen in the figure, reflects the nanoparticle nature of the sample. X-ray diffraction shows that metal oxide is pure -Fe_2O_3 having rhombohedral structure.

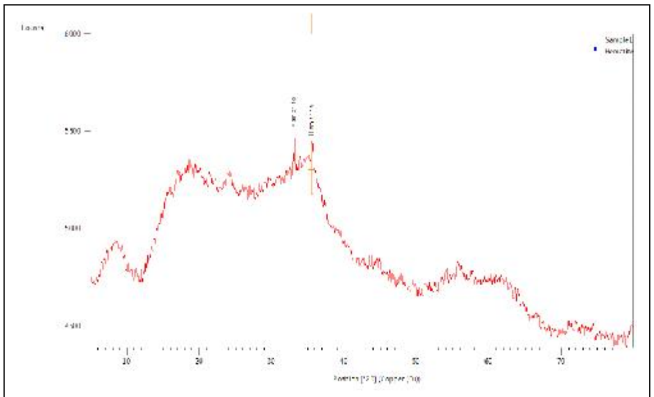

Fig. 2 : XRD septra of synthesis iron oxide nanoparticles

Scanning electron microscopy - Energy dispersive spectroscopy :

A scanning electron microscopy was employed to analyze the structure of nanoparticles that were formed. The images obtained by scanning electron microscopy revealed the particles with spherical shape and 10-50nm in size, as displayed in Fig. 3A and B. EDS analysis was carried out to determine the elemental composition and stoichiometry of the synthesized iron oxide nanoparticles. Iron, oxygen signals and other signals also have been detected. The other signal may be coming from the bio

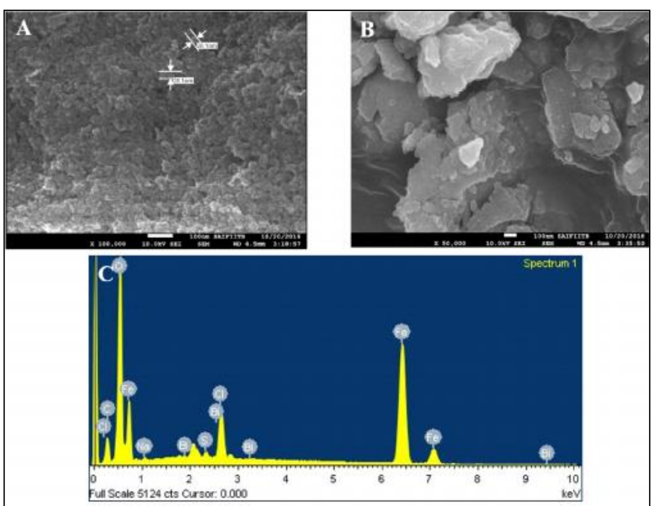

Fig. 3 A & B :SEM images of IONPs and C – EDS spectra of IONPs D - AAS of IONPS

Table 1 : AAS of IONPs	
Sample	Fe content (%)
Plant extract (Hydroponics)	0.0007
Iron oxide nanoparticles (Hydroponics)	40.34

active molecules present in Spinach leaves extract. It implies that the nanoparticles are mainly indeed made up of only Fe and O (Markova *et al.*, 2014, Hussain *et al.*, 2014; Kulkarni *et al.*, 2016; Matheswaran *et al.*, 2014; Omnidvari *et al.*, 2014; Mahnaz *et al.*, 2013; Pattanayak and Nayak, 2013 a and b and Latha and Gowri, 2014).

The EDS study has identified various elements (C, O, Al, Si, P, S, Cl, Fe and Bi) with its composition. The elemental composition of Fe was found to be 58.30% in biosynthesized IONPs Fig. 3C.

Atomic absorption spectroscopy is a quantitative estimation of elements using the absorption of optical radiation (light) by free atoms in the gaseous state. The IONPs synthesized from hydroponically grown spinach extract has estimated 40.34% of Fe content whereas the corresponding plant extracts has shown Fe content in ppm level. Mechanism behind the nanoparticles synthesis from plant leaf extract is not yet clear, however studies shows that biomolecules especially phenolic compounds in plant products leads the production of metallic iron nanoparticle (Luo *et al.*, 2014).

Conclusion :

Plant materials look more feasible as material for production of iron nanomaterials due to its environmentally friendly characteristics and economic value as an alternative to the large-scale production of nanoparticles. However, the mechanism has not yet been clearly described and there is need to explore the phytochemistry behind the synthesis of IONPs.The rapid biological synthesis of IONPs using spinach leaves extract provides environmental friendly, simple and efficient route for synthesis of benign nanoparticles. The colour change of the solution was due to the surface plasmon resonance during the reaction with the ingredients present in the spinach leaf extract, resulting in the formation of iron oxide nanoparticles. The synthesized nanoparticles were of spherical shape and the estimated sizes were 10-50 nm confirmed by SEM. The size and metal confirmation were found from the characterization using AAS, XRD and FTIR techniques. All these techniques it was proved that the concentration of plant extract to metal ion ratio plays an important role in the shape determination of the nanoparticles. The EDS analysis has showed higher iron content in hydroponics IONPs (58.3%) The AAS has estimated Fe content for hydroponics IONPs (40.34%). Success of such a rapid time scale synthesis of metallic nanoparticles is an alternative to chemical synthesis protocols which is also of low cost.

To achieve the sustainability of nanomaterial synthesis, more research is needed to explore. Understanding the biochemical mechanisms for nanoparticle synthesis is a prerequisite to the success of any new methodology, and any solution must be economically competitive with conventional methods. In order to reduce the cost for synthesis of nanoparticle local resources should be utilized. In future research, more detailed study will provide a clear description of biomolecules and their role in mediating the synthesis of nanoparticles. The goal is to influence the rate of synthesis and improve nanoparticle stability. Few studies confirm that biosynthesized nanoparticles are less toxic. In conclusion, green nanotechnology processes, as described in this paper, provide a strong foundation for the production of biochemical or functionalized nanoparticles that can serve as building blocks in the development of new products that can be applicable in antianemia formulation.

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