Biogenic Synthesis of Iron oxide Nanoparticles from Marine Algae

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Abstract

Green nanotechnology, deals with the use of biological systems such as bacteria, fungi, algae, plants, etc. for the synthesis of Nanoparticles (Nps). The aim of our study was to undertake Green synthesis of Iron oxide nanoparticles/magnetite (Fe_3O_4-Nps) from marine algae such as Chaetomorpha antennina (green algae), and Turbinariaturbinata (brown algae). Two types of Iron oxide Nps were synthesized and characterized – bare Iron Oxide Nps and citrate coated Iron Oxide Nps. This biogenic or green synthesis is actively pursued in recent years as an alternative, efficient, inexpensive, and environmentally safe method for producing nanoparticles with specified properties.

Keywords: marine algae; Iron oxide nanoparticles (Fe_3O_4); Chaetomorpha antennina (green algae); Turbinariaturbinata (brown algae)

1. Introduction

Synthesis of nanoparticles involves numerous methods like physical, chemical, biological as well as hybrid methods. The subsequent properties of nanoparticle depend on the methods used for their synthesis [1]. Physical and chemical methods are extensively employed for production of metal and metal oxide nanoparticles. However, this production requires the use of very reactive and toxic reducing agents that in turn cause undesired detrimental impacts on the environment. Also, some of the physical methods are expensive and labor intensive, requiring high temperature, vacuum and expensive equipments [2]. However, with the advent of green nanotechnology, better alternative, efficient, inexpensive as well as environment friendly methods are now available for producing nanoparticles with specified properties [3] and can be easily scaled up for large scale synthesis too. Green nanotechnology uses biological systems such as plants, bacteria, fungi, yeast, actinomycetes and algae.

The iron oxide nanoparticles have immense potential usage such as in targeted drug delivery [4], as biosensors [5] and also for various other medical [6] as well as environmental applications [7], [8]. The biogenic synthesis of Iron Oxide Nps is undertaken because of its unique properties, such as being superparamagnetic and yet biocompatible and biodegradable [9]. Green synthesis of iron nanoparticles has been reported from marine algae - brown algae [1],[10] and red algae [11] and has also been employed for bioremediation [10].

2. Materials and Methods

2.1. Green Synthesis of Nanoparticles

2.1.1. Preparation of Algal Extract

The seaweed Chaetomorpha antennina (green algae), and Turbinariaturbinata (brown algae) were washed with water, dried, pulverized and stored. To prepare the extract, 0.1 g of algal powder was added to 100 ml of Ultrapure water and was heated to 70-80°C. The crude algal extract was stirred continuously, then filtered and the supernatant thus obtained was used as the seaweed bioextract.

2.1.2. Synthesis of Magnetite (Fe_3O_4)

0.1M FeCl_2.4H_2O and 0.1% algal extract were added in the ratio 2:3, in four different sets, each with a different pH. The pH was adjusted to 6, 8, 10 and 12 using NaOH. These reactions were maintained at temperature ranging from 60-70°C. The synthesized Fe_3O_4-Nps were washed thrice using 70% ethanol, dried in hot air oven for 24 hrs. To evaluate the effect of temperature on the nanoparticle synthesis, two different sets of reactions were performed at temperature ranging from 60°C and 27°C. The green synthesized bare Iron oxide Nps were stored till further use. The bare Iron oxide Nps were coated with Tri sodium citrate. Requisite amount of prepared citrate solution (0.001 M) is added to the synthesized Iron oxide Nps, sonicated and then dried. The resultant capped Iron Oxide Nps were further characterised along with uncapped Iron oxide Nps.
2.2. Characterization

2.2.1. UV–Visible Absorption Spectra

UV–Visible absorption spectroscopy measurements were performed in aqueous buffer using a Shimadzu double beam monochromator spectrometer (UV-2540).

2.2.2. X-ray Diffraction

The Fe₃O₄ nanoparticles were analysed for phase composition using X-ray powder diffractometer (Rigaku Miniflex 600) over the 2θ range from 20–60° at rate of 50/min, using Cu- Kα radiation (λ = 1.54060 Å"). The average particle size of Fe₃O₄-Nps were estimated using the Debye-Scherrer equation, d = kλ/(β•cosθ), where d is the particle size of the crystal, k is Sherrer constant (0.9), λ is the X-ray wavelength (0.15406 nm), β is the width of the XRD peak at half-height, and θ is the Bragg diffraction angle.

2.2.3. FTIR

Fourier transform infrared spectrometer (Shimadzu Affinity 1S) was performed to analyze the structural characteristics of the Iron Oxide Nps.

2.2.4. Scanning Electron Microscopy

The Iron Oxide Nps synthesized at pH 8- both bare and citrate capped, were analyzed using SEM and morphological data were studied.

2.2.5. Charge Characterization of Iron Oxide Nps

Agarose gel electrophoresis was performed to analyze the charge of the synthesized Iron Oxide Nps. 2.5% agarose gel was prepared in 1x TAE buffer. The samples were loaded and the gel was run at 100V.

3. Results and Discussion

3.1. Iron Nanoparticle Synthesis

The Chaetomorpha antennina (green algae) bioextract, as well as Turbinaria turbinata (brown algae) bioextract was used for synthesizing the green Iron Oxide Nps. Varying pH, temperature, precursor concentration (FeCl₃.4H₂O) as well as algal extract concentration resulted in green synthesized Iron Oxide Nps having differing features, as shown in Table 1 and Table 2. In the present study, we focused on obtaining very small green synthesized Iron Oxide Nps that could be easily uptaken by plants. The Iron Oxide Nps were prepared using ferrous chloride as iron precursor and Algal extract as reducing agent and stabilizer. The addition of ferrous chloride solution as an iron precursor to the Algal extract containing sulphated polysaccharides as a major component which has sulphate, hydroxyl and aldehyde group may cause the oxidation of Fe²⁺ and stabilization of the nanoparticles. A decrease in pH during the formation of Iron Oxide Nps signifies the involvement of OH group in the reduction process. Optimal pH conditions were maintained by using concentrated NaOH. The proposed green synthesis method for Iron Oxide Nps was found to be constructive and extremely reproducible.

3.2. Factors Affecting Synthesis

Many factors were noted to affect synthesis of Iron Oxide Nps. In the present study, we have focussed on few parameters such as biological material, concentration of reactants, pH and temperature. See Table 1 and Table 2 for details. Biogenic synthesis of Nanoparticles from algae is a bottom up approach where the main reaction occurring is reduction/oxidation. The antioxidant or reducing properties of algal extracts, are usually responsible for the reduction of metal compounds into their respective nanoparticles.

3.2.1. Biological material

Bioextracts of different Algal species (Chaetomorpha antennina Turbinaria turbinata) can give variations in particle size, morphology, property etc. We observed that concentration of algal extract (0.1%) is very important for nanoparticles synthesis. Higher concentration of algal extract (1%) leads to higher concentration of reducing and capping agents, hence resulting in bulky sized Iron Oxide Nps.

3.2.2. Concentration of FeCl₃.4H₂O

Since FeCl₃.4H₂O is employed as the precursor in this synthesis, its higher concentration results in agglomeration; further leading to bulky sized Iron Oxide Nps. Hence 0.1M FeCl₃.4H₂O was standardised for further experiments.

3.2.3. Effect of pH on green synthesized Iron Oxide Nps

The data (Table 1) represents the variation in properties of Nps observed with respect to changes in pH. Irrespective of the algae used, it is observed that the Nps synthesized at pH 8 shows a higher remanence. The magnetic properties of the Nps show a huge variation; Nps synthesized at pH 6 shows the least magnetism. Nps synthesized at pH 8 were used for further evaluations in plants and published elsewhere [13].

3.2.4. Effect of temperature on green synthesized Iron Oxide Nps
The effect of temperature on green synthesis was evaluated in the two algal extracts and it was observed that high temperature 60°C (in comparison to low temperature 20°C) resulted in smaller Iron Oxide Nps. The properties of Np were observed to change with respect to temperature. For the synthesis of Np with higher remanence and better magnetism, a higher temperature is preferred. Thus, throughout the experiment, 60-70°C temperature was maintained.

3.3. Iron Nanoparticle Characterization

3.3.1. UV−visible absorption spectra

The characteristic surface plasmon band (SPR) of Fe₃O₄ is centered at 190-250 nm, and hence the iron oxide formed is Fe₃O₄ (Figure 1a). Since there is near infrared absorption, the complex is confirmed as Fe₃O₄ because Fe₂O₃ which also has two ionic species does not show absorption at near-infrared region. UV−visible absorption spectroscopy measurements were performed in aqueous buffer using a Shimadzu double beam monochromator spectrometer (UV-2540) equipped with an integrated sphere assembly ISR-240A in the range of 200-800 nm (Figure 1a). UV−visible absorption spectra of the algal extracts were also undertaken. Reaction kinetics is as follows:

Algae/Fe²⁺ +8 OH⁻ →[Green synthesised Fe₃O₄]↓(s)+4H₂O(aq)

Table 1: The table shows the variation in Green synthesized Iron Oxide Nps properties obtained from Chaetomorpha antennina (green algae) with respect to varying pH conditions.

<table>
<thead>
<tr>
<th>pH</th>
<th>Precipitation</th>
<th>Amount of Precipitation</th>
<th>Magnetism (Ferromagnetism)</th>
<th>Remanence</th>
<th>Particle size</th>
<th>Yield (g)</th>
<th>Nano Size (XRD Data)</th>
<th>Charge of Np</th>
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<tr>
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<td>+</td>
<td>+++</td>
<td>+</td>
<td>0.086g</td>
<td>9nm</td>
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<td>+++</td>
<td>++</td>
<td>++</td>
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<td>13nm</td>
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<td>+</td>
<td>++</td>
<td>+++</td>
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<td>+++</td>
<td>10nm</td>
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Table 2: The table shows the variation in Green synthesized Iron Oxide Nps properties obtained from Turbinaria turbinata (brown algae) with respect to varying pH conditions.

<table>
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<th>pH</th>
<th>Precipitation</th>
<th>Amount of Precipitation</th>
<th>Magnetism (Ferromagnetism)</th>
<th>Remanence</th>
<th>Particle size</th>
<th>Yield (g)</th>
<th>Nano Size (XRD Data)</th>
<th>Charge of Nps</th>
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<td>14</td>
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3.3.2. FT-IR

Fourier transform infrared spectrometer (Shimadzu Affinity 1S) was used to analyse the characteristics of the Fe₃O₄ nanoparticles (Figure 1(b)). Signals at 3,319 cm⁻¹ correspond to OH stretching. The band at 1,641 cm⁻¹ was attributed to the binding of a C=O group with the nanoparticles [12]. The formation of Fe₃O₄ is characterized by two absorption bands at 535 and 307 cm⁻¹ which correspond to the Fe–O bond in magnetite[1].

Figure 1(a) UV-Vis Spectra; (b) XRD spectrum; (c) FTIR spectra; SEM image of bare Fe₃O₄Np (d) and citrate capped Fe₃O₄Np (e) obtained from Chaetomorpha antennina (green algae).
3.3.3. Scanning Electron microscopy

Morphology of iron oxide nanoparticle was visualized with help of Scanning Electron microscopy. See, Figure 1d, 1e, 2d, 2e for details.

Fig. 2.(a) UV-Vis Spectra; (b) XRD spectrum; (c) FTIR spectra; SEM image of bare Fe₃O₄Nps (d) and citrate capped Fe₃O₄Nps (e) obtained from Turbinariaturbinata(brown alga).
3.3.4. X-ray diffraction

The phase identification and crystalline structures of the nanoparticles was characterized by X-ray powder diffraction (Figure 2(b)). The X-ray diffraction patterns obtained for the Fe₃O₄-NPs synthesized using BS extract is shown in Figure 3. It is found that there exist strong diffraction peaks with 2θ values of 30.4°, 35.8°, 43.5°, 54.1° and 57.4°, corresponding to the crystal planes of (200), (311), (511) and (440) of crystalline Fe₃O₄-NPs, respectively. The results show the spinel phase structure of magnetite and are in agreement with the XRD standard for the magnetite nanoparticles. (JCPDS file No. 19-0629). Using the Scherrer equation the average crystallite sizes of the magnetic Fe₃O₄-NPs are found to be in the range of 8–16 nm and was comparable with results obtained by other researchers, working with algal extract [1].

3.3.5. Charge characterization of Iron Oxide Nps

In the present study it was observed that the Iron Oxide Nps were negatively charged when citrate capping was undertaken. However, uncoated Iron Oxide Nps were positively charged when observed on 2.5% Agarose gel electrophoresis. Hence the negatively charged citrate Iron Oxide Nps certainly differs with respect to uncoated Iron Oxide Nps and could be employed selectively as per the needs, see Figure 3.

Fig. 3: Electrophoresis of citric acid coated and bare magnetic Iron Oxide Nps in TAE buffer. (Sample 1-Chaetomorpha citrate capped, Sample 2-Turbinaria citrate capped , Sample 3-Chaetomorpha bare Iron Oxide Nps, Sample 4-Turbinaria bare Iron Oxide Nps).

Conclusion

In summary, we demonstrated that Iron Oxide Nps can be synthesized from both Chaetomorpha antennina (green algae), as well as Turbinaria turbinata (brown algae). Bioextracts of different Algal species (Chaetomorpha antennina/ Turbinaria turbinata) can give variations in particle size, morphology, property etc. We observed that concentration of algal extract (0.1%) is very important for nanoparticles synthesis as higher concentration of algal extract (1%) results in bulky sized Iron Oxide Nps. Nanoparticles synthesized at pH 8 shows a higher ferromagnetism, while the Nps synthesized at pH 6 shows the least magnetism. The Yield of Nps was also low at pH 6 when compared with higher pH conditions.

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