Full Length Research Paper

Investigation of carrier oil stabilized iron oxide nanoparticles and its antibacterial activity

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Iron oxide nanoparticles were synthesized by co-precipitation method. The polyunsaturated carrier oil (flaxseed oil) is used as a stabilizing agent for iron oxide nanoparticles. Kirby Bauer method was used to investigate the antibiotic sensitivity of carrier oil stabilized and uncoated SPIONs at 10 and 20 μg/L on Gram-positive bacterium Bacillus cereus (vegetative cell). The nanoparticles were characterized by X-ray diffraction method (XRD), Fourier transform infrared red (FTIR) analysis, particle size analyzer and Transmission Electron Microscopy. Structure of magnetite nanoparticles was confirmed by XRD analysis and the estimation of nanoparticle size was confirmed with TEM. The attachment of functional groups of oil was predicted using FTIR spectroscopy. This comparison study revealed that carrier oils stabilized iron oxide nanoparticles show more antibacterial activity than the bare iron oxide nanoparticles.

Key words: Iron oxide nanoparticles, flaxseed oil, Bacillus cereus

INTRODUCTION

The iron oxide nanoparticles can be synthesized by co-precipitation method. The nanoparticles of iron oxide such as Fe₃O₄ and γ-Fe₂O₃ are very prominent materials in biomedical applications (Sun et al., 2007; Lee et al., 2004; Pan and Yu, 2009). The prevention of agglomeration is critical a factor during the synthesis and it can be controlled by appropriate stabilizer, but the stabilizing agent should ensure its function and not to have any effect on the toxicity of the nanoparticles. In this work, flaxseed oil (linseed oil) is used as a stabilizing agent. Flaxseed oil is a colourless to yellowish oil obtained from the dried, ripened seeds of the flax plant (Linum usitatissimum, Linaceae). Flax-based oils are sought after as food because of their high levels of polyunsaturated α-linolenic acid (C18:3) (Figure 1), which is a one form of omega-3 fatty acid. The other fatty acids, oleic acid (monounsaturated - omega -6 - C18:1), saturated palmitic acid (C16:0) and stearic acid (C18:0) are also constituted (Angerer and von Schacky, 2000; Balk et. al., 2006; Barceló-Coblijn et al., 2008; Barre et al., 2009). Linseed oil is an edible oil marketed as a nutritional supplement (Bassett and Rodriguez-Leyva, 2009). Some

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The synthesis of iron oxide nanoparticles was carried out in a two-step process. Initially, a solution of 0.4 mol/L iron(III) chloride (FeCl₃) and 0.2 mol/L ferrous sulphate (FeSO₄) was prepared. This solution was then mixed with deionized water and heated to 80°C under continuous stirring using a magnetic stirrer for 1 h. The pH was maintained between 10 and 11 with the addition of 10% NaOH. A dark solution was obtained, which was then heated to 80°C in a hot air oven and allowed to cool down.

For stabilization, the precipitated iron oxide particles were washed several times with double distilled water and filtered. Then, 20 μL of flaxseed oil was added to the precipitated solution for 30 min. Continuous stirring using a magnetic stirrer was used to ensure uniform distribution of the oil. The suspension was then heated to 80°C in a hot air oven to evaporate the water, leaving a fine powder. The iron oxide nanoparticles were then dried at 150°C for 2 h and ground to a fine powder.

MATERIALS AND METHODS

All the chemicals used were of analytical reagent grade and used without further purification. Ferric chloride (FeCl₃, 99%), ferric chloride (FeCl₃, 99%), and Sodium hydroxide (NaOH) were obtained from Merck (India). Flaxseed oil was obtained from Falcon Industries, India. The Gram-positive bacterium B. cereus was purchased from Institute of Microbial Technology (Chandigarh, India), India.

Coprecipitation method was adopted for making the iron oxide nanoparticles. A 100 ml of 0.4 mol/L solution FeCl₃ and 100 ml of 0.2 mol/L FeSO₄ were mixed and dissolved in deionized water. Then 2 mol/L of sodium hydroxide was added into the above solution and the pH value between 10 to 11 was maintained with continuous stirring using a magnetic stirrer for 1 h and a dark precipitation was formed. 5 ml of flaxseed oil was taken and heated to 80°C in hot air oven and added in the precipitated solution for stabilization. The precipitated iron oxide particles were washed several times with double distilled water and filtered. Then it was dried at 150°C for 2 h and ground to fine powder. Then the same procedure was followed for preparing bare iron oxide nanoparticles without adding of flaxseed oil.

X-Ray diffraction (XRD) patterns were recorded with a Philips analytical X-ray diffractometer using CuKα radiation (λ= 1.5406 Å). FTIR spectra were performed and recorded with a Fourier transform infrared spectrophotometer of type Nicolet 870. TEM was recorded using Philips CM12 model. Particle size analysis was done by Malvern (U.K.) Make 2000E model.

Determination of antibacterial activity by well-diffusion method

Antimicrobial assay for the synthesized iron oxide nanoparticles were performed against Gram-positive B. cereus by Kirby-Bauer disk diffusion method. The pure cultures of organisms were sub-cultured in Müller-Hinton broth at 121°C at 15 psi for 45 min in an autoclave. The medium is poured into sterile Petri plate and incubated at 37°C for 24 h to check the plate sterility. The overnight grown B. cereus (4×10⁹) count was taken and swabbed on three dimensional lawn types on Mueller Hinton agar (MHA) plates. The sterile disc was coated with 10 μg/L each nanoparticle fix on the top surface of the medium. The plates were incubated at 37°C for 24 h and observed for every 4 h. It was observed that the zone of lysis was increasing on prolonged incubation. After 24 h incubation the plates were examined for the appearance of zone of inhibition. The zone of inhibition was measured in mm and recorded.

RESULTS AND DISCUSSION

XRD analyses confirmed that the synthesized nanoparticles were iron oxide nanoparticles as shown in Figure 2. The characteristic peaks were marked by their 2θ angles and compared with JCPDS data. The planes (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1) and (4 4 0) were observed for the corresponding angles of both the samples. The particle sizes were evaluated from the XRD data using the Debye - Scherrer equation and the average particle sizes of the flaxseed oil stabilized iron oxide nanoparticles were 32 and 52 nm for the uncoated sample.

The surface modification of the iron oxide nanoparticles with their stabilization was confirmed by Fourier transform infrared spectroscopy measurements (FTIR) (Figure 3). The presence of absorption peaks in the region of wave numbers 550 to 630 cm⁻¹ corresponding to the Fe-O vibration. The peaks at 3434 cm⁻¹ in Figure 2a and 3442 cm⁻¹ in Figure 2b were related to the vibrations of -OH and the peaks at 1707, 1629, 1706 and 1631 cm⁻¹ were due to the overlapping of the absorption bands of the carboxyl groups and double bond of oleic acid and α-linolenic acid, respectively (Lu et al., 2012). The other peaks observed in the region of 882 to 1366 cm⁻¹ in sample (b) were due to the additional compounds (polyphenols, peroxides and polycyclic aromatic hydrocarbons (PAHs), vitamin K and E) present in the oil.

The particle size and the morphology of iron oxide nanoparticles were observed by transmission electron microscopy (TEM). Figure 4 shows TEM micrographs of samples of uncoated and flaxseed oil stabilized iron oxide nanoparticles and these images indicate that the particles are not aggregated and the mean physical sizes were obtained by Debye-Scherer formula. The antibacterial activity of iron oxide nanoparticles of uncoated (a) and flaxseed oil mediated samples were performed against Gram- positive bacterium B. cereus (vegetative cell) at 10 μg/L and 10 μg/L concentrations using agar well diffusion method. The values of zone of inhibition (mm) of these nanoparticles were presented in the Table 1.

Figures 5 and 6 show the zone of inhibition of bacterial growth on agar plates with two different concentrations of flaxseed oil stabilized and bare iron oxide nanoparticles. It was observed from the images and pie chart that the growth of bacteria was inhibited gradually with increase in
concentration of iron oxide nanoparticles. Further the results clearly demonstrate that Flaxseed oil mediated iron oxide nanoparticles could promise a better antimicrobial agent than the bare iron oxide nanoparticles.

**Conclusion**

In the field of nanotechnology, the development of reliable and eco-friendly processes for synthesizing metal oxide nanoparticles is very essential. Here, we have reported a simple, eco-friendly and low-cost approach for preparation of magnetite nanoparticles by reduction of ferric chloride solution with a green method using flaxseed oil as the stabilizing agent and compared with the uncoated iron oxide nanoparticles. The characteristics of the obtained iron oxide nanoparticles were studied using FTIR, XRD and TEM techniques. The antibacterial effects of flaxseed oil stabilized iron oxide nanoparticles and bare iron oxide nanoparticles against gram- positive bacterium bacillus cereus (vegetative cell) were investigated.

The results exhibit that the flaxseed oil stabilized iron oxide nanoparticles were less in size, more antibacterial activity than the bare iron oxide nanoparticles.
Figure 4. TEM photographs of uncoated (a) and flaxseed (b) SPIONs.

Table 1. Zone of inhibition (mm) for various concentrations of ncoated (a) and flaxseed (b) SPIONs.

<table>
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<th>S/N</th>
<th>Nanoparticle</th>
<th>Zone of inhibition (mm)</th>
<th>S/N</th>
<th>Nanoparticle</th>
<th>Zone of inhibition (mm)</th>
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<td>1</td>
<td>Flaxseed oil stabilized SPIONs</td>
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REFERENCES


