

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 poly unsaturated α-linolenic acid (C18:3) (Figure 1), which is a one form of omega-3 fatty acid. The other fatty acids, oleic acid (monosaturated - 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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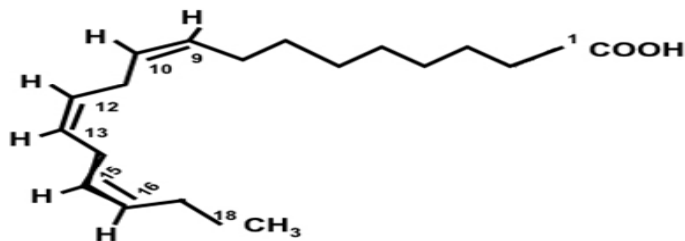


Figure 1. Structure of α -linolenic acid.

studies suggest that α -linolenic acid (a substance found in flaxseed and flaxseed oil) may benefit people with heart disease (Bays 2007; Hou et al., 2007; Ye et al., 2006).

The antibacterial test on *Bacillus cereus* (*B. cereus*) was studied using this flaxseed oil stabilized iron oxide nanoparticles. *B. cereus* is a bacterium that is commonly associated with large outbreaks of food borne illness. *B. cereus* has a wide distribution in nature, frequently isolated from soil and growing plants, but it is also well adapted for growth in the intestinal tract of insects and mammals.

MATERIALS AND METHODS

All the chemicals were of analytical reagent graded and used without further purification. Ferrous sulphate (FeSO_4 , 99%), ferric chloride (FeCl_3 , 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 FeCl_3 and 100 ml of 0.2 mol/L FeSO_4 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 grinded 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 $\text{CuK}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$). 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* (4×10^9) count was taken and swabbed on three dimensional lawn types on Mueller Hinton agar (MHA) plates. The sterile disc was coated with 10 $\mu\text{g/L}$ of each nanoparticle fix on the top surface of the medium. In another case 20 $\mu\text{g/L}$ of each nanoparticle was coated and fixed 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-Scherrer 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 $\mu\text{g/L}$ and 10 $\mu\text{g/L}$ 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

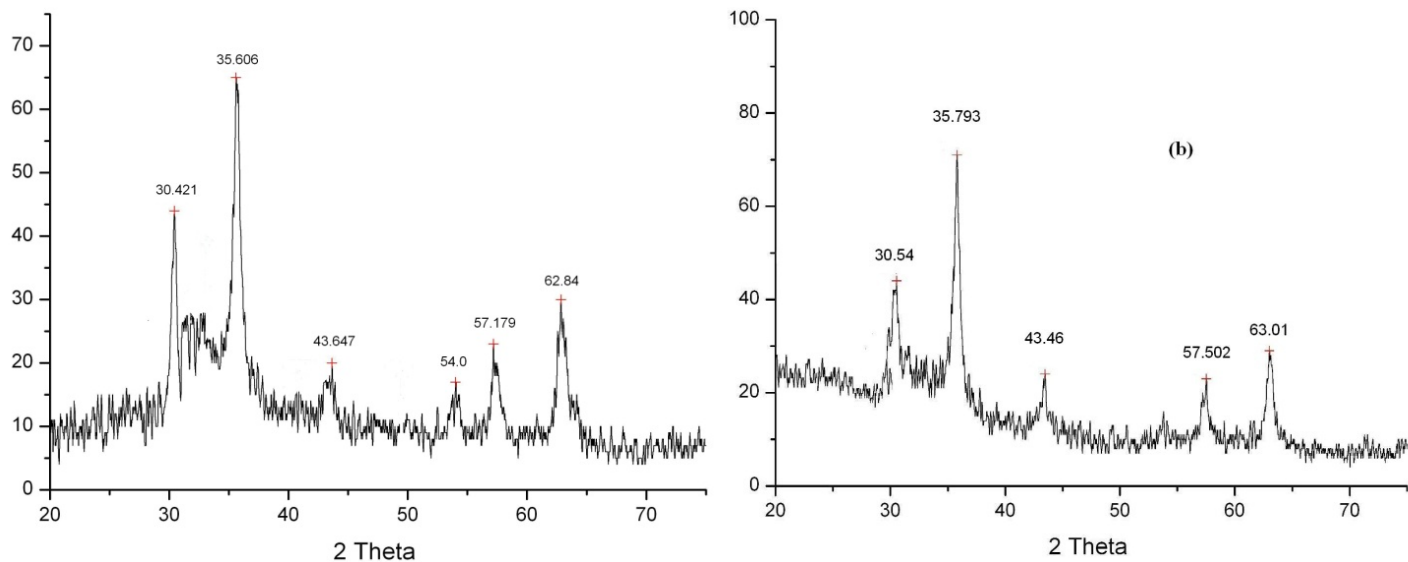


Figure 2. XRD patterns of uncoated (a) and flaxseed (b) iron oxide nanoparticles.

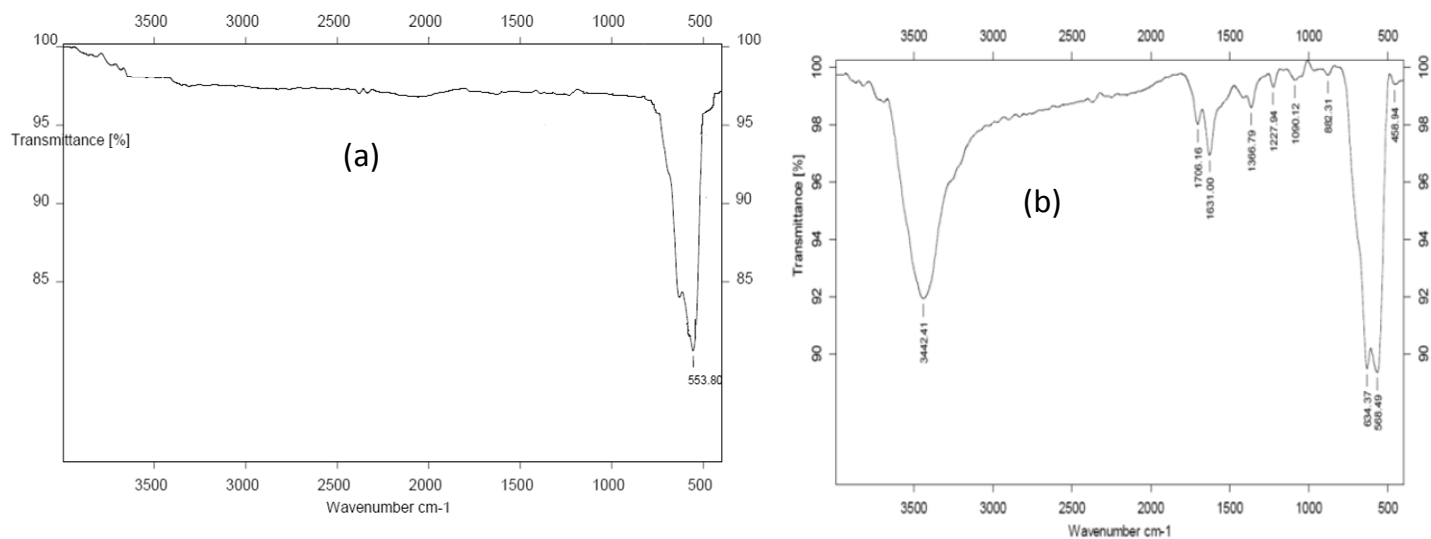


Figure 3. FTIR analysis of uncoated (a) and flaxseed (b) iron oxide nanoparticles.

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 of 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.

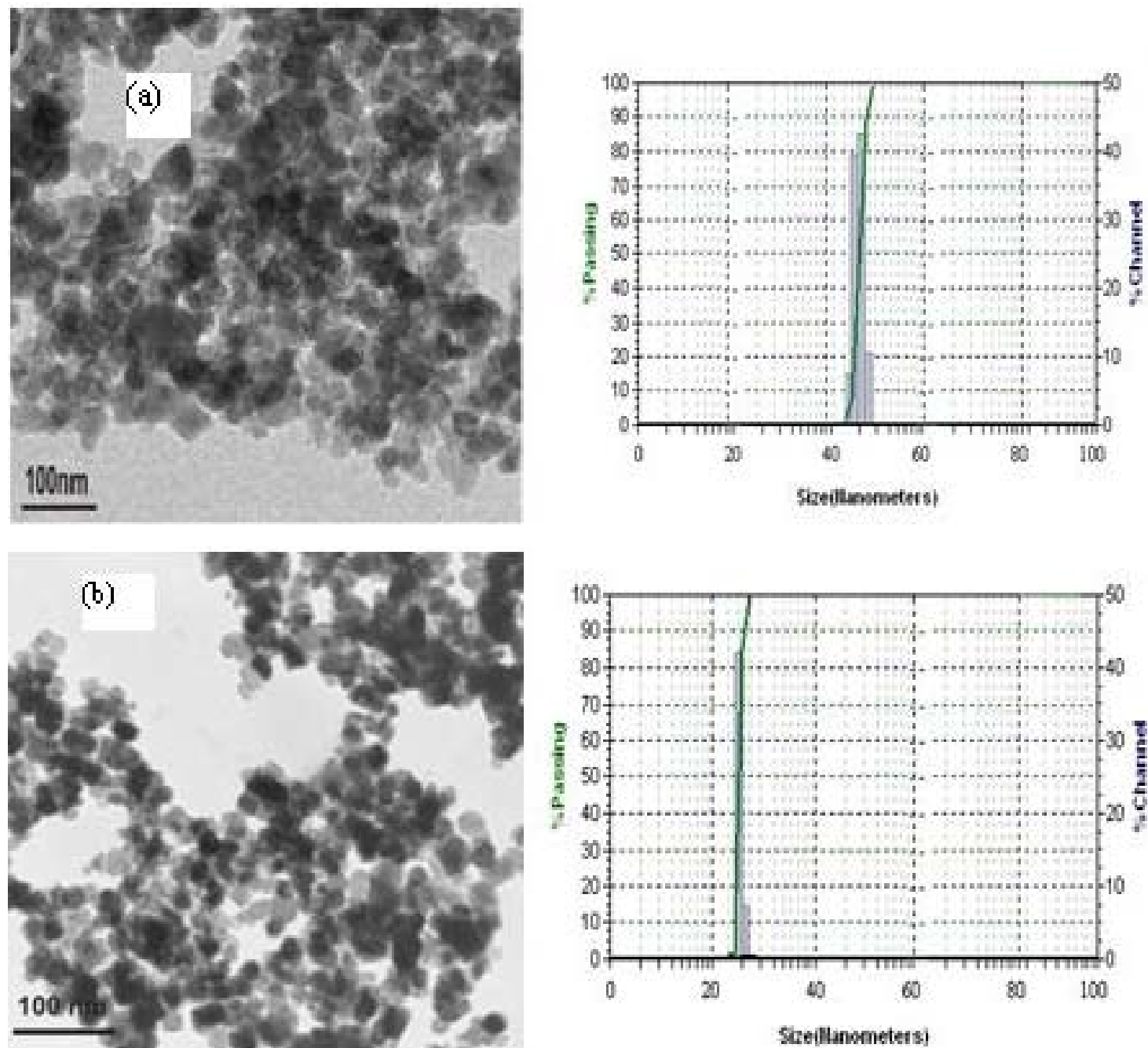


Figure 4. TEM photographs of uncoated (a) and flaxseed (b) SPIONs.

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

10 µg/L of nanoparticle coated			20 µg/L of nanoparticle coated		
S/N	Nanoparticle	Zone of inhibition (mm)	S/N	Nanoparticle	Zone of inhibition (mm)
1	Flaxseed oil stabilized SPIONs	3.75	1	Flaxseed oil stabilized SPIONs	8.5
2	Uncoated SPIONs	1.5	2	Uncoated SPIONs	3.5

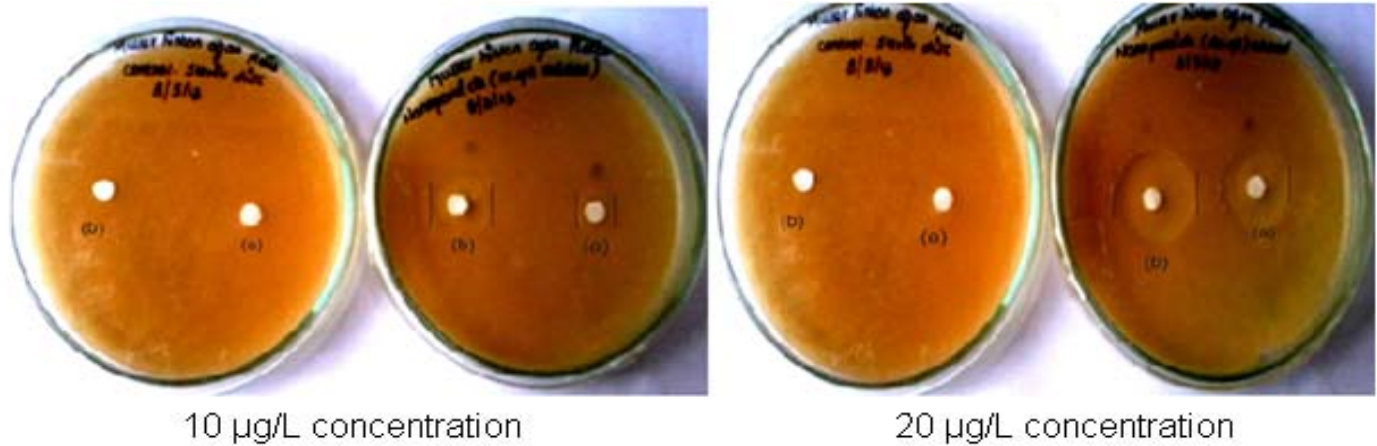


Figure 5. Zone of inhibition (mm) of uncoated (a) and flaxseed (b) iron oxide nanoparticles

10 µg/L of nanoparticle coated

20 µg/L of nanoparticle coated

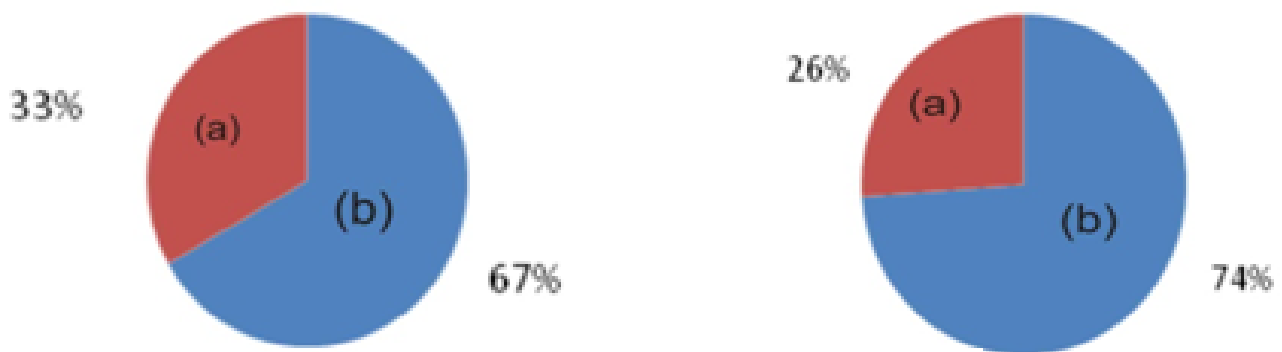


Figure 6. Pie chart zone of inhibition (mm) of uncoated (a) and flaxseed (b) iron nanoparticles.

REFERENCES

Sun J, Zhou S, Hou P, Yang Y, Weng J, Li X, Li M (2007). Synthesis and Characterization of biocompatible Fe₃O₄ Nanoparticles, Wiley periodicals, Inc. J. Biomed. Mater. Res. 333-341.

Lee S, Jeong J, Shin SC, Kim JC, Kim JD (2004). Synthesis and characterization of superparamagnetic maghemite nanoparticles prepared by co-precipitation technique, J. Magnetism and Magnetic Mat. 282:147- 150.

Pan A, Yu D (2009). Demark-Wahnefried W. Meta analysis of the effects of Flaxseed interventions on blood lipids, Am. J. Clin. Nutr. 90(2):288-297.

Angerer P, von Schacky C (2000). polyunsaturated fatty acids and the cardiovascular system. Curr. Opin. Lipidol. 11(1):57-63.

Balk EM, Lichtenstein AH, Chung M (2006). Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: A systematic review. Atherosclerosis. 189(1):19-30.

Barceló-Coblijn G, Murphy EJ, Othman R, Moghadasian MH, Kashour T, Friel JK (2008). Flaxseed oil and fish-oil capsule consumption alters human red blood cell n-3 fatty acid composition: a multiple-dosing trial comparing 2 sources of n-3 fatty acid. Am J Clin Nutr. 88(3):801-809.

Barre DE, Mizier-Barre KA, Griscti O, Hafez K (2009). Flaxseed oil supplementation increases plasma F1-phytosteranes in healthy men. J. Nutr. 139(10):1890-1895.

Bassett CM, Rodriguez-Leyva D, Pierce GN (2009). Experimental and clinical research findings on the cardiovascular benefits of consuming flaxseed. Appl Physiol Nutr Metab. 34(5):965-974.

Bays HE (2007). Safety considerations with omega-3 Fatty Acid therapy. Am. J. Cardiol. 99(6A):S35-43.

Hou Y, Xu Z, Sun S (2007). Controlled Synthesis and chemical Conversions of FeO Nanoparticles, Angew. Chem. Int. Ed. 46:6329 6332X.

Ye R, Daraio C, Wang C, Talbot JB, Jin S (2006). Room temperature solvent-free Synthesis of Monodisperse Magnetite Nanocrystals, J. Nanoscience Nanotechnol. 6:852-856.

Lu C, Bhatt LR, Jun HY, Park SH, Chai KY (2012). Carboxyl-polyethylene glycol-phosphoric acid: a ligand for highly stabilized iron oxide nanoparticles, J. Mater. Chem., 22:19806-19811.