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ANTIBACTERIAL STUDY OF OLIVE OIL STABILIZED SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES

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ABSTRACT

This work presents the synthesis, characterization and implication of magnetic nanoparticles in identifying the antibiotic sensitivity on gram positive bacteria with one of the important carrier oil (Olive oil), which is so useful in biomedical applications and here used as stabilized agent. The iron oxide nanoparticles were synthesized using co-precipitation method and stabilized with bio-surfactant such as olive oil. These nanoparticles were characterized by X-ray diffraction method, FTIR analysis, particle size analyzer and Transmission Electron Microscopy. Structure of initial magnetite nanoparticles synthesized was confirmed by XRD analysis and the estimation of nanoparticles size with the value of 20-50 nm and it was confirmed with TEM. The attachment of functional groups of oils was predicted using FTIR spectroscopy. Studies indicate that olive oil stabilized iron oxide nanoparticles show effective antibacterial activity toward the gram- positive bacterium bacillus cereus compared to sample which was prepared without any stabilizer. The results suggest that iron oxide NPs with surface coating of olive oil could potentially be used as an effective antibacterial agent.

Keywords: Super paramagnetic Iron oxide nanoparticles, Olive oil and bacillus cereus.

INTRODUCTION

The nanotechnology has exposed a wide range of biomedical applications with various approaches by taking into consideration the needs of greener bioprocesses and novel enhancers for synthesis using microbial processes, biosurfactants, and/or biosurfactant producing microbes are emerging as an alternate source for the rapid synthesis of nanoparticles. It is an alternative greener approach to reduce the costs without sacrificing too much quality. Biosurfactants are natural surfactants derived from microbial origin composed mostly of sugar and fatty acid. They have higher biodegradability, lower toxicity, and excellent biological activities. The biosurfactant mediated process and microbial synthesis of nanoparticles are now emerging as clean, nontoxic, and environmentally acceptable "green chemistry" procedures. The biosurfactant-mediated synthesis is superior to the methods of bacterial- or fungal-mediated nanoparticle synthesis, since biosurfactants reduce the formation of aggregates due to the electrostatic forces of attraction and facilitate a uniform morphology of the nanoparticles. In this review, we highlight the biosurfactant mediated synthesis of nanoparticles with relevant details including a greener bioprocess, sources of biosurfactants, and biological synthesized nanoparticles based on the available literature and laboratory findings [1-2].

Magnetic iron oxide nanoparticles (MION) have been used in various fields owing to their unique properties including large specific surface area and simple separation with magnetic fields. MIONs present many potential possibilities in biomedicine. Also, the interest in the potential application of the magnetic technique and in food related applications such as enzyme immobilization, protein purification, and food analysis in pharmacy is notably growing. It is currently being recognized that this magnetic nanotechnology could play an important role in this area. Fe_3O_4 (Magnetite) and γ- Fe_2O_3 (Maghemite) iron oxides are biocompatible, but there is question of potential impact of these nanoparticles on the environment and human health. Lot of investigations has been carried out using iron oxide nanoparticles linked to their high mobility and specific reactivity with cells. Magnetite is readily oxidized to maghemite in the presence of oxygen. They are commonly referred to as superparamagnetic, because while on a microscopic level they are ordered, they are magnetized as a whole in the presence of an extremely applied magnetic field.

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It is very important to control the size and shape and to keep the thermal and chemical stability of the nanoparticles. Stable colloidal suspension of magnetic nanoparticles is challenging owing to both Vander Waals forces and magnetic dipolar interactions. Thus, it is essential to coat magnetic nanoparticles with a surfactant during chemical synthesis in order to prepare well-dispersed nanoparticles. Oleic acid is a well-known surfactant in stabilizing colloid and in addition to this some other long chain carboxylic acids, such as erucic acid and linoleic acid also have been used. Oleic acid is typically preferable because it is easily available inexpensive natural sources (e.g., olive oil). Hence the usage of these carboxylic acids can be substituted with everyday chemicals. Edible oils show a potential for replacing oleic acid with a mixture of fatty acids. The carrier oil chosen here is Olive Oil the stabilization of the iron oxide nanoparticles and also these oils have the antimicrobial properties. The table 1 shows the percentage of various fatty acids constituted in olive oil.

The olive oil used here is a natural stabilizer and which is derived without any chemical reaction. The olive oil is unlikely to cause allergic reactions, and as such can be used in preparations for lipophilic drug ingredients. Further the olive oil is very effective in controlling of heart disease, stroke, cholesteral level and the usage of iron oxide nanoparticles with its coating during the treatment may have the advantage to the blood cells. Olive oil is predominantly a triacylglyceride of long chain fatty acids with free fatty acids (FFA), Polyphenols (Antioxidants), Peroxides, Polycyclic Aromatic Hydrocarbons (PAHs),vitamin K and vitamin E. The Primary fatty acids in olive oil are oleic acid [8], linoleic acid and linoolenic acid. Oleic acid is monosaturated and makes up 85% of olive oil ($C_{17}H_{35}COOH$) or CH_3-$(CH_2)_7$ – CH = CH – $(CH_2)_7$ – COOH and linoleic acid is polyunsaturated which makes up 15% of olive oil [1-5] . The olive oil mediated syntheses of particles were successfully demonstrated and shows that the nanoparticles were stable for several months without any decomposition and favours particles homogeneity [2-4].

It was thought that the iron oxide is not considered as an antibacterial agent, a few studies predict that the effect of iron oxide nanoparticles on bacteria and its inhibition of bacteria activity have been appreciated. Iron oxide, are not antibacterial in their bulk form but may exhibit antibacterial properties in nanoparticulate form. Iron oxides are very prominent in biomedicine not only due to their inherent antibacterial properties, but also due to their superparamagnetic nature. Hence these MIONs coating with some type of antimicrobial agents possibly permit to get inside the body with a magnetic field. Here the carrier oil coating on IONPs can enhance both biocompatibility and antibacterial activity. Several reports suggests that the various nanoparticles viz. Al_2O_3, Fe_3O_4, CeO_2, ZrO_2 and MgO have the antibacterial potential against various gram positive and gram negative bacterias. In this work, we studied the antibacterial test on Bacillus cereus (B. cereus). Bacillus 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. There are two types of illnesses associated with B. cereus: emetic and diarrheal. The emetic illness (vomiting) is caused by a heat and acid stable toxin produced by B. cereus in food before the food is consumed. The diarrheal illness is associated with a less stable toxin. This toxin is released when a large number of B. cereus cells are broken down by the stomach's enzymes. B. cereus is widely distributed in the environment and is found almost everywhere including soil, dust, water, air, and decaying matters also found on animals [5-7].

MATERIALS AND METHODS

All the chemicals were of analytical reagent grade and used without further purification. Ferrous sulphate ($FeSO_4$, 99%), ferric chloride ($FeCl_3$, 99%) and Sodium hydroxide (NaOH) were obtained from Merck (India). Olive Oil was received from Falcon (Exporters of Essential Oils), Bangalore, India. Deionized water was used for the reactions at all stages of the synthesis. All the equipments and materials for antibacterial study were received and conducted in Microcore research Lab, Erode. The gram-positive bacterium bacillus cereus was purchased from Institute of Microbial Technology (Chandigarh, India), India.

Synthesis and Characterization

The co-precipitation method was adopted for synthesis of MIONs. 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 was maintained between 10-11 with continuous stirring using a magnetic stirrer for 1 hour and a dark precipitation was formed. Similarly four samples were prepared and kept separately. Each 5 ml of Olive oil is taken and heated to 80^0C in hot air oven. Then this oil was added slowly in the four samples and stirred continuously for 48 hrs. The resulting ferrosferric hydroxide dehydrates yielded precipitation of the iron oxide particles and they were washed several times with double distilled deionized water and then filtered. Finally it was dried at 150^oC for 2 hr and grinded to fine powder.

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 between 4000 and 400 cm-1 with a resolution of 4cm-1. The morphologies and compositions of the Fe_3O_4 nanoparticles were examined by Scanning Electron Microscopy (SEM) using a LEO 1455 VP equipped with energy dispersive. TEM photograph was taken by Philips CM12 model. VSM studies carried out by Lakeshore, USA; Model 7404, particle size analysis were done by Malvern (U.K.) Make 2000E model [8].

Antibacterial assay

Muller Hinton Agar 3.8 % concentration is prepared in reverse Osmosis water and sterilized at 121 deg C at 15 psi for 45 minutes under Autoclave. The medium is poured in to sterile petriplate and incubated at 37 deg for 24 hrs to check the plate sterility. It was found that the complete MHA plates are clean and sterile further used for the antibacterial sensitivity test by disk diffsuion method (Kirby Bauer method). The overnight grown Bacilli cereus ($4x10^9$) count a loop full of inoculums of was taken and swabbed on three dimensional lawn type on MHA plates. To the plates the sterile discs are fixed and the plate was considered as control. The sterile disc was coated with 10µg of each nanoparticle fix on the top surface of the medium. In another case 20µg of each nanoparticles was coated and fixed on the top surface of the medium. The plates were incubated at 37 deg C for 24 hrs and observed for every 4 hrs. It was observed that the zone of lysis was increasing as the incubation on prolonged incubation. After 24 hrs incubation the plates were examined for the appearance of zone of inhibition. The zone of inhibition was measured in mm and recorded [9].

RESULTS AND DISCUSSION

The iron oxide powder sample with olive oil as surfactant is prepared and characterized by XRD studies. The XRD patterns reveal that the powders are in nanosize and the nanoparticles of iron oxide prepared by wet chemical route are crystalline. The fine particles nature of the sample is reflected in the X-ray line broadening. The X-ray diffraction analysis shows that the sample prepared by the co-precipitation method resulted the formation of mixed phase of Fe_3O_4 and γ- Fe_2O_3 nanoparticles. The presence of γ-Fe_2O_3 nanoparticles is due to the oxidation of Fe_3O_4 nanoparticles during synthesis. The eight characteristic peaks for (220), (221), (311), (400), (422), (511), (440) and (533) correspond to iron oxide spinel structure of magnetite and maghemite. Fig.1 shows the XRD pattern of the mixed phase of nanocrystalline iron oxide (Fe_3O_4 and γ- Fe_2O_3) and it matches well with the JCPDS data (JCPDS cards #75-0033 (Fe_3O_4), # 39-1346 (γ- Fe_2O_3).The application of Debye- Scherrer's formula to the major peak intensities reveals the information of Fe_3O_4 and γ- Fe_2O_3 nanoparticles are with average size of 70 - 80 nm [6-8].

The TEM image and particle size distribution of the olive oil coated iron oxide nanoparticles are shown in fig.2. The TEM samples were prepared by placing a drop of dilute suspension of iron oxide nanoparticles in ethanol on a carbon coated copper grid and allowed the solvent to evaporate slowly at room temperature. It reveals that the particles are in spherical shape and its average size obtained from TEM analysis is almost same as it is estimated from XRD analysis.

FTIR spectra were recorded in solid phase using the KBr pellet technique in the regions of 3500–400 cm^{-1}. FTIR spectra of iron oxide nanoparticles are shown in Figure 3. FTIR spectra of iron oxide nanoparticles exhibited vibrations in the region 400–600 cm^{-1} which can be attributed to the vibrations of Fe−O which confirms the formation of iron oxide nanoparticles. The band appearing at 1629 cm^{-1} can be attributed to the angular deformation of water δH−OH, while the band appearing at 3434 cm^{-1} can be assigned to the O−H stretching of water. The present findings agree well with the values reported in the available literature. This FTIR analysis of the magnetically separated MNP's demonstrated the covalent binding of the carboxylic group of the particle surface in a bidentate / briding manner.

The magnetic properties of the olive oil coated iron oxide nanoparticles was measured by Vibrating-sample magnetometer. Fig.4 shows the magnetization of the coated iron oxide nanoparticles with reference to the external field at room temperature. Its saturation magnetization is predicted as 40 emu/g and it exhibits superparamagnetic behavior [9].

Antibacterial properties

Antibacterial activity results revealed that olive oil stabilized iron oxide nanoparticles acted as excellent antibacterial agents against gram- positive bacterium bacillus cereus. The table 2 shows the zone of inhibition (mm) of olive oil stabilized iron oxide nanoparticles and the Figure 4 shows the zone of inhibition produced by two different concentrations of iron oxide nanoparticles. The concentration of 20µl of olive oil stabilized nanoparticles exhibited large zone of inhibition (3.5mm) on bacterial growth against bacillus cereus than concentration of 10µl of olive oil stabilized nanoparticles (2mm). It appears that the antibacterial activity of the nanomaterials increased with increase in concentrations of iron oxide nanoparticles.

Table 1. Various fatty acids in Olive oil

FattyAcids (%)	Olive oil
Oleic acid	63-81 %
Linoleic acid	5-15 %
Palmitic acid	7-14 %
Stearic acid	3-5 %

Table 2. Zone of inhibition of various concentrations of Oliveoil stabilized iron oxide nanoparticles

S.No	Olive oil stabilized Nanoparticle Concentrations	**Zone of inhibition in(mm)**
1	10 µl	2
2	20 µl	3.5

Fig 1. XRD of nano particles of Magnetite(Fe_3O_4) and Maghemite (γ- Fe_2O_3)

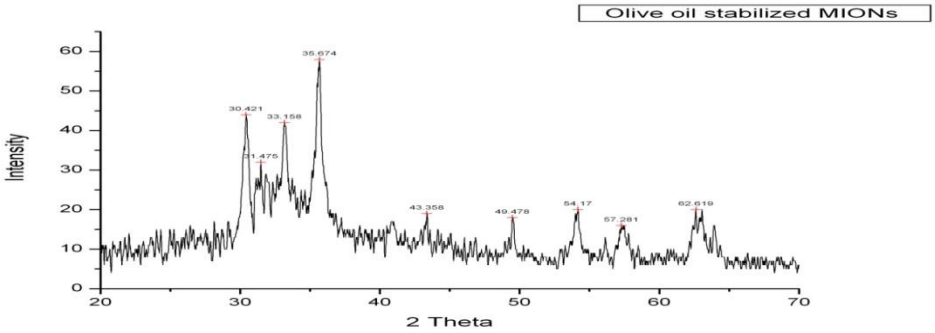
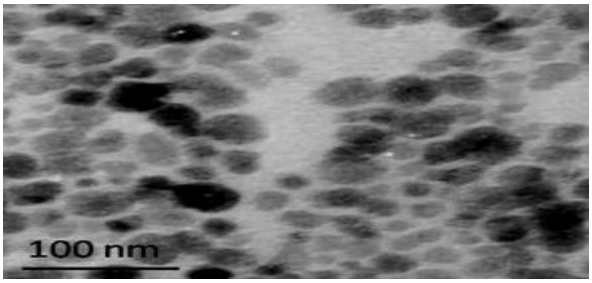


Fig 2. TEM image and Particle Size distribution of olive oil mediated iron oxide nanoparticles



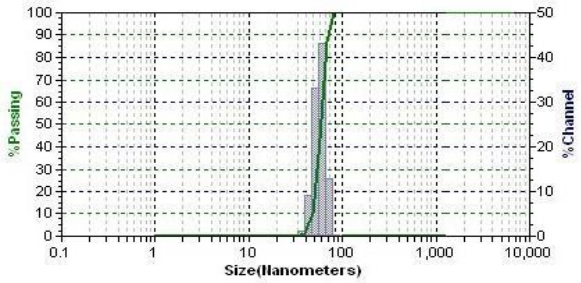


Fig 3. FTIR spectra of olive oil stablized iron oxide nanoparticles

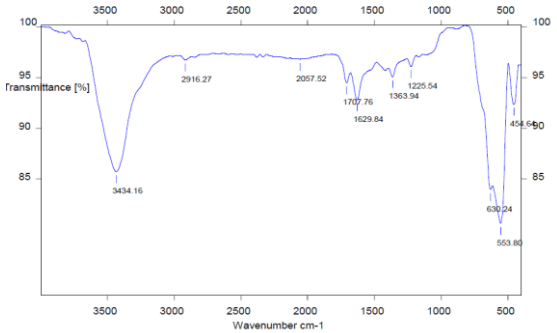


Fig 4. Magnetization curve at room temperature for olive oil encapsulated iron oxide nanoparticles

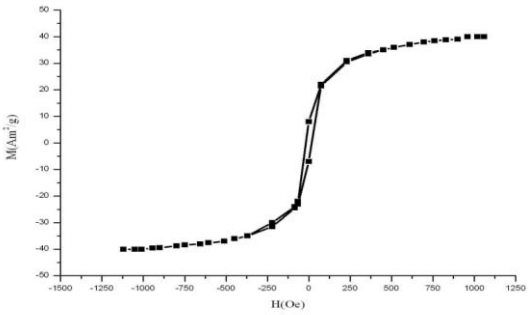


Fig 5. Zone of inhibition of various concentrations of Olive oil stabilized iron oxide nanoparticles

CONCLUSION

The nanosized Iron oxide particles of were synthesized by the co-precipitation method. A non-toxic chemical synthesis of iron oxide nanoparticles has been developed by olive oil as capping and stabilizing agent. It has been shown that the as-prepared nanoparticles are in good homogeneity with mixed phases of iron oxides. XRD and TEM results showed that these nanoparticles were around 70 – 80 nm in size. M-H characteristics show the super paramagnetic behavior at room temperature of the iron oxide nanocrystallites. Furthermore, the antibacterial activity of the synthesized nanomaterial was compared and varied considerably with two different concentrations. Our result indicates that the antimicrobial activity is increased with increase in concentration of nanoparticles.

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