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Research Article

SYNTHESIS CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF IRON OXIDE NANOPARTICLES AGAINST *STAPHYLOCOCCUS EPIDERMIDIS*

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ABSTRACT

Objective: This study deals with the synthesis of iron oxide nanoparticles by sol-gel technique, their characterization and antibacterial activity of these nanoparticles against *Staphylococcus epidermidis.*

Methods: Hematite (α-Fe_2O_3) nanoparticles were successfully synthesized by sol-gel method using tetraethyl orthosilicate as a precursor. The structural morphology, size, and chemical state of synthesized iron oxide nanoparticles have been investigated by X-ray diffractometer (XRD), transmission electron microscopy, Fourier transform infrared spectroscopy, and ultraviolet-visible spectroscopy. The antibacterial activities of these iron oxide nanoparticles were investigated on a pathogenic bacteria *S. epidermidis*, by measuring the zone of inhibition and colony-forming units on solid medium and by measuring the optical density of the culture solution. Antibacterial activity of iron oxide nanoparticles was also compared with well-known standard antibiotics.

Results: It was confirmed from XRD data that the synthesized iron oxide nanoparticles were hematite (α-Fe_2O_3) nanoparticles. Average particle size of the Fe_2O_3 nanoparticles was found to be 38.57 nm by XRD characterization. The antibacterial activity of Fe_2O_3 nanoparticles was almost comparable to the most of the standard antibiotics (taken for comparison), but Fe_2O_3 nanoparticles were found to be more effective than antibiotic ampicillin and sulfatriad toward *S. epidermidis*.

Conclusion: This study shows that Fe_2O_3 nanoparticles possess good antibacterial properties; therefore, these metal nanoparticles may be used in place of antibiotics. These inorganic metal nanoparticles can be used by pharmaceutical industries for further research regarding the toxicity study for its use in human being.

Keywords: Fe_2O_3, Sol-Gel, X-ray diffractometer, Transmission electron microscopy.

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INTRODUCTION

Nanosize metallic nanoparticles have been the subject to research in recent years because these materials represent an intermediate dimension between bulk materials and atoms/molecules. In recent years, the field of nanoscience and nanotechnology has resulted in the production of different kinds of metal and metal oxide nanoparticles with antibacterial effects due to their high stability and non-toxic nature [1]. Among these metal nanoparticles, iron oxide nanoparticles have received special consideration because of their numerous scientific and technological applications such as biosensor [2,3], antimicrobial activity [4], ferrofluids, magnetic storage media, magnetic refrigeration, magnetic resonance imaging [5], cancer treatments [6,7], cell sorting, and targeted drug delivery. Iron oxide nanoparticles have also been widely used in biomedical research because of their biocompatibility and magnetic properties [8,9]. The synthesis of these IO nanoparticles is carried out by different chemical approaches such as sol-gel [10,11], hydrothermal [12], co-precipitation [13] surfactant mediated/template synthesis, microemulsion [14], electrochemical, and laser pyrolysis. The development of new resistant strains of bacteria to current antibiotics has become a serious problem in public health; therefore, there is a strong incentive to develop new bactericides from various sources. Recent advancement in the field of nanotechnology has provided an attractive method for synthesizing alternative antimicrobial agents and reducing biofilm formation. Although nanoparticles have long been known to exhibit a strong toxicity to a wide range of microorganisms [15,16], very little is known about the toxicity of iron oxide nanoparticles toward these microorganisms. In the present study, an attempt has been made to synthesize iron-oxide nanoparticles by sol-gel technique and these particles were characterized by various techniques along with the evaluation of their antibacterial activity against human pathogenic Gram-positive bacteria with a view to explore their pharmaceutical applications.

MATERIALS AND METHODS

Materials

All of the chemicals used in the experiment were of analytical grade and obtained from standard chemical sources. The *Staphylococcus epidermidis* (microbial type culture collection [MTCC] NO. 3382) was obtained from MTCC, Institute of Microbial Technology, Chandigarh.

Synthesis of Fe_2O_3 nanoparticles

Fe_2O_3 nanoparticles were synthesized using sol-gel method. The procedure uses $FeSO_4.7H_2O$ solution of pH 1-2, ethanol, and tetraethyl orthosilicate (TEOS) as the precursor material. The Fe_2O_3 nanoparticles were prepared by mixing $FeSO_4.7H_2O$ solution drop by drop into the flask containing 1:4 TEOS and ethanol solution with continuous stirring. The resulting solution was heated at 70.0°C with continuous stirring in a closed container for 6.0 h. The resulting solution was then kept in the oven at 100.0°C for 10–15 days and after that, the particles were kept in muffle furnace at 400.0°C for 4.0 h. Reddish-brown Fe_2O_3 nanoparticles were obtained.

Characterization techniques

The size, structure, morphology, and magnetic properties of as prepared metal nanoparticles were characterized by Fourier transform infrared (FT-IR) (Shimadzu corp-02014) in the wavelength range

400–4000/cm, ultraviolet (UV)-visible spectroscopy (Shimadzu 1800) in the wavelength range 200–1000/cm, X-ray diffractometer (XRD) (Rigaku mini-2 using Cuα1, λ=0.15406 nm radiations), and transmission electron microscopy (TEM) (FEI-Philips, Morgagni 286D with magnification up to 2,80,000x, Acc. Voltage: 100 Kv).

Antibacterial study

Antibacterial activity of the Fe_2O_3 nanoparticles against a Gram-positive *S. epidermidis* was investigated by measuring zone of inhibition (ZOI), colony-forming unit (CFU), and optical density (OD). The antibacterial activity of these Fe_2O_3 nanoparticles was also compared with well-known antibiotics. The bacterial test organisms *S. epidermidis* was grown in nutrient broth at 37°C for 24 h. The ZOI was measured by agar well diffusion method. A100 μl of the bacterial culture was spread onto the solidified agar plates. The wells were prepared in the agar medium with the help of a cork borer. These wells were subsequently loaded with different concentrations of Fe_2O_3 nanoparticles suspension in double-distilled water. The CFU of the bacterial culture was evaluated with different concentrations (10, 15, and 20 mg/ml) of Fe_2O_3 nanoparticles using the standard broth dilution method. The growth behavior of the *S. epidermidis* was investigated by measuring the OD through the administration of different concentrations of Fe_2O_3 nanoparticles into the dilute solution of the broth.

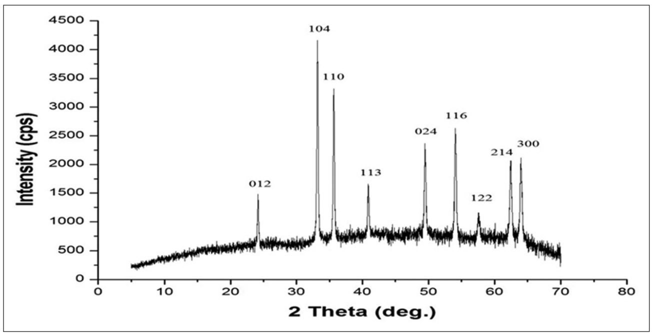

Fig. 1: X-ray diffractometer pattern of iron oxide nanoparticles synthesized by sol-gel technique

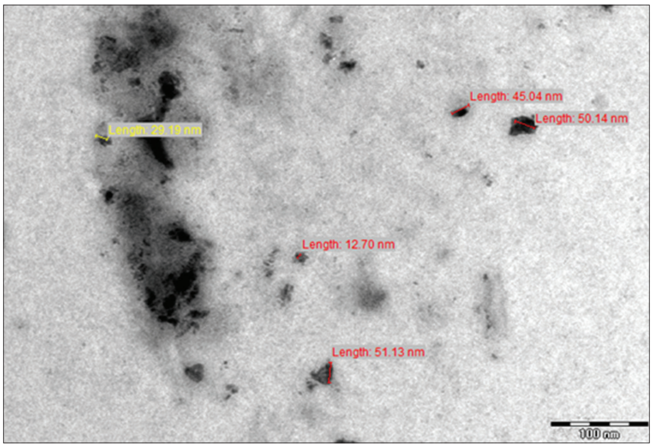

Fig. 2: Transmission electron microscopy image of iron oxide nanoparticles synthesized by sol-gel technique

RESULTS AND DISCUSSION

Characterization of iron oxide nanoparticles

Fig. 1 shows the X-ray diffraction pattern of the iron oxide nanoparticles synthesized by sol-gel method. The X-ray diffraction pattern revealed major peaks at 2θ values of 24.13 (012), 33.17 (104), 35.65 (110), 40.86 (113), 49.46 (024), 54.04(116), 57.6 (122), 62.49 (214), and 63.98 (300), respectively [17,18]. These XRD peaks correspond to pure α-Fe_2O_3 (hematite) nanoparticles. The XRD patterns were indexed to pure hexagonal structure with lattice parameter of a = 5.038 Å and c = 13.772 Å. The diffraction peaks correspond with JCPDS card no. 01-1030 and 87-1164, indicating that the α-Fe_2O_3 nanoparticles are crystalline structure. Average particle size of the Fe_2O_3 nanoparticles was found to be 38.57 nm using Scherrer's formula $d = K\lambda/\beta \cos\theta$.

Fig. 2 shows the TEM images of synthesized iron oxide nanoparticles. The particles appeared to be almost rod shaped. It can be seen from Fig. 2 that there is a uniform distribution of particle with mean particle size 37.64 nm which is nearly in close agreement with the XRD result.

Fig. 3 shows the FT-IR spectra of iron oxide nanoparticles synthesized by sol-gel method. The characteristic absorption bands at 557, 520, 482, and 434 cm^{-1} corresponds to the Fe-O stretching and bending vibration mode of α-Fe_2O_3 nanoparticles [19,20]. The peak centered at 3425 cm^{-1} corresponds to the stretching vibration of intermolecular hydrogen bond (O–H) existing between the adsorbed water molecules and indicates some amount of hydroxyl group.

Fig. 4 shows the UV-visible spectra of iron oxide nanoparticles synthesized by sol-gel method. An absorption band at 296 nm is characteristic of iron nanoparticles.

Antibacterial activity of iron oxide nanoparticles

Fig. 5a shows the ZOI produced by different concentrations (25, 26, 27, 28, and 29 mg/ml) of the Fe_2O_3 nanoparticles against *S. epidermidis*. It was observed that the ZOI increases with an increase in the concentration of the Fe_2O_3 nanoparticles. Fig. 5b and c shows the antibacterial activity

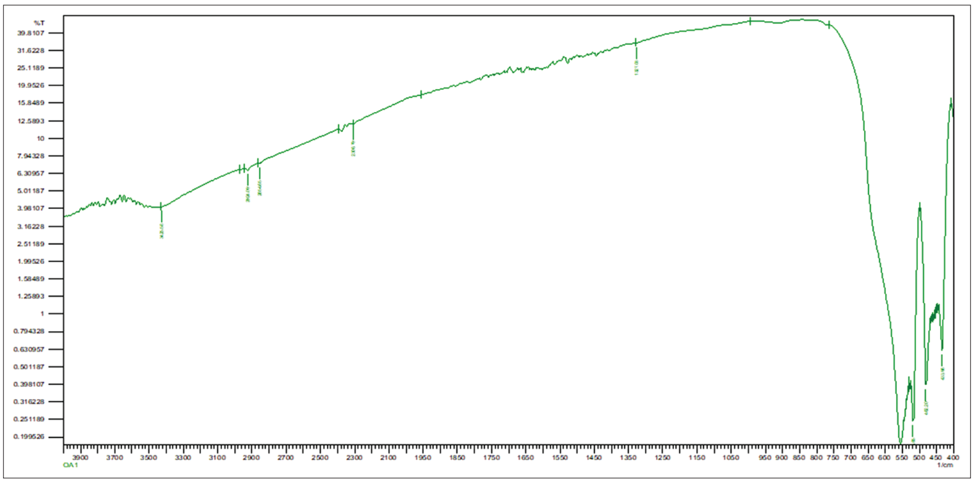

Fig. 3: Fourier transform infrared spectra of iron oxide nanoparticles synthesized by sol-gel technique

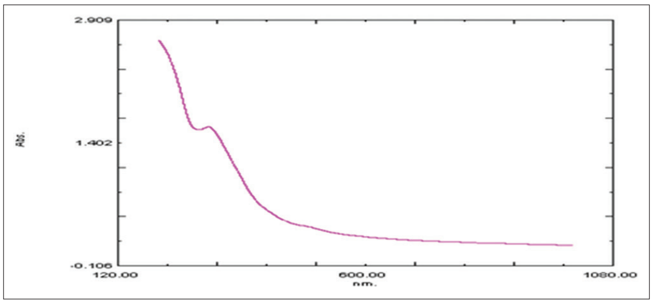

Fig. 4: Ultraviolet-visible absorption spectra of iron oxide nanoparticles synthesized by sol-gel technique

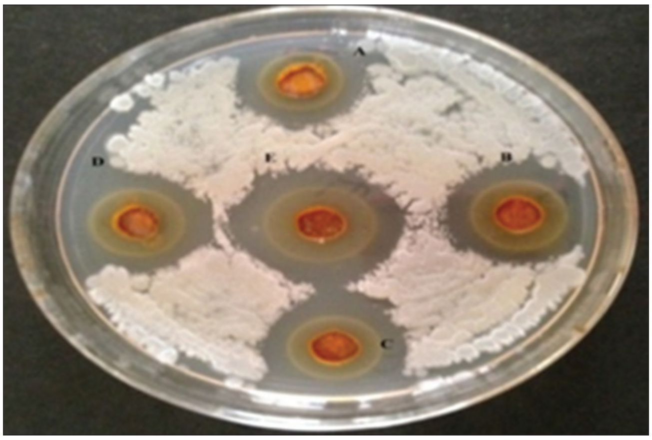

Fig. 5a: Antibacterial activity of iron oxide nanoparticles at different concentration against *Staphylococcus epidermidis* (A=25 mg/ml, B=26 mg/ml, C=27 mg/ml, D= 28 mg/ml, E=29 mg/ml)

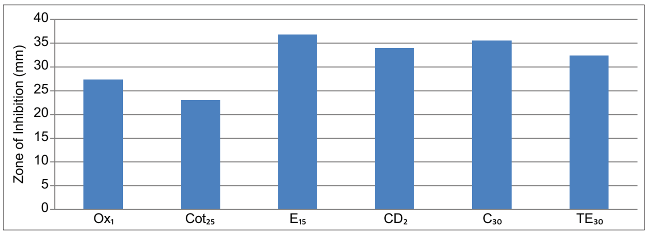

Fig. 5b: Zone of inhibition produced by oxacillin (ox_1), cotrimoxazole (Cot_{25}), erythromycin (E_{15}), clindamycin (CD_2), chloramphenicol (C_{30}), and tetracycline (TE_{30})

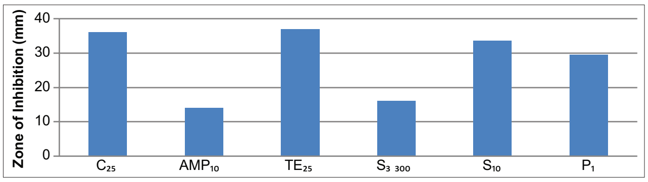

Fig. 5c: Zone of inhibition produced by chloramphenicol (C_{25}), ampicillin (AMP_{10}), tetracycline (TE_{25}), sulfatriad ($S_{3\ 300}$), streptomycine (S_{10}), and Penicillin (P_1)

of standard antibiotics against *S. epidermidis*. The antibiotics were taken in the form of hexa discs. Hexa Disc HX-022 consists of sulfatriad (300 mcg), tetracycline (25 mcg), ampicillin (10 mcg), chloramphenicol (25 mcg), penicillin G (1 unit), streptomycin (10 mcg) and hexa disc HX-034 consists of co-trimoxazole (25 mcg), clindamycin (2 mcg), oxacillin (1 mcg), erythromycin (15 mcg), tetracycline (30 mcg), and chloramphenicol (30 mcg). It was found that all of these antibiotics

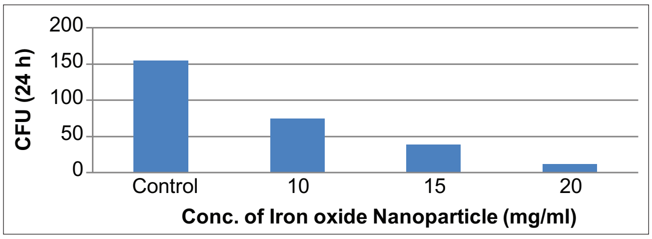

Fig. 5d: Colony-forming unit characterization of iron oxide nanoparticles against *Staphylococcus epidermidis*

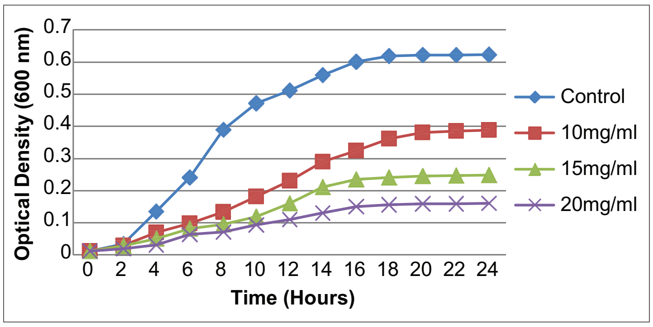

Fig. 5e: Effect of iron oxide nps on the growth of *Staphylococcus epidermidis* in terms of optical density

were effective against *S. epidermidis.* The Fe_2O_3 nanoparticles were also showed good antibacterial activity against *S. epidermidis*. Fig. 5d shows the CFU/ml of the dilute bacterial broth supplemented with different concentrations (0, 10, 15, and 20 mg/ml) of Fe_2O_3 nanoparticles. It was found that the CFU has been reduced significantly with an increase in the conc. of the Fe_2O_3 nanoparticles. Fig. 5e shows the time-dependent changes in the bacterial growth monitored at a regular interval of 2 h (up to 24 h) by measuring OD of the control and bacterial solutions supplemented with different concentrations (10, 15, and 20 mg/ml) of Fe_2O_3 nanoparticles. It is clear that slope of the bacterial growth curve is continuously decreased with increasing concentration of the nanoparticles.

CONCLUSION

In this study, iron oxide nanoparticles of mean size 38.57 nm were synthesized using easy and economical sol-gel technique. Antibacterial activity of these Fe_2O_3 nanoparticles against a Gram-positive bacterium, *S. epidermidis* was investigated by measuring ZOI, CFU, and OD. Antibacterial activity of these Fe_2O_3 nanoparticles was also compared with standard antibiotics. The minimum inhibitory concentration of these nanoparticles was found to be 22 mg/ml for the investigated bacteria. The CFUs decrease with an increase in the conc. of the iron oxide nanoparticles. The results obtained from the ZOI, CFU, and OD measurements were in close conformity with each other. It was found that antibacterial activity of Fe_2O_3 nanoparticles was almost comparable to the most of the standard antibiotics (taken for comparison), but Fe_2O_3 nanoparticles were found to be more effective than antibiotics ampicillin and sulfatriad toward *S. epidermidis*. This study shows that Fe_2O_3 nanoparticles possess good antibacterial properties. In recent times due to excessive use of antibiotics, the pathogens become resistant to most of the antibiotics and the excess use of these antibiotics adversely affects our immune system. Consequently, the metal nanoparticles can be used in pharmaceutical industries and provides a path for further research regarding the toxicity study for its use in human being.

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AUTHOR'S CONTRIBUTIONS

Poonam Sangwan was involved the synthesis, characterization, and antibacterial study of iron oxide nanoparticles.

Harish Kumar guided this research at each and every step.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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