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Characterization and Evaluation of Antibacterial Activities of Chemically Synthesized Iron Oxide Nanoparticles

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ABSTRACT

The iron oxide nanoparticles have been synthesized in co-precipitation method using aqueous solution of ferric and ferrous ions with sodium salt. The synthesis of iron-oxide nanoparticles were validated by UV-Visible spectroscopy which showed higher peak at 370 nm as valid standard reference. An average size of iron oxide nanoparticle found by diffraction light scattering (DLS) particle size analyser, ranges approximately between 10 nm to 120 nm with mean particle size of 66 nm. The X-ray power diffraction (XRD) analysis revealed the crystallographic structure of magnetic particles. Characterization of the mean particle size and morphology of iron oxide nanoparticles confirmed that the iron oxide nanoparticles are nearly spherical and crystalline in shape. Further the antibacterial effect of iron oxide nanoparticles was evaluated against ten pathogenic bacteria which showed that the nanoparticles have moderate antibacterial activity against both Gram positive and Gram negative pathogenic bacterial strains and retains potential application in pharmaceutical and biomedical industries.

Keywords: Iron Oxide; Co-Precipitation; Nanoparticles; Antibacterial Activity

1. Introduction

Nanometer-size metallic nanoparticles have been the subject to research in recent years because these materials represent an intermediate dimension between bulk materials and atoms/molecules [1]. Among these metallic nanoparticles, iron oxide (IO) have received special attention because of their variety of scientific and technological applications such as biosensor [2], antimicrobial activity [3], food preservation [4], magnetic storage media, ferrofluids, magnetic refrigeration, magnetic resonance imaging, hyperthermic cancer treatments, cell sorting and targeted drug delivery [5-7]. Besides, it has also been widely used in biomedical research because of its biocompatibility and magnetic properties [8]. The synthesis of these IO nanoparticles are carried out by different chemical approaches such as coprecipitation, Sol-gel and forced hydrolysis, hydrothermal, surfactant mediated/template synthesis, microimulsion, electrochemical and laser pyrolysis. Among these, the co-precipitation technique is probably the simplest and most efficient chemical pathway through which a larger amount of nanoparticles can be synthesized [9].

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 bacteriocides from various sources [10]. Recent advancement in the field of nanotechnology has provided attractive method for synthesizing alternative antimicrobial agents and reducing biofilm formation [11]. Although nanoparticles have long been known to exhibit a strong toxicity to a wide range of micro-organisms [10, 12], very little is known about the toxicity of iron oxide nanoparticles towards these microorganisms.

In the present study, an attempt has been made to synthesize iron-oxide nanoparticles in co-precipitation method and characterize it by absorption spectrophotometer (UV-VIS), particle size analyzer (PD), X-ray diffraction (XRD), and scanning electron microscope (SEM) along with the evaluation of their antibacterial activity against ten human pathogenic Gram positive and Gram negative bacteria with a view to explore their pharmaceutical applications.

2. Materials and Methods

2.1. Materials

All the chemicals used in this work were analytical reagent grade from commercial market. Distilled water was used for preparation of the solutions after deoxygenation

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with dry N_2 for 10 min. The divalent ($FeCl_2 \cdot 4H_2O$), trivalent ($FeCl_3 \cdot 6H_2O$) iron salts, 2 M HCl solution and aqueous NaOH (25% - 28%, w/w) were also deoxygenated with dry nitrogen before use.

2.2. Synthesis of Iron-Oxide Nanoparticles

Iron-oxide (IO) nanoparticles were synthesized by co-precipitation method as reported by Predoi [13]. The synthesis was carried out by coprecipitation of ferrous and ferric ion salts in aqueous solution by adding base at room temperature with flowing N_2 gas. Briefly, 4.0 ml of 1 M $FeCl_3$ and 1.0 ml of 2 M $FeCl_2$ solution were dissolved in deionised deoxygenated (DD) water followed by adding 200 ml of 0.02 M HCl solution under vigorous stirring at 8000 rpm for about 30 min. The resulting brown precipitate was added with 200 ml of 1.5 M NaOH solution, the color of the mixture then turned from brown to black.

$$2Fe^{3+} + Fe^{2+} + 8OH^- \rightarrow Fe_3O_4 \downarrow + 4\ H_2O$$

The Fe_3O_4 (IO) nanoparticles were finally collected as power after oven dried at 50˚C (**Figure 1**).

2.3. Characterization Techniques

2.3.1. UV-VIS Spectra Analysis (UV-VIS)

The reduction of pure Fe^{3+} ions was monitored by measuring the UV-VIS spectrum of the reaction medium after diluting a small aliquot of the sample into distilled water at wave length 330 - 450 nm. UV-VIS spectral analysis was done by using UV-VIS spectrophotometer (Systronis-117).

2.3.2. Particle Size Analysis (PD)

In order to determine the average particle size distribution, the milled powder of iron oxide nanoparticles was measured by ZETA Sizer Nanoseries (Malvern instruments Nano ZS). Initially, the liquid dispersant containing 500 ml of deionized water and 25 ml of sodium hexametaphosphate was kept in the sample holder and then iron oxide (IO) nanoparticles were dispersed in deionised water followed by ultrasonication.

2.3.2. X-Ray Diffraction (XRD)

In order to obtain the structural information of the product, the crystallographic structure of magnetic particles was analyzed by X-ray power diffraction (XRD). The crystallographic analysis of samples in diffraction patterns were recorded from 10˚ to 70˚ with a panalytical system diffractometer (Model: DY-1656) using Cu Kα (λ = 1.542 Ao) with an accelerating voltage of 40 KV. Data were collected with a counting rate of 1˚/min. The Kα doublets were well resolved.

Figure 1. Synthesized ironoxide nanoparticles.

2.3.3. Scanning Electron Microscope (SEM)

To characterize mean particle size and morphology of Iron oxide nanoparticles, SEM (scanning electron microscope) was performed using Jeol JSM-6480 LV SEM machine of 20 KV of accelerating voltage.

2.3.4. Screening of Antimicrobial Activity

Ten pathogenic bacteria viz. *Staphylococcus aureus* (MTCC 1144), *Shigella flexneri* (Lab isolate), *Bacillus licheniformis* (MTCC 7425), *Bacillus brevis* (MTCC 7404), *Vibrio cholerae* (MTCC 3904), *Pseudomonas aeruginosa* (MTCC 1034), *Streptococcus aureus* (Lab isolate), *Staphylococcus epidermidis* (MTCC 3615), *Bacillus subtilis* (MTCC 7164) and *E. coli* (MTCC 1089) used in the study were obtained from Institute of Microbial Technology, Chandigarh or lab isolates. The organisms were maintained on nutrient agar (Hi Media, India) slopes at 4˚C and subcultured before use.

Agar cup plate method of Khalid *et al.* [14] was carried out to establish the antibacterial activity of the iron oxide (IO) nanoparticles against the test pathogens. Wells of 6 mm diameter were punched over the agar plates using sterile gel puncher (cork borer) 100 µl (50 mg/ml) of nanoparticle powder in sterile distilled water were poured into the wells. The plates were incubated at 37˚C for 24 h. The zone of the clearance around each well after the incubation period, confirms the antimicrobial activity of the IO nanoparticle extract. Neomycin (30 µg/disc) was taken as standard.

3. Results and Discussion

The iron oxide nanoparticles (Fe_3O_4) synthesized by co-precipitation of ferric and ferrous chloride was validated by UV-Visible spectroscopic analysis and their scanning absorbance vs wave length (λ) has been established (**Figure 2**). The characteristics peaks of IO nanoparticles were observed at 370 nm, which is due to charge transfer spectra. The particle size distribution of the iron oxide nanoparticles determined by laser diffraction method with a multiple scattering technique revealed that the particle size distribution of iron oxide nanoparticles ranges approximately from 10 nm to 120 nm with mean particle size of 66 nm and the distribution of oxide nanoparticle is more uniform with a narrow distribution range (**Figure 3**).

The XRD analysis of IO nanoparticles shown in **Figure 4**, were made to detect the diffraction angles at 31.5˚, 35˚, 37˚, 45.2˚ and 53˚ which implies the diffraction surfaces of the nanoparticle crystal. The diffraction angles of different peaks are corresponds to Fe_3O_4 nanoparticles.

This data is very close to the American Society for Testing and Materials (ASTM) data of iron oxide [(Fe_3O_4)] nanoparticles, which could be a good evidence to prove that the prepared nanoparticles, was made of iron oxide. The X-ray power diffraction (XRD) results of nanoparticles confirmed that the synthesized product was a magnetite (Fe_3O_4) [15].

Further analysis of the SEM image of synthesized iron oxide nanoparticles, showed a clear image of highly dense IO nanoparticles which are almost spherical in size (**Figure 5**). The size of most of the nanoparticles ranges from 30 nm to 110 nm. However the percentage of nanoparticles beyond 100 nm is very less. The average percentage of nanoparticles present in our synthesized sample is 66 nm. From the image it is confirmed that the sample contains various sizes of nanoparticles which are indeed agreement with the result obtained from DLS particle analyser. Similar results on SEM analysis of IO nanoparticles has also been reported by other workers [7].

The antibacterial activities of the iron oxide nanoparticle evaluated against ten pathogenic bacteria (six Gram positive and four Gram negative) are presented in (**Table 1** and **Figure 6**). The result of antibacterial activity of IO nanoparticle showed moderate antimicrobial activity against eight pathogenic strains (six gram positive and two gram negative) with zone of inhibition ranging from 9 mm to 22 mm (**Table 1**).

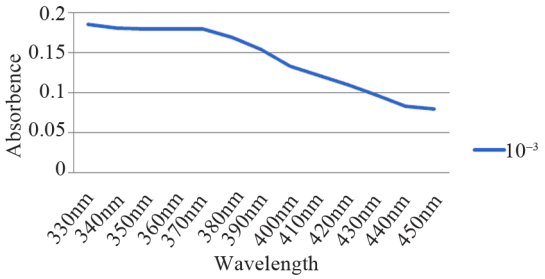

Figure 2. The UV-VIS spectrum of Fe_3O_4 naoparticles.

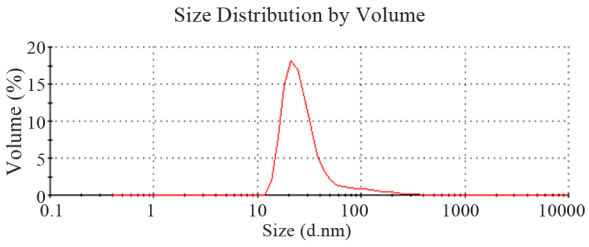

Figure 3. DLS particle size analysis curve of iron oxide nanoparticles.

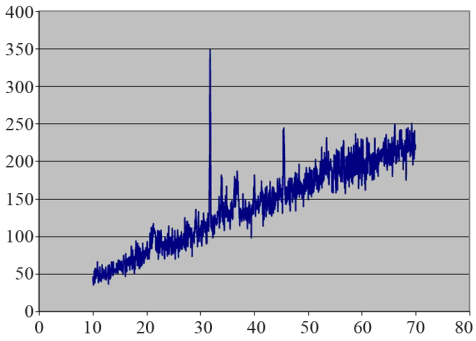

Figure 4. XRD of Fe_3O_4 nanoparticles.

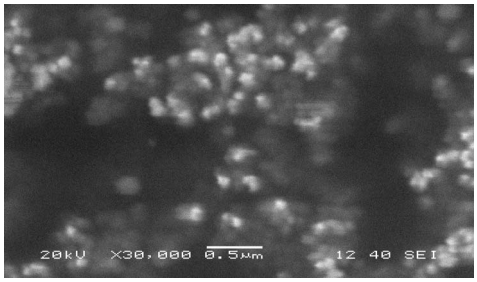

Figure 5. SEM image of synthesized iron oxide nanoparticles.

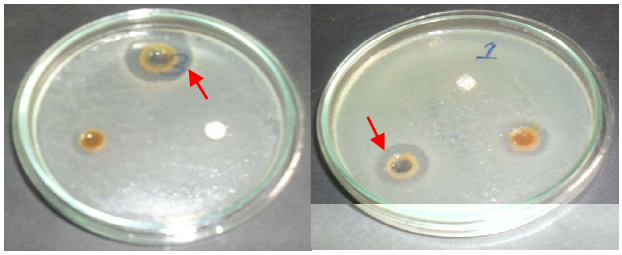

Figure 6. Study of antibacterial activity (zone of inhibition) of Fe_3O_4 nanoparticles.

Table 1. Antibacterial activity of iron oxide nanoparticle and standard antibiotics.

Strains	Iron oxide nanoparticles (50 mg/ml)	Standard antibiotics neomycin (30 µg/disc)
Staphylococcus aureus	12 ± 0.35	17 ± 0.70
Shigella flexneri	0 ± 0.0	18 ± 0.35
Bacillus licheniformis	22 ± 0.70	21 ± 1.4
Bacillus brevis	9 ± 0.15	27 ± 0.35
Vibrio cholerae	9 ± 0.0	18 ± 0.70
Pseudomonas aeruginosa	0 ± 0.0	18 ± 0.35
Streptococcus aureus	12 ± 0.35	16 ± 0.35
Staphylococcus epidermidis	14 ± 0.44	15 ± 0.07
Bacillus subtilis	20 ± 1.11	16 ± 1.4
Escherichia coli	11 ± 0.44	14 ± 0.07

The present results are comparable with that of the standard antibiotic Neomycin (30 µg/disc). The IO nanoparticles do not show any activity against two Gram negative bacteria viz. *Shigella flexneri* and *Pseudomonas aeruginosa* (**Table 1**). There are many factors responsible for the antibacterial activity of iron oxide nanoparticles.

The main mechanism by which these particles showed antibacterial activity might be via oxidative stress generated by ROS [10,12]. ROS, including superoxide radicals (O^{2-}), hydroxyl radicals (–OH), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$), can cause damage to proteins and DNA in bacteria. In the present study, metal oxide (FeO) could be the source that created ROS leading to the inhibition of most of the pathogenic bacteria including *Staphylococcus aureus*. A similar process was also described by Kim *et al.* (2007) in which Fe^{2+} reacted with oxygen to create hydrogen peroxide (H_2O_2). This H_2O_2 consequently reacted with ferrous irons via the Fenton reaction and produced hydroxyl radicals which are known to damage biological macromolecules [16].

Some authors have demonstrated that the small size of nanoparticles can also contribute to bactericidal effects. For example, Lee *et al.* [17] reported that the inactivation of *Escherichia coli* by zero-valent iron nanoparticles [17] could be because of the penetration of the small particles (sizes ranging from 10 - 80 nm) into *E. coli* membranes. Nano scale zero valent iron (NZVI) could then react with intracellular oxygen, leading to oxidative stress and eventually causing disruption of the cell membrane. Studies on ZnO and MgO nanoparticles have also shown that antibacterial activity increased with decreasing particle size [18,19]. In the present study, the concentration of nanoparticles was a major factor for antibacterial activity of the nanoparticle. A similar concentration-dependent behavior was observed by Kim *et al.* [20] when they investigated the antimicrobial effects of Ag and ZnO nanoparticles on *S. aureus* and *E. coli* [18,19]. Similarly, in a study of bactericidal effects of iron noxide nanoparticles on *S. epidermidis*, Taylor and Webster [21], also reported concentration dependent bacterial inhibition. It is also important to note that IO nanoparticles do not negatively influence all cells and thus it can be said that with an appropriate external magnetic field, FeO nanoparticles may be directed to kill bacteria as needed throughout the body.

4. Conclusion

Application of Iron Oxide nanoparticle shows zone of inhibition comparable to that of other nanoparticle (Ag) of topical use. Furthermore it shows better bactericidal activity in Gram-positive bacteria as compared to Gram-negative bacteria. The present study highlights the potential application of IO nanoparticles as antibacterial agents which can be explored for its topical application in pharmaceutical and biomedical industries and opens the path for further research regarding the toxicity and carcinogenicity properties for its use in human being.

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