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Antibacterial Fe_3O_4 nanoparticles: synthesis and characterization

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ABSTRACT
The current article deals a simple and one step method for the synthesis of Fe_3O_4 nanoparticles and its antibacterial application. Fe_3O_4 nanoparticles have been synthesized by solvothermal reduction of $FeCl_3$ in the presence of oleylamine. Nanoparticles thus obtained have been characterized by using TEM, XRD and UV-Vis spectroscopy. Antibacterial test explicitly demonstrate that Fe_3O_4 synthesized using solvothermal method, is an excellent antibacterial material for *E coli*and *Streptococcus Pneumonia*. This is a dose dependent effect and has different effects on gram positive and gram negative bacteria.
Keywords: Fe_3O_4, magnetite, half metal, biocompatible, antibacterial

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I. INTRODUCTION

Nano sized materials owe superior physical and chemical properties due to their mesoscopic effect, small object effect, quantum size effect and surface effect, compared to their atomic or bulk counterparts. Nanoparticles are very exciting and have been attention of much seek recently as they have attractive properties which could see potential role in wide range of biomedical applications such as cell therapy, Magnetic resonance imaging (MRI), targeted drug transportation, photo thermal effect, tissue engineering, regenerative medicines etc (1-7). In the area of antibacterial agents, metal nanoparticles are of a significant interest as they have large surface area with highly potential active sites. A very distinct class of metal oxide with superior magnetic properties and unique biocompatibility has been observed in iron oxide (Fe_3O_4) nanoparticles. In the recent past, Fe_3O_4 nanoparticles have been intensively investigated due to some of its excellent properties (8-11). Further Fe_3O_4 nanoparticles have drawn much attention because of its biology related properties (12-16). In addition to these, multiple oxidation state of Fe_3O_4 nanoparticles provides an extra edge over other materials for physicochemical applications (17-19). A number of strategies have been developed in last several years, to synthesize high quality monodispersed magnetic nanoparticles. The choice of preparation route of magnetic nanoparticle is dependent of their prospective applications. The magnetic carrier in nanotechnology and medicine require a well defined composition and size, stability in aqueous conditions and lack of aggregation in organic as well as aqueous solvent in a wide range of pH. The composition and dispersion stability is essential regardless of a change of the ionic strength of a solution and the presence of other substances. Therefore various preparation methods for monodispersed magnetic Fe_3O_4 nanoparticles have been developed i.e. precipition, solvothermal, hydrothermal,microemulsion, thermal decomposition, decomposition of organometallic precursors, sol-gel method and polyol methods (20-29). Amstad and his coworkers (30) produced Fe_3O_4 nanoparticles with ethylene glycol with excellent stability. Microwave plasma chemical vapor deposition technique has also been deployed for the synthesis of Fe_3O_4nanopyramid arrays (31). Because of the high ratio of surface to volume and magnetization, Fe_3O_4 nanoparticles are prone to agglomeration especially for the large scale synthesis. Various methods other have been applied to resolve the mentioned problem. Functionalization, and coating with ligands, or silica are the most common techniques to prevent the agglomeration of nanoparticles. Along with its benefits, this surface modification also offers some undesirable changes in their chemical and physical properties. Therefore there is all time surge for the developing new methods for the synthesis of such novel materials. In this paper, we have demonstrated a novel, simple and one step process for the synthesis of Fe_3O_4 nanoparticles. Solvothermal reduction of $FeCl_3$ was done in the presence of oleylamine in an autoclave chamber for 12 hours. Nanoparticles of Fe_3O_4 were obtained by dissolving and precipitating several times. Various characterization tools were deployed to confirm the formation of Fe_3O_4 nanoparticles. Once we get confirmed about the formation of Fe_3O_4,

antibacterial test of this material was done using MacConkey agar and Luria-Bertani (LB) media. Two different (1 gram positive and 1 gram negative) types of bacterial strains were used for this purpose. Our results show that, even at very low concentration of Fe_3O_4 nanoparticles, it is very effective in inhibition of growth of bacteria.

II. MATERIALS AND METHODS

Oleylamine and $FeCl_3$ with high purity of 99.9, procured from sigma Aldrich and Methanol and toluene procured from Merck have been used as raw materials. The obtained chemicals were used without further purification.

Synthesis of Fe_3O_4 nanoparticles

1.2 mmol $FeCl_3$was dissolved in 25 ml oleylamine and heated up to 100ºC under vigorous magnetic stirring at 400 rpm for 30 minutes. Material thus turned into dark brownish suspension. Further this suspension was transferred into 100 ml Teflon-lined stainless steel autoclave for solvothermal reaction at 200ºC for 24 h. After the 24 hours of reaction time, the mixture was allowed to cool at room temperature. Purification of quantum dots was done by two step dissolution and precipitation process using toluene and Methanol respectively. In this method, 5 ml of toluene was mixed in reaction mixture followed by sonication for 10 minutes. Further 15 ml of methanol is added and centrifuge for 10 minutes at 4000 rpm. This process was repeated for 3 times to remove unreacted ion in the solution. The solution was then allowed to dry in vacuum at 60ºC.

Bacterial culture

Escherichia coli (E. coli) and *Streptococcus Pneumonia* were chosen as the model pathogen for antibacterial activityexperiments. These pathogens wereharvested in LBmedia at 37ºC for 24 h to get the exponential growth phase.The cells were cultivated by centrifugation and washed withsaline solution (0.9%NaCl) to remove residual macromolecules.The cells were re-suspended in a saline solution to maintain theconcentration of 10^5 colony forming units (cfu mL^{-1}).

Characterizations of Fe_3O_4 nanoparticles

Structural and microstructural characterizations of synthesized nanomaterials were made by XRD and TEM. XRD measurement was done by placing one drop of reaction mixture on a clean glass slide and allowed it to dry. Further glass slide was exposed to X-Ray to record XRD pattern. This XRD pattern was recorded using Rigaku Miniflex-600 diffractometer using Cu (Kα, λ=1.5418Å). TEM images were captured with the help of JEOL JEM-2100F microscope operated at 200 kV. Sample for TEM was prepared by placing one drop of reaction solution on a carbon coated copper grid with 300 meshes. For studying the optical property of as prepared nanoparticles, the UV-Vis absorption spectra were recorded using UV-Vis spectrophotometer (Perkin Elmer Lambda 750S) in 200-600 nm range.

Antibacterial activity of Fe_3O_4 nanoparticles

Antibacterial activity of Fe_3O_4 nanoparticles were studies over two bacterial strains (one gram negative viz. *E. coli*, and one gram positive i.e. *Streptococcus Pneumonia*). Strains were generously provided by Dr. Manish Tripathi, department of microbiology, IMS, BHU. Both bacterial cells were injected in saline solution having five different concentrations namely 0, 20, 50, 70 and 100 µg/mL of Fe_3O_4 nanoparticles. The mixture was kept for 3 h at 37ºC under mild shaking. Further this mixture was used to apply on different LB media plates. These plates were incubated for 24 h at 37ºC. At an interval of 1 h, reaction mixture was taken out to measure the time course of antibacterial activity of samples.

III. RESULTS AND DISCUSSIONS

XRD study of Fe_3O_4 nanoparticles

Inorder to validate the formation of Fe_3O_4 nanoparticles XRD pattern of synthesized material was recorded. Recorded pattern of XRD shows diffraction peaks at 30.16°, 35.49°,43.01°, 53.78°, 57.21°, and 62.73° of 2θ value (Fig.1). These diffraction peaks can easily be indexed as (220), (311), (400), (422), (511), and (440) of the cubic structure (Fd3m space group) of Fe_3O_4 nanoparticles

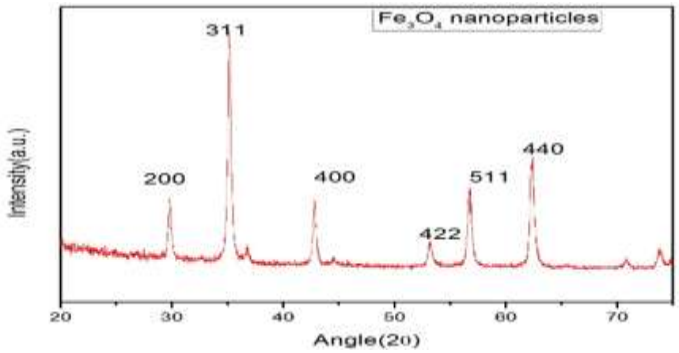

Fig.1XRD pattern of Fe_3O_4 nanoparticles

TEM analysis

Intensive TEM analysis was done to find out information regarding shape, size and crystallinity of synthesized nanoparticles. Fig.2 is a representative TEM image of Fe_3O_4 nanoparticles. In this image, particles seem to be agglomerated. Particles are almost spherical in nature with average particles size lying between 15-30 nm. Inset of this figure shows a high resolution image of Fe_3O_4 nanoparticles. From inset, it is quite clear that particles with good crystallinity have been formed.

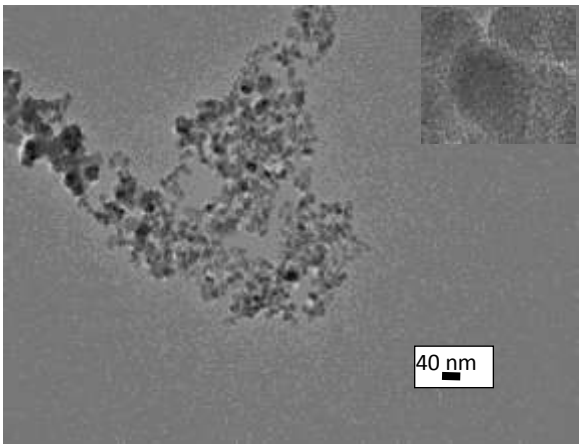

Fig.2 TEM image pattern of Fe_3O_4 nanoparticles

UV-Vis Spectra

Fig.3 depicts the optical absorbance spectrum of the as synthesized Fe_3O_4 nanoparticles at ambient conditions. It shows an absorption band in the region of 330-450nm in the visible range of the wavelength, which originates from the

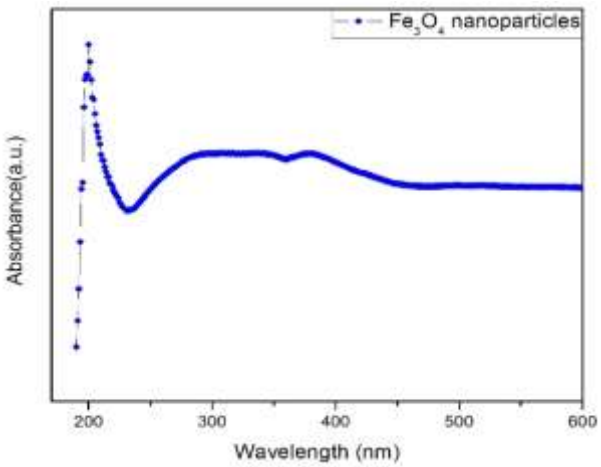

Fig 3. UV-Vis spectra of as synthesized Fe3O4 nanoparticles. absorption and scattering of UV radiation by magnetic nanoparticles, which is in accordance with the previously reported literature [32].

Antibacterial activity of Fe_3O_4 nanoparticles

The antibacterial activity of Fe_3O_4 nanoparticles was evaluated by colony forming count method. Our result shows a remarkable reduction of cell count with increasing concentration of Fe_3O_4 nanoparticles. Further, by comparing the growth curves for both bacterial, we can say that gram positive bacteria was found more resistant and can tolerate even higher concentration of Fe_3O_4 nanoparticles. The dynamics of bacterial growth was monitored in liquid LB media. For this study, we took LB media with 0µg/mL as control. Both bacterial strains were inoculated (separately) to the different concentrations of Fe_3O_4 nanoparticles mixed in LB media. For all concentrations, these cells show decrease in growth with time. In the case of gram positive bacteria this growth was relatively small as compared to gram negative bacteria. The readings for bacterial growth were taken after an interval of one hour. Increasing concentration of nanoparticles progressively inhibited the growth of bacteria.

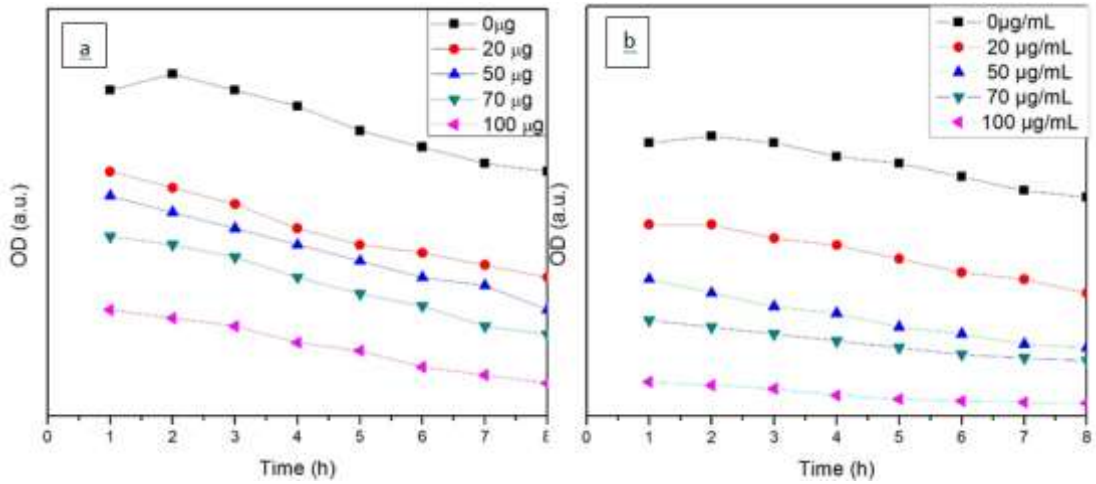

Fig.4 Growth curve for bacterial dynamic in LB media having different concentrations of Fe_3O_4 nanoparticles for (a) Streptococcus, and (b) E. Coli

IV. CONCLUSIONS

A simple, one step solvothermal method for the synthesis for Fe_3O_4nanoparticles have been demonstrated. XRD pattern of synthesized nanomaterial can easily be indexed with known lattice parameter of Fe_3O_4which confirms the formation of the material we desire. A good estimate of particle size and shape of nanoparticles was monitored using TEM. Different amounts of Fe_3O_4 nanoparticles have been added to LB media, in order to explore the anti-bacterial properties of Fe_3O_4nanoparticles. Our results show that Fe_3O_4 nanoparticle behaves as a very effective antibacterial material.

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