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Chemical synthesis of magnetic iron nanoparticles and their antibacterial activity

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ABSTRACT

The synthesis of novel metal nanoparticles is the new trend in nanotechnology. Magnetic nanoparticles comprises of iron (Fe) has been synthesized by chemical procedure using ferric chloride and ferrous sulphate. The synthesized magnetic nanoparticles were attracted towards an external magnet. The size of the nanoparticles was obtained from the TEM analysis, which showed that the size of nanoparticles were in the range of 30-100nm. As the magnetic nanoparticles have wide applications in diagnostics and therapeutics, they were checked for the antibacterial activity against various gram positive and gram negative bacteria.

KEYWORDS

Chemical synthesis;
Iron nanoparticles;
TEM;
MIC;
Antibacterial activity.

INTRODUCTION

Nanotechnology is the rapidly progressing branch of science projecting into various fields with its novel applicative material properties and their applications[1]. The nanoparticles have been synthesized extensively in the past decade following different processes like co-precipitation, thermal decomposition and reduction, micelle synthesis and hydrothermal synthesis[2]. The magnetic nanoparticles are one of its kind which are identified as the potential particles. At first the magnetic nanoparticles were isolated from the bacterium *Magnetotactic bacteria*. The isolated particles were spherical in shape and were surrounded by a protein coat. Usually the magnetic nanoparticles come across various defects like corrosion when exposed to external environment, which makes the magnetic nanoparticles get degraded and these finally lost their identity[2]. Therefore protective measures like utilization of surfactant or thin layer coating of the magnetic iron nanoparticles with Silicon, Platinum etc. These measures keep the actual iron particle away from the corrosion. The iron as a core material ensures the magnetic properties of such protected and functionalized nanoparticles. In case of the magnetic nanoparticles isolated from the *magnetotactic bacteria* the protein coat around the particle protect it from the corrosion.

Magnetic nanoparticles have wide applications in various fields such as ultra high density magnetic recording, magnetic fluids and biomedical materials[3-6]. Magnetic nanoparticles have attracted many researchers and they have attempted to prepare magnetic nanoparticles with high functionality[3]. On the other hand, the surface modification of nanoparticles by organic molecules is also important for their functionalization not only for nanocomposite fabrication[7] but also for

biological applications[8,9]. They offer a high potential for numerous biomedical applications, such as cell separation[10], automated DNA extraction[11], gene targeting[12], drug delivery[13], magnetic resonance imaging[14] and hyperthermia[15]. When coated with, for example, an antibody, they can be applied for highly sensitive immunoassays[16-18] or small substance recoveries[19]. Furthermore, single-stranded DNA or oligonucleotide immobilized on magnetic particles were successfully used for DNA hybridization analyses with the aim of identifying organisms[20,21] and single-nucleotide polymorphism analyses for human blood[22,23]. The applicative aspects of the magnetic nanoparticles are mainly due to their magnetic iron core and their specific binding or adhesion property by functionalization.

In this paper we wish to add one more potential application to the list of applications of magnetic nanoparticles. We have synthesized iron magnetic nanoparticles and they were checked for the antibacterial inhibition against various strains of gram positive and gram negative bacteria. The MIC (Minimum Inhibitory Concentration) of the bacteria towards the magnetic nanoparticles also found.

EXPERIMENTAL

Preparation of magnetic nanoparticles

The magnetic nanoparticles were prepared by the chemical co-precipitation of Fe^{+2} and Fe^{+3} ions in an alkaline solution, followed by a treatment under hydrothermal conditions[24]. 100ml solution of 1M $FeSO_4 \cdot 7H_2O$ (Merck Specialties Pvt Ltd., Mumbai, India) and 2M $FeCl_3$ (Qualigens Fine Chemicals, Mumbai, India) were thoroughly mixed and added to ammonium hydroxide (Merck Specialties Pvt. Ltd., Mumbai, India) with constant stirring at 80°C. The particles thus obtained exhibited a strong magnetic response. Impurity ions were removed by washing the particles several times with hot double distilled water. The yield of precipitated magnetic nanoparticles was determined by removing known aliquots of the suspension and drying to a constant mass in an oven at 60°C. The magnetic nanoparticles prepared were stable at room temperature (25-30°C) without getting agglomerated. The particles were characterized for size using transmission electron microscopy (TEM). The particle size was determined from transmission electron microscopy (Hitachi X-700).

Preparation of active bacterial cultures

For the antibacterial assay the young bacterial cultures are activated. For this process, a loop full of inoculum of pure bacterial cultures were inoculated into individual 100mL of Nutrient Broth (Himedia, Mumbai) taken in 250mL Erlenmeyer conical flasks. The flasks were kept under shaking in a shaker cum incubator (LJE model, Scigenics Biotech Pvt. Ltd, Chennai, India) at 37°C for 24hours of incubation.

Antibacterial assay

Antibacterial assay was conduced with several gram positive and gram negative bacteria like *Staphylococcus sps, Bacillus sps, E.coli, Klebsiella sps* and *Pseudomonas sps.* obtained from the Microbiology division, Sri Venkateswara University, Tirupati. The pure cultures of these bacteria were maintained on the nutrient agar slants. The magnetic nanoparticles synthesized were made into several dilutions using distilled water having the concentrations of 1000, 100, 50 and 10ppm. The antibacterial activity was performed in petriplates by following the disc diffusion method. For this method, nutrient agar media (Himedia, Mumbai) was prepared and the bacteria of each strain were seeded separately in different petriplates by spread plate technique. Later Whatmann No.1 filter paper discs of 6mm diameter were made and sterilized and were incubated with 15µL of the different dilutions of the magnetic nanoparticles. These discs were placed over the medium and were incubated in an incubator at 37°C for 24hr.

RESULTS AND DISCUSSION

The magnetic iron nanoparticles were synthesized by following the co-precipitation method. During their formation the nanoparticles were checked for their magnetic behavior using an external magnet. By keeping the external magnet closer to them they got attracted towards the magnet through the beaker. The size of the magnetic nanoparticles was estimated by TEM analysis (Figure 1).

The TEM micrograph showed the magnetic nanoparticles of different sizes and shapes though they

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TABLE 1 : Antibacterial activity of iron nanoparticles on various bacterial strains at 1000, 100, 50 and 10 ppm.

S.No	Bacterial strain	Zone of Inhibition (in mm)			
1	*Staphylococcus sps*	11	9	10	9
2	*E.coli*	11	7	7	8
3	*Bacillus sps*	13	-	-	-
4	*Pseudomonas sps*	8	8	7	-
5	*Klebsiella sps*	9	8	8	8

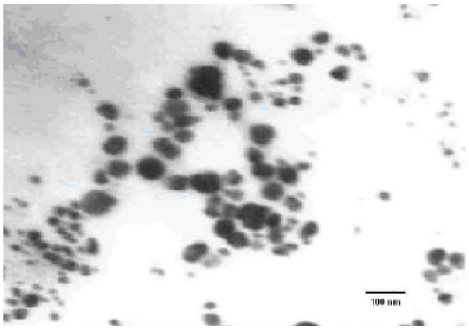

Figure 1 : TEM micrograph of magnetic iron nanoparticles

appear roughly spherical. The TEM analysis revealed that the size of the magnetic nanoparticles synthesized were within the range of 30-100nm. The polydispersed nature of the magnetic nanoparticles is due to the experimental conditions. The experimental conditions and several other parameters have to be optimized to get specific sized magnetic nanoparticles.

The antibacterial activity against a wide range of the gram negative and gram positive bacteria showed some optimal results. The antibacterial assay performed for the different bacterial strains with magnetic nanoparticles were provided in TABLE 1.

The assessment of the results showed that the magnetic nanoparticles of 1000, 100, 50 and 10ppm showed activity against *Staphylococcus sps* (gram positive bacteria) by producing a zone of 11, 9, 10 and 9mm diameter respectively. For the same concentrations of magnetic nanoparticles the gram negative bacteria *E. coli* showed 11, 7, 7 and 8mm diameter of zone of inhibition. For *Klebsiella sps* it is 9, 8, 8 and 8mm diameter of zone of inhibition. The bacterial strain *Bacillus sps* showed inhibition only at 1000ppm of concentration and no zone was obtained at the remaining concentrations. The *Pseudomonas sps* showed inhibition zones of 8 and 7mm of diameter at 1000,100 and 50ppm concentrations respectively and without zone of inhibition at 10ppm concentration. These results showed that the bacteria *Staphylococcus sps*, *E.coli* and *Klebsiella*

TABLE 2 : MIC of various bacteria towards magnetic nanoparticles

S.No	Bacterial Strain	MIC (in ppm)
1	*Staphylococcus sps*	10
2	*E.coli*	10
3	*Bacillus sps*	1000
4	*Pseudomonas sps*	50
5	*Klebsiella sps*	10

sps were inhibited at all concentrations. While bacterial strains like *Bacillus sps* and *Pseudomonas sps* were inhibited at particular concentrations only 1000, 50 ppm respectively. Among the five bacterial strains are used in the present study, *Staphylococcus sps* highly inhibited, and *Bacillus sps* was the least, which is only at 1000ppm concentrations. When the results were assessed and compared it is known that the gram positive bacteria are more susceptible to the low concentrations of the magnetic nanoparticles while the bacterium *Bacillus sps* inhibited by the magnetic nanoparticles only at the higher concentrations. From this study MIC (Minimum Inhibition Concentration is also calculated for each bacterial strain and listed in the TABLE 2.

It is well known that the metals have toxicity towards bacteria[25]. The novel metal nanoparticles like Ag and Au have already proved for their antimicrobial activity. The silver nanoparticles were widely used for the application of the antimicrobial activity. The silver nanoparticles were shown potent against bacteria[26-29], fungi[30] and even viruses like HIV[31]. The silver nanoparticles of less than 5nm were shown to be active against the HIV virus by binding to the gp-120 protein of the virus. The antibacterial activity of these novel metal nanoparticles is due to the small size. Because of their small size these can easily penetrate through the cell membrane and cause the malfunction of the bacterial cell. For silver nanoparticles it is showed that they got attached to the membrane and there by neutralizing the proton motive force the bacteria got killed[32]. One other mechanism of killing is the binding to DNA and proteins. This binding causes the blocking of the active sites of protein and blocking of the binding of transcription factors incase of DNA[30]. The antibacterial activity shown by the magnetic nanoparticles it is inferred that any of the above mechanisms may apply. To find out the actual reason for the antibacterial activity of the magnetic nanoparticles is our future objective.

CONCLUSION

In the present study the magnetic iron nanoparticles were synthesized by co-precipitation method and their antibacterial activity was assessed. The possible reason for the antibacterial activity of the magnetic nanoparticles was discussed. Based on the above investigation we conclude that the magnetic nanoparticles also have the antibacterial activity that can be used for therapeutic applications.

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