Journal of Scientific and Innovative Research 2014; 3(5): 495-498
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2014; 3(5): 495-498

Received: 15-09-2014
Accepted: 05-10-2014

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Effects of core iron oxide nanoparticles on microbial control and bacteriostatic activity against *Escherichia coli, Staphylococcus aureus* and *Mycobacterium smegmatis*

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Abstract

The anti-bacterial activity of the Fe3/citric acid/cephalosporin nanoparticles (core) on *Staphylococcus aureus* (MTCC737), *Mycobacterium smegmatis* (MTCC994) and *Escherichia coli* (MTCC443) was tested using this system as an active compound carrier. We have describe the simple method to obtain iron nanoparticles with uniform size distribution by utilizing citric acid and as surface-capping agents the dimensions of Fe nanoparticles were in the 5-20 nm range and they were characterized by High Resolution Transmission Electron Microscopy (HRTEM).The particle size was tailored by tuning the concentration ratio of iron ions to carboxylic acid groups. Fe nanoparticles assisted by citric acid to analyze the interaction different type of cephalosporin against the these bacteria The antibacterial activity was observed in both, citric acid core nanoparticles/cephalosporin and core nanoparticles alone against these pathogen.

Keywords: Nanofluid, Magnetic, Fe nanoparticles, Antibacterial, Cephalosporin, HRTEM.

Introduction

Magnetic nanoparticles have drawn much scientific interest by using magnetic nanoparticles for biological and medical purposes for a variety of studies.[1] Magnetic iron oxide based inorganic nanostructures materials have been synthesized and tested for various applications in medicine: as imaging agents, as heat mediators in hyperthermia treatments, in tissue repair, immunoassay, detoxification of biological fluids, cell separation, as magnetic guidance in drug delivery. The advantages of using these materials come from their magnetic properties, high surface area that provides higher sensitivity, better targeting and improvement of the colloidal stability of the nanostructures.[2] Important properties of the nanoparticles required for biomedical applications[3, 4] which are derived from a precise control of particle size, shape, dispersion and conditions that affect these properties. One area that is particularly promising is the use of magnetic nanoparticle systems for probing and manipulating biological systems.[5] Coating nanoparticles with natural or synthetic polymers or surfactants is a method that provides stability of the ferrofluid colloidal suspensions. Use of surfactants such as: citric acid, oleic acid, hexaldehyde or sodium carboxymethyl cellulose leads to highly dispersed and high quality nanoparticles with good biocompatibility and smaller particle size.[4]

Coated nanoparticles are important for their lower toxicity due to the presence of the biocompatible coating, and also due to the lower adsorption sites for proteins, ions and other components in medium.[6] Usually iron oxides Fe_3O_4 or γ- $Fe_3O_4$are synthesized through the co-precipitation of Fe^{2+} and Fe^{3+} aqueous salt solutions[5], by addition of a base. Properties of nanoparticles such as size, shape and composition, are influenced by the type of salt, pH, ions ratio and ionic strength of the medium.[7] Some methods use magnetotactic bacteria (MTB)[8] that are able to internalize Fe and convert it into magnetic nanoparticles, in the form of either

magnetite (Fe_3O_4) or greigite (Fe_3S_4)[9], Electrochemical preparation has also been used in situ for synthesis of core-shelled Fe_3O_4 nanoparticles.[10] Sun *et al*.[11, 12] developed a thermal decomposition method that uses a Fe/acac salt, 1, 2-hexadecanediol, oleic acid, oleylamine, and biphenyl ether mixture to obtain nanoparticles that are further used for silver coating in order to improve bacterial activity and paramagnetic properties of the nanostructures.[13] A system that uses a combination of magnetron sputtering and gas–aggregation techniques produces Fe nanoclusters of variable controlled mean size (diameters from 2 to 100nm) and high magnetic moments for biomedical applications.[14] Magnetic temperature sensitive liposomes based on Fe_3O_4 could be used to generate heat to achieve a certain temperature and during this time encapsulated anticancer drugs are released.[15]

In this study, new magnetic core-shell iron oxide base nonmaterial was obtained, adapting the coprecitated method in order to improve colloidal dispersion and to control particles size.[16] Small size of nanoparticles was also on purpose, due to the possibility of targeting through blood barriers.[17] Citric acid was used as surfactant for coating the Fe_3O_4 nanoparticles, followed by adsorption-coating with four different cephalosporins. The bacterial activity was tested on three different bacteria: *E coli*, *S. aureus* and *M. smegmatis*

Materials and Methods

Materials

Staphylococcus aureus (MTCC737), *Mycobacterium smegmatis* (MTCC994) and *Escherichia coli* (MTCC443) has been brought from IMTECH Chandigarh. India

Iron (Fe) nanoparticles synthesis

Fe nanoparticles were synthesized at room temperature from $FeCl_3$ using $NaBH_4$ as a reducing agent and $PdCl_2$ which serves as a nucleating agent. The concentration ratio (R+/−) of iron ions (Fe^{3+}) to citric acid monohydrate (CA) was systematically varied and its effect on the resulting nanoparticles size was studied. The concentration ratio (R+/−) is defined as the ratio of total positive (Fe^{3+}) to negative charges (CA and OA) which can be present in solution. The R+/− is calculated to be $3[Fe^{3+}]/([OA] + 3[CA])$. In a typical nanoparticles synthesis procedure (R+/− = 0.86), 0.16 mmol $FeCl_3$, 0.16 mmol CA, 0.08 mmol OA and 0.1 ml PdCl2 solution (0.01 M) were dissolved in a mixed solvent (150 ml water and 40 ml ethanol). After purging with a gas mixture [95% argon (Ar) and 5% hydrogen (H_2)] for 30 min, 0.06 g $NaBH_4$ of 10 ml water solution was added in one shot and after that mixture immediately became black, indicating the formation of Fe nanoparticles.

Characterization of Iron oxide

Transmission Electron Microscopy (TEM) images were taken on a Phillips-CM200 electron microscope at 200 kV. One drop of suspended samples (solvent-ethanol) was deposited on carbon coated copper grid and dried. TEM was used to observe the morphology of the nanoparticles. The particle size is reported as the average observed size (Davg), which is the average particle size of approximately 1000 individual particles from multiple TEM images.[18, 19]

Determination of anti-bacterial activity

The qualitative antibiogram interpretation process was done according to international standard.[20] Incolumum preparation has been performed by making suspensions from 2-3 colonies isolated in physiological serum; the suspension turbidity has been done either nephelometric controlled or by comparison with standard tubes. Seeding was done by proper medium was chosen according to the tested bacterial species. Incubation has been depending upon the bacterial species: in normal atmosphere, 37°C, 20-24 hours for *E.coli*, *M. smegmatis* and *S.aureus*; *Interpretation:* a confluent bacterial culture appeared; inhibition zones appeared around the micro pills (the lack of the bacterial growth); the diameters of the inhibition zones were read, taking into account the used antibiotic, the quantity of the antibiotic in the pill and the tested bacterial species. The sensitivity diameters were compared with the standard ones.

Results and Discussion

Fe nanoparticles synthesis

Citric acid [CA] has been traditionally utilized as surface capping agent to control the formation of metallic nanoparticles such as cobalt[18], gold[21], palladium[22], and silver[23]. In our Fe nanoparticles synthesis we also utilized $PdCl_2$, Which serves as a nucleating agent, along with CA to control Fe nanoparticles formation.[24] Figure 1(a) shows that the Fe particle size ranges from 200 to 20 nm as the concentration of Fe^{3+} to CA remained at 10. This result revealed that the amount of CA cannot control Fe nanoparticles formation when there is more Fe^{3+} than CA present in the solution. When the amount of CA was increased and R+/− became 0.7, uniform Fe nanoparticles with an average size of 11 nm were successfully synthesized (figure 1(b)). These results suggest that a greater amount of CA (or lower R+/−) leads to smaller particles with more uniform size distribution, which is consistent with other reports.[19, 22, 23]

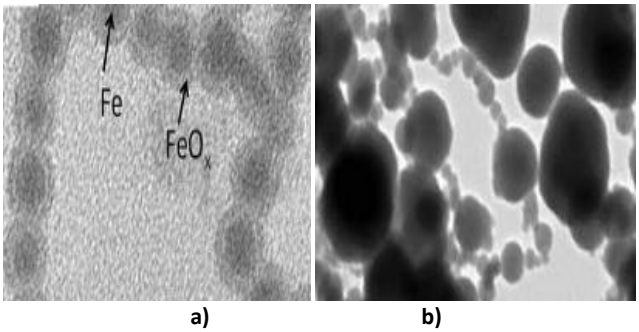

Figure 1: TEM images of Fe nanoparticles synthesized at formulation ratios of Fe3+ to citric acid

Determination of anti-bacterial activity

Bacteriostatic activity by cephalosporin drug alone (table 1) and with nanoparticles core alone (table 2) were tested with three different bacteria, *E. coli M. smegmatis and B. subtilis*. It was observed that, for the same time interval, the inhibition zone diameters for cephalosporins conjugated with nanoparticles (Table 3) were higher from both the cephalosporin and nanoparticles core alone. This leads that the nanofluid increase potency of drug as it act as carrier and itself act as bacteriostatic activity.

Table 1: Inhibition zone diameter on *E. coli*, *Mycobacterium smegmatis* and *S. aureus*

Cephalosporins	**Inhibition zone diameter [mm] on *Escherichia coli***	**Inhibition zone diameter [mm] on *Staphylococcus aureus***	**Inhibition zone diameter [mm] on *Mycobacterium smegmatis***
Cefoperazone	21	22	17
Cefotaxime	26	28	16
Ceftriaxone	28	27	19
Cephachlor	19	29	16

Table 2: Inhibition zone diameter on *E. coli* and *S. Aureus* on Fe_3O_4 /citric acid (Core)

Nanofluid	**Inhibition zone diameter [mm] on *Escherichia coli***	**Inhibition zone diameter [mm] on *Staphylococcus aureus***	**Inhibition zone diameter [mm] on *Mycobacterium smegmatis***
Fe_3O_4-citric acid core/shell	15	12	10

Table 3: Inhibition zone diameters of cephalosporins extra-shelled Fe_3O_4

Cephalosporins adsorption-shelled Fe_3O_4	**Inhibition zone diameter [mm] on *Escherichia coli***	**Inhibition zone diameter [mm] on *Staphylococcus aureus***	**Inhibition zone diameter [mm] on *Mycobacterium smegmatis***
Cefoperazone	24	27	15
Cefotaxime	28	26	13
Ceftriaxone	31	24	16
Cephachlor	21	22	11

Conclusions

We have demonstrated the feasibility of preparing low loss, Fe nanoparticles polymer composites. We have design a simple method to obtain iron nanoparticles with uniform size distribution by utilizing citric acid and as surface-capping agents. The particle size was fabricated by tuning the concentration ratio of iron ions to carboxylic acid groups. The Fe nanoparticles were successfully coated by a layer of silica to prevent Fe cores from oxidizing. Adsorption process is a convenient way to obtain a different type cephalosporin drug carrier's nanoparticles core. These core nanoparticles drug carrier not only useful in bacteriostatic effect against *E. coli, M. smegmatis and S. Aries* but it also enhances the efficacy and potency against cephalosporin antibiotic. The small size of the core nanoparticles makes possible the delivery of the antibiotic when targeting certain organs like the brain, liver and kidney. The method of drug encapsulation with core nanoparticles will be very useful for drug delivery system against visceral leishmania[25] and biomedical research. It also applicable where the potency of drug is very low that can not able to elicit the enough response to inhibit the growth of pathogen.

Acknowledgment

We gratefully acknowledge the department and center of SAIF and CRNTS, IIT Bombay, India for providing instrumental facilities

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