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Antimicrobial activity of Chitosan Coated Iron Oxide Nanoparticles

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Abstract : Synthesis and coating of superparamagnetic iron oxide nanoparticles was carried out by wet chemical synthesis method. Controlled co-precipitation DS method was used to prevent critical oxidation of iron oxide. The precursors used for the preparation of iron oxide nanoparticles are Ammonium Ferrous Sulphate and Ferric Chloride. Cetyl Trimethyl Ammonium Bromide is used as asurfactant. As a reducing agent, Liquor Ammonia is used. The extracts of *Cuminum cyminum* and *Ocimum tenuiflorum* in the ratio 2:1 were also used instead of chemicals as surfactant and reducing agent.The size of the iron oxide nanoparticles obtained by HORIBA SZ-100 Zeta Sizer was 239.7 nm and the peak obtained by Analytek Jena UV Spectroscopy was around 320 nm. The nanoparticles were coated with chitosan and placed in a bacterial culture. It acted as an antimicrobial agent on the applied sites on the bacterial culture. This will act as a potential source for antimicrobial actions and in treatment in biomedical applications.
Keywords: Superparamagnetic iron oxide nanoparticles,Cuminum cyminum, Ocimum tenuiflorum, antimicrobial activity, biomedical applications.

Introduction

Nanotechnology is one of the most emerging field in the recent years. The advantages in nanotechnology makes us to apply the concepts in a variety of fields. Especially, in the field of medicine, many forms of applications like antimicrobial activity, drug delivery system and diagnostics are made available with the help of nanotechnology. The iron oxide nanoparticles have a large functional surface to bind, carry and coat other materials[1,2]. This enables coating of a spectrum of antimicrobial agents on its surface. The antimicrobial coating of iron oxide nanoparticles is a possible way to destroy and deactivate the microbes. This work describes how the iron oxide nanoparticles were prepared with the help of natural plant extracts and their antimicrobial activity.

Preparation of Plant Extracts

5g each of *Cuminum cyminum* and *Ocimum tenuiflorum* are added with 20 ml of distilled water in a beaker and heated separately on a stirrer to a temperature of 90^{o}C and then cooled down. Then the solutions in the beaker were filtered with Whatmann filter paper to get the extract.

Synthesis of iron Oxide Nanoparticles

The synthesis was carried out by wet chemical synthesis and Co-precipitation methods(1). One part of0.1M ferrous ammonium sulphate($(NH_4)_2Fe(SO4)_2$) is taken in a beaker and magnetically stirred for 20 min and two parts of 0.2M ferricchloride(Fe_2Cl_3)is then added and stirred for10 min. In chemical synthesis, Cetyl Trimethyl Ammonium Bromide(C-TAB) of 0.2 gin 20 mlis usedassurfactant and0.5ml of sodium borohydride is used as a reducing agent. In the case of green synthesis technique[3], the extracts of *Cuminum cyminum*(10 ml) and *Ocimum tenuiflorum*(5 ml) was mixed and added instead of C-TAB and sodium borohydride. Immediately after the addition of reducing agent, Iron oxide nanoparticles were formed and were indicated by the characteristic change in color from light brown to black.

Purification of Iron Oxide nanoparticles

After the formation of nanoparticles, the solution was removed and their on oxide nanoparticles were taken in distilled water and centrifugedat16000rpmfor5minutes. Then it is dried in an oven to obtain the pure solid iron oxide nanoparticles and coated with Chitosan.

Antimicrobial Assay

The iron oxide nanoparticles of various concentrations like 1,3,5 µg respectively are taken in solution form and were coated in small discs of Whatmann filter paper and kept on the bacterial cultures of common microbes like *E. coli*. The setup was left for 12 hours and positive result was obtained in an increasing manner with respect to concentration.

UV-Spectroscopy and particle Size Analysis

The iron oxide nanoparticle sizes at pH 5.78 were obtained with the HORIBA SZ-100 particle size analyzer and Analytek Jena UV-VISIBLE spectrometer.

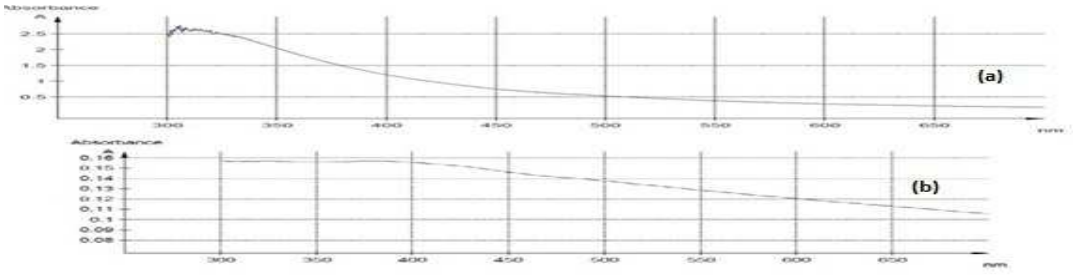
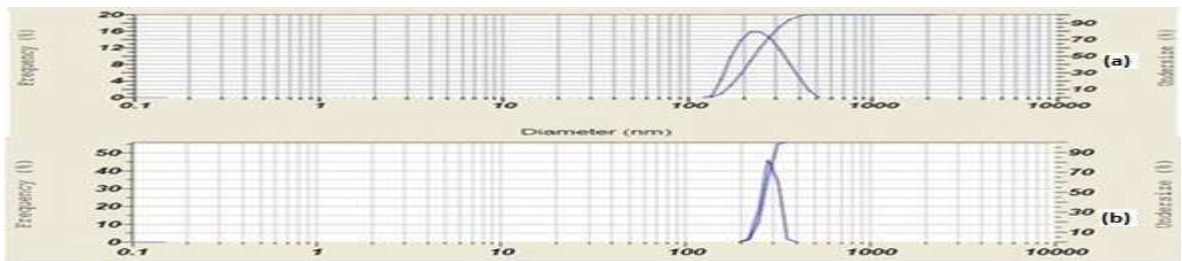


Fig-1: UV-VISIBLE spectroscopy of Iron Nanoparticles

UV Peak obtained for green synthesized nanoparticles (a)is 310 nm and chemically synthesized nanoparticles (b) is 365 nm



The size of the nanoparticles obtained by particle size analyser for (a) is239.7 nm and (b)is 269.1 nm

Fig-2: Particle size analysis of iron oxide nanoparticles with zeta sizer

Results and Discussion

The iron oxide nanoparticles synthesized by both chemicals and by green synthesis method like *Cuminum cyminum* was found to exhibit a good antimicrobial activity at higher concentrations. UV Peak obtained by Green and Chemical Synthesis were 310 nm and 365 nm respectively.The size of the nanoparticles obtained by particle size analyser for Green and Chemical Synthesis were 239.7 nm and 269.1 nm. The iron oxide nanoparticles synthesized by natural materials were showing a high antimicrobial activity dueto antimicrobial alkaloids present in them. In the presence of microbes the solution appears denser. But, when Ironoxide nanoparticles were introduced to the solution containing microbes, the optical density of the solution decreases due to antimicrobial activity of Iron oxide nanoparticles. With its superparamagnetic properties, it will be a vital means for targeted antimicrobial activities in the human body in the near future. After 90 minutes, the optical density of the bacterial culture in solution was found to be high, but after the introduction of Iron oxide nanoparticles the optical density of the solution tends to reduce.

Fig-3: Pictures in Sequence in clockwise direction - Iron oxide nanoparticles in Solution and Powdered form; Preparation of Inoculum; Transfer of Bacterial Culture.

Table : Measurement of Optical Density before and after Antimicrobial Assay

TIME (min)	OD at 600nm (culture alone)	OD at 600nm (culture + ironoxide)
90	0.0294	0.0226
180	0.0340	0.0289
270	0.0351	0.0306
360	0.0415	0.0368

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