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DNA-assisted synthesis of chitosan/α-Fe_2O_3 nanocomposites for antioxidant and antimicrobial activities

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Abstract. Novel nanocomposites, based on chitosan/α-Fe_2O_3 (C/FD), have been successfully synthesized. The FD nanoparticles were prepared by co-precipitation method using DNA as the capping agent. The samples were characterized by XRD, EDAX, SEM and TEM. The hematite nanoparticles that were prepared using DNA were compared with the samples prepared using EDTA and CTAB as capping agents. The effect of C/FD nanocomposites on the growth of few common water pathogens were studied to explore its use as an antibacterial agent to be used in water purification. The antioxidant activity of samples was tested using a DPPH assay at two different concentrations of FD. It provides the possibility of improving radical scavenging activity by varying the preparation conditions. The results also showed that the C/FD being a bio-compatible, eco-friendly and low cost material is used to inhibit the growth of several fungi and bacteria that can be useful for a number of applications in the food and pharmaceutical industry.

Keywords. Metal oxide nanoparticles; nanocomposites; antioxidant; antibacterial activity.

1. Introduction

Metal oxide nanoparticles are used for a large variety of applications including catalysis, sensors, optoelectronic materials and environmental remediation [1]. The antimicrobial properties of metal oxide nanoparticles is coming up as the current interest in researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains. A number of studies have concentrated on the synthesis and utilization of metal oxide or their dual nanocomposites for versatile applications [2,3]. Nanoparticles of α-Fe_2O_3 (FD) have received much attention due to their unique antifungal, antibacterial and UV filtering activities. FD is a stable, biocompatible and non-toxic material. The properties of FD are highly affected by the morphology, crystallite size and crystallinity of the material. Many synthetic methods for preparing FD have been investigated. In this work, we describe a simple co-precipitation method for synthesizing nano-sized FD particles.

The factors that influence the incorporation of hematite nanoparticles in various applications include low degree of agglomeration and homogeneous arrangement of particles, which greatly rely on the method of synthesis. To obtain the final product with required properties, a co-precipitation process is required to control the concentration, pH, temperature and stirring speed of the solution. Moreover, capping agents/stabilizers are essential factors in the synthesis of nanostructures. Adding surface capping organic material to the solution is one of the ways to achieve size restriction. There is always search for a reliable and environment friendly process to manufacture metals and metal oxide nanoparticles for minimizing or even eliminating the use of hazardous chemicals. Though physical and chemical methods are trendier for nanoparticle synthesis, the biogenic synthesis fabrication is a better choice due to ecofriendliness. DNA 'the molecule of life' has been attracting attention of researchers in diverse areas of science and technology. It can be used as a data storage medium. Another application of DNA is being explored in the field of nano biotechnology. Establishing a tie between biotechnology and nanotechnology resulted in a new field called nano biotechnology. DNA can be used as a bio template to grow inorganic quantum-confined structures like quantum dots, quantum wires, metallic nanoparticles, metal oxides and sulphides, etc. Here, we have adopted a biogenic synthesis route using DNA as the capping agent.

Chitosan (CH) is an alkaline, non-toxic, hydrophilic, biocompatible and biodegradable polymer. CH(N-deacetylated derivative of chitin) is a linear co-polymer of glucosamine and N-acetylglucosamine units and naturally found in the exoskeleton of crustaceans (shrimp), fungal cell walls and in other biological materials. Among other reported properties, CH has received attention for enzyme immobilization. CH is a natural non-toxic biopolymer, which is a major component of the shells of crustaceans such as crab, shrimp and crawfish. CH has different types of reactive functional groups. By modification of these groups various materials for different fields of application can be achieved [4]. Nowadays, the preparations of magnetic nanoparticles encapsulated in CH are of great

interest [5]. Nanocomposites are multiphase materials with one of their components between 1 and 100 nm in size. These materials have physical and mechanical properties including high strength, toughness and heat resistance at a wide range of temperatures. CH as well as CH oligomers have been shown to inhibit the growth of several fungi and bacteria, especially pathogens [6–8]. This property of CH as an antimicrobial agent could be useful for a number of applications in the food and pharmaceutical industry.

The high surface to volume ratio of nanostructures makes it viable candidates to act as free radical scavengers than their bulk counterparts [9]. The antioxidant assessment of nonmaterial has become a crucial study in pharmaceutical science as well as nanotechnology. The aim of this study is to synthesize novel bio-nanocomposites based on hematite coating by CH. The hematite nanoparticles are prepared by a simple co-precipitation method. Finally, the antibacterial properties of the obtained hematite and hematite/CH nanocomposites are evaluated against *Escherichia coli* (*E. coli*) (ATCC 25922), *Salmonella typhimurium* (*S. typhimurium*) (MTCC 98), *Shigella flexneri* (*S. flexneri*) (ATCC 29508) and *Candida albicans* (*C. albicans*) (ATCC 2091). To the best of our knowledge, this is the first work on the synthesis of chitosan/α-Fe_2O_3 (C/FD) nanocomposites for antimicrobial and antioxidant activity using DNA as the stabilizer.

2. Materials and methods

The chemicals used for the synthesis of (C/FD) nanoparticles, namely ferric nitrate, sodium hydroxide and DNA powder were obtained from MERCK and CH was obtained from matsyafed chitin and CH plant, Neendakara, Kollam.

2.1 *Synthesis of α-Fe_2O_3 nanoparticles*

Hematite nanoparticles were prepared by arrested co-precipitation method using ferric nitrate, sodium hydroxide and DNA under constant stirring. Here, the aqueous solution of DNA powder was used as a capping agent to control the particle size. The obtained red coloured precipitate was separated from the reaction mixture and washed several times with distilled water and alcohol to remove impurities and traces of chemicals used. The wet precipitate was allowed to dry naturally and then thoroughly ground to obtain a precursor in the form of fine powder. Then the sample was annealed at a temperature of 500°C. Similarly, we adopted ethylenediaminetetraacetic acid EDTA and cetyltrimethylammonium bromide CTAB as capping agents instead of DNA for comparing the particle size of hematite and the procedure was repeated with the same molar solutions of ferric nitrate and sodium hydroxide. Henceforth, the samples obtained from DNA, EDTA and CTAB will be referred as FD, FE and FC.

2.2 *Synthesis of chitosan/α-Fe_2O_3 nanoparticles*

1.25 g of CH flake was dissolved in a solution of 150 ml (0.1 M) acetic acid and 20 ml (0.2 M) NaCl. The viscous solution was stirred continuously for 12 h to fully dissolve the CH flake. Then, 0.625 g of the hematite powder was added into the solution, subsequently, another 25 ml of (0.1 M) acetic acid was added. The slurry was stirred continuously for 24 h to obtain the final solution [10].

2.3 *Antioxidant activity test*

The radical scavenging activity of C/FD nanoparticles is determined using DPPH assay. The DPPH radical scavenging activity was measured as per the process described by Blois *et al* [11]. Briefly, 0.1 mM of DPPH solution in methanol was prepared and 1 ml of this solution was mixed with 3 ml of sample solutions in methanol at different concentrations. The deep violet colour of DPPH radical solutions becomes colourless in the presence of nanoparticles. Finally, after 30 min, the absorbance was measured at 517 nm. Butylated hydroxyanisole is used as a standard. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. DPPH radical-scavenging activity was calculated according to the following equation:

$$\%\ \text{Inhibition} = ((A_0 - A_1)/A_0 \times 100), \quad (1)$$

where A_0 was the absorbance of the control (1 ml of DPPH + 3 ml of methanol) and A_1 was the absorbance of the sample solution.

2.4 *Antimicrobial activity test*

Antimicrobial activity of hematite nanoparticles was analysed using filter paper disc diffusion technique against *E. coli* (ATCC 25922), *S. typhimurium* (MTCC 98), *S. flexneri* (ATCC 29508) and *C. albicans* (ATCC 2091). Mueller-Hinton agar and Sabouraud's agar medium were used for culturing the microbes and the required volume of the medium (20 ml) was poured into the sterile petri dishes and allowed to solidify. The different samples (FD and C/FD) were dissolved in 0.5 ml of N-N dimethyl formamide and 30 µl of the dissolved solution was poured into the discs (Himedia sterile 6 mm disc) using aseptic technique and then placed with centres at least 24 mm apart. These plates were incubated at 37°C for 24 h but for *C. albicans*, the plates were kept for 48 h at 25°C.

3. Characterization

X-ray diffraction (XRD) spectra of samples were recorded with a PAN analytical Model X'pert pro X-ray diffractometer employing Cu kα radiation at 40 kV and 100 mA at a scanning rate of 8° min^{-1} in the range 20−70°. The surface morphology of the samples was recorded with a Hitachi-model

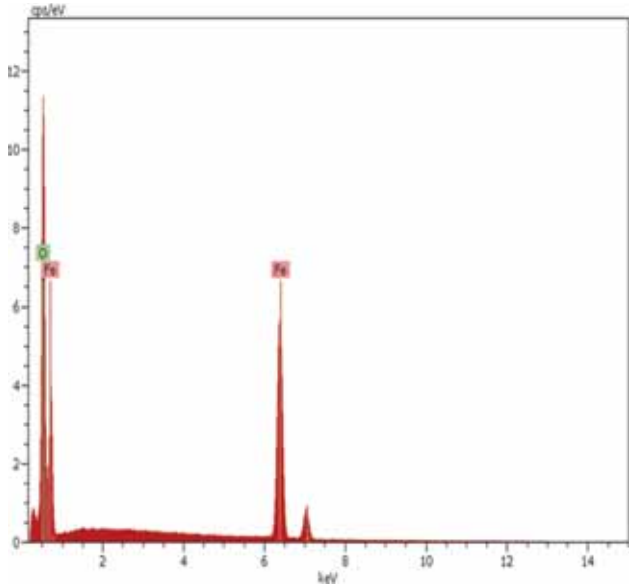

Figure 1. EDAX spectrum of sample FD.

Table 1. Results of EDAX analysis showing the percentage of Fe and O in the sample.

Element	Mass %	Atom %
O	35.04	65.31
Fe	64.96	34.69
Total	100	100

S-3000H scanning electron microscopy and transmission electron microscopy (TEM). The radical scavenging activity of C/FD composite is elucidated spectrophotometrically using UV-1601 Shimadzu spectrometer.

4. Results and discussion

4.1 *Morphological studies*

The energy dispersive analysis (EDAX) gives information about the elements present in the sample as well as approximate stoichiometry. The result of EDAX of the sample FD (figure 1) shows that there is no impurity in the sample and the prepared samples obey the stoichiometry. The prepared nanoparticles contain only FD particles. The role of DNA is to control the particle size during the synthesis technique. The percentages of iron and oxygen in the sample are given in table 1.

The crystal phase identification of the samples is done by comparing the X-ray diffraction spectra with reported crystal diffraction data by JCPDS. The XRD pattern of the samples is shown in figure 2. The XRD studies reveal that the nanoparticles formed by the chemical method are crystalline. The diffraction pattern of the samples are in close agreement with

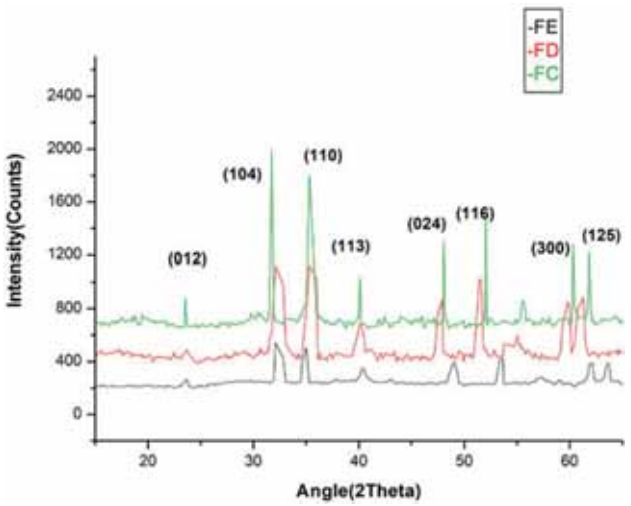

Figure 2. XRD spectrum of samples FD, FE and FC.

reported values in JCPDS card 88-2359 (lattice parameters, $a = 5.112$ and $c = 13.820$) and they are matching with the rhombohedral phase of FD (hematite) with no characteristic peaks of other impurities. The most intense peak (intensity 100) is from the (1 0 4) plane, which corresponds to an angle of $2\theta = 32.810^{\circ}$. The fine particle nature of the oxide samples are reflected in X-ray broadening. The crystalline sizes are determined from the XRD line broadening using the Scherrer equation (2)

$$d = k\,\lambda\,/\beta\cos\theta, \tag{2}$$

where λ is the wavelength of X-ray, β the full-width at half-maximum and θ the Bragg's angle of diffraction. Using equation (2) the average particle size obtained for Hematite nanoparticles at 500°C is 17.86 nm by using the bio template DNA as capping agent while the size of nanoparticle obtained using EDTA and CTAB at 500°C is 19.12 and 27.82 nm. This indicates that the size of crystallites can be adjusted by using different capping agents.

The surface morphology of FD and C/FD using DNA as capping agents were studied using TEM and SEM. TEM images of the samples FD, FE, FC and C/FD are shown in figure 3. The morphology of the hematite nanoparticles was not uniform, formation of cluster and particle agglomeration resulting in increased particle size was observed [12,13]. The average particle size of the samples FD, FE and FC are 13.6, 17.10 and 35.82 nm, respectively. Both XRD and TEM studies revealed that DNA can be used as an effective biogenic capping agent to reduce the size of hematite nanoparticles. Therefore, further studies in this work are carried out using DNA enhanced hematite samples. Figure 3b shows the TEM images of C/FD. Interestingly, the size of C/FD was nearly maintained as those before doping CH particles. Such high surface/volume ratio might benefit for the surface chemical reaction. Moreover, the diffraction ring recorded by the

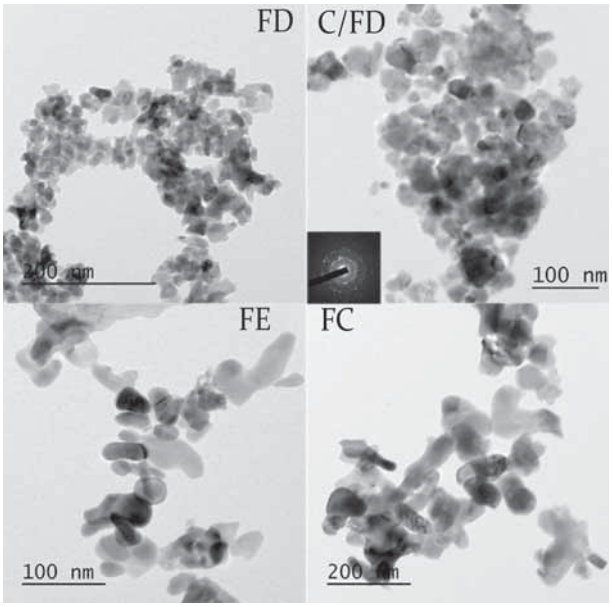

Figure 3. TEM images of samples (**a**) FD, (**b**) C/FD, (**c**) FE and (**d**) FC.

selected area electron diffraction (inset of figure 3b) indicated that C/FD are of crystalline structure.

For microstructure analysis the as synthesized samples of FD and C/FD were directly transferred to the chamber of SEM without disturbing the original configuration of the reaction products. The SEM images of the samples are shown in figure 4. The nanoparticles were aggregated into larger irregular structures with no well defined morphologies. The agglomeration of nanoparticles is generally explained as a common way to minimize their surface free energy; however, some authors have reported that the agglomeration is assigned to the existence of organic radicals that act as binders [14].

4.2 *Antioxidant activity*

The antioxidant activity of two sets of C/FD composites (C1 and C2) are validated using DPPH assay. In sample C1 the concentration of hematite is 0.625 g and in C2 it is 1.25 g. Anti-radical activity assay is based on the reduction of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Owing to the presence of an odd electron it gives a strong absorption maximum at 517 nm. The scavenging effect of C1 and C2 on

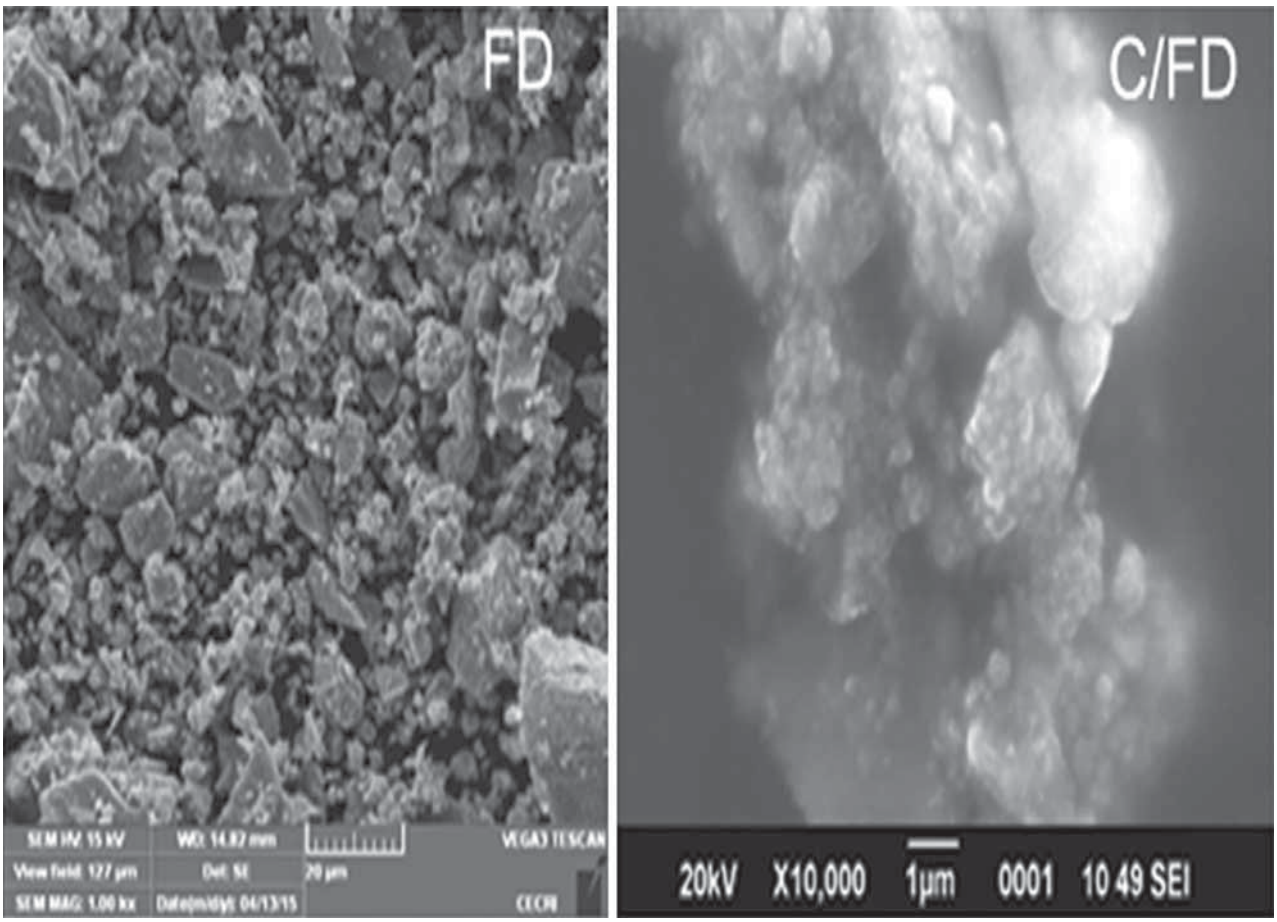

Figure 4. SEM images of samples (**a**) FD and (**b**) C/FD.

Table 2. Antioxidant activity of C/FD composite.

Sample C1		Sample C2	
Concentration (μg)	Antioxidant activity (%)	Concentration (μg)	Antioxidant activity (%)
10	2.7	10	3.1
30	3.7	30	4.1
50	4.4	50	4.7
70	6.2	70	6.7
90	7.6	90	11.9
100	8.6	100	13.3

Table 3. Results obtained for the microbial activity of prepared samples.

	Diameter of clear inhibition zone in mm	
Parameters	FD (mm)	C/FD (mm)
Escherichia coli	7	15
Salmonella typhimurium	6	16
Shigella flexneri	9	9
Candida albicans	7	12

DPPH radical is investigated and the antioxidant efficiency is found to increase with increase in sample dosage for both samples as shown in table 2. The antioxidant activity also increases with an increase in concentration [15,16]. It can be noticed that the sample C1 exhibits lower scavenging activity when compared to C2 samples. The higher concentration of hematite nanoparticles attributed to the higher scavenging activity of C2 samples. The scavenging activity exhibited by C/FD nanocomposite can also be attributed to the ability of Hematite nanoparticles to transfer its electron density towards the free radical located at nitrogen atom in DPPH.

4.3 *Antimicrobial activity*

The antibacterial activity against three water pathogens, namely *E. coli*, *S. typhimurium* and *S. flexneri* are analysed for the samples FD and C/FD and results are given in table 3. The antifungal activity of the samples against *C. albicans* (yeast) are also given in table 3. Figures 5 and 6 show inhibition zones obtained for *E. coli, S. flexneri, C. albicans* and *S. typhimurium*. In sample FD maximum activity was obtained for *S. flexneri* and minimum for *S. typhimurium*. Interestingly the activity of C/FD was reversed, which means maximum activity for *S. typhimurium* and minimum for *S. flexneri*. The

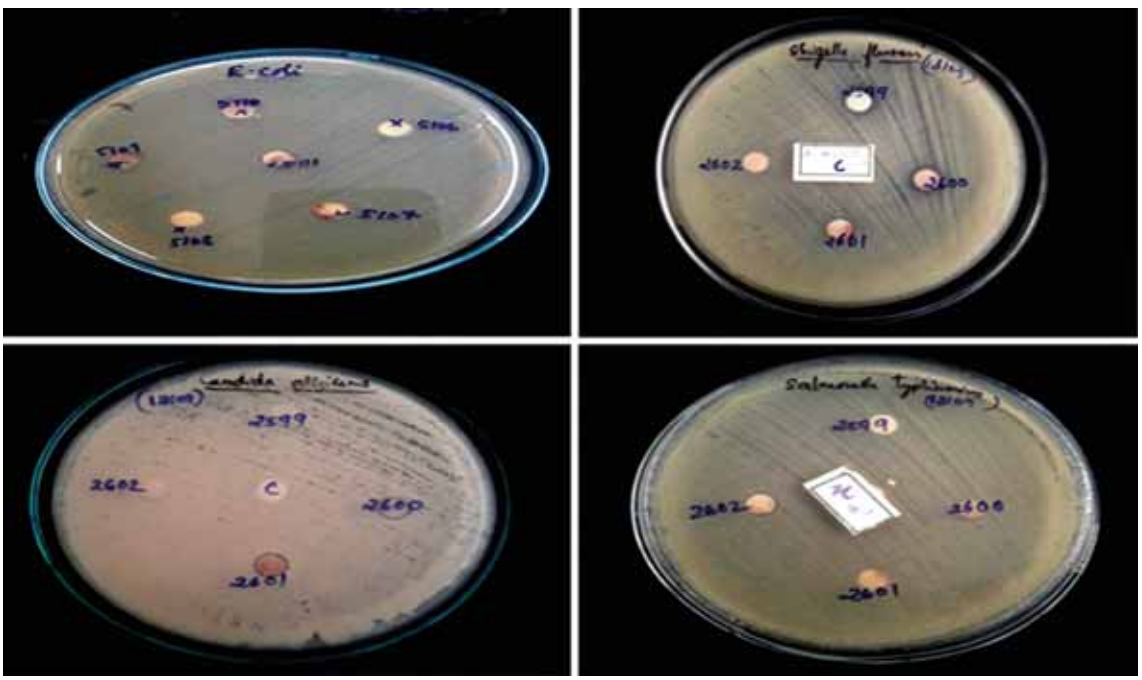

Figure 5. Photograph showing the inhibition zones obtained for the sample FD.

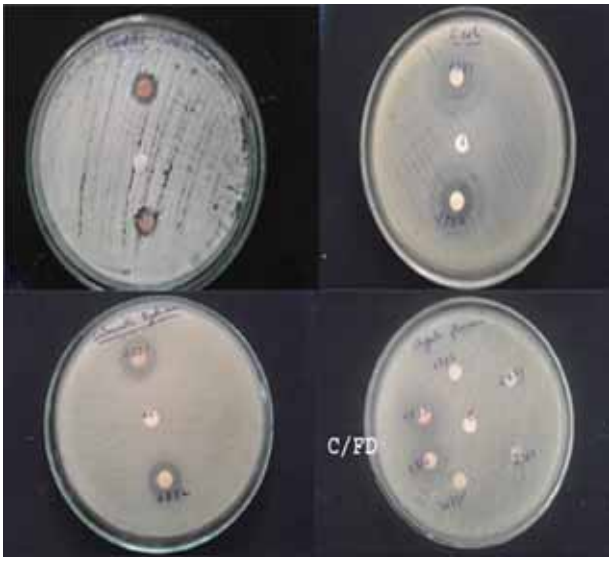

Figure 6. Photograph showing the inhibition zones obtained for the sample C/FD.

sample C/FD exhibit higher anti-bacterial activity than the sample FD on account of the special character of the CH. Various studies have been reported to inhibit the growth of microorganism using iron oxide nanoparticles [17,18]. The biological activities of metal complexes depend on the charge, the nature of the counter anion, the geometrical configuration and the oxidation state of the central metal ion [19]. The interaction of iron oxide nanoparticles with the cell generates reactive oxygen species (ROS), such as superoxide ($^{\bullet}O_2$) and hydroxyl radicals. This ROS cause cytotoxic reactions by inhibition of DNA synthesis and destruction of cell viability [20].

As a particle approaches near the membrane, a potential called zeta potential is generated. Zeta potential that is, surface charge, can greatly influence particle stability in suspension through the electrostatic repulsion between particles. It can also determine nanoparticles interaction with cell membrane of bacteria, which is usually negatively charged. The negatively charged surface of the bacteria cell is the target site of the polycation [21]. Therefore, the polycationic CH nanoparticles with higher surface charge density provide higher affinity with bacteria cells for a quantum-size effect. The C/FD composites exhibit greatly higher antibacterial activity than FD. The higher surface charge density of C/FD composite enhances the affinity with the negatively charged bacteria membrane, which is probably responsible for their higher bacterial activity.

5. Conclusion

Quantum confined C/FD nanocomposites was synthesized using DNA as the capping agent. The formation of FD nanoparticles with size 17.86 nm was confirmed from XRD. TEM confirmed the dumbbell nature of the iron oxide nanoparticles. The antibacterial and anti-oxidant activity of the prepared nanocomposites proves it to be a potential member of bioactive materials. The antioxidant activity of composites can be improved by varying the concentration of FD in the sample. The obtained composites in the present study have small particle size, which may improve their stability in the presence of biological cations and their antibacterial activities due to the interaction with the negatively charged biological membrane. Even though an effort to elucidate mechanisms responsible for antibacterial and antioxidant activities of C/FD nanocomposites is done, still there is scope for further investigation.

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