International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.7, No.2, pp 769-779, 2014-2015

ICONN 2015 [4th -6th Feb 2015]
International Conference on Nanoscience and Nanotechnology-2015
SRM University, Chennai, India

Facile Synthesis of Fe_3O_4@Ag Magnetic Nanoparticles and Their Application in Detection of Pathogenic Microorganism

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Abstract: Magnetic nanoparticles with a few nanometers to micrometer diameter are largely used in biomedical application such as diagnosis, drug delivery, and treatment of numerous pathogens because of its biocompatibility and inertness. In the present investigation we are interested in synthesis of core shell magnetic nanoparticle in order to attach various biomolecules and bioconjugation. Here the silver coated magnetic nanoparticle is of our choice of nanoparticles in which they can be further derivative with biomolecules and drugs so that they can be further used in imaging and preconcentration of pathogenic microorganism in dilute concentration. Synthesis of these nanoparticles can be achieved by co-precipitation method or by thermolysis of iron oleate with different experimental condition followed by reaction with respective metal salt in order to synthesis of core shell system. The reactivity of core shell magnetic nanoparticles with fluoroquinolone antibiotics has been tested and their combined system can be utilized to study the Antimicrobial actions of drugs protected nanoparticles. The drugs protected core shell nanoparticles shows enhanced antimicrobial action against various microorganisms than the pure drug. This method can be further extended for the preconcentration of microorganism from drinking water samples. The proposed method is found to be easier way detection of microorganism at low level concentration.

Keywords : Drug delivery, Core-Shell, Bioconjugation and Fluoroquinolone.

Introduction

Nanotechnology is beginning to allow scientists, engineers and physician to work at the cellular and molecular levels to produce major advances in the life sciences and healthcare. Real applications of nanostructured materials in life sciences are uncommon at the present time. However, the excellent properties of these materials when compared with their bulk counterparts provide a very promising future for their use in this field. Nanoparticles have high surface to volume ratio and thus mass transfer and heat transfer properties are better than bulk materials[3]. The properties of the nanomaterials are device and cannot be generalized even though the particles under comparison might be made of similar material and comparison[7].

Nanoparticles that possess magnetic properties offer exciting new opportunities in biomedical field including improving the quality of magnetic resonance imaging (MRI)[8], hyperthermic treatment for malignant cells[2], site-specific drug delivery[12], detection of protein[6], separation and purification[14] of biological molecules and cells, and also for measuring low concentrations of bacteria[4]. Magnetic nanoparticles show remarkable new phenomena such as superparamagnetism, high field irreversibility, high saturation field, extra anisotropy contributions or shifted loops after field cooling[9]. These phenomena arise from finite size and surface effects that dominate the magnetic behavior of individual nanoparticles. The surface can be functionalized for selective interaction and their magnetic properties make them controllable by an external magnetic field.

Among the magnetic material with suitable properties magnetite (Fe_3O_4) is the only one that has up to now been allowed for use in humans[13]. Nanocrystalline silver has been proved to be the most effective antimicrobial agent since silver and its compounds have powerful antimicrobial capability and broad inhibitory biocidal spectra for microbes including bacteria viruses and eukaryotic microorganisms[5]. Biofunctional magnetic iron oxide / silver (Fe_3O_4@Ag) core/shell nanoparticles are so versatile that they can couple with other analytical means for pathogen defection. They have super paramagnetic and antibacterial properties[3].

In the present study, the Fe_3O_4@Ag core shell nanoparticle is coated with fluroquinolone drugs such as ciprofloxacin and gatifloxacin. Ciprofloxacin drug is a very useful answer to the treatment of an array of bacteria. In addition, it also renders less potential side effects compared to others in its class. It is a broad-spectrum antibiotic effective against Gram-negative and Gram-positive bacteria[6]. It directly acts on enzymes to prevent bacterial cell division. Gatifloxacin is an antibiotic of the fourth-generation fluoroquinolone family, that like other members of that family. It is a synthetic broad-spectrum 8-methoxyfluoroquinolone antibacterial agent for oral or intravenous administration. The bactericidal action of the above two drugs are to block bacterial DNA proliferation and spread by binding to the DNA gyrase enzyme. This enzyme is a type II topoisomerase and important in separating replicated DNA[12]. The binding action will cause double-stranded breaks in the chromosome of the bacteria which stops cell division. The drug also directly acts on any existing bacteria under its coverage, killing them immediately. It shows the enhanced antimicrobial activity than the palin Fe_3O_4@Ag nanoparticles. The drug capped Fe_3O_4@Ag nanoparticles were proved to have excellent antibacterial activity against *Staphylococcus aurous (S. aureus), Escherichia coli (E.Coli) and Micrococcus leteus (M. Leteus).*

Material & Methods

The synthetic work was conducted in room temperature and N_2 atmosphere. All chemicals were obtained from Zigma Aldrich Co. SEM and EDAX images were taken with field emission scanning electron microscopy (JSM-6500 F, Jeol, Enserberg, Germany). X-ray diffraction of the samples was performed on a Philips PW 1800 X-Ray Diffractometer. Particle size analysis was performed at room temperature or set to 25°C in Nanosizer ZS experiments. The UV-vis spectrum was recorded on a PerkinElmer UV-Lambda 25 scanning spectrophotometer operating at a slit width of 1.0 nm.

Antibacterial activity of the drug coated iron oxide @ silver (Fe_3O_4@Ag) core-shell nanoparticle is analyzed by studying the Zone of inhibition by disc diffusion method and minimum inhibition concentration (MIC) is studied by agar streak dilution method. Cytotoxicity of the synthesized compound is studied by MTT assay.

Exprimental Details

a) Synthesis of Iron Oxide (Fe_3O_4) Nanoparticles

Firstly, Fe_3O_4 nanoparticles are synthesized by coprecipitation and Thermolysis methods. The two methods are explained below

a) Coprecipitation Method

2.7g (10mmol) of $FeCl_3.6H_2O$ and 1.0g (5mmol) of $FeCl_3.H_2O$ are dissolved in 15ml of 0.3M HCl solution while stirring under N_2, the mixture is titrated to PH 10-11 by the dropwise addition of 6.0M NH_4OH. Black precipitation formed immediately and the reaction continued for 30minutes. The precipitation is isolated via magnetic decantation and washed with water. The large aggregates are removed by filtration. The Fe_3O_4 nanoparticles are dissolved in 100ml of 0.01M tetramethylammonium hydroxide pentahydrate[10]. Finally, black 40mM Fe_3O_4 nanoparticle solution is stored in air under benchtop condition for future use.

b) Thermolysis Method.

In this method Iron-Oleate complex was dissolved in the mixture of oleylamine and oleic acid (volume ratio 3:1) at 200°c for 2h. During heating nitrogen gas was gently blown through the reaction system to remove the trace hydrate vapour, the Fe_3O_4 nanoparticle were precipitated by adding ethonol.

c) Synthesis of Fe_3O_4@Ag Core-ShellNanoparticles

Fe_3O_4@Ag nanoparticles are synthesized by the reverse micelle method 100ml of 40mM Fe_3O_4 nanoparticles are mixed with a W/O microemulsion containing 1.4ml of Triton X-100, 1.4ml of n-hexonol and 7.5ml of cyclohexane with vigorous stirring. Then 200µl of 0.10M $AgNO_3$ are added after 30minutes 200µl of 0.20M $NaBH_4$ are added to the solution. The mixture is stirred at room temperature for 4h. The black Fe_3O_4@Ag nanoparticles are precipitated by adding excess acetone and then centrifuged and repeatly washed with ethanol and water to remove surfactant and unreacted materials[10]. The nanoparticles obtained are suspended in water for future use. Figure 1 shows the structure of functionalized magnetic core-shell nanoparticles.

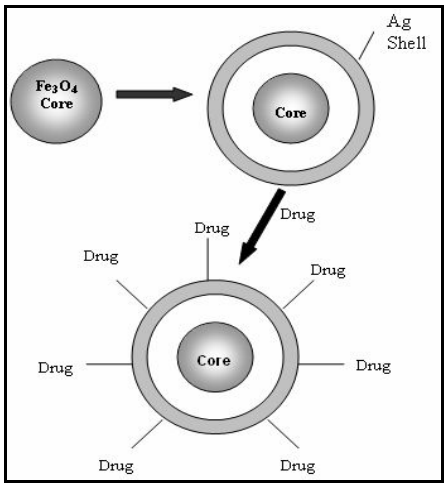


Figure 1. **Functionalized Magnetic Core-Shell Structure**

C. Synthesis Of Antibiotic Drug Coated Core-Shell Nanopartilces

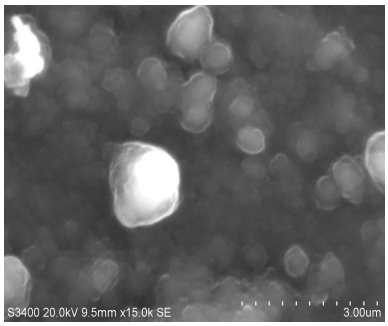
The drug capped Fe_3O_4 @ Ag Nanoparticles was synthesized by chemical adsorption method. Ciprofloxacin and Gatifloxacin belong to a class of drugs called fluroquinolones antibiotics which function by Figurehting bacteria that invade the body. 10 mM of drug was dissolved in 10 ml water, this solution is added with 1 ml of Fe_3O_4@Ag nanoparticles and above mixture is stirred for 2 hours.

Results and Discussion

The functionalized iron oxide @ silver (Fe_3O_4@Ag) core-shell nanoparticles is characterized by Scanning electron microscopy (SEM), X-ray diffraction analysis (XRD), UV-Visible spectroscopy (UV-Vis), Particle size ananlyzer(Dynamic light scattering instrument), Vibrating sample magnetometer (VSM).

A. Scanning Electron Microscopy (Sem)

Scanning electron microscopy (SEM) is coupled with energy dispersive X-ray spectroscopy (EDAX). The pictures are taken with the field emission scanning electron microscopy (JSM-6500 F, Jeol, Enserberg, Germany). It is a method for high resolution imaging and characterization of functionalized surfaces of the nanoparticle with spatial resolution[14]. The images are taken at 3µm and 5µm and 10µm spatial resolution. It gives the surface morphology of the core-shell nanoparticle. Figure 2A, 2B shows the surface morphology of Fe_3O_4 and Figure 3A, 3B shows the surface morphology of Fe_3O_4 @ Ag nanoparticles.



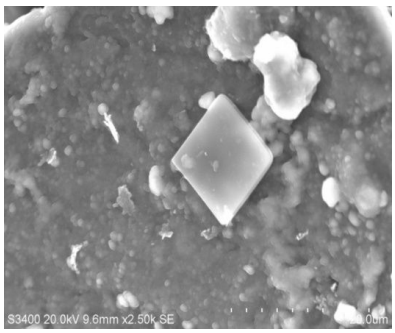
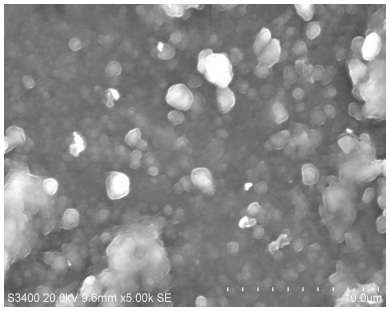


***Figure 2A*. SEM image 1 of Fe_3O_4** ***Figure 2B.* SEM image 2 of Fe_3O_4**



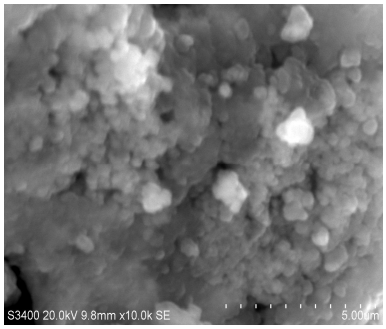


***Figure 3A*. SEM image 1 of Fe_3O_4 @ Ag** ***Figure 3B.* SEM image 2 of Fe_3O_4 @ Ag**

B. Energy Dispersive X-Ray Spectroscopy (Edax)

EDAX analysis supports identification of material phase composition at atomic levels, even for extremely small particles. It gives the chemical composition of each nanopartilce present in core-shell structure. Figure 4A and 4B gives the EDAX peaks pattern of Fe_3O_4 and Fe_3O_4 @Ag nanoparticles. It confirms the presence of iron oxide(Fe_3O_4) and silver (Ag) in the synthesized nanopartilce system.

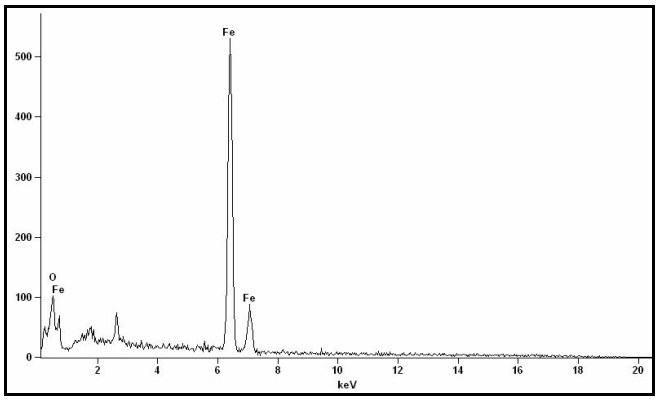


***Figure 4A*. EDAX peak pattern of Fe_3O_4 nanoparticle**

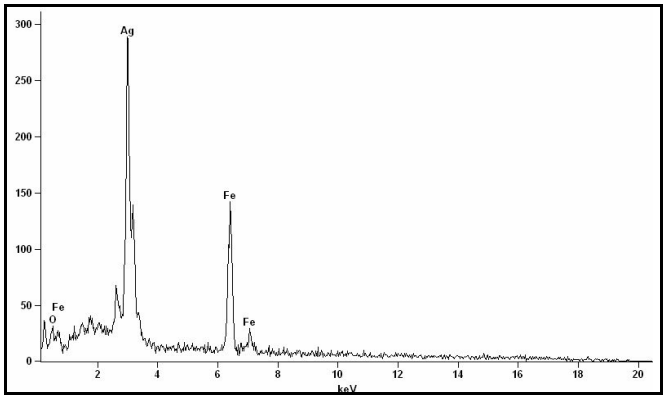


***Figure 4B*. EDAX peak pattern of Fe_3O_4 @Ag nanoparticle**

C. X-Ray Diffraction Analysis (Xrd)

To confirm the composition of the particles, the XRD patterns for Fe_3O_4 and Fe_3O_4@Ag nanopartilcles is measured. Form this analysis we can have details about the crystallography structure and lattice constants of the particular nanoparticles**.** X-ray diffraction of the samples is performed on a Philips PW 1800 X-Ray Diffractometer.

XRD patterns in Figure 5A shows the characteristic peaks (at $2\Theta = 35.5^0, 63.1^0$) marked with their indices (311), (422). This indicates that the nanoparticles are pure Fe_3O_4 with an inverse cubic spinal structure. The peak at $2\Theta = 35.5^0$ is the only peak corresponding to the indices (311) for Fe_3O_4. The occurrence of the most intense peak of Fe_3O_4 indicates the presence of Fe_3O_4 as the core[2]. In Figure 5B shows peaks (at $2\Theta = 38.1^0, 44.3^0, 64.4^0, 77.4^0$) reveal indices corresponding to (111), (220), (311), (400) for pure silver. This indicates the presence of silver (Ag) as the shell.

D. Particle Size Analyzer (Dynamic Light Scattering Measurements)

Particle size analyzer result for Fe_3O_4@Ag nanoparticles in water is made to access the stability of the colloidal suspension.

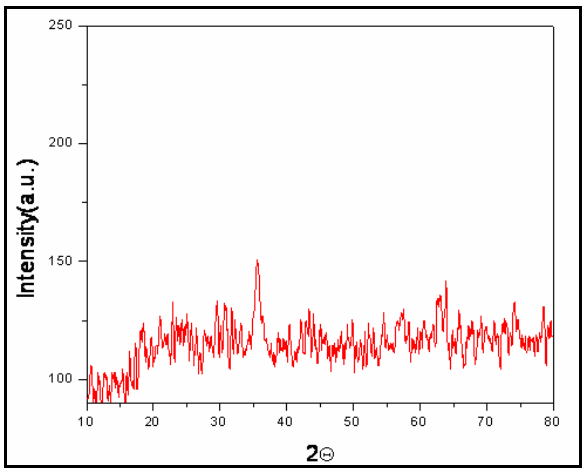


Figure 5A. **XRD pattern of Fe_3O_4 nanoparticle**

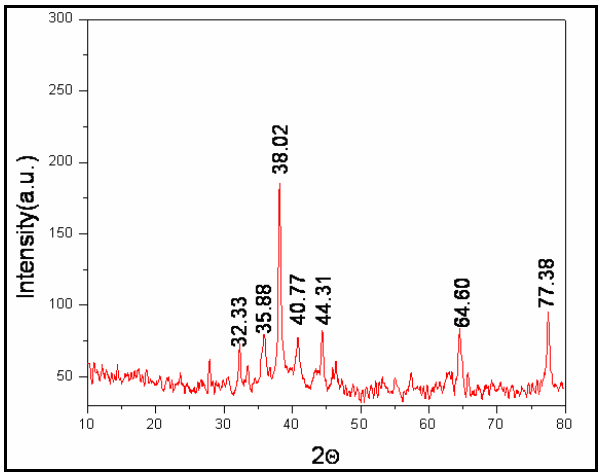


Figure 5B. **XRD pattern of Fe_3O_4 @Ag nanoparticle**

This analysis is performed at room temperature or set to 25°C in Nanosizer ZS experiments. Figure 6A shows the size distribution of Fe_3O_4 and Figure 6B shows the histogram curve, likewise Figure 7A shows the size distribution of Fe_3O_4@Ag nanopartilce and Figure 7B shows the histrogram curve for the Fe_3O_4@Ag nanoparticle[3].The mean diameter determined for Fe_3O_4 is 120.1 nm and for Fe_3O_4@Ag is 144.2 nm. Sedimentation could occur when the nanoparticles with high concentration in water are left for more than one day.

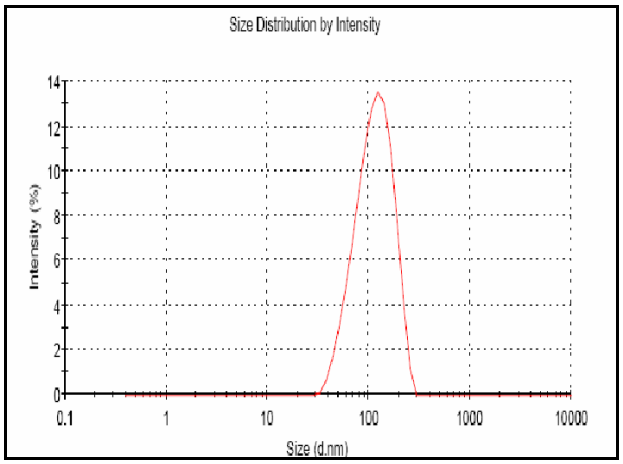


Figure 6A **Size distribution curve for Fe_3O_4 nanoparticle**

A). Measurement Of Antibacterial Properties Or Fe_3o_4@Ag And Antibacterial Drug Coated Fe_3o_4@Ag Nanoparticles

In the present study, the paper disc agar diffusion method is used to evaluate the antibacterial activity of the synthesized compounds *in vitro*. The antibacterial activity of the nanopartilce and drug capped nanoparticle system is studied against three different microorganism *Staphylococcus aureus, Micrococcus leteus,* and *Escherichia coli*. Level of Zone of inhibition is larger for drug capped nanoparticle than the uncapped plain nanoparticle system. Similarly the level of zone of inhibition value of standard drugs is smaller when compared with the drug capped nanoparticle system[8,11].

The drug capped nanoparticle system shows higher level of zone of inhibition value than the uncapped plain nanoparticles and the standard drugs. Figureure 10A, 10B, 10C shows the area of zone of inhibition picture for three different bacterial species. Table 1 shows the diameter of zone of inhibition value in mm for the standard drug, and Table 2 shows the diameter of zone of inhibition value in mm for the plain nanoparticle and drug coated nanoparticle system.

E. Uv-Visible Spectrometer Analysis

The UV-visible absorption spectrum of Fe_3O_4@Ag nanoparticles solution is shown in Figureure 8. The absorption spectrum was recorded on a PerkinElmer UV-Lambda 25 scanning spectrophotometer operating at a slit width of 1.0 nm. The absorbance of Fe_3O_4 nanoparticle occurs at 273 nm, a typical surface plasmon resonance band at 412 nm is observed for silver nanoparticles. It confirms the presence of iron oxide and silver in the core-shell nanoparticle system.

F. Vibrating Sample Magnetometer (Vsm)

The magnetic property of Fe_3O_4@Ag nanoparticles are examined using VSM magnetometry. Figure 9 shows the magnetization of nanoparticles versus the magnetic field at 300 K. It can be seen the Fe_3O_4 and Fe_3O_4@Ag nanoparticles shows the typical linear hysteresis loop for superparamagnetic materials. This shows that the iron oxide and iron oxide@silver core-shell nanopartilces show superparamagnetism at 300 K and it can be separated from water by applying magnetic field

G. Fluroquinoloid Antibiotic Drug Coated Fe_3o_4@Ag Core-Shell Magnetic Nanoparticles For Pathogen Detection

Fe_3O_4@Ag Core-Shell nanoparticle and antibiotic drug (ciprofloxacin and Gatifloxacin) coated Fe_3O_4@Ag Core-Shell nanoparticle can be used as a system for detection of microorganism. Here Fe_3O_4 is used as a magnetic drug carrier, and Silver (Ag) enhances the antibacterial efficiency.

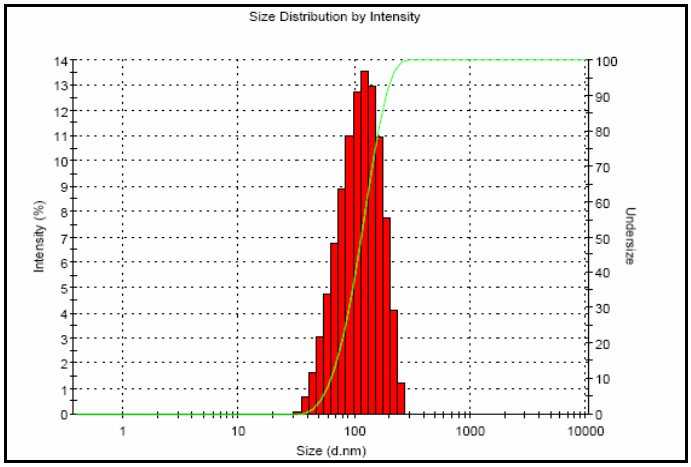


Figure 6B. **Histogram of Fe_3O_4 nanoparticle**

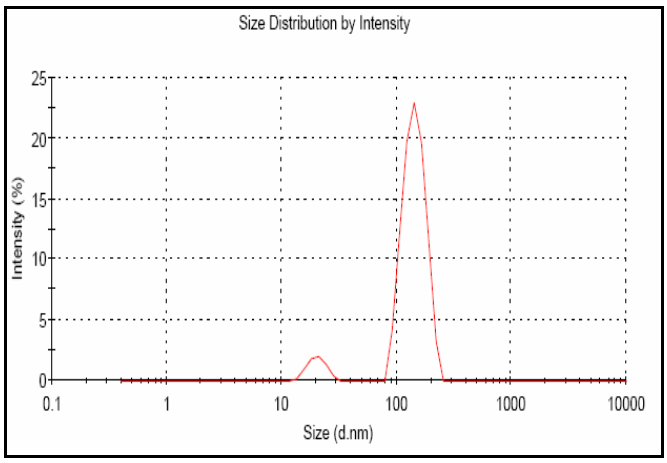


***Figure 7A*. Size distribution curve of Fe_3O_4@Ag nanoparticle**

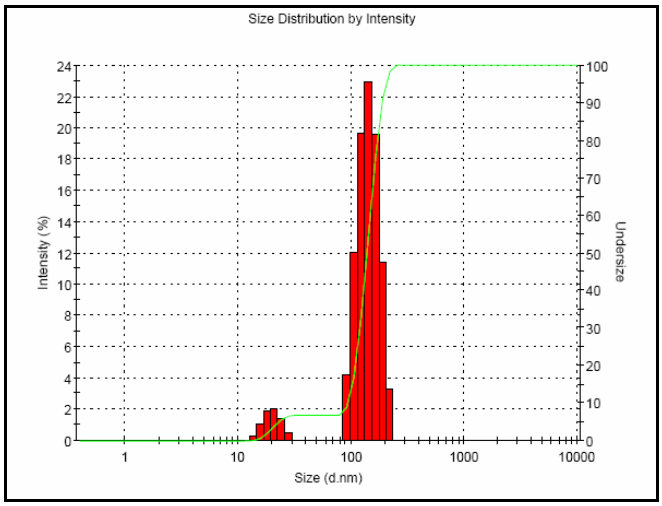


***Figure 7B*. Histogram of Fe_3O_4@Ag nanoparticle**

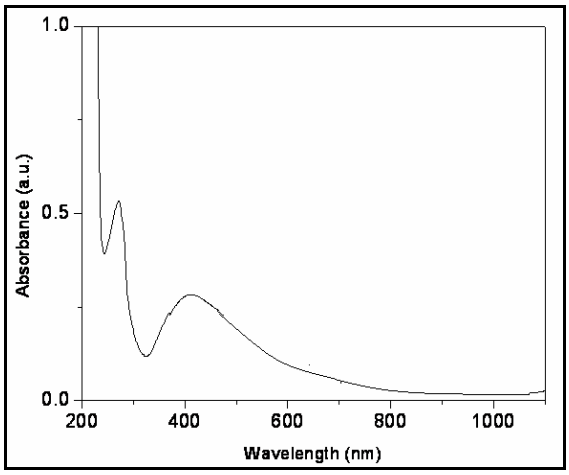


Figure 8. UV-vis spectra of Fe_3O_4 and Fe_3O_4@Ag nanoparticles

H .Minimum Inhibition Concentration Measurements (Mic)

The MIC is considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria on the plate. This is done by using agar streak dilution method. This experiment is done against three different microorganism *Staphylococcus aureus, Micrococcus leteus,* and *Escherichia coli*. The MIC values of

drug capped nanoparticle system is very small when compared with the MIC values of plain nanoparticles. These values are given in µg/ml. The MIC value for drug capped nanoparicles and uncapped plain nanoparticles system is given in table 3.

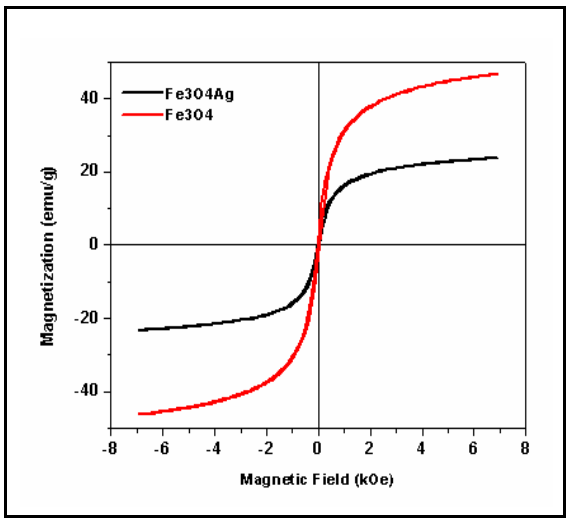
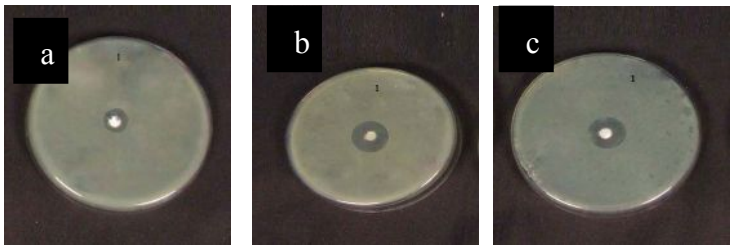


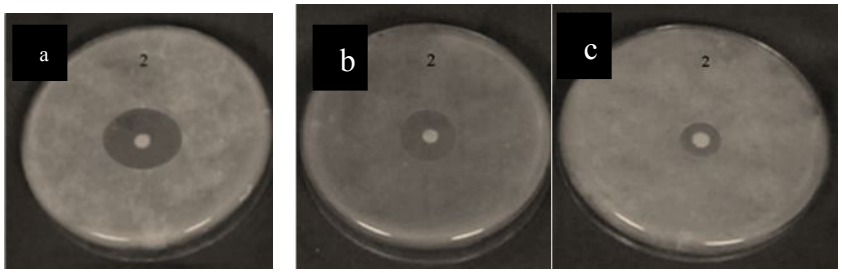
Figure 9. Hysteresis curve of Fe_3O_4 and Fe_3O_4@Ag nanoparticles



Staphylococcus aureus

Figure.10A Area of zone of inhibition 1

(a) Ciprofloxacin capped Fe_3O_4 @ Ag, (b)Gatifloxacin capped Fe_3O_4 @ Ag, (c)Fe_3O_4 @ Ag



Micrococcus leteus

Figure.10B Area of zone of inhibition 2

(a) Ciprofloxacin capped Fe_3O_4 @ Ag

(b) Gatifloxacin capped Fe_3O_4 @ Ag

(c) Fe_3O_4 @ Ag

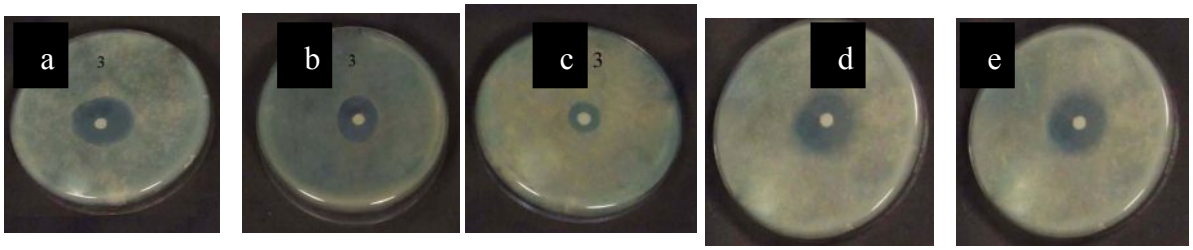


Figure 10C - Zone of inhibition by samples against Escherichia coli

(a) Ciprofloxacin capped Fe_3O_4 @ Ag.

(b) Gatifloxacin capped Fe_3O_4 @ Ag.

(c) Fe_3O_4 @ Ag

(d) Ciprofloxacin Plain drug

(e) Gatifloxacin Plain drug

Table 1. **Zone of inhibition value for Standard drug**

Microorganism	Zone of Inhibition in mm	
	Ciprofloxacin	Gatifloxacin
Escherichia coli	*28*	*24*

Table 2 Zone of inhibition value for plain nanoparticle and drug capped nanoparticles

Microorganism	Zone of Inhibition in mm		
	Ciprofloxacin capped Fe_3O_4 @ Ag	Gatifloxacin capped Fe_3O_4 @ Ag	Fe_3O_4 @ Ag
Staphylococcus aureus	39	38	14
Micrococcus leteus	37	34	15
Escherichia coli	42	35	17

can be used for biomedical purposes. The CC50 (µg/ml) value of the nanoparticle and drug capped nanoparticle is shown in table 4.

Table 3. Minimum Inhibitory concentration (MIC) value of Fe3O4 @ Ag and antibiotics capped Fe3O4 @ Ag Nanoparticles

Micro organism	MIC in µg/ml		
	Ciprofloxacin capped Fe_3O_4 @ Ag	Gatifloxacin capped Fe_3O_4 @ Ag	Fe_3O_4 @ Ag
Staphylococcus aureus	2.4	3.6	24
Micrococcus leteus	3	12	28
Escherichia coli	1.8	12	33.6

Table.4 Cytotoxicity Concentration CC_{50} values

Test Extract	CC_{50}(µg/ml)
Fe_3O_4@Ag	6.2275±0.3459
Ciprofloxacin capped Fe_3O_4@Ag	80.0046±2.0569
Gatifloxacin capped Fe_3O_4@Ag	57.2241±3.4282

Conclusion

From the M-H curve (hyteresis loop), it is confirmed that the core-shell nanoparticles system has superparamagnetic behavior, it is capable of separating nanoparticle at room temperature by applying magnetic field. Antibacterial measurements of nanoparticles and drug capped nanoparticles gives the area zone of inhibition value. The drug capped nanoparticles system shows more antibacterial efficiency than the plain nanoparticles and the standard drug. The combined effect of drug and nanoparticles give a good antibacterial property for the synthesized compound. This is because of the high surface to volume ratio property of the nanoparicle.

And also Minimum inhibition concentration value for drug capped nanoparticles is very small compared with uncapped nanoparticles sysem. From this, at very lower dosage level itself we can achieve the antibacterial efficiency.

From the cytotoxiciy measurements, we can understand that, the uncapped plain nanoparticles achieve the cytotoxicity CC_{50} value at very lower concentration itself. Cytotoxicity value for antibacterial drug coated nanoparticles is small. Antibiotic drug coated nanopartilces shows less cytotoxicity and shows more antibacterial activity (MIC is low). Fe_3O_4@Ag shows higher cytotoxic value than the drug coated nanoparticles, and also shows less antibacterial activity (MIC is high).

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