

FABRICATION AND EVALUATION MAGNETIC NANOPARTICLES LOADED WITH CEFIXIME

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ABSTRACT: Background. Bacterial infections are an important cause of serious health issues worldwide. Various antibacterial drugs have been developed but they have numerous side effects. Development of drug loaded magnetic nanoparticles will help to achieve targeted drug delivery while sustaining the release of drug. It will also enhance its antibacterial activity by using iron oxide. **Method.** Drug loaded iron oxide nanoparticles were developed to sustain and enhance the antibacterial activity of drug. Chitosan was used as a polymer. The method adopted to prepare magnetic nanoparticles was co-precipitation. Formulated magnetic nanoparticles were tested for drug release, surface morphology, antibacterial activity and FTIR. **Results.** It was observed from the findings that both formulations were effectively loaded with drugs. The SEM results showed the semi spherical nanoparticles effectively loaded with drug. FTIR spectrum revealed characteristic peaks related to functional groups. Raman spectroscopy showed characteristic bands of both drugs and drug loaded iron oxide nanoparticles. Antibacterial assay results showed an enhanced antibacterial activity by using iron oxide nanoparticles. **Conclusion.** It was concluded from the study that magnetic nanoparticles could be one of the best approaches to load antibacterial drugs while sustaining and enhancing their antibacterial activity.

Keywords: Magnetic nanoparticles, Chitosan, Cefixime, Sustained release, Antibacterial activity.

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INTRODUCTION

Novel drug delivery systems serve as the means to carry drug molecules to a particular site of the body in an adequate quantity via a particular route so that they can produce their specific biological effects and finally maintaining their appropriate amount in the body in a specific range for a particular period of time in a controlled and targeted way keeping in view the safety and efficacy parameters. They overcome the challenges encountered while dealing with conventional dosage forms enabling sustained biological effect, minimized drug degradation, less drug related toxicity, improved patient compliance, enhanced drug efficacy, better comparable safety and improved drug bioavailability [1].

Nanotechnology is an emerging era of research yielding small particles of nanoscale size (1 to 100nm) that have revolutionized the whole scenario by altering the physical and chemical characteristics of medicinal substances which resultantly influence their biological effects. Depending upon their particular properties, there are various types of nanoparticles (differing in size, shape or function) attracting researcher's attention in multidisciplinary fields. Nanoparticles have enabled delivery of drugs in the most appropriate dosage range thus elevating therapeutic efficacy

and improving patient compliance [2]. It has made it possible to increase the bioavailability of poorly soluble drugs by increasing their water solubility via incorporating them in the form of nanoparticles. Magnetic nanoparticles made from the use of various polymers are being employed as drug delivery carriers to ensure the controlled and targeted release of drug at particular sites of body. They are important carriers to deliver peptide drugs and gene therapy [3].

In the past few years, there has been an extensive use of nanotechnology in the medical field, especially for drug delivery. Nanoparticles can be used as a carrier system for drugs and molecules which are bioactive and hence their usage has been surveyed to accomplish the goal of decreasing the harmful effects and increasing the therapeutic effects of drugs. Variety of nanostructures which includes, silicon or carbon materials, liposomes, magnetic nanoparticles and polymers can be used for delivering the drug. The use of MNP's and super paramagnetic iron oxide nanoparticles (SPIONs) has gained a massive attention in developing delivery systems, for release of drugs in controlled manner.

They have two essential features: Nano-scale dimensions and Magnetic properties. Nanoparticles can pass through the narrow blood vessels because of their nano sizes and also enter through cell membranes. Their magnetic properties allow them to get influenced by the external magnetic field, aiding them to reach the target organs where active biomolecules, which binds to these nanoparticles' surfaces are then released. Magnetic nanoparticles offer the advantage of super paramagnetism, not keeping magnetized after the action of magnetic field, which reduces the chances of particle aggregation. Drug targeting through nanoparticles decrease the wastage of drug, drug administration frequency as well as reduce the side effects by providing prolonged and sustained drug release [4]. Super paramagnetic iron oxide nanoparticles are extensively used for applications such as magnetic resonance imaging, immunoassay, detoxification of biological fluids, drug delivery, tissue repair, hyperthermia and cell separation. Hyperthermia provides a striking method for cancer treatment; it is linked with less harmful effects in contrast to radiotherapy and chemotherapy [5]. Upon locating a malignancy or lesion, external magnetic fields control the direct particle assembling to provide therapeutic effects [6]. To make the therapeutic treatments effective, transition metals (e.g. Fe, Co, Ni) or metal oxides (e.g. Fe_3O_4 , $\gamma\text{-Fe}_2\text{O}_3$) are used to achieve magnetization. Small iron oxide NP's are used for diagnosis in vitro, over more than 50 years. Nanoparticle surfaces must be modified to improve biocompatibility and reduce aggregation [7].

Antibacterial activity, is linked to, compounds which in the vicinity kill bacteria and decrease their growth. The increase of resistance of bacteria to antibacterial drugs is a usual occurrence, and is a main problem. The infectious diseases are the extreme healthcare challenges all over the world because bacteria have become resistant against various antibacterial agents. Nano materials have appeared as novel, antimicrobial agents. Numerous, classes of the antimicrobial NPs and nano carriers for the delivery of antibiotics have proved their efficacy, for handling infectious diseases which includes the ones resistant to antibiotics [8].

The magnetic core and surface coating of magnetic nanoparticles determines their prospective in drug delivery systems. The coatings are known to eradicate or diminish its aggregation under bodily conditions. Chitosan, as a derivative of chitin, is a striking natural biopolymer from renewable resources because of the presence of functional groups of amino and hydroxyl within its structure. Because of the enormous surface to volume ratio, compared to bulk form of chitosan, chitosan nanoparticles have exceptional, biological, physico-

chemical and antimicrobial properties. These exclusive properties make chitosan NPs a favorable biopolymer for use of DDSs [9].

The current study involves the development and characterization of Cefixime loaded chitosan-magnetic nanoparticles. Drug loaded magnetic nanoparticles were produced by co precipitation method

Components of magnetic nanoparticles

Magnetic nanoparticle consists of following parts:

- 1) A magnetic core
- 2) Protective coating
- 3) Organic linker
- 4) Active molecule

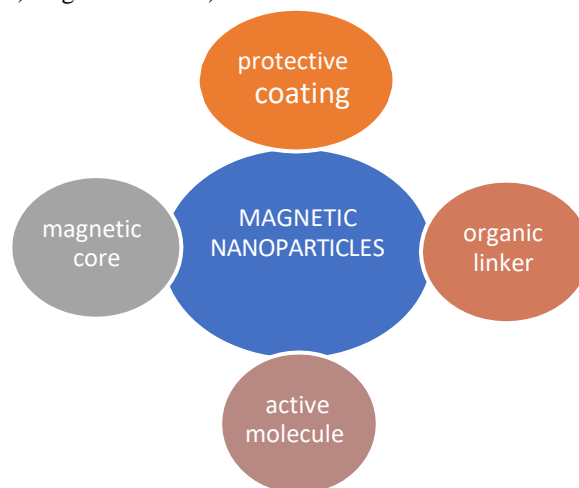


Figure 1: Components of magnetic nanoparticles.

MATERIAL AND METHOD

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ferric chloride hexahydrate and $\text{FeCl}_3 \cdot 4\text{H}_2\text{O}$ ferric chloride tetrahydrate purchased from Universal Chemicals Lahore. Sodium Hydroxide NaOH was purchased from Panreac Quimica SLU Barcelona. Chitosan and cefixime was gifted by Saffron Pharmaceuticals. Glacial acetic acid ethanol was purchased from Sigma-Aldrich UK. Phosphate buffer saline (PBS) was self-prepared using potassium monobasic phosphate and NaOH. Fresh deionized and distilled water bottles were purchased from Batteries Care Enterprises Lahore and Super Scientific Store Faisalabad.

Preparation of Magnetic Nanoparticles

Firstly, a solution of Ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) 4g and ferric chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) 4g was prepared in 50 mL of water. The above solution of ferric and ferrous ions was added gradually into a solution of 2 M NaOH with constant stirring, having a room temperature pH of less than 10 [10]. At room temperature, solution was sonicated for 60 minutes. Finally the filtration of particles, and three times washing with deionized water was performed. The resultant particles were dried on hot plate at 70-90 °C for 3-4 hours [11, 12].

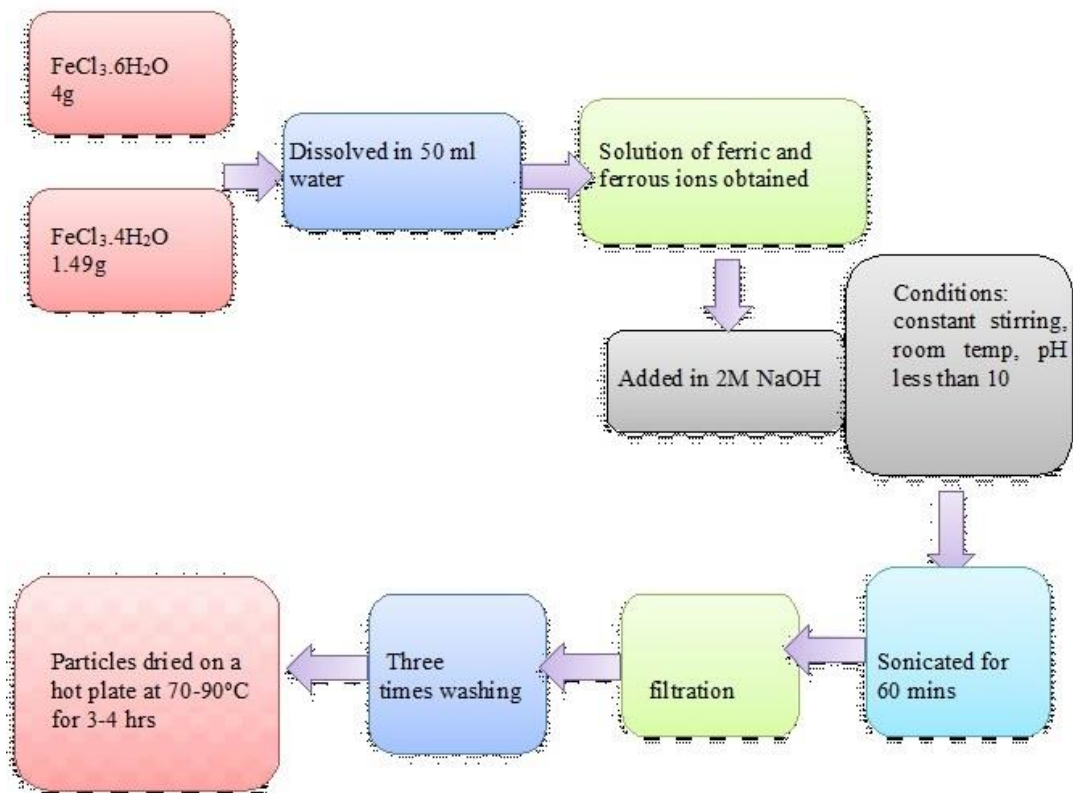


Figure 2: Preparation of iron oxide nanoparticles.

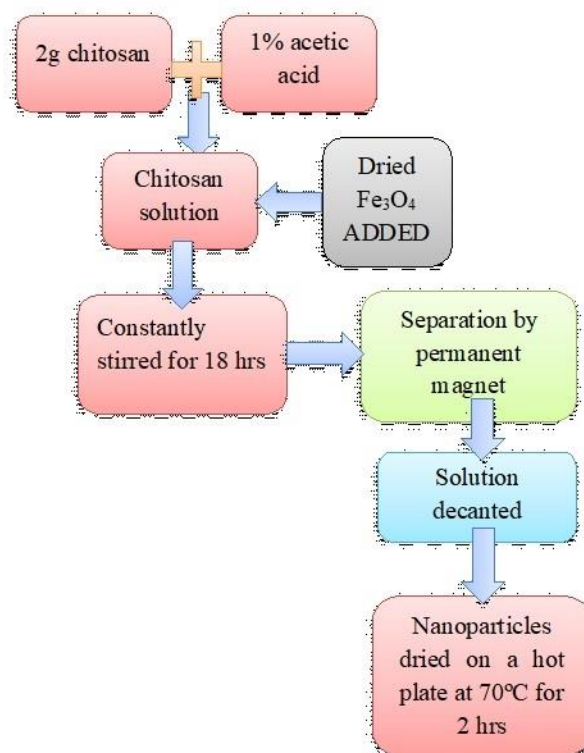


Figure 3: Preparation of chitosan coated MNP.

Preparation of Chitosan-Coated Magnetic Nanoparticles

2g of chitosan was added in a 1% acetic acid solution and dissolved [13]. Acetic acid solution 1% was prepared by adding 1ml acetic acid to 99 ml water. The dried Fe_3O_4 were mixed with chitosan solution in a beaker, and constantly stirred for 18 hours. The separation of MNP's coated with chitosan was done by placing a permanent magnet under the beaker, the MNP's settle down while the remaining solution is decanted. The separated MNP's were dried on a hot plate at a temperature of 70°C for 2 hrs [11].

Preparation of Drug-Chitosan-Magnetic Nanoparticles

Drug loading in chitosan- MNP's was performed by adding the drug solution (0.58g drug in 50 ml

deionized water) drop wise with constant stirring into the aqueous dispersion of MNP's. The aqueous dispersion was prepared by suspending formed MNP's in 50 ml deionized water. The drug solution containing the mixture of CS-MNP was stirred magnetically. The stirring was carried out at room temperature for duration of 18 hours, to assist uptake of drug. Separation of the final product which is drug coated with CS-MNP, was achieved by placing permanent magnet under the beaker. After decanting the top layer drug loaded MNP's were obtained and dried on a hot plate for 30 mins to one hour (Fig. 4 & 5).

For cefixime the solvent used was ethanol because it is insoluble in water. In the whole procedure, all solutions were prepared using deionized water [11].

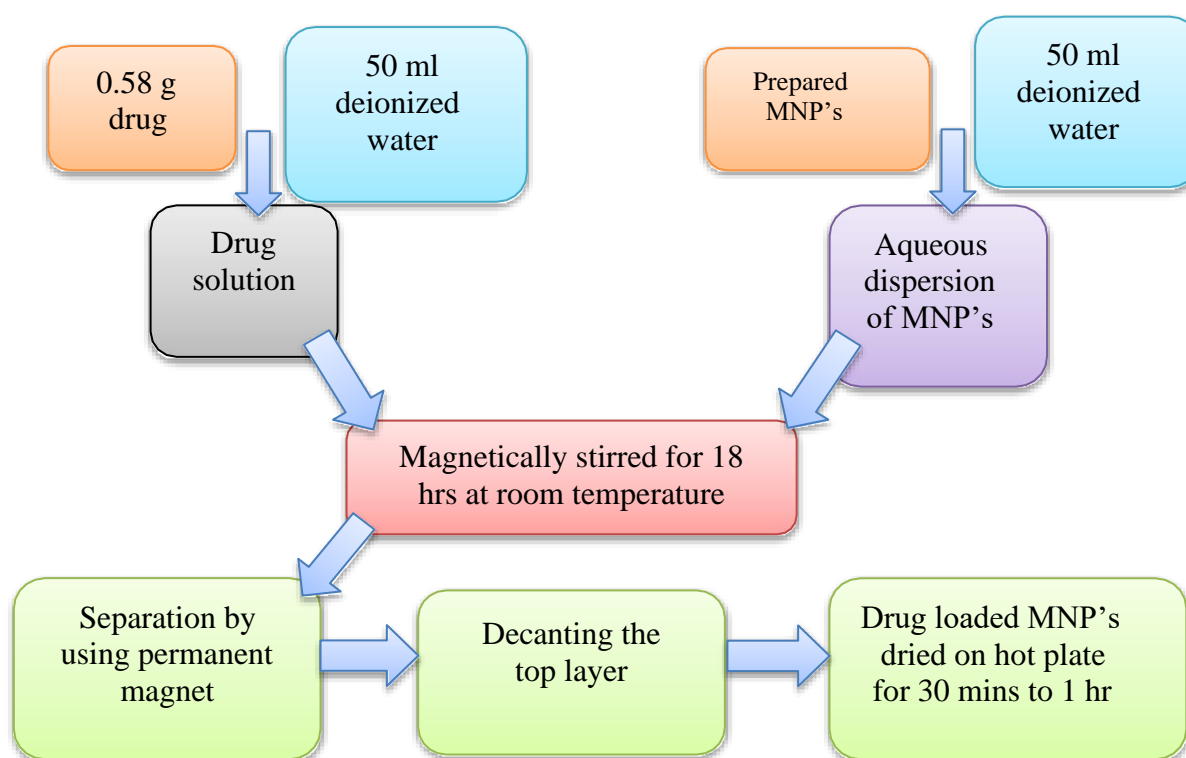


Figure 4: Flow chart for preparation of drug coated chitosan MNP.

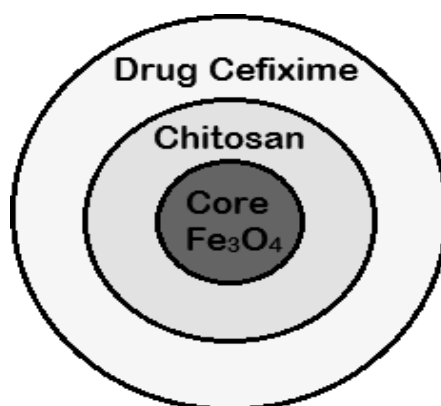


Figure 5: Conceptual image of drug loaded MNP.

Preparation of Phosphate Buffer Saline pH 7.4

0.2 M potassium monobasic phosphate (PMP) and 0.2 M NaOH solutions were required. To prepare 0.2M PMP solution, 2.72g PMP was dissolved in 100 ml DI water. Whereas to prepare 0.2 M NaOH 0.8g NaOH was dissolved in 100 ml DI water. Then 50 ml of PMP solution was taken and mixed with 39.1 ml NaOH solution. Then by adding DI water their volume was made up to 200 ml. The pH of final solution was adjusted using a pH meter. To decrease the pH, PMP solution was added drop wise and to increase the pH, NaOH solution was added if required [14, 15].

Physical Appearance of Magnetic Nanoparticles

The drug loaded, chitosan coated magnetic nanoparticles were evaluated with naked eye to check the appearance.

Magnetic Properties of Magnetic Nanoparticles

The magnetic properties of MNP's were analyzed by dispersing the MNP's in water inside a glass container. Then that glass container was placed in front of the magnetic field produced by a permanent magnet, to see how the magnetic nanoparticles behave [16].

Fourier Transform Infrared Spectroscopy

An (FT-IR) spectroscopy study was carried out with the help of nicolet 6700 Fourier transform infrared spectroscope. The transmission mode was used in the wavelength range of 4000-650. The gas used was nitrogen. The numbers of scans performed were 128 having a resolution of 8. The spectra obtained from the spectroscopic study are shown in the results [17].

Scanning Electron Microscopy

SEM of magnetic nanoparticles was performed using TESCAN Vega 3 LMU – Variable pressure Scanning Electron Microscope. This scanning electron microscope is capable of imaging and elemental characterization of metals, ceramics, polymers and biological specimens. Detectors used in this microscope include secondary electron, back scattered electron and energy dispersive detectors (EDX) [18].

Particle size analysis using zeta sizer

Particle size was analyzed by using Zeta sizer (Malvern Instruments, UK). Suspension of particles (to be analyzed) was prepared by using water that was filtered through a filter of 0.45 μm . Then this aqueous suspension was placed in clear disposable zeta cell. Evaluation was done by dynamic light scattering method. Particle size was measured by dynamic light scattering method and recorded [19].

Raman Spectroscopy

Molecular vibration, crystal structure, chemical composition of the prepared magnetic nanoparticles was determined by Raman spectroscopy with model in Via Raman Microscope by Renishaw UK [20].

For the preparation of 200 ml PBS, 0 **Antibacterial Assay**

Firstly, Muller Hilton agar was prepared by adding agar 23g in 750ml distilled water. Then the prepared agar media was poured in conical flask.

All the apparatus including petri plates, test tubes and prepared agar media were placed in autoclave for sterilization at 121°C for 90 mins. After 90 mins, temperature was reduced to 50 °C. All the apparatus was taken out.

All petri plates were placed under UV lamp. Then 20-30 ml agar media was poured in each petri plate and allowed to solidify for 20 mins.

Bacterial strains were refreshed by adding them in 5-10 ml sterilized water and allowed to stand for 30 mins. Streaking of refreshed bacterial strains was performed by dipping the cotton bud in refreshed bacterial culture. Then it was streaked out in both directions on petri plates containing solidified agar media.

Using a cork borer, a small hole was created in the agar media. Then our samples solutions were poured inside the wells. After 24 hrs of incubation at 37°C, the zones of inhibition were checked and measured [21].

RESULTS AND DISCUSSION

Physical Appearance of Magnetic Nanoparticles

The fabricated MNP's were physically evaluated with naked eye. They were small particles being black in color due to the presence of ferric chloride.

Magnetic Properties of Magnetic Nanoparticles

The magnetic properties of drug loaded iron oxide nanoparticles were determined by placing their solution in front of a permanent magnet. It was seen that all the magnetic nanoparticles stick to the walls of the glass container facing the permanent magnet.

Fourier Transform Infrared Spectroscopy

To evaluate structural properties, confirm presence of specific functional groups and assure chemical cross-linking within network structure, polymer (chitosan), active drug (cefixime), blank magnetic nanoparticles and drug-loaded magnetic nanoparticles of cefixime; physical mixture of all these were characterized by Fourier transform infrared spectroscopy by scanning them in range of 4000 to 500 cm^{-1} to obtain their respective spectra.

The spectrum of pure chitosan shows several characteristic peaks at 3280, 2865, 1616, 1541 and 1374 cm^{-1} . A peak at 3280 cm^{-1} corresponds to N-H and O-H stretching. The absorption band at 2865 cm^{-1} is attributed to C-H stretching vibrations. A band at 1616 cm^{-1} is due to the N-H bending vibrations. 1374 cm^{-1} is due to C-N stretching vibrations and 1004 cm^{-1} is due to C-O-C stretching vibrations

FTIR of CFM showed the following characteristic peaks: peak at 3259.75 cm^{-1} due to vibration of –OH group, peak at 1769.26 cm^{-1} due to vibration of –CO group. Both peaks at 1382.54 cm^{-1} and 1591

cm⁻¹ are due to bending of –CH₂ group. Peak at 1225.22 cm⁻¹ is due to bending of CH group.

FTIR spectrum of synthesized iron oxide nanoparticles show various well-defined peaks at 3724, 3261, 1617 and 1540 cm⁻¹. The peak at 3724 cm⁻¹ is present in all spectra of magnetic nanoparticles which shows that this peak is specific to MNP's. The peak positioned at 3261 cm⁻¹ is due to bending vibration of OH group. Two peaks at 1617 and 1540 cm⁻¹ are due to chitosan coating of iron oxide nanoparticles. Few smaller peaks between 500 to 600 cm⁻¹ are due to the presence of Fe-O, which confirms the synthesized iron oxide nanoparticles [22].

The peak carboxylic group appears as it is and on the same wavenumber 3257 cm⁻¹ but the alkane group is absent. The peak of C=O shifted to a slightly higher wave number from 1715.53 cm⁻¹ to 1767.82 cm⁻¹ which shows an increase in vibration of the bond. The wave number corresponding to C-N group is increased greatly from 1288.07 to 1337.91 cm⁻¹. The halogen group is present as it is. These functional groups confirm the presence of drug LFX in MNP's. FTIR of CFM MNP's shown in Fig. 7, shows a shift of peak from 3259 to 3724 cm⁻¹ which shows an increase in vibration of –OH group. A very slight decrease in wavenumber is observed in case of –CO group from 1769.26 to 1746 cm⁻¹ which is due to slight decrease in vibration of –CO group. The peak of –CH₂ group remains unchanged. Whereas the peak of –CH group is absent. These functional groups confirm the presence of CFM in MNP's [23].

Scanning Electron Microscopy

Surface properties of iron oxide MNP's and drug loaded chitosan magnetic nanoparticles were evaluated by scanning electron microscope. For this purpose, photographs of high resolution were taken at magnifications of 50kx, 25kx, 10kx and 5kx as depicted in Fig 8.

The SEM images of cefixime loaded MNP's show large, irregular and agglomerated particles. The effective drug loading can be seen by large white spots. The drug loaded magnetic nanoparticles show hollow and porous, smooth and wrinkled appearance [24].

Particle Size Analysis

Particle size of prepared magnetic nanoparticles were evaluated by dynamic light scattering method and recorded. The particle size of optimum formulation of magnetic nanoparticles is shown in Fig. 9. Average hydrodynamic diameter of magnetic nanoparticles was evaluated using Zetasizer (Malvern Instruments, UK) and size found to be 200 nm that is within the range of nanoparticle size (0- 200 nm).

Antibacterial Assay of Cefixime Magnetic Nanoparticles

Antibacterial assay of drug loaded MNP's and blank drug was performed by agar well diffusion

method. Their antimicrobial activity was determined against two types of bacteria: *Staphylococcus aureus* and *Escherichia coli*. The drug solutions were used in four different concentrations i.e. 500 µg/mL, 250 µg/mL, 125 µg/mL, 75 µg/mL. The diameter of the zones of inhibition was measured and results were interpreted.

The results show that cefixime alone has good antibacterial activity against *Staphylococcus aureus* and *E. coli*. Cefixime ZOI against *S. aureus* was 10 mm and *E. coli* was 12 mm. An increase in the ZOI of CFM MNP's was observed. The ZOI of CFM MNP's was 14 mm against *S. aureus* and 15 mm against *E. coli*.

This improved antibacterial activity of cefixime was due to the presence of chitosan and iron oxide in the nanoparticles. Both chitosan and iron oxide possess antibacterial activities of their own, thus enhancing the activity of cefixime. So, we can say that CFM MNP's possess better antibacterial activities than the drugs alone due to chitosan. It was also found out that in case of *E. coli* the positively charged surface of metal oxide NPs may bind to cell membrane which is negatively charged through electrostatic interactions which disrupt bacterial functions thus enhancing the antibacterial effects of iron oxide nanoparticles [25].

Raman Spectroscopy

The drug cefixime iron oxide nanoparticles and cefixime loaded iron oxide nanoparticles were characterized by Raman spectroscopy and various peaks were obtained by scanning them in the range of 200-4000 cm⁻¹.

Fig. 11 shows the nature of the iron oxide core determined by Raman spectroscopy. The irradiation of energy on the sample produces molecular effects which lead to Raman effect. The Raman spectrum peaks of magnetite were investigated where the peak at 707 cm⁻¹ was identified as band characteristic and is related to e.g. phonon modes whereas the peak at 339 cm⁻¹ is related to Al_g phonon mode.

Fig.11 shows that cephem nucleus in cefixime is characterized by a weak intensity peak at 804 cm⁻¹ which represents combined scissoring and deformation of the carboxyl group and beta-lactam ring. The medium intensity peak at 1785 cm⁻¹ represents the Raman spectral feature of the lactam ring indicating stretching vibrations of C=O. The scissoring vibration of methyl group appears at 1430 cm⁻¹. The wagging vibration of CH₂ is shown by the peak at 1281 cm⁻¹. In cefixime loaded iron oxide nanoparticles the peak at 1785 cm⁻¹ which indicated the lactam ring moved to higher wavenumber of 1813 cm⁻¹ showing an increase in stretching vibrations. The scissoring vibrations of methyl group also increased as the band shifted to 1524 cm⁻¹ in CFM MNP's. These peaks confirm the presence of drug cefixime in MNP's [26].

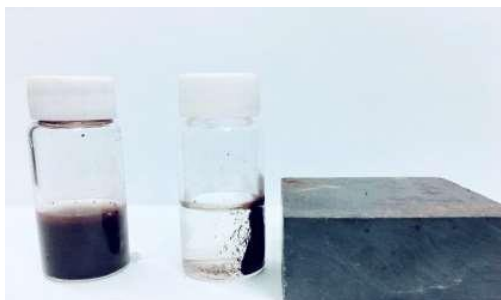


Figure 6: Magnetic properties of MNP.

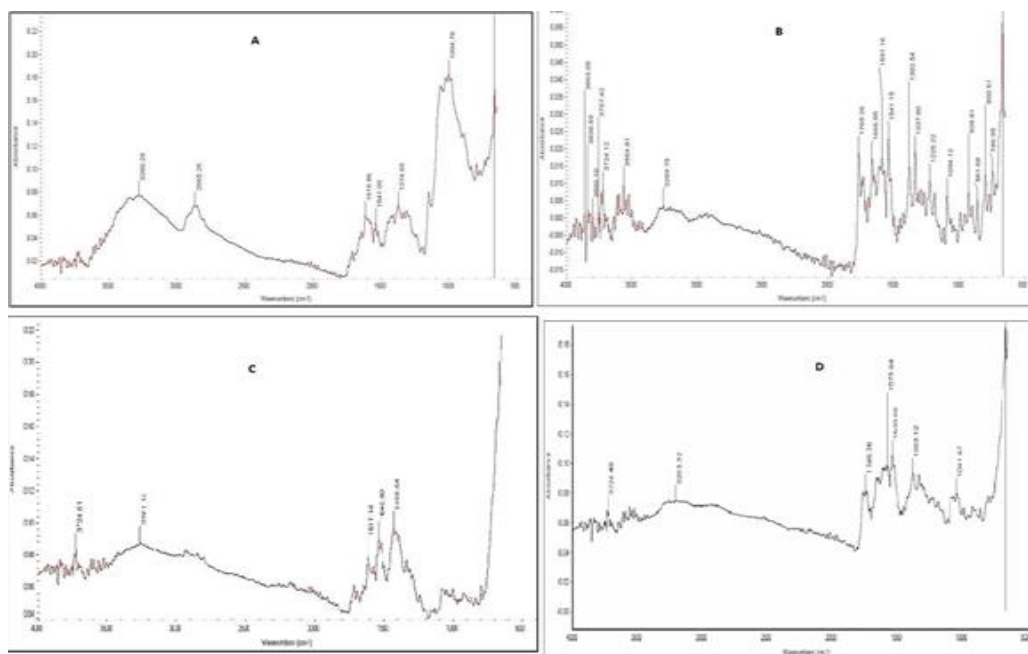


Figure 7: FTIR spectrum of CFM MNP.

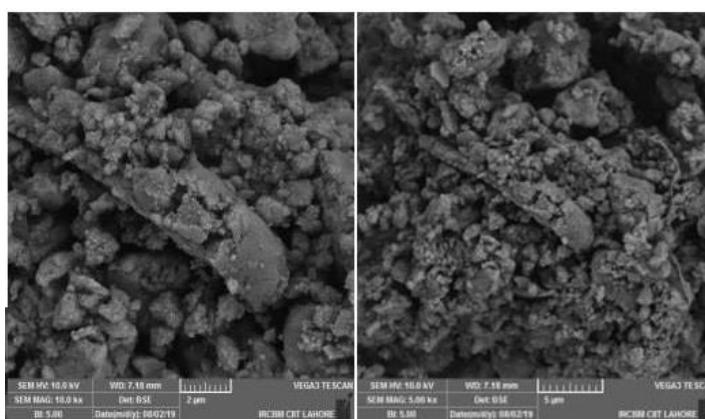


Figure 8: Scanning electron microscopy photographs of cefixime loaded MNP's at different magnifications.

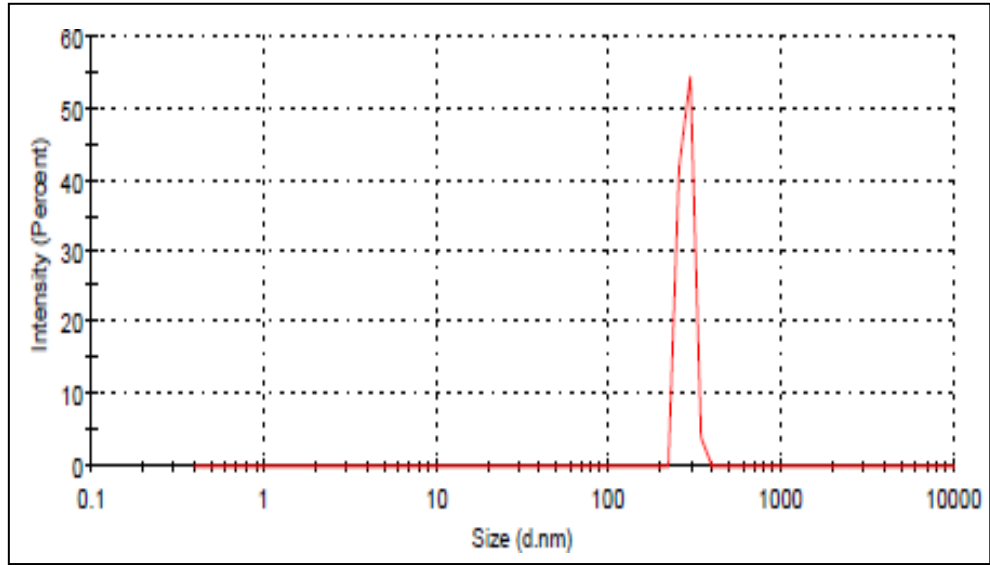


Figure 9: Particle size of magnetic nanoparticles.

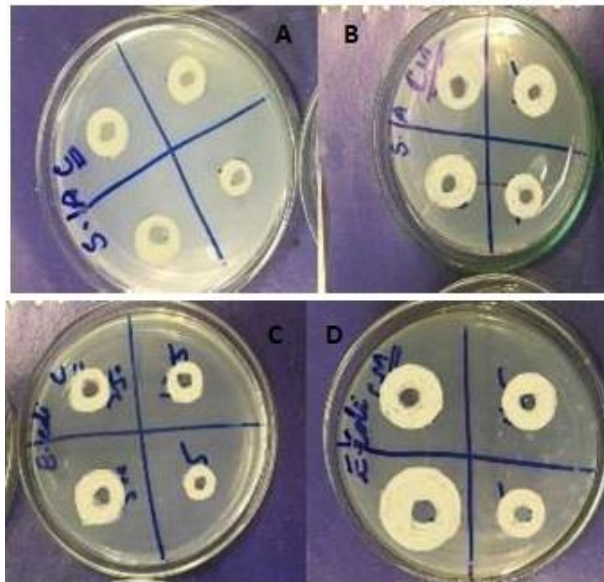


Figure 10: Antibacterial activity of (A) drug CFM (B) CFM MNP against *S. aureus* bacteria and *E. coli* (C, D).

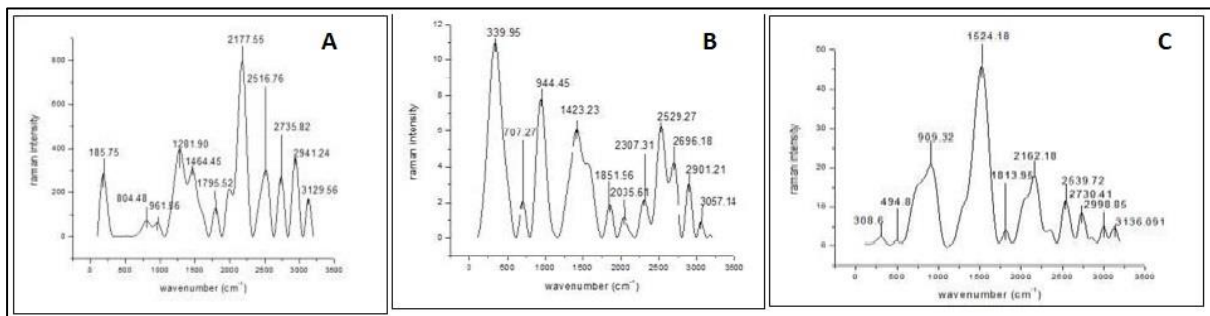


Figure 11: Raman spectra of (A) Drug cefixime (B) iron oxide nanoparticles (C) cefixime MNP.

CONCLUSION

Magnetic iron oxide nanoparticles have shown the excellent capacity for association of drug cefixime using the polymer chitosan. The objective of this research was to formulate magnetic iron oxide nanoparticles by co-precipitation method. Cefixime has antimicrobial drugs having activity against wide range of bacteria for example *Staphylococcus aureus* and *E. coli*. They are used for upper respiratory tract infections, urinary tract infections etc. The prepared

magnetic nanoparticles were checked for different parameters such as magnetic properties, particle size, FTIR, SEM, Raman spectroscopy, in- vitro release and antibacterial activity. Results of all parameters were satisfactory and confirmed that prepared formulation can be effectively used for loading of cefixime. The study showed that iron oxide nanoparticles can be used to enhance the antibacterial activity drug.

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