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Formulation and evaluation of chitosan coated magnetic nanoparticles of Amoxicillin trihydrate

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ABSTRACT

The present investigation is designed to formulate and evaluate chitosan coated Magnetic nanoparticles (MNPs) containing Amoxicillin trihydrate as a model drug for controlled drug delivery. MNPs were prepared by using chemical co-precipitation method and these uncoated MNPs were further coated by chitosan and Amoxicillin trihydrate. The prepared coated MNPs were then evaluated by various techniques such as FT-IR, TGA and XRD. FTIR spectra depicted that chitosan and Amoxicillin trihydrate (AMT) were successfully coated on to iron oxide MNPs by electrostatic interaction. Thermogravimetric analysis (TGA) of the formulation established that MNPs improved the thermal stability of the drug. XRD studies revealed that the Iron oxide MNPs were having inverse spinal structure. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) photomicrographs represented that the uncoated MNPs were rhombohedral in shape and had the average diameter of 35-40nm which after coating increased to 40-45nm. The in vitro drug release study showed that MNPs have sustained release profile of drug delivery with burst release of AMT in first 2 hours followed by sustained release. Antimicrobial activity of the formulation was confirmed by agar plate diffusion method and Minimal inhibitory Concentration was assessed by using tube dilution method against suitable microbial strains.

Keywords: Amoxicillin trihydrate, Antimicrobial activity, Chitosan, Magnetic nanoparticles

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INTRODUCTION

Nanoscience is considered as the most vital research area in modern science which includes nanoparticles, nanotubes, nanocolloids, nanocrystals, nanofilms etc. Nanoparticles are offering foremost advantages owing to its small size, greater surface areas, drug delivery characteristics and atypical electronic, magnetic and optical properties [1,2,3,4]. Magnetic nanoparticles is one among these nanoparticles,having size less than 100 nm and they are made to move under the influence of externally applied magnetic field. In MNPs the core elements are magnetic elements such as Iron (Fe), nickel (Ni), cobalt (Co), manganese (Mn), etc and their derivatives. With the advancement in the field of nanobiotechnology, magnetic nanoparticles have achieved escalating consideration in various biomedical applications such as MRI, virus detection, Magnetic cell separation, enzyme catalysis, gene therapy, targeting chemotheraphy and radiotherapy [5]. MNPs can be synthesized by various techniques such as co-precipitation, condensation methods, microemulsion or reverse micelles synthesis, thermal decomposition, laser pyrolysis, Annealing etc but among all these co-precipitation is the oldest simplistic facile and expedient method and it doesn't require much special facilities for production [6,7]. However MNPs are intrinsically unstable and get demagnetized because of corrosion which poses major limitation in their use. Various strategies are employed to overcome these problems such as encapsulating the MNPs with biocompatible polymers to prevent certain changes that may occur when they get exposed to biological system [8,9,10]. Many MNPs are pharmacologically inert and have no considerable LD50 and as soon as they get access to the blood stream, plasma proteins coat them by a process named opsonization [11,12,13]. Reticuloendothelial system and macrophages recognizes these opsonised particles and try to remove these particles by phagocytosis. These particles can be made unseen to RES by altering

the nanoparticles surface for enhancing the circulation time [14]. Polymer Coated Nanoparticles allow binding of the drug by entrapment on the particles, covalent or adsorbent attachment and enhance compatibility with ingredients, reduce propensity to leaching, and protect particle exterior from oxidation. However, functionalized nanoparticle can be used as adjunct to magnetic targeting that could further augment the target specificity. Toxicity and biocompatibility of MNPs can be determined by various factors such as nature of the magnetic component (i.e iron, cobalt, magnetite and nickel etc), final particle size, coating and their core. Iron oxide NPs are considered as suitable for biomedical applications whereas cobalt and nickel are toxic and more prone to oxidation and therefore they are of less importance [15].

Based on these observations present investigation was designed to fabricate magnetic nanoparticles stabilized by chitosan coating containing suitable model drug (amoxicillin trihydrate) and to evaluate them in terms of particle size, zeta potential, *in vitro* drug release study etc.

MATERIALS AND METHODS

Materials

Amoxicillin trihydrate (AMT) was generously gifted by Indchemie Health Specialities Pvt. Ltd., Solan (HP, India). Iron (II) sulfate heptahydrate ($FeSo_4$.7H2O, 99%) and iron (III) chloride hexahydrate ($FeCl_3.6H_2O$, 99%) and epichlorhydrin were obtained from HPLC. Pvt. Ltd., Mumbai,India. Chitosan pure polymer (low molecular weight, deacetylation 75%–85%) was procured from Sisco research lab Pvt. Ltd., India. Ammonium hydroxide sol. (30%w/v) and acetic acid glacial (99.8%) were obtained from C.D.H. Pvt. Ltd.,India. All the chemicals used in study were of suitable analytical grade.

Methods

Synthesis of Magnetic Nanoparticles:

The method employed for the preparation of Magnetic nanoparticles was Chemical Co-precipitation [16]. Accurately weighed amount of $FeSo_4.7H_2O$ and $FeCl_3.6H_2O$ were dissolved in distilled water with continuous stirring under N_2 for 1 hour at 80^oC. After that, 30% w/v Ammonium Hydroxide solution was introduced into the solution rapidly and stirring was further continued for 1 hour in N_2 atmosphere. Refluxing nitrogen was necessary to prevent oxidation. Resultant mixture was then placed in hot air oven for next 5 hours at 60^0C and then cooled down to room temperature. The precipitate particles were washed with hot water and magnetic nanoparticles were separated by using permanent magnet as shown in Fig. 1.

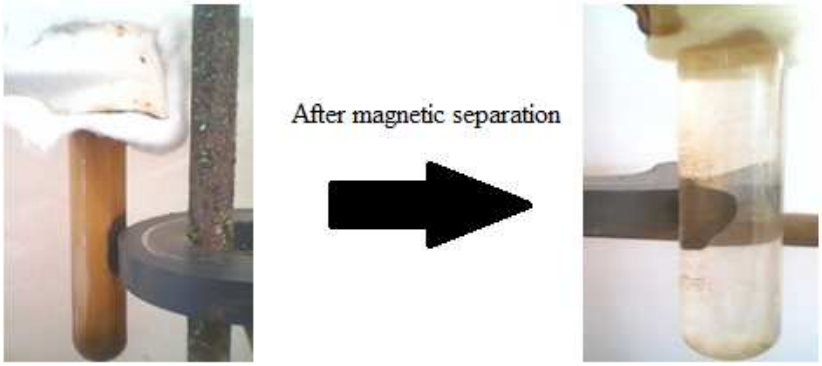

Fig. 1 Separation of MNPs by permanent magnet

Coating of Magnetic Nanoparticles with Chitosan & Drug

Preparation of Chitosan Solution:

For preparing chitosan solution, acetic acid(0.5 ml) was made up to 100 ml with distilled water and chitosan(1 gm) was added to the solution followed by dropwise addition of 10 ml epichlorhydrin (as a cross linker) under vigorous mechanical stirring for 3 hours [17].

Coating Procedure:

Weighed amount of dried magnetic nanoparticles were dispersed in distilled water and mixed with above prepared chitosan solution (1%) and subsequently with 25 ml of Amoxicillin solution. The mixture was then stirred for 24 hours [18], small amount of ammonium hydroxide was added to make the mixture basic again. Chitosan was coated on to the surface of nanoparticles by electrostatic interaction and stirring was necessary for uniform deposition of chitosan. The black precipitate was collected by permanent magnet and lyophilized and then freeze dried product was stored in desiccator.

Drug Loading:
Suitable quantity of drug loaded nanoparticles were first ground into powder form, out of which precisely weighed 10 mg were taken and placed into a 150 ml conical flask having 100 ml distilled water. The suspension was shaked for 24 hours on mechanical shaker and then filtered through a 0.2 µm filter (Minisart syringe filter). Concentration of the drug was determined by using UV-visible Spectrophotometer. Drug loading capability was represented in form of Entrapment Efficiency (EE %) and calculated as below:

$$EE\% = \frac{\text{Amount of loaded amoxicillin in nanoparticle}}{\text{Amount of drug used in formulation}} x \ 100$$

***In-Vitro* Drug Release Study:** Dissolution studies for Amoxicillin loaded MNPs was carried out using a 6-station Dissolution Rate Test Apparatus with a rotating paddle stirrer at 50 rpm and 37±0.5^{0}C. Dissolution media consisted of 900 ml N/10 HCl solution (pH 1.2) and phosphate buffer of pH 7.4 [16] (pH 1.2 and 7.4 for simulation of stomach and intestine pH environment, respectively) A sample of MNPs equivalent to 5 mg of Amoxicillin was used in each test. At predetermined time intervals, 5 ml of samples were withdrawn from the external buffer solution and were replaced with fresh buffer solution. The released amount of Amoxicillin in the samples was analyzed at 230 nm and 273 nm respectively using UV-Visible Spectrophotometric method.

Infra-Red (IR) Spectra analysis: To study the interaction between Amoxicillin and components of MNPs as well as the polymer chitosan IR spectroscopy has been utilized. IR spectra of pure Amoxicillin, uncoated iron oxide MNPs, iron oxide MNPs coated with Chitosan and drug loaded iron oxide MNPs were obtained by KBr Pellet method using Infra-red spectrophotometer (Perkin Elmer).

Morphological Study of MNPs:
a) Scanning Electron Microscopy:
Surface morphology of the MNPs was observed by Scanning Electron Microscopy (SEM). SEM was performed by placing the lyophilized sample of MNPs on a double stick tape above aluminium stubs in order to have even level of particles. After that samples were gold coated by means of sputter gold coater and cooled over liquid nitrogen for avoiding melting of sample when they get exposed to electron beam.

b) Transmission Electron Microscopy(TEM):
The mean particle size, size distribution, shape and uniformity of the particles of the samples were obtained by High Resolution Transmission Electron Microscopy (HRTEM). For performing TEM, suspension of MNPs in aqueous solution was prepared and a drop was put onto a copper grid having carbon film coating over it and kept for drying. TEM of uncoated iron oxide MNPs and AMT loaded MNPs was performed at an accelerating voltage of 120 kV.

Particle size and Zeta Potential: The size of nanoparticles was determined by particle size analyzer using dynamic light scattering technique. Zeta potential (ζ) an indicator of surface charge, is an electric potential at the shear surface of double layer of MNPs which determines particles stability in dispersion. Particle size & Zeta potential of the formulation was measured with Zetasizer (Malvarn ZS90) based on the principle of electrophoretic mobility under an electric field.

X-Ray Diffraction Characterization: Powder X-ray Diffraction patterns were employed for determining the crystal structure of the samples in the range of 25-70 degrees on XRD Diffractometer using CuK_α radiation. The XRD patterns of pure drug, uncoated iron oxide MNPs, iron oxide MNPs coated with Chitosan and drug loaded iron oxide MNPs were obtained.

Thermogravimetric Analysis: Thermogravimetric analysis is one of the important techniques used to determine complex formation and evaluation of physicochemical properties of compound by percentage weight loss during heating. Thermogravimetric analysis of pure Amoxicillin, uncoated iron oxide MNPs, chitosan coated iron oxide MNPs, pure chitosan and AMT loaded chitosan coated iron oxide nanoparticles was performed by TGA/SDTA 851 Mettler Toledo instrument. The sample was heated in platinum pan at 15^{0}C/min from 0 to 900^{0}C in atmospheric conditions. Percentage weight loss with change in temperature was recorded for all the samples.

Antibacterial Activity of the Formulation:
The antimicrobial assay of Chitosan coated MNPs of AMT was performed using agar plate diffusion method [19]. This method is dependent on antibiotic diffusion in a solidified agar layer from a vertical cylinder in a Petri dish or plate so that growth of the microorganism added is prevented completely in a zone surrounding the cylinder containing antibiotic solution.

Procedure: 2.8 gms of nutrient broth was poured into 100 ml of distilled water, the media was boiled and properly mixed for avoiding any lump formation. Further, the media was autoclaved at 121^0 C for 15 minutes. Four Petri plates were prepared and inoculated with four different bacterial strains (*Bacillus subtilis* (MTCC 16), *Staphylococcus aureus* (MTCC 3160) (Gram positive) and *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa* (Gram Negative) under laminar air flow for avoiding contamination. After inoculation followed by solidification, three cavities were built into it. In all the plates three cavities were marked as 0, 1, and 2. Cavities named '0' were kept blank,'1' were filled with drug's standard solution (10 µg/ml) and '2' marked cavities were filled with chitosan coated MNPs of AMT (equivalent to 10 µg/ml). Petri plates were then placed in BOD incubator for 24 hours at 37± 0.5^0C. After incubation the zone of inhibition (ZOI) for Chitosan coated MNPs of AMT and standard solution of antibiotic were measured.

Minimal Inhibitory Conc. of Formulation: The Minimal Inhibitory Concentration (MIC) of the formulation is determined by tube dilution (turbidimetric) method [20] which is based on impeding the augmentation of a microbial culture in a uniform antibiotic solution in a medium that favours its rapid growth in the absence of the antibiotic. The MIC is the smallest amount of an antimicrobial agent that prevents the growth of the organism. MIC value was calculated for formulation using Amoxicillin trihydrate as the standard drug for antibacterial activity.

Method

Preparation of standard and test solution: Test compounds and standard compound (Amoxicillin trihydrate) were dissolved in dimethylsulfoxide to give a concentration of 10 mg/ml.

Preparation of double strength nutrient media: Nutrient broth was suspended in 1000 ml of distilled water, boiled to dissolve and sterilization of the media was done by autoclaving at 121^0C for 15 minutes.

Preparation of suspension of micro-organisms: Microorganism Suspension was prepared by relocating the organism from culture to 10 ml of sterile normal saline solution.

Determining of Minimal Inhibitory Concentration (MIC): Sterilized media (1 ml) was transferred into sterilized test tubes. 1 ml of 0.1 µg/ml test solution was poured in one tube and successively dilutions were made to give a concentration of 50, 25, 12.5, 6.25, 3.125 and 1.562 µg/ml. 0.1 ml bacteria suspension was inoculated into all these test tubes and they were incubated at 37±0.5^0C for 24 hours. Visual inspection of the tubes was done for the growth of bacteria that is indicated by turbidity. MIC was evaluated by the least concentration of the sample that stopped the evolution of turbidity. The activity was compared with standard (Amoxicillin trihydrate).

RESULTS AND DISCUSSION

Drug Entrapment Efficiency:

Amount of drug entrapped in chitosan coated MNPs was found to be dependent on composition of polymer. Amoxicillin trihydrate was physically adsorbed on the chitosan coated MNPs. About 37.54 % of the incubated drug was loaded into the chitosan coated MNPs.

% EE = 0.0627/1.667x 100= 37.54%

***In-vitro* drug Release from MNPs:** The release profile for AMT from MNPs was studied for 8 hours in pH 7.4 phosphate buffer solution and pH 1.2 hydrochloric acidic solution. The % of AMT release from MNPs is shown in Fig. 2. It is evident that the AMT release from MNPs is dependent on the pH, with the release rate at alkaline pH 7.4 is lower than that at acidic pH 1.2. The cumulative percentage release of the drug from the formulation was found to be 40.24% and 70.42% at pH 7.4 and pH 1.2 respectively. Burst release of AMT was observed in both pH medium for the initial 2 h which is due to the release of free drug present on the polymer surface along with physically adsorbed drug. About 51% of AMT entrapped in the coated MNPs was released in 4 hours in the pH 1.2 medium, while in phosphate buffer solution at pH 7.4 not more than 33%.

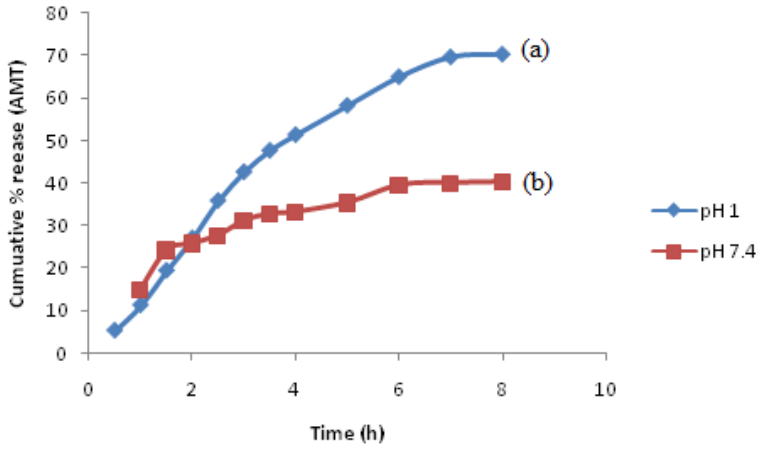

Fig. 2 AMT release curves from chitosan coated MNPs in different medium: pH 1.2 hydrochloric acid and pH 7.4 phosphate buffer

Release kinetics of AMT from loaded MNPs: For investigating the release kinetic behavior of AMT from the MNPs, zero order, first order, Higuchi model and Korsemeyer Peppas model were considered. After studying these four models it was found that the Higuchi model [21] describes the AMT release behavior better than the other models. The values of R^2 (0.982 & 0.926) shown in Table 1 indicate that Higuchi equation is the best fit mathematical model for AMT release in both medium, suggesting that the drug is released from polymeric matrix (insoluble in the solvent) by diffusion, the drug extraction from the matrix could result in sharp interface. The drug was leached out in a section between the interface and the solvent by Fickian's diffusion with linear gradient and drug was in a 'pseudo steady-state'. *Higuchi's square-root Eqn*:

$M_t/M_\infty = kt^{1/2}$

Here M_t and M_∞ are respectively the accumulative and the maximal amounts of drug released, *t*, the time, *k* is constants. Fig. 3 representing the release kinetics of formulation by Higuchi model in N/10 HCl sol. (pH 1.2) & Phosphate buffer sol. (pH 7.4).

Table 1 Correlation coefficient (R^2) and slope obtained by fitting the data of the release of AMT from AMT loaded chitosan coated MNPs in N/10 HCl (pH 1.2) & Phosphate buffer (pH 7.4)

Sr. No.	Model	N/10 HCl		Phosphate Buffer (pH 7.4)	
		Slope	R^2	Slope	R^2
1.	Zero order	8.982	0.924	0.052	0.852
2.	First order	0.123	0.718	0.0038	0.731
3.	Higuchi equation	4.355	0.982	1.639	0.926
4.	Korsemeyer Peppas equation	0.951	0.965	0.424	0.914

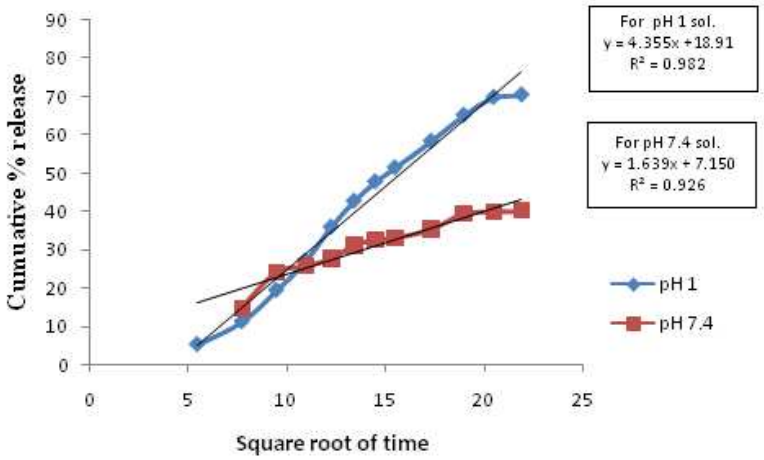

Fig. 3 Release kinetics of formulation by Higuchi model in N/10 HCl sol. (pH 1.2) and Phosphate buffer sol. (pH 7.4)

IR Spectroscopy:

Fig. 4 represents the FTIR Spectra of uncoated iron oxide MNPs (a), Chitosan coated iron oxide MNPs (b), AMT loaded iron oxide MNPs(c). The peak at 3430 cm^{-1} was appeared in all the three FTIR spectra which correspond to -OH .In all the spectra at about 570 and 616 cm^{-1} the characteristic peak for Fe_3O_4 was appeared which relates to the Fe-O bond [22]. Spectra (b) represent the characteristic peak at 1636 cm-1 which relates to N-H bending vibration and can also indicates that chitosan react with glutaraldehyde to form Schiff base . Absorption band at 1414 cm^{-1} relates to –C-O stretching of the primary alcoholic group. On comparing the spectra of Fe_3O_4 coated with chitosan with AMT loaded chitosan coated iron oxide MNPs, a shift in band from the 1636 cm^{-1} for N-H bending vibration to 1625 cm^{-1} was seen and 617 cm^{-1} peak indicated Fe-O bond. A sharp peak is observed at 2362 corresponding to C-H stretching. Above observations signifies that chitosan and AMT were coated on the Fe_3O_4 MNPs successfully. As iron oxide carries the –ve charge on its surface therefore it has an affinity for chitosan to protonate it. Therefore, the protonated chitosan could be coated onto the MNPs by electrostatic interaction and chemical reaction occurs by glutaraldehyde cross-linking.

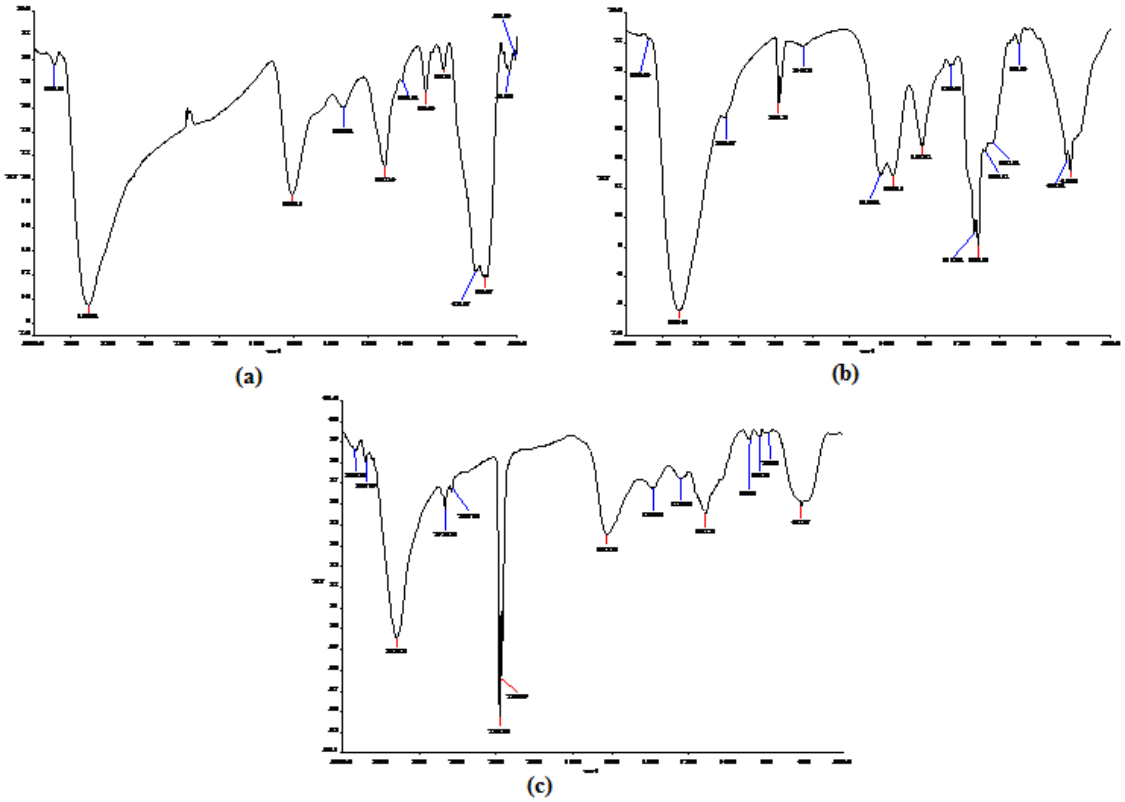

Fig. 4 FTIR Spectra of uncoated iron oxide MNPs (a), Chitosan coated iron oxide MNPs (b), and AMT loaded iron oxide MNPs(c)

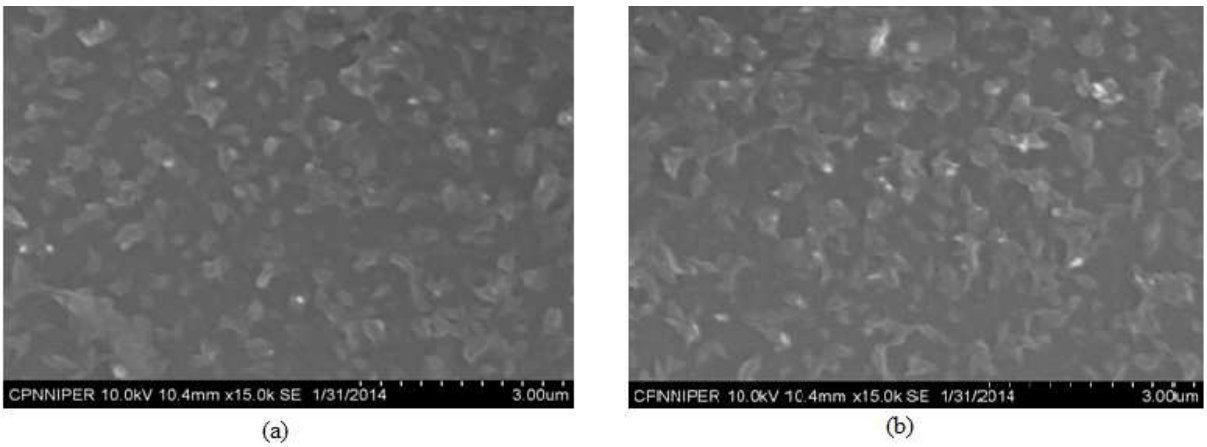

Fig. 5 SEM micrograph of (a) Uncoated Iron oxide MNPs and (b) AMT loaded chitosan coated MNPs

Morphological Study (Scanning Electron Microscopy): SEM was used for observing the surface morphology of the synthesized uncoated and drug loaded chitosan coated MNPs. Fig. 5 (a) and (b) show typical images of uncoated Iron oxide and coated MNPs respectively. Micrograph demonstrates that the Iron oxide MNPs had a rhombohedral shape, and were changed into an agglomerated structure without having any definite shape.

Determination of Mean Particle Size and Size Distribution Properties by Transmission Electron Microscopy & Particles Size Analyzer:

TEM was performed for determining the particle size, shape and particles uniformity of the synthesized uncoated and drug loaded chitosan coated MNPs under optimal conditions. The coated as well as uncoated MNPs appeared to be in nanosize range. The TEM image of uncoated iron oxide MNP (Fig. 6a), established that the MNPs were monodispersed and had a very small size around 35-40 nm in diameter. The image of chitosan coated MNPs (Fig. 6b) manifested that the chitosan has successfully coated the MNPs structure which was also indicated by slight increase in particle size of coated MNPs to 40-45 nm. The TEM imaging also indicated that coated MNPs are uniformly dispersed and agglomeration is not affected by coating process. Although, the aggregative phenomenon has been observed in the AMT loaded chitosan MNPs which requires further optimization of process.

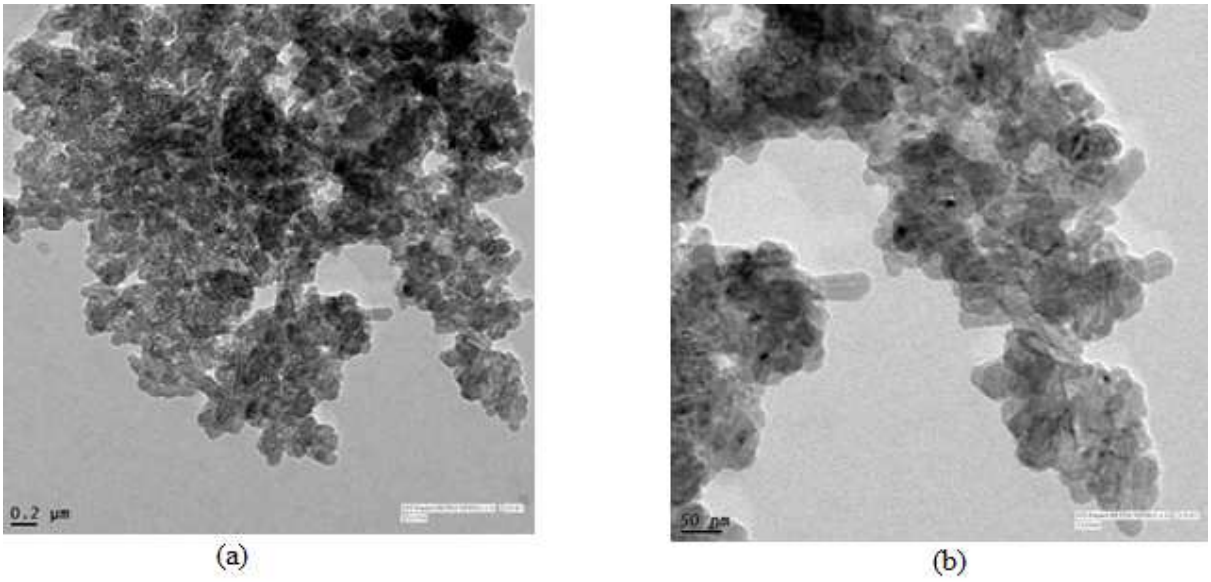

Fig. 6 TEM Micrograph of (a) uncoated Iron oxide MNPs with 0.2 µm bar and (b) AMT loaded chitosan coated iron oxide MNPs with 50 nm bar

Distribution pattern of the MNPs was further confirmed by particle size analysis studies. The average particle size (hydrodynamic diameter) was observed to be 183.7±11.22 nm and 230.6±15.95 nm for uncoated iron oxide MNPs and AMT loaded chitosan coated MNPs, respectively, as shown in Fig. 7(a) and 7(b). As the average particle size of the uncoated and AMT loaded iron oxide MNPs obtained by PSA technique was higher than reported by TEM, this might had occurred owing to hydration [23] of MNPs in distilled water during PSA studies followed by agglomeration of particles.

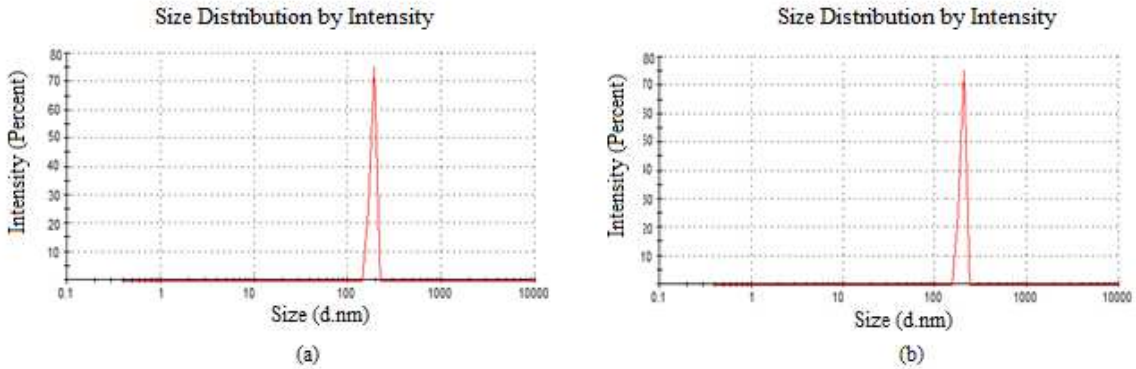

Fig. 7 Particles size distribution of (a) uncoated iron oxide MNPs and (b) AMT loaded chitosan coated MNPs by Particle Size Analyzer

Zeta Potential Study:
Zeta potential of the formulation is dependent upon the concentration of electrolytes in soluble medium. Zeta potential of uncoated iron oxide MNPs was about +19.5±0.78 mV due to the presence of positively charged Fe^{2+} and Fe^{3+} ions and their precipitates. Zeta potential of the chitosan coated MNPs was about -27.3±4.02 mV as negatively charged chitosan polymer made a coating layer around the core magnetic particles and the zeta potential of the final formulation containing AMT loaded chitosan coated MNPs was about -22.1±5.67 mV as AMT was physically adsorbed on the chitosan layer ,therefore no significant change in the zeta potential of the final formulation was recorded. The zeta potential of particular MNPs should be higher than a critical value as if the potential is lower, MNPs may get precipitate or aggregate.

XRD Study: The XRD patterns for the uncoated, AMT loaded MNPs and pure Amoxicillin trihydrate drug are shown in Fig. 8(a), 8(b) and 8(c) respectively. In sample (a) and (b), six characteristic peaks of Fe_3O_4 were observed at 2θ = 30.7°, 35.6°, 43.5°, 52.4°, 58.3°, and 64.9°, respectively named as (220), (311), (400), (422), (511) and (440). It also reveals that the resulting nanoparticles were pure Fe_3O_4 and having inverse-spinel structure (21). XRD diffraction pattern present the broad nature of the diffraction bands which indicates that Fe_3O_4 have small particle sizes.

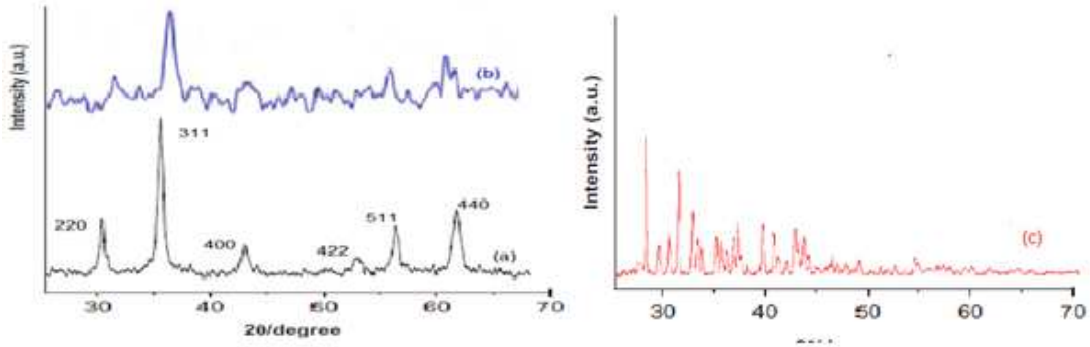

Fig. 8 X-ray diffraction patterns for (a) uncoated iron oxide MNPs, (b) AMT loaded chitosan coated MNPs and (c) pure amoxicillin trihydrate

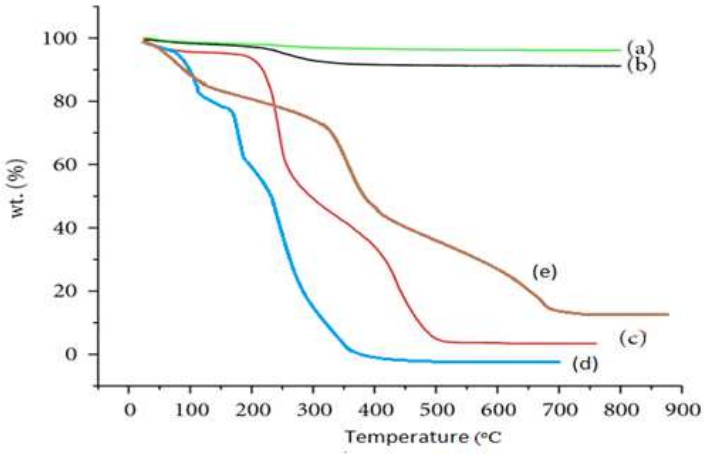

Fig. 9 Thermal degradation curves of nanoparticles prepared by co-precipitation method: (a) uncoated iron oxide MNPs (b) chitosan coated iron oxide MNPs, (c) pure chitosan, (d) pure AMT (e) AMT loaded chitosan coated iron oxide MNPs

Thermogravimetric study: The TGA curve for uncoated iron oxide MNPs in Fig. 9(a) shows a straight line without any dipping indicating that the sample is pure inorganic salt and not having any free and chemically adsorbed water molecules. The TGA curve for only chitosan coated MNPs in Fig. 9(b) shows a minor drop of curve at 270^0C, which might have taken place due to elimination of water physically or chemically bound to chitosan. The TGA curve for pure chitosan in Fig. 9(c) affirm the elimination of water at 270^0C and also shows second stage at 450^0C where weight loss occurred due to decomposition of chitosan molecule. The thermogravimetric analysis curve for pure AMT Fig. 9(d) shows the first drop of curve at 84.27^0C asserting the water molecules removal from Amoxicillin trihydrate. After reaching at 182^0C the drug molecules attains sufficient energy for moving in an ordered agreement for crystallization and immediately melting of the drug molecules occurs at 194^0C and it is followed by molecules degradation. The TGA curve for AMT loaded chitosan coated MNPs in Fig. 9(e) shows that the curved shape of the AMT curve is transformed because of coating. The 1st weight loss occurred around 73^0C

corresponding to the minimal loss of free and chemically adsorbed water. At 310^0C commencement of degradation of iron oxide and dehydroxylation AMT occurred which is greater than the decomposition temperature of pure AMT. Slow mass reduction was observed which may be attributed to decomposition of chitosan at 630^0C. The curve also indicated that no complex formation took place between drug molecules and polymer but they bind due to the electrostatic interactions.

Antibacterial Activity of Formulation: Agar plate diffusion method established the antibacterial activity of the MNPs containing AMT as shown in Fig. 10 and 11, depicting the antibacterial activity of the formulation against gram +ve and –ve bacterial strains respectively. Data obtained by measuring the ZOI is presented in Table 2 for formulation and standard drug. The presence of well defined boundary in all cavities affirmed the antibacterial activity of the AMT loaded MNPs.

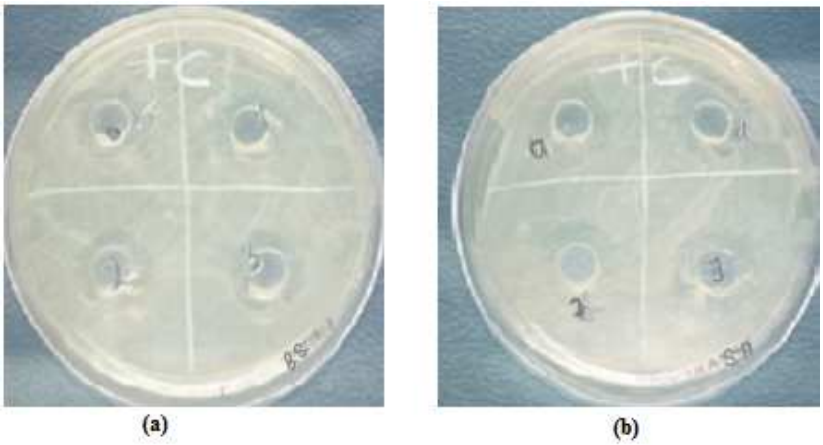

Fig. 10 The pictures depicting zone of inhibition of standard solution of AMT (10 µg/mL in 0 and 1 labeled cavities) and AMT loaded chitosan coated MNPs (10 µg/mL in 2 and 3 labeled cavities) against gram + bacteria (a) *Bacillus subtilis* (b) *Staphylococcus aureus*

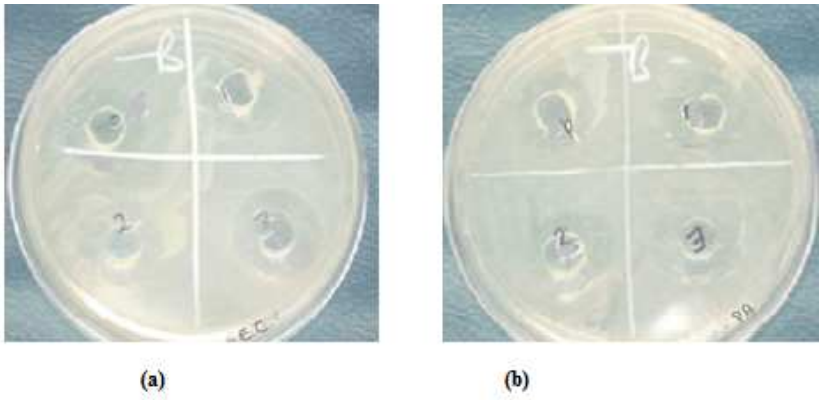

Fig. 11 The pictures depicting zone of inhibition of standard solution of AMT (10 µg/mL in 0 and 1 labeled cavities) and AMT loaded chitosan coated MNPs (10 µg/mL in 2 and 3 labeled cavities) against gram –ve bacteria (a) *Escherichia coli*, (b) *Psedomonas aeruginosa*

Table 2 Zone of Inhibition for prepared MNPs vs standard drug solution

S.No.	Bacterial stain	Zone of inhibition (cm ± S.D.) for MNPs	Zone of inhibition (cm ± S.D.) for standard drog
1.	*Bacillus subtilis* (MTCC 16)	2.7±0.20	2.67±0.20
2.	*Staphylococcus aureus* (MTCC 3160)	2.65±0.20	2.62±.20
3.	*Escherichia coli* (MTCC 40)	2.72±0.20	2.73±0.20
4.	*Psedomonas aeruginosa*	2.68±0.20	2.57±.20

Minimal Inhibitory Concentration: The Minimal Inhibitory Concentration (MIC) of the formulation is determined by tube dilution (turbidimetric) method and the results are shown in Table 3. Antimicrobial activity was observed at higher concentration for all the bacterial strains. Amoxicillin trihydrate is effective against all strains with 1.562 to 3.125 µg/ml concentration. As above table shows that formulation has a significant antibacterial activity against tested strains. Formulation was effective against *Bacillus subtilis* and *Escherichia coli with 6.25 µg/ml* (MIC) and

against *Staphylococcus aureus with 3.125 µg/ml* (MIC). As a result all the formulations were found to have antimicrobial activity against all the tested microbial strains.

Table 3 Antibacterial activity of formulation and standard drug (-) no activity, (+) antibacterial activity

Sr. No.	Conc. (µg/ml)	*Bacillus subtilis* (MTCC 16)		*Staphylococcus aureus* (MTCC 3160)		*Escherichia coli* (MTCC 40)	
		Formulation	*Std. drug*	*Formulation*	*Std. drug*	*Formulation*	*Std. drug*
1.	50	- -	- -	- -	- -	- -	- -
2.	25	- -	- -	- -	- -	- -	- -
3.	12.5	- -	- -	- -	- -	- -	- -
4.	6.25	- -	- -	- +	- -	- -	- -
5.	3.125	- +	- -	+ +	- -	- +	- -
6.	1.562	+ +	- +	+ +	- +	+ +	- +

CONCLUSION

Amoxicillin loaded chitosan coated MNPs were successfully formulated by co-precipitation technique and characterized by various techniques. FTIR spectra represented that chitosan and Amoxicillin trihydrate were successfully coated on to iron oxide MNPs by electrostatic interaction & by chemical reaction through glutaraldehyde and Amoxicillin trihydrate was loaded by physical adsorption. Thermogravimetric analysis demonstrated that MNPs augmented the thermal stability of the drug and no extra peak appeared in the graph representing that there was no complex formation. Morphological study of formulation by SEM showed that the iron oxide MNPs had a rhombohedral shape that was changed into an agglomerated structure lacking any definite shape and TEM showed that average size of uncoated MNPs was about 35-40 nm which increased to 40-45 nm after coating. The XRD studies established that the Iron oxide MNPs were inverse spinal structure. Percentage entrapment efficiency of the formulation was observed to be 37.4%. The *in-vitro* dissolution studies demonstrated that the AMT release rate from MNPs was dependent upon the pH of the medium. The release rate at pH 7.4 is slower than at pH 1.2 and the cumulative percentage release from the formulation was found to be 40.24% and 70.42% respectively. Zeta potential of the uncoated Iron oxide MNPs and AMT loaded chitosan coated MNPs were found to be +19.5 mV and -22.1 mV respectively. Antibacterial activity of the formulation determined by Agar plate diffusion method affirmed the anti bacterial activity. Tube Dilution Method was used for determining the MIC value of MNPs against *Bacillus subtilis* and *Escherichia coli*, and against *Staphylococcus aureus, which was found to be 6.25 µg/ml* (MIC) *with 3.125 µg/ml* (MIC) respectively. The magnetic behavior of the MNPs was confirmed by using a permanent magnet for separation of the nanoparticles. Therefore, the formulation could deliver the drug to the targeted tissue under the influence of externally applied magnetic field. The formulated MNPs delivered the drug in a controlled way and the polymer which was used for preparation of MNPs was biocompatible, nontoxic and serves as a best carrier for antibiotic drug. Magnetic chitosan nanoparticles developed in this study may serve as a potential device for the delivery of antibiotic drug to the targeted tissue under the influence of externally applied magnetic field.

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