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Research Article

Preparation and Evaluation of Cefixime Nanoparticles Prepared Using Fenugreek Seed Mucilage and Chitosan as Natural Polymers

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ABSTRACT

Objectives: The present investigation was attempted to develop a natural polymer based cefixime nanoparticles using *Trigonella foenum-graecum* (Fenugreek) seed mucilage and chitosan as drug carrier to facilitate controlled drug release at the target site. Methods: The Cefixime nanoparticles were prepared by the modified coacervation method. The prepared drug nanoparticles were evaluated for particle size, zeta potential, surface morphology, entrapment efficiency, *in-vitro* drug release studies and also *in-vitro* antimicrobial efficiency studies for the selected ideal batch. Results: The prepared drug nanoparticles was found to be the average mean particle size in range from 133.8±8.5 to 446.3.2±20.3nm and zeta potential of greater than +30 mV or less than -30 mV indicate the better physical stability. The surface morphology of the prepared nanoparticles was found to be spherical with smooth surface and entrapment efficiency was found to be 70.4±1.2 to 83.4±2.0. The *in-vitro* drug release showed a biphasic pattern with initial burst release followed by the sustained release of the drug up to 24h. The MIC50 values of pure drug and prepared nanoparticles for *Salmonella* Typhi isolates was found to be 250 µg/ml and 125 µg/ml respectively. The disc agar diffusion test revealed that pure drug yielded 9mm clear zone surrounding the disc, whereas the prepared formulation discs produced 12mm clear zone at 125 µg/ml. Conclusions: The developed natural polymer based cefixime nanoparticles facilitate the controlled drug release profile and better zone of inhibition with minimum concentration thereby improving the patient compliance followed by declining the limitations associated with antibiotics.

Key words: Fenugreek seed mucilage, Chitosan, Controlled drug release, Antimicrobial efficiency.

INTRODUCTION

Typhoid fever is a severe illness caused by the bacterium *Salmonella enterica* serotype Typhi normally transmitted through the faecal contamination of water or food of an infected person[1,2]. According to WHO, an estimated 21 million cases of typhoid fever and 2, 22,000 deaths occur annually throughout the world. Flouroquinolones like ciprofloxacin are the drug of choice for the treatment of multidrug-resistant (MDR) (resistant to chloramphenicol, trimethoprim-sulfamethoxazole and ampicillin) strains of *Salmonella* Typhi. However, Flouroquinolones were contraindicated in paediatric patients and pregnant women due to its damage to the articular cartilage. Moreover, wide usage of quinolones in the treatment leads to the development of resistance to these antibiotics. Due to the development of MDR *Salmonella* Typhi in endemic countries an alternative drug for the treatment of typhoid fever is essential[3]. In the search for alternative antibiotics for the treatment of MDR typhoid fever, the third generation cephalosporins have shown good activity against *Salmonella* Typhi[4]. Cefixime is an oral third generation cephalosporin was reported to be more effective in treating MDR typhoid fever in children and adults showing good antimicrobial activity against *Salmonella* Typhi. Unfortunately, the major limitation with cefixime was its poor bioavailability (40-50%), poor protein binding (60%) and with a short half-life of 2-3 h lead to the administration of 200mg twice daily for 7-14 days[5]. To overcome the limitations associated with current conventional dosage form a novel drug delivery approach is necessary. Nanoparticulate drug delivery systems has emerged as prevalent drug delivery systems for the treatment of enteric infectious diseases over the past few decades by improving drug bioavailability, sustained pharmacological effect with decreased emergence of antibiotic resistance and side effects there by reducing health care costs leading to better patient compliance[6]. The biodegradable polymeric nanoparticles have been extensively used as controlled drug delivery carriers in the pharmaceutical and medical fields with better encapsulation and significant therapeutic potential with less toxic properties[7,8]. The advances in drug delivery have urged the discovery of novel excipients which are safe and fulfill specific functions that influence the rate and extent of drug release and absorption[9]. Natural plant based material can also be tailored to meet the necessities of novel drug delivery systems and thus can compete with the synthetic polymers that are

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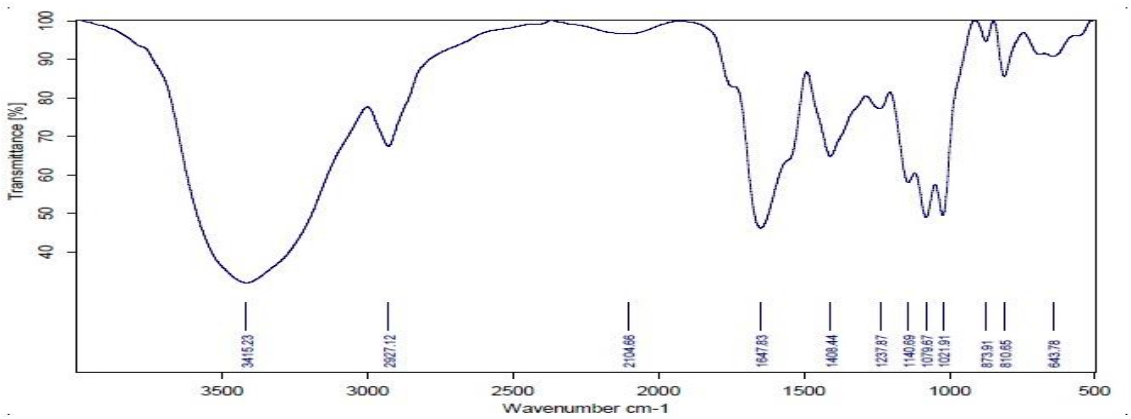

Figure 1: FT-IR spectra of Fenugreek seed mucilage

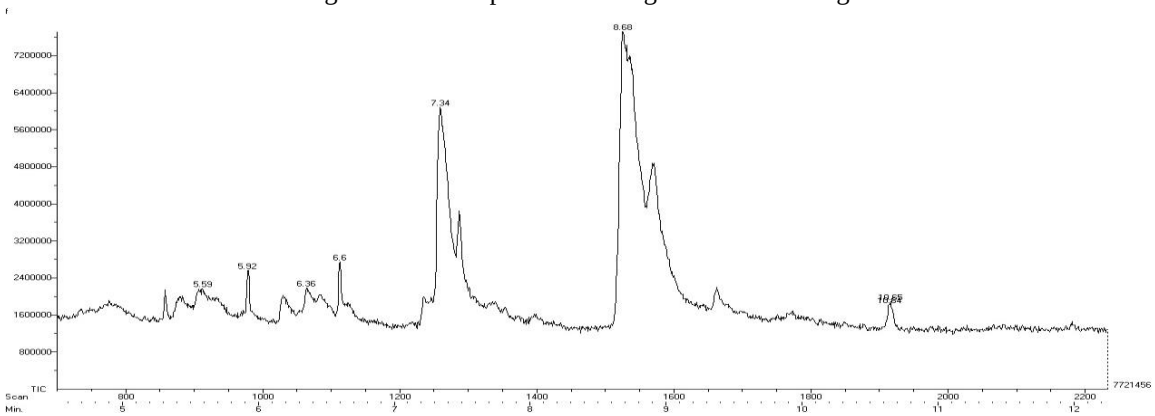

Figure 2: GC-MS analysis of acid base hydrolysed fraction of Fenugreek seed mucilage

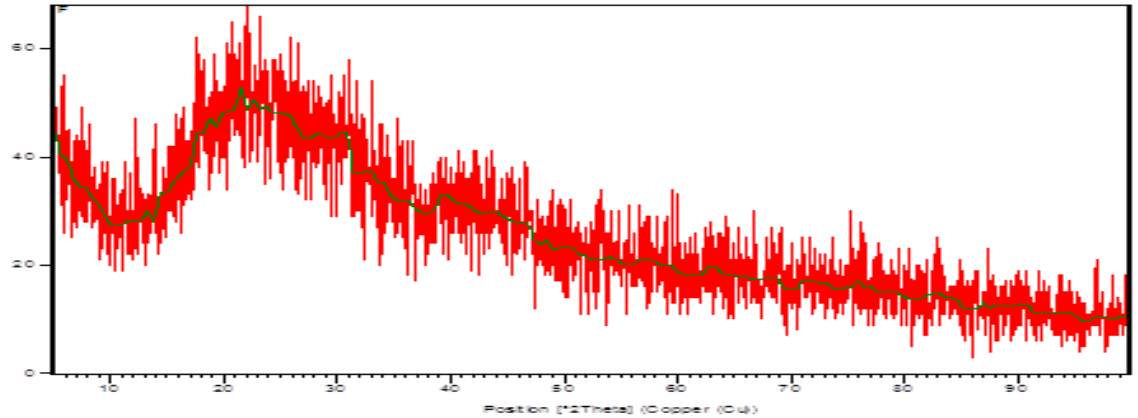

Figure 3: X ray diffraction pattern of Fenugreek seed mucilage

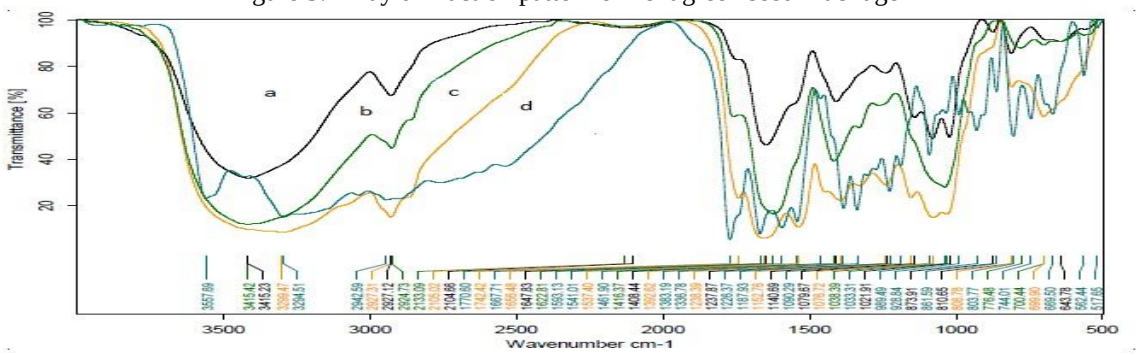

Figure 4: FT-IR spectra of physical mixture containing drug and polymers: a) Fenugreek seed mucilage b) chitosan c) physical mixture d) cefixime

available in the market. The plant based polymers have been studied for their wide application in various pharmaceutical dosage forms like transdermal patches, nanoparticles, microspheres, buccal films. Mucilages are extensively used biodegradable polymeric materials for conventional and novel dosage forms and have a wide variety of applications in pharmacy as they modify the drug release and absorption. These natural polymers have benefits over synthetic polymers, as they are stable chemically, biocompatible, less toxic, economical, biodegradable, and easily available[10,11]. *Trigonella foenum-graceum*, commonly known as Fenugreek, is an herbaceous plant belonging to family Fabaceae[12]. Various phytochemical studies revealed that the main constituents present in the Fenugreek seeds were the carbohydrates and mucilages (galactomannans)[13]. Fenugreek seeds produce high viscosity mucilage at low levels of concentration which does not dissolve in water and forms a viscous tacky mass when exposed to fluids[14]. It can be used as binders, suspending agents and matrix formers for controlled drug delivery due to its gelling property[15]. Chitosan is a deacetylated form of chitin, an abundant polysaccharide present in crustacean shells. Chitosan is a biodegradable and biocompatible cationic polymer having good mucoadhesive and membrane permeability properties. Hence, chitosan has been broadly investigated for its potential in controlled drug delivery[16]. Upon mixing of anionic and cationic polymers the interactions result in their spontaneous association of forming polyelectrolyte complex with reversible electrostatic bonds. The present investigation was aimed to prepare and to characterize the natural polymer based cefixime nanoparticles prepared using chitosan and *Trigonella foenum-graecum* seed mucilage as matrix formers to sustain the drug release inside the infected cells to attain better therapeutic concentration at the targeted site with improved bioavailability and half-life.

MATERIALS AND METHODS

Cefixime was obtained as a gift sample from Kniss laboratories Pvt Ltd, Chennai, India. Fenugreek seeds were procured from local market, Chennai. Chitosan was procured from Sigma-Aldrich Chemical Co. Ltd. All other chemicals used were of analytical grade and double distilled water was used throughout the experiments.

Extraction and purification of Fenugreek seed mucilage

The high quality Fenugreek seeds were obtained from the local market and washed with water to remove the dirt and debris. The Fenugreek seeds (250g) were soaked in double distilled water (500 ml) overnight and then heated

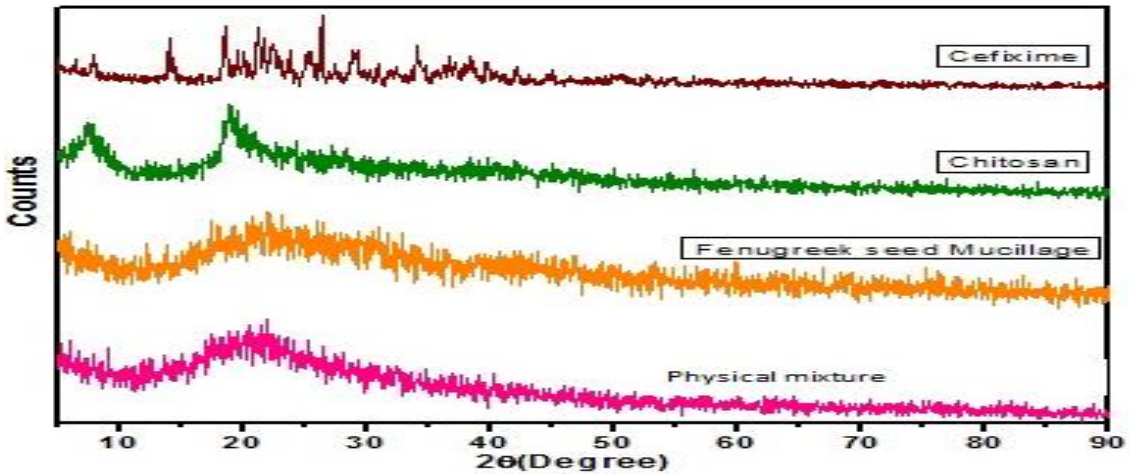

Figure 5: XRD Pattern of cefixime, chitosan, Fenugreek seed mucilage and physical mixture containing drug and polymers

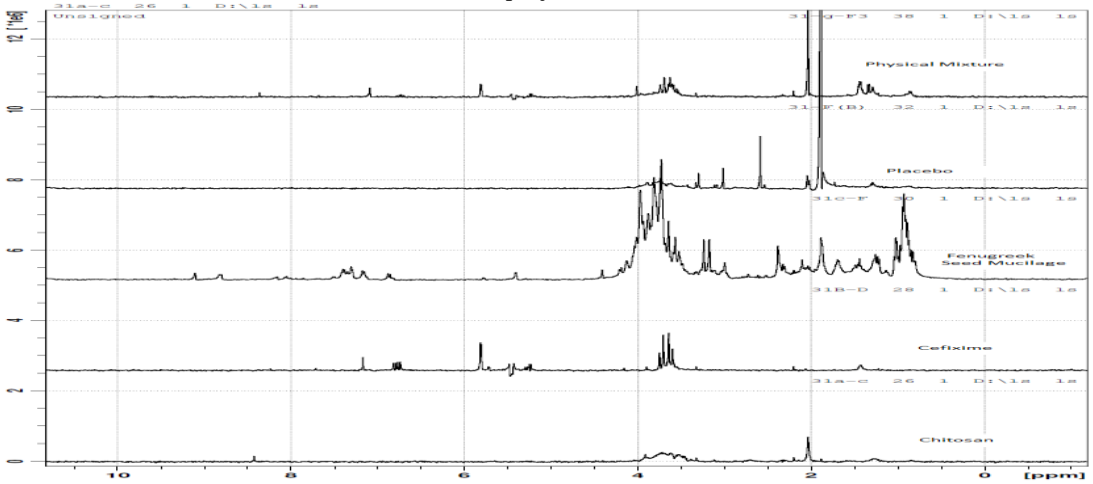

Figure 6: NMR spectra of cefixime, chitosan, Fenugreek seed mucilage and physical mixture containing drug and polymers

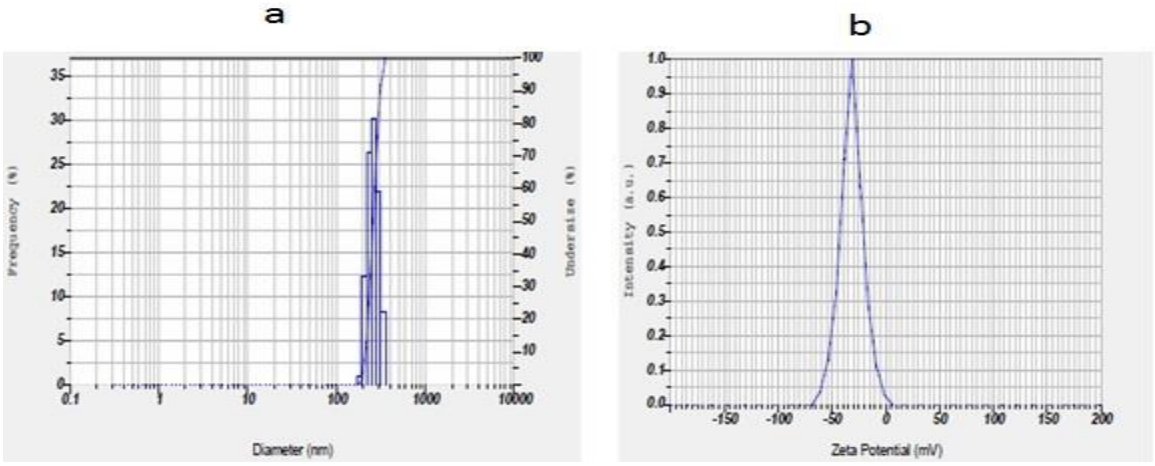

Figure 7: (a) particle size distribution of formulation (F2) (b) zeta potential measurement of formulation (F2)

Table 1: Characterization of formulations for average particle size, poly dispersity index and zeta potential*

Formulation	Average size mean (nm)	Polydispersity index	Zetapotential (mV)
F1	133.8±8.5	0.19±0.04	15.3±1.51
F2	178.6±8.5	0.25±0.08	-31.7±0.96
F3	260.3±17.0	0.49±0.07	30.9±1.25
F4	446.3±20.3	0.62±0.03	12.9±0.99

*Data represented as mean ± SD (n=3)

at 50°C for 2h. The mucilage is extracted using a multi-layer muslin cloth bag to remove the marc from the solution. Acetone was added to the above filtrate to precipitate the mucilage and the mucilage is separated, dried in an oven at 50°C stored in desiccator till use. The obtained powder was re-dissolved in 100 ml of water, filtered and centrifuged for 20 min at 3000 rpm. The supernatant clear solution was evaporated and dried. This process of purification was repeated thrice. The purified solid mass was dried by freeze drying and obtained powder was stored in an airtight container[17].

Structural characterization of Fenugreek seed mucilage by FT-IR, GC-MS, XRD

The FT-IR spectrum of sample was recorded on FT-IR spectrophotometer. The sample were mixed with KBr in ratio of (1:4) and pressed into pellets under mechanical pressure using hydraulic press. The scans were obtained at a resolution of $2cm^{-1}$ from 4000 to $400cm^{-1}$[18].The GC-MS analysis was carried out using HP-5 conventional capillary column (30m x 0.25mm with internal diameter of 0.25µm) coupled to ion trap mass spectrometry functioned at 70ev. The columns were automated from 50 to 250 ^{0}C at 50^0C/min[19]. X-ray diffraction patterns of mucilage was carried out using Schimadzu, XRD 6000 equipment, with nickel filtered tube CuKα1 at a voltage of 45 kV and current of 45 mA, The scanned angle was set at 2Θ from 5º to 90º and scanned rate was 1º/min to determine the crystallinity of the sample[20].

Drug-polymer compatibility studies

FT-IR, proton NMR and XRD studies were carried out to determine the possible interaction between the drug and excipients used. FT-IR and XRD analysis was carried out as reported earlier. Proton NMR spectra of sample were recorded in an NMR spectroscopy. 100 mg of sample was dissolved in deuterium oxide and chemical shifts were reported in ppm relative to an internal standard TMS (tetramethylsilane) for ^{1}H NMR. NMR spectrum was attained at a base frequency of 400MHz, with 16 transitions and delay time 1.5s using deuterium oxide as solvent (samples in tubes of 0.5 cm id). The spectral width was 200 ppm, chemical shifts were expressed in δ (ppm) relative to the resonance of internal TMS. The presence of an interaction is identified by the alteration, shift or disappearance of a characteristic absorption peak of the drug.

Preparation of cefixime nanoparticles

The cefixime nanoparticles were prepared by using coacervation method reported earlier with slight modification[21,22]. The anionic polymer solution was prepared by dissolving purified mucilage (0.04% w/v) in distilled water and kept under magnetic stirring and pH of the solution was adjusted to 5.2 using 0.1N HCl. The cationic polymer solution was prepared by dissolving chitosan (0.02% w/v) in 0.1% v/v acetic acid was kept under magnetic stirring and pH of the solution was adjusted to 5.5 using 1N NaOH, a constant amount of cefixime 0.05% (w/v) was added to the cationic polymer solution with constant stirring at 3500 rpm. The anionic polymer solution was added slowly drop wise to the mixture of chitosan and cefixime at different polymeric ratios to give four different formulations i.e., F1 (1:1), F2 (2:1), F3 (1:2), F4 (2:2) then centrifuged at 12000 rpm for 45 min and lyophilized. The obtained nanoparticles were stored in desiccator until further use.

Physicochemical characterization of cefixime nanoparticles

Particle size, poly dispersity index and zeta potential

Nanoparticles size distribution and zeta potential was determined using Nanoparticle analyser SZ-100. The size distribution analysis was performed at a scattering angle of 90° and at a temperature of 25°C using samples

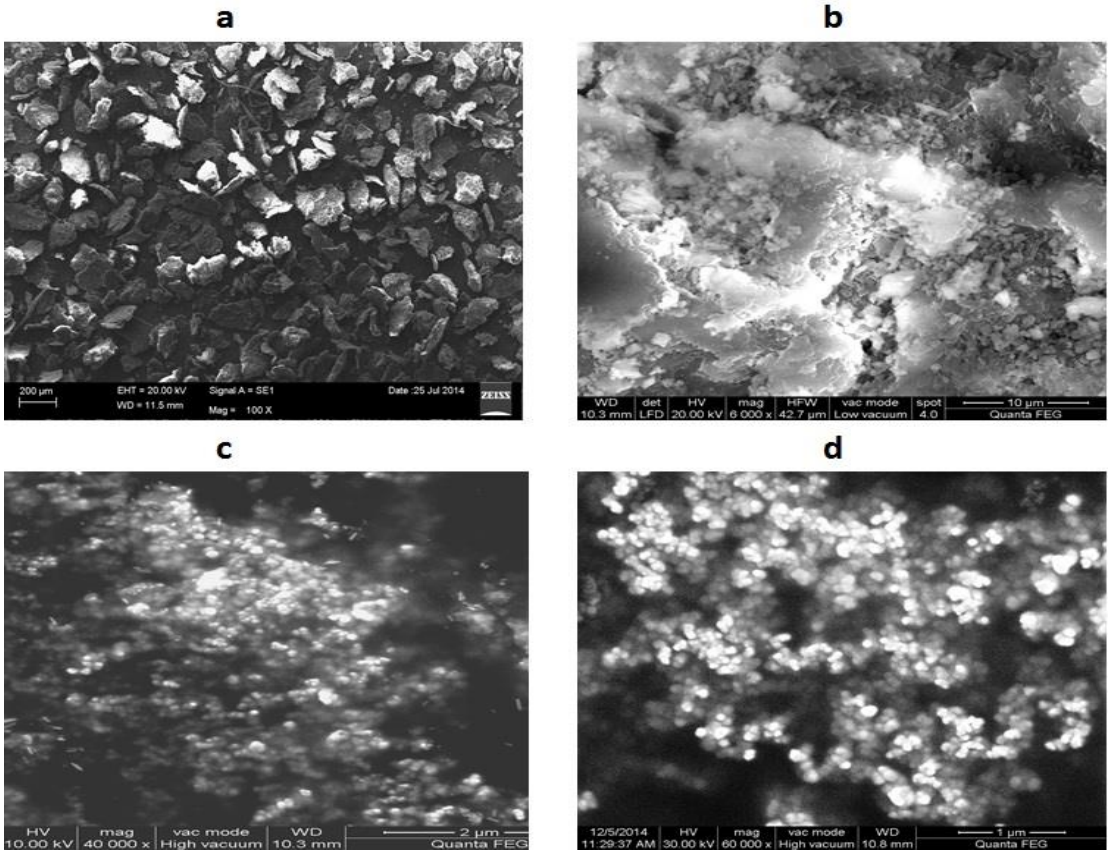

Figure 8: SEM micrograph of a) Fenugreek seed mucilage b) chitosan c) cefixime nanoparticles at lower magnification d) cefixime nanoparticles at higher magnification

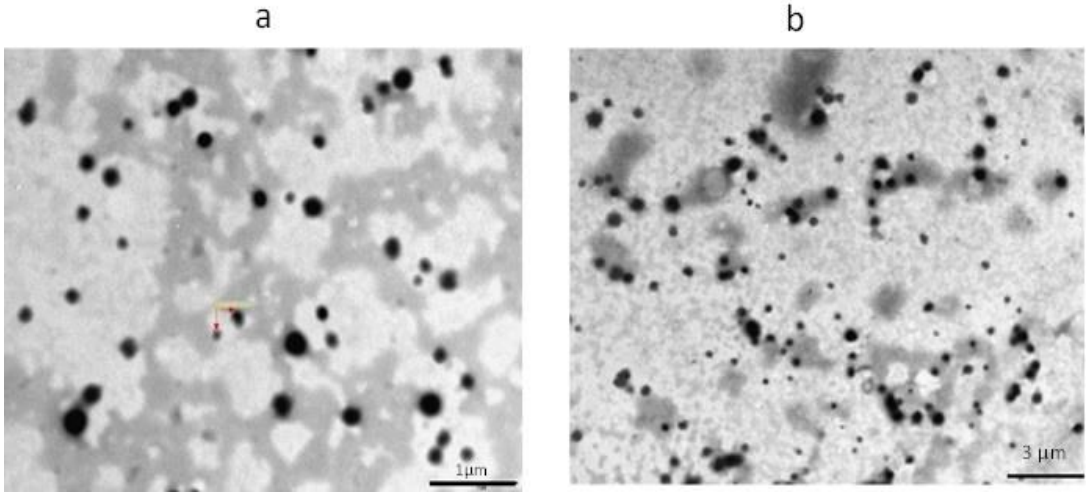

Figure 9: TEM micrograph of cefixime nanoparticles

appropriately diluted with isopropanol. The poly dispersity index (PDI) is a measure for the broadness of a particle size distribution and can be used for the determination of nanoparticle dispersion. PDI between 0.03 - 0.06 as monodisperse, 0.1 - 0.2 as narrowly distributed and 0.25-0.5 as broadly distributed and value above 0.7 indicated extremely broad size distribution that cannot be described by means of PDI. The zeta potential was measured using a disposable zeta cuvette using samples appropriately diluted with double distilled water.

Morphological analysis

The morphology of nanoparticles was studied using a scanning electron microscope (SEM) (Hitachi S 3000H, Japan). The sample was fixed to the plate surface with double-sided adhesive tape and sputtered coated with gold as the samples were non-conducting and surface morphological features were observed. Transmission electron microscopy (TEM) was used to study the surface morphology of nanoparticles. A small aliquot of nanoparticles suspension were dropped onto formvar-coated copper grids and dried in hot air oven for 45 min then the samples were stained using 2% w/v

Table 2: Characterization of formulations for % entrapment efficiency and % drug content

Formulation	%Entrapment efficiency	%Drug content	% Yield
F1	73.0±1.5	81.1±1.8	40.1±1.2
F2	83.4±2.0	85.1±1.6	44.5±0.9
F3	79.4±1.8	82.7±1.5	43.6±1.4
F4	70.4±1.2	64.0±1.2	47.4±0.8

*Data represented as mean ± SD (n=3)

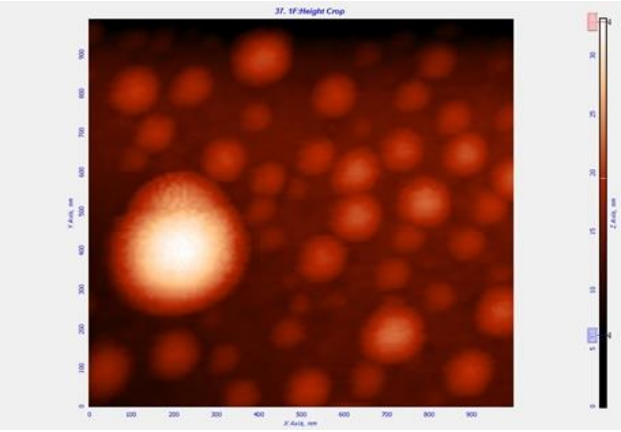

Figure 10: AFM micrograph of cefixime nanoparticles

phosphotungstic acid at room temperature. The picture was captured using digital micrograph and soft imaging viewer software (Olympus, Germany) was used for capturing and analysis. The morphological characteristics of cefixime nanoparticles were observed using Atomic Force Microscopy (AFM). The small quantity of sample was dissolved suitably in isopropyl alcohol was deposited on a glass slide and dried overnight to form a thin film and was analysed by AFM (NT-MDT).

Determination of % encapsulation efficiency and % drug content

The percentage encapsulation efficiency of drug loaded nanoparticles was determined by separating unentrapped drug from the nanoparticles by centrifugation at 12,000 rpm at 5^0 c for 45 min. The supernatant was filtered through whatmann filter paper and the amount of cefixime in the clear supernatant was determined spectrophotometrically by measuring the absorbance in UV-Vis spectrophotometer at 287 nm[23, 24].

$$\% \text{ Entrapment efficiency} = \frac{(\text{Amount of the total drug added} - \text{Amount of free drug})}{\text{Amount of total drug added}} \text{X } 100$$

$$\% \text{ Drug content} = \frac{\text{(Weight of drug in nanoparticles)}}{\text{(Weight of nanoparticles recovered)}} \text{ X } 100$$

$$\%\text{Yield} = \frac{\text{(Weight of nanoparticles recovered)}}{\text{(Weight of the polymer and drug fed initially)}} \text{ X } 100$$

In-vitro drug release by diffusion bag technique

In-vitro drug release studies for the cefixime nanoparticles were performed using diffusion bag technique. The cefixime nanoparticles (equivalent to 10mg) dispersed in 5ml of dissolution medium was placed in a dialysis bag (MWCO: 12–14 kDa, surface area of 22.5 cm^2) immersed in USP Apparatus I with a dissolution medium (300ml) of 0.05 M potassium phosphate buffer of pH 7.4 stirred at 37 ±2°C maintained at 100 rpm. 1ml of sample was withdrawn at regular intervals of time and an equal volume of buffer solution was added to maintain the constant volume of dissolution medium. The amount of drug released was measured spectrophotometrically using UV at 287nm. All measurements were performed in triplicate (n=3) and SD was calculated[25, 26].

Drug release kinetics

*T*he *in-vitro drug* release data obtained were extrapolated by various mathematical models such as Zero order, First order, Higuchi, Koresmeyer-Peppa's and Hixson-Crowell equation to know the mechanism of drug release for all the formulations. The equation with high regression coefficient (r^2) for formulation will be the best fit of release data. Zero order equation describes that the system where the release rate is independent of the concentration of the dissolved species. The first order equation describes the release from the system where the dissolution rate dependent on the concentration of the dissolving species. The Higuchi square root equation describes the release from the systems where the solid drug is dispersed in a insoluble matrix, and the rate of drug release is related to the rate of drug diffusion. The Hixson-Crowell cube root law describes the release from the systems where there is change in surface area and diameter of the particle. Koresmeyer-Peppa's equation is used when the release mechanism is not well known or when more than one type of release phenomenon could be involved. If n = 0.5 indicates pure fickian diffusion, n = 0.5-1 indicates anamolous non-fickian diffusion and n=1 indicates zero order release[27-29].

In-vitro antimicrobial efficiency

Stock cultures were kept at 4°C on slant. The active cultures required for the experiment were prepared by transferring a loop full of culture from the stock cultures to test tubes containing nutrient broth and incubated for 24 h at 37ºC.

Disc agar diffusion technique

The antibiotic-resistant profile of sample was determined by disc agar diffusion method to determine the zone of inhibition. The study was carried out on Muller Hinton Agar (MHA) medium prepared by weighing 3.8 g and dissolved in 100ml of distilled water to this 1gm of agar was added and kept for sterilization. After sterilization the MHA media was poured in to sterile petriplates and were allowed to solidify for 30 minutes. Then the inoculums were spread on to the solid plates with sterile swab moistened with the bacterial suspension. 20 µl of pure drug (cefixime) and cefixime nanoparticles were serially diluted to a different concentration of 62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml and 1000 µg/ml was added to respective disc placed on MHA plates. These plates were incubated for 24 h at 37ºC. Then the activity was determined by measuring the diameter of zone of inhibition[30].

Broth dilution technique

Table 3: Curve fitting data of all formulations

Formulations	Zero order Equation	First order Equation	Higuchi Equation	Koresmeyer Equation	Peppa's	Hixson-Crowell Equation
	r^2	r^2	r^2	n	r^2	r^2
F1	0.990	0.950	0.983	0.51	0.973	0.954
F2	0.996	0.838	0.98	0.52	0.988	0.930
F3	0.990	0.871	0.979	0.59	0.98	0.898
F4	0.978	0.877	0.974	0.53	0.983	0.921

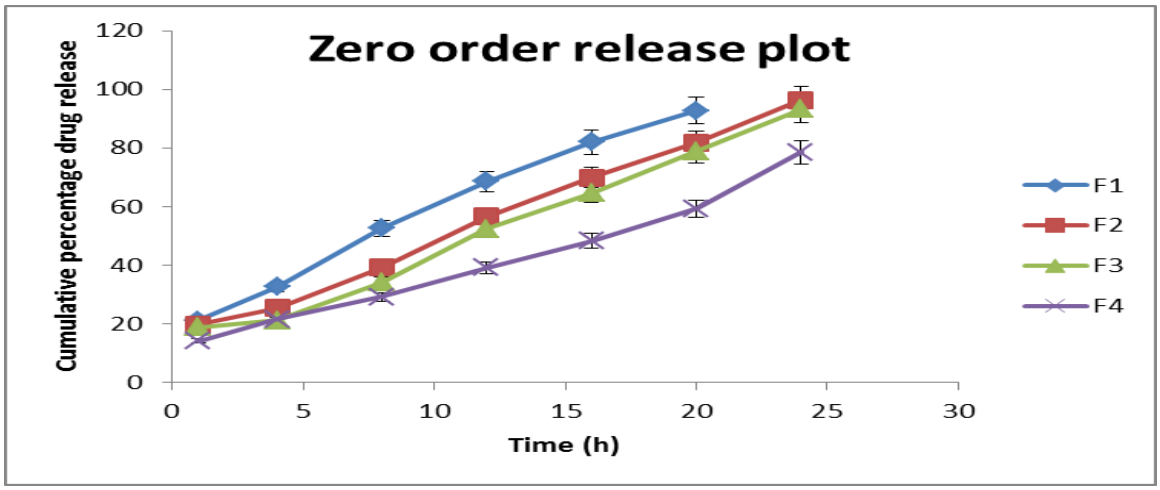

Figure 11: *In-vitro* drug release profile of prepared formulations

5 ml sterilized nutrient broth was taken in each tube. To this 100 µl of culture was added and then 100 µl of different concentration of samples was added. Optical density (OD) was measured at 620 nm[31].

RESULTS AND DISCUSSION

Structural characterization of Fenugreek seed mucilage by FT-IR, GC-MS, XRD

The major peaks present in the FT-IR of Fenugreek seed mucilage at 3415.23cm^{-1} indicates the Stretching vibrations of free –OH groups and N-H groups, characteristic peak at 2927.12 cm^{-1} corresponds to C-H stretching vibrations of carboxyl group, absorption band at 2104.66 cm^{-1} represents C≡C stretching band, , the peak at 1647.83 cm^{-1} represents stretching mode of keto groups, the band at 1408.44 cm^{-1} represents C-H bending vibrations, the peak at 1237.87 cm^{-1} indicates O-H bending mode, the peaks at 1079.67 cm^{-1} and 1021.91 cm^{-1} corresponds to stretching vibrations due to C-O, C-N, C-C groups, the bands at 873.91 cm^{-1} represents N-H, C-H rocking mode, the frequency at 643.78 cm^{-1} indicates rocking vibrations of C-H group (Fig 1). The GC-MS of hydrolysed fraction showed seven peaks at retention time (RT) 10.65 min, 8.68 min, 7.34 min, 6.6 min, 6.36 min, 5.92 min and 5.59 min, with molecular ion peaks of mass/charge (m/z) ratio 318, 193.77, 170.85, 117, 154.77, 164.26 and 140.94 respectively confirmed the presence of major neutral sugars such as Rhamnose, D-Galactose, D-Arabinose and uronic acid such as D-galacturonic acid (Fig 2). From XRD pattern no characteristic peaks were observed indicates that the mucilage was found to be in amorphous form (Fig 3).

Drug-polymer compatibility studies

The FT-IR spectra of cefixime, Fenugreek seed mucilage, chitosan and its physical mixture containing drug and polymer were analysed (Fig 4). There were no changes in the peak shape and peak position of drug in the physical mixture containing drug and polymer used. Hence the spectra indicate that there were no incompatibility between drug and excipients used. The X-ray diffraction pattern of drug and the physical mixture were carried out. The pure drug confers crystalline nature with sharp peak between 7.96^0 $^o2\Theta$ to 26.37^0 $^o2\Theta$ a characteristic of cefixime that represent the crystalline nature of the drug, the same diffraction was also observed in the physical mixture with decreased intensity of signal indicating no signs of incompatibility between the drug and polymers (Fig 5). The proton NMR spectra of cefixime and its physical mixture with polymers were carried out. There were no significant differences in the characteristic proton assignments of the drug compared to its physical mixture with the polymers. Hence the drug was compatible with the drug (Fig 6).

Physicochemical characterization of cefixime nanoparticles

Particle size and zeta potential

The average particle size and PDI was found to be increased with increase in polymer concentration. Zeta potential is of critical importance in monitoring the particle dispersion and determining the stability of a nanoparticle suspension. A higher value of zeta potential results in greater electro-static repulsion between the particles thus minimizing aggregation/ flocculation (Fig

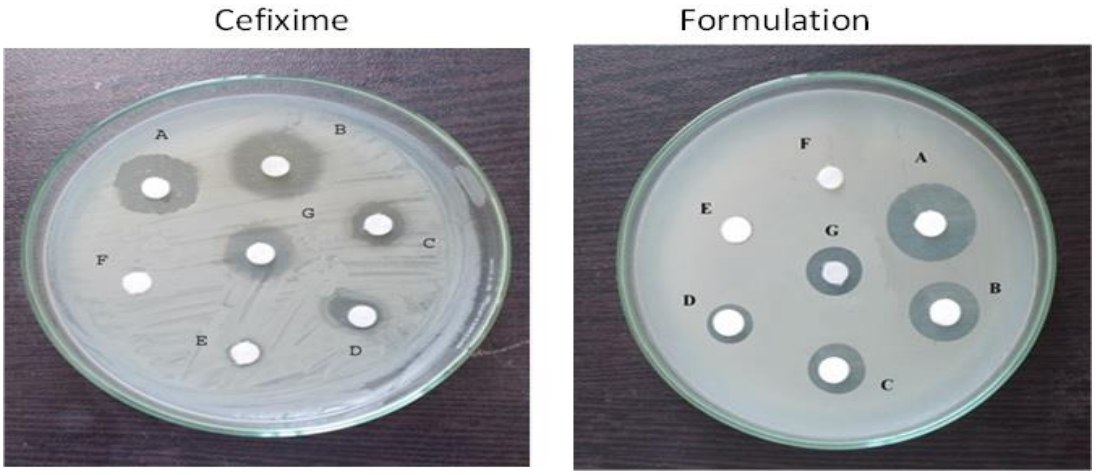

Figure 12: Determination of zone of inhibition (mm) by disc agar diffusion technique of the selected formulation (F2) at different concentrations. A. 1000 µg/ml B. 500 µg/ml C. 250 µg/ml D. 125 µg/ml E. Blank F. DMSO (Negative control) G. Streptomycin (10µg/ml) to respective disc.

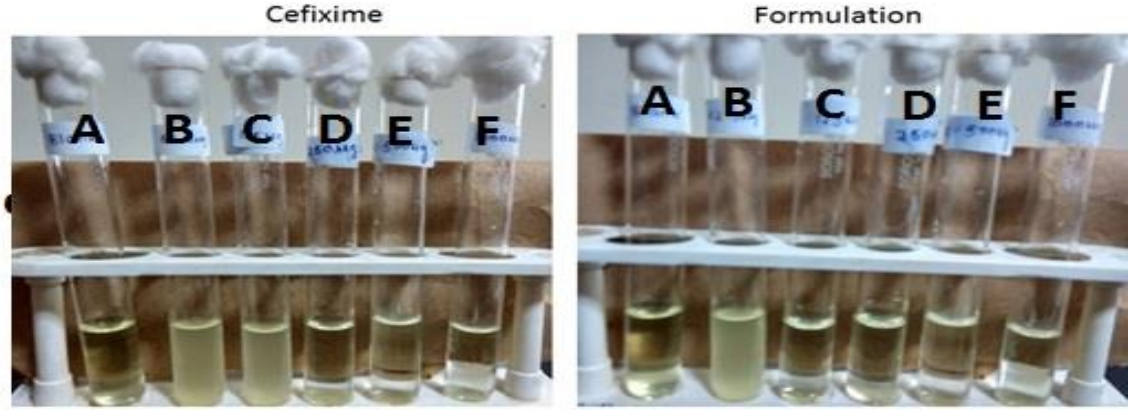

Figure 13: Determination of MIC by broth dilution technique of the selected batch (F2) at different concentration. A. DMSO (Negative control) B. 62.5 µg/ml C.125 µg/ml D. 250 µg/ml E. 500 µg/ml F.1000 µg/ml

7). The Formulations F2 and F3 with Zeta Potential < -30 mV and > +30mV respectively, shows high degrees of stability with no aggregation. In addition, from the zeta potential measurement, the dominated component on the particles surface for formulation F2 was predicted as mucilage, being negatively charged polymer imparts anionic nature to nanoparticles, whereas for F3 the dominated component on the particles surface was predicted as chitosan which imparts cationic nature to the nanoparticle. The zeta potential value for F1 and F4 formulations was found to be approximately neutral (Table 1).

Morphological analysis

The SEM studies, surface morphology of the prepared formulation was found to be spherical, smooth surface with solid dense structure (Fig 8) The TEM images indicates that the nanoparticles with spherical shape in size range 100-150 nm and appeared to be in same diameter as compared to average particle size measured using nanoparticle analyser (Fig 9). The AFM studies confirmed the presence of spherical and dense solid nanoparticles (Fig 10). and three-dimensional view of the nanoparticles showed that the nanoparticles are discrete with average particle size of 150 nm.

Determination of % entrapment efficiency, % drug content and % yield

The % entrapment efficiency of the cefixime nanoparticles was found to in the range from 70.4±1.2 to 83.4±2.0. The % drug content was found to be in the range from 64.0±1.2 to 85.1±1.6 and % yield was found to be in the range from 40.1±1.2 to 47.4±0.8. The % entrapment efficiency and % drug content was found to be increased with increase in polymer concentration, but in formulation F4 due to increase in size the surface area of nanoparticles was decreased which in turn decreased the drug entrapment efficiency and drug content. The % yield was found to be increased with increase in polymer concentration (Table 2).

In-vitro drug release by dialysis diffusion bag technique

From *in-vitro* drug release study by dialysis bag diffusion technique showed a biphasic pattern with initial burst release followed by sustained release of drug up to 24 h. The F2 showed sustained drug release compared to other formulations. The amount of drug release was decreased with increase in polymer concentration due to increase in the thickness of the polymeric membrane which decreases the diffusion of drug through it (Fig 11).

Drug release kinetics

The results obtained from the data extrapolated by using different kinetic models revealed that the zero order plots were linear for all the formulations and first order plots were non linear for all the formulations (Table 3). Based on the highest regression coefficient value (r^2) the best fit model for all formulations was found to be Higuchi model (r^2: 0.97-0.98). The release of drug from the polymer matrix containing hydrophilic polymers involves diffusion. To confirm the diffusion mechanism the data was fitted into Koresmeyer-Peppa's equation, as 'n' values indicates between 0.5-1, the mechanism of drug release was found to follow anomalous non-fickian diffusion i.e. the increased diffusivity of drug from the matrix by solvent-induced relaxation of the polymers.

In-vitro antimicrobial efficiency

The Disc Agar Diffusion technique reveals that the diameter of zone of inhibition of pure drug and prepared formulation (F2) was found to be 9mm and 12 mm at 125 µg/ml which confers that the prepared formulation was found to be showing better zone of inhibition against the pure drug solution. There by customising the drug release profile of the cefixime nanoparticles, an effective therapeutic concentration was attained which may overcome the limitations associated with conventional dosage forms (Fig 12)**.** From the broth dilution technique the MIC50 values of pure cefixime and prepared formulation (F2) for cefixime sensitive *Salmonella* Typhi isolates were determined. The MIC50 value of cefixime nanoparticles and pure cefixime was found to be 125 µg/ml and 250 µg/ml respectively. The MIC50 of prepared cefixime nanoparticles was one fold lesser compared to pure cefixime solution which reveals the better antimicrobial activity of the prepared formulation against pure drug this may be due to the increased bacterial adhesion of cefixime nanoparticles at the site of target **(**Fig 13).

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