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DEVELOPMENT AND CHARACTERIZATION OF CEFUROXIME AXETIL LOADED NANOPARTICLES

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ABSTRACT Cefuroxime axetil (CA) is a second generation cephalosporin antibiotics used for respiratory tract infections. It's having poor solubility, poor bioavailability and short biological half-life. The present study was attempted to develop a natural polymer based CA nanoparticles using Fenugreek seed mucilage and chitosan to facilitate drug release at target site. Nanoparticles prepared by modified coacervation method and evaluated for particle size, zeta potential, morphological studies, entrapment efficiency, in-vitro drug release studies, antimicrobial efficiency and stability studies. Particle size of nanoparticles was found from 180.5±18.2nm to 348.3±20.3nm and zeta potential was neutral and indicates better physical stabilities with no aggregation. The surface morphology was spherical with smooth surface using SEM and TEM images. Entrapment efficiency was 76.37±0.49% to 88.84±0.74%. *In-vitro* drug studies shown controlled release up to sufficient hours and F2 formulation exhibits least percentage drug release. An *in-vitro* antimicrobial efficiency proven better zone of inhibition than pure drug. Stability studies showed good results with no-observable physical changes. Developed nanoparticles support to tailor better drug release profile and antimicrobial efficiency for improving the patient compliance.

KEYWORDS : Cefuroxime Axetil, Nanoparticles, Fenugreek seed, Chitosan.

INTRODUCTION

Some antibiotics have poor bioavailability, poor protein binding and short half-life. To overcome this, nanoparticles (NPs) has used as a prevalent drug delivery systems (DDS)[1] for infectious diseases by improving bioavailability, sustained pharmacological effect, enhancement of cellular uptake, reduction of toxic effects and reducing health care costs[2]. Natural plant based material can be tailored to meet necessities of DDS especially for NPs. Mucilage's used promising natural biodegradable polymeric materials[3]. *Trigonella foenum-graceum*, known as Fenugreek, belonging to *Fabaceae* family[4], the plant mainly shows presence of saponin (4.8%) and alkaloids[5] and seed are a rich source of fiber and protein, main constituents were carbohydrates and mucilages[6]. Fenugreek seed mucilage used as binders and matrix formers and gelling property[7]. Chitosan is biodegradable and biocompatible cationic polymer[8] and limitation is faster dissolution in stomach and low capacity for controlled release[9]. To overcome limitations, polyelectrolyte complexes are formed by interactions between molecules that carry oppositely charged ionisable groups[10].

Cefuroxime axetil (CA) is a second-generation cephalosporin antibiotics[11] used for respiratory tract infections. CA is oral prodrug that shows 30-40% bioavailability in fasted and 50-60% in fed states[12] and rapidly hydrolysed by esterases in intestinal mucosa and portal blood to produce Cefuroxime. The 1-acetoxyethylester group in position 4 of CA ensures lipophilicity and promotes intestinal absorption but compromise on solubility, prodrug shows limits its effective absorption and bioavailability[13], poor protein binding (33%) and half-life of 3.5hrs lead to administration twice for 5-10 days. NPs are known to improve dissolution rate of poorly water-soluble drugs owing to increased surface area[14-15].

The present investigation, to prepare NPs using chitosan and fenugreek seed mucilage as matrix formers to control drug release inside an infected cells to attain effective therapeutic concentration at targeted site with improved bioavailability and half-life. An objective is to develop NPs by modified coacervation method and evaluate the characterizations.

MATERIALS AND METHODS

Cefuroxime axetil (CA) obtained from Orchid Pharma Pvt ltd, Chennai, Chitosan received from Qualikems Fine Chem. Pvt Ltd, New Delhi, Sodium hydroxide from Chemdays Chemicals, Ahmadabad, Disodium hydrogen phosphate from Linco Scientific Chemicals, Haryana. All other chemicals used were as analytical grade.

METHODS

Preformulation studies

Compatibility studies - Fourier transforms infrared spectroscopy (FTIR)

Preformulation were performed for compatibility studies**.** FTIR spectrum was recorded using Schimadzu instrument. Drug alone and combination with polymers (1:1) was taken and subjected to studies[16]**.**

Preparation of nanoparticles

CA nanoparticles were prepared by modified coacervation technique using different ratios (Table-01). Anionic polymer solution was developed by dissolving seeds mucilage (0.04%w/v) in distilled water and kept under magnetic stirring and pH adjusted using 0.1N Hcl. The cationic polymer solution were prepared by dissolving chitosan (0.02%w/v) in 0.1% v/v acetic acid in same way, then pH adjusted using 1N NaOH. CA (0.05% w/v) was added to cationic solution with continuous stirring at 3500rpm. An anionic polymer solution was added drop wise into chitosan and CA mixture, follow centrifuged then lyophilized and NPs were stored until further use[17].

Table - 01: Composition of Cefuroxime axetil nanoparticles

Ingredients*	Formulations Code			
	F1	F2	F3	F4
Cefuroxime axetil	50	50	50	50
Fenugreek seed	20	40	20	40
Chitosan	20	20	40	40

*** (Equivalent to mg)**

Characterization of nanoparticles

Particle size, Poly dispersity index (PDI) and Zeta potential (ZP)

Particle size of NPs was determined by photon correlation spectroscopy (PCS) using Zeta sizer Nano ZS (Malvern Instruments Ltd, Worcestershire, UK). PDI was determined by PCS for the broadness of a particle size distribution and determination of dispersion. ZP was measured using Zeta sizer at a fixed angle of 90°C and 25°C, using a disposable zeta cuvette with water[18].

Determination of entrapment efficiency (EE)

Percentage EE of NPs was determined by cooling centrifuge at 12000 rpm for 45min. The supernatant was filtered and amount of CA was determined spectrophotometrically at 281nm[19].

$$EE\ (\%) = \frac{\text{Amount of total drug added - Amount of free drug}}{\text{Amount of total drug added}} \times 100$$

In-vitro drug release studies

In-vitro drug release was performed using dialysis technique[20]. NPs suspension are taken in a diffusion tube which tied with dialysis bag (MWCO: 12-14kDa) (pore size: 2.4nm) containing donor compartment. Receptor compartment are filled with 50ml of PBS (pH 7.4). Drug release through membrane to the outer compartment, which is agitated with a magnetic stirrer at 37±2°C, and maintained at 100rpm. 1ml sample withdrawn adequately and equal volume of buffer was replaced. Drug released measured spectrophotometrically and cumulative percentage drug release was calculated.

Kinetics analysis of drug release studies

In-vitro drug release data obtained were extrapolated by various mathematical models such as Zero order, First order, Higuchi, Koresmeyer-Peppa's equation to know mechanism of drug release. The equation with high regression coefficient (R^2) for the formulations will be best fit of release data[21].

Morphological studies

Scanning electron microscopy (SEM)

NPs suspension 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 using SEM, Tescan vega3 sb, USA[18].

Transmission electron microscopy (TEM)

NPs suspension was drop cast onto copper grids and dried in hot air for 45min, and then samples were stained using 2%w/v of phosphotungstic acid at room temperature before loading into microscope, maintained at 80kV. The picture of particle shape and surface topography was captured using digital micrograph and soft imaging viewer software was used for capturing and analysis using TEM (Olympus, Germany)[22].

In-vitro antimicrobial efficiency

Antibiotic-resistant was determined by disc agar diffusion method. After sterilization, an agar media was poured into sterile petridish plates and allowed to solidify for 30min and then it was spread on solid plates with sterile swab moistened with bacterial suspension *S. Aureus* and *E.coli*. Pure drug and NPs were taken separately, then serially diluted to a different concentration and it was added to respective disc placed on agar plates, incubated for 24hrs at 37ºC. Then antimicrobial efficiency was determined by measuring zone of inhibition.

Stability studies

Best formulations were placed in borosilicate screw capped glass containers and stored in stability chamber (45±2^{0}C, 70±5% RH) and maintained at 30 days under ICH and WHO guidelines to assess their potency. At end, the samples were withdrawn and determined ZP and particle size[23].

RESULTS AND DISCUSSION

Compatibility studies - Drug polymer interaction

Compatibility studies were determined by IR spectroscopy using KBr method. FT-IR spectra of prepared sample were taken in the wavelength region was 600-3800cm^{-1} at ambient temperature and resolution was 4cm^{-1}. IR spectrum of drug was found be similar to the standard which indicates that obtained sample was pure. From results (Figure-01 & Figure-02), it has been observed that the characteristic C-H stretching, C-O stretching, C=C stretching, O-H stretching, C-N stretching of drug was unchanged in the physical admixtures spectra, and depicts that main peaks in the functional group and finger print region are identical which indicates no modification. There were no significant differences were observed and gave same kind of peaks and proving intactness of drug in physical admixtures and stable in nature.

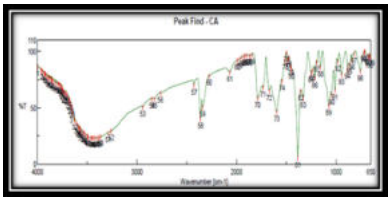


Figure-01: IR spectra of pure cefuroxime axetil

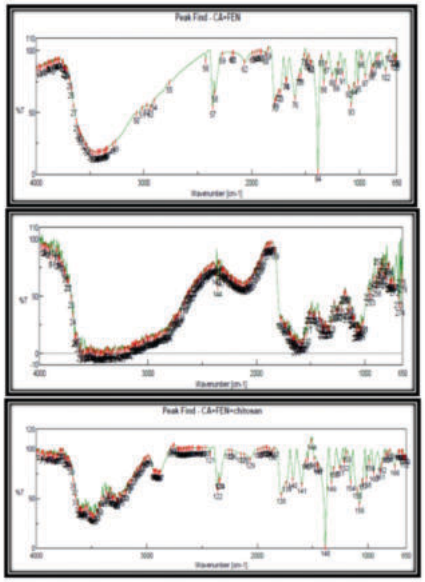
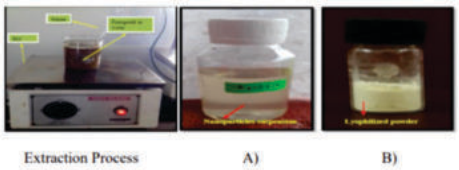


Figure-02: IR spectra cefuroxime axetil physical admixtures (CA + Fenugreek, CA + Chitosan, CA + Fenugreek + Chitosan)

Preparation of Nanoparticles

Prepared NPs were lyophilized and characterized by various parameters.



Photograph -1: A) Formulated nanoparticles B) Lyophilized powder

Characterization of Nanoparticles

Particle Size, Poly dispersity index (PDI) and Zeta potential (ZP)

The particle size of NPs was found from 180.5±18.2nm to 348.3±20.3nm. The mean particle size of all formulations was increased depends on drug polymer ratios. Size distribution curves were shown in F1 to F4 (Figure-03).

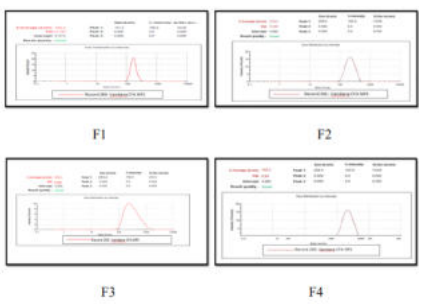


Figure-03: Particle Size of Nanoparticles (F1 – F4)

PDI values of NPs were 0.162 to 0.540, which indicates a relative homogenous dispersion and expected to maintain homogeneous dispersion with PDI at or lowers than 0.5 (Table-02).

Table - 02: Polydispersity index of nanoparticles

Formulations Code	Polydispersity Index
F1	0.162
F2	0.242
F3	0.406
F4	0.540

ZP was found -30mV to +30mV. F2 formulation shown -30.3mV indicate higher stability and optimum. It has been observed that, negatively charged seed mucilage imparts anionic nature and chitosan which imparts cationic properties to NPs. ZP value was found to be approximately neutral[21] (Figure-04).

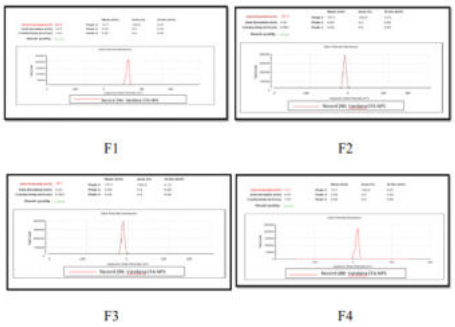


Figure-04: Zeta potential of nanoparticles (F1-F4)

Determination of entrapment efficiency (EE)

Nanoparticles EE was found between 76.37±0.49% to 88.84±0.74% (F1-F4). F2 formulations proved high EE due to increasing an anionic polymer concentrations and higher viscosities. It's due to decrease in particle size and higher stability[21] based on ZP (Figure-05).

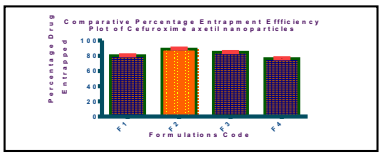


Figure-05: Percentage entrapment efficiency of nanoparticles

***In-vitro* drug release studies**

In-vitro studies of NPs were determined using phosphate buffer and drug released found from 82.34% to 95.23%, and proven a biphasic pattern with initial burst release followed then controlled release. The percentage drug releases of F2 formulation were found to be least at end of dissolution due to increasing concentration of primary polymer and decrease in concentration of secondary polymer (2:1 ratios). The dense network of drug-polymer increases the tortuisity, thus delaying drug release (Figure-06).

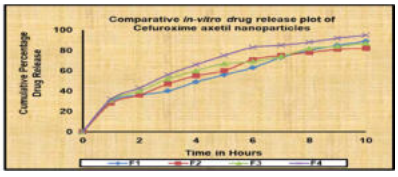


Figure-06: Comparative in-vitro drug release of nanoparticles

An *in-vitro* kinetics data (Table-03) indicated that, drug release was best fitted with zero order kinetics and formulated batches compliance with Higuchi's plot, then follows Fickian diffusion mechanism and also confirmed that controlled release pattern for sufficient hours.

Table - 03: Kinetics analysis of *in-vitro* drug release data

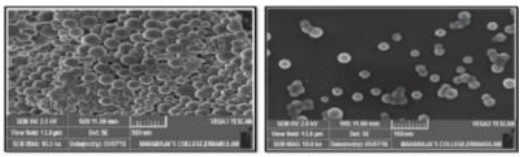
Formulations Code	Release model							
	Zero order		First order		Higuchi's		Koresmeyer and peppa's	
	R	S	R	S	R	S	R	S
F1	0.962	7.97	0.952	-0.089	0.968	29.49	0.954	0.506
F2	0.985	7.45	0.900	-0.075	0.983	27.09	0.989	0.498
F3	0.986	7.50	0.886	-0.084	0.989	26.44	0.991	0.455
F4	0.991	8.40	0.877	-0.122	0.979	30.22	0.987	0.492

Correlation coefficient (r), Slope(s)

Morphological studies

Scanning Electron Microscopy (SEM)

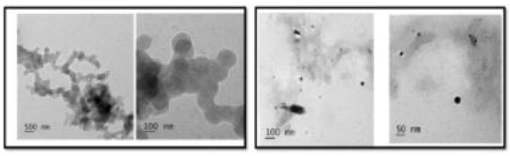
The surface morphology of best formulation (F2) assessed by SEM, was discrete in nature and almost spherical with smooth outer surface having size range of 500nm and 100nm in different angles (Photograph - 2 A & B), and confirmed nanoparticles have uniform size distribution, spherical in shape.



Photomicrograph-2: SEM images of nanoparticles (A & B)

Transmission Electron Microscopy (TEM)

TEM analysis of F2 also had shown small, spherical, individual surface morphology and exhibits size range of 100-500nm (before sonication) and 50-100nm (after sonication). From results (Photomicrographs-3), it was further confirmed that the formation of Nps.



Photomicrograph-3: TEM images of nanoparticles

In-vitro antimicrobial efficiency

Best NPs (F2) shown better zone of inhibition than standard drug due to the controlled release (Photomicrograph-4) and effectively inhibiting the growth of microorganisms and proven potential for bacterial infections treatment.

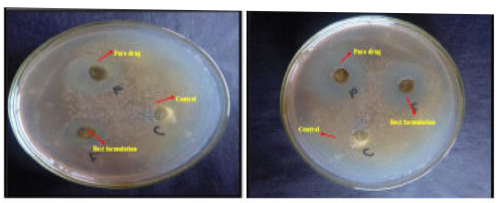


Photo micrograph-4: Antimicrobial efficiency of CA nanoparticles

Stability studies

Stability studies of selected formulations (F2) were analyzed in stability chamber and results (Figure-07) were shown good potency and there is no change in physical appearances including no significant changes using ZPs and particle size measurements after storage periods, which proven stability of Nps.

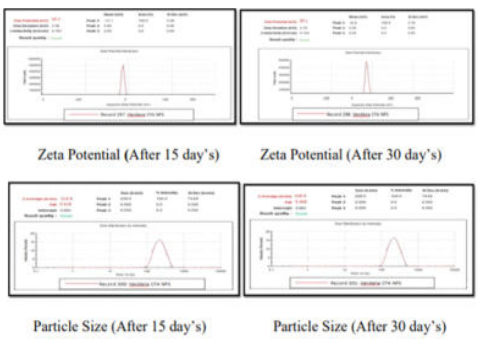


Figure-07: Zeta potential and particle size of NPs

CONCLUSION

NPs were developed using natural mucilage and formulations found controlled drug release with enhanced *in-vitro* antimicrobial efficiency and proven better zone of inhibition, thereby achieving good therapeutic efficacy and improve patient compliance. Over all, delivery of CA NPs has potential to combat drug resistance against bacterial infections.

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