

PREPARATION AND CHARACTERIZATION OF CEFTRIAXONE SODIUM ENCAPSULATED CHITOSAN NANOPARTICLES

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ABSTRACT

Objective: The objective of this study was to prepare ceftriaxone sodium chitosan nanoparticles (CS-NP) from different drug and polymer ratios and analyze their physicochemical characteristics.

Methods: Ceftriaxone sodium loaded chitosan nanoparticles were prepared using chitosan as a polymer and tri sodium polyphosphate (TPP) as cross linking agent by ionic cross linking and coacervation with the aid of sonication. Various trials have been carried out for the confirmation of nanoformulation. Parameters such as the zeta potential, polydispersity, particle size, entrapment efficiency, *in vitro* drug release Thermo gravimetric analysis and scanning electron microscope of the nanoparticles were assessed for confirmation of nanoformulation.

Results: The formulated nanoparticles showed mean particle size, polydispersity index and zeta potential to be 183.1±8.42 nm, 0.212±0.05, +38.5±1.6 mV respectively and the drug loading was found to be 46.42±10 %. *In vitro* drug release was showed a biphasic release pattern with initial burst release followed by sustained release of formulated nanoparticles. The cumulative percentage of drug release was about 83.08 %.

Conclusion: Formulation F2 was found to be the best formulation with a higher cumulative percentage of drug release. Modified ionic gelation method can be utilized for the development of chitosan nanoparticles of ceftriaxone sodium. Polymer and crosslinking agent concentrations and sonication time are rate-limiting factors for the development of the optimized formulation. The chitosan nanoparticles developed would be capable of sustained delivery of ceftriaxone sodium.

Keywords: Ceftriaxone sodium, Chitosan nanoparticles, Zeta potential analysis, Scanning Electron Microscope Analysis, Particle size

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INTRODUCTION

Ceftriaxone sodium is a semisynthetic antibiotic that can effectively treat several types of bacterial infections. It is a β -lactamase-resistant cephalosporin with an extremely long serum half-life up to 4-to 10-times longer than the other antibiotics in this class. Because of its poor cellular penetration the effectiveness of antimicrobial treatment is limited especially third-generation cephalosporin. The poor cellular penetration of the antibiotic was associated to its high molecular weight (661.6 kg) as well as its hydrophilicity ($\log P -0.6$) [1].

In recent years, it has become more and more evident that the development of new drugs alone is not sufficient to ensure progress in drug therapy. A promising strategy to overcome these problems involves the development of suitable drug carrier systems. Solid nanoparticles were introduced at the beginning of the 1990s, as an alternative to solid nanoparticles, emulsions and liposomes in cosmetic and pharmaceutical preparations [2-5].

Chitosan is a promising nano-carrier to release the encapsulated drug to the targeted tissues. Chitosan nanoparticles can be prepared by the interaction of oppositely charged macromolecules [3]. Compared with other biological polymers, chitosan is one of the most extensively used polymers. This is because chitosan possesses some ideal properties of polymeric carriers for nanoparticles such as biocompatible, biodegradable, nontoxic, and inexpensive [4]. The interaction can be controlled by the charge density of TPP and chitosan, which is dependent on the pH of the solution. Nano particulate systems show their promise as a potential ideal drug delivery system for poorly soluble, poorly absorbed and labile substances [5]. To overcome the limitation of synthetic drug, targeted drug delivery systems are designed. Nanoparticles have unique functional characters such as small particle size, high stability, lower toxicity, tuneable hydrophilic-hydrophobic balance

and the ability to bear surface features for target specific localization, etc. The aim of the present study was to prepare and characterize ceftriaxone sodium loaded chitosan nanoparticles and produced release of ceftriaxone sodium.

MATERIALS AND METHODS

Ceftriaxone sodium, chitosan (CS) (MW= 60-90 k Da; degree of deacetylation 85%) and sodium tripolyphosphate (TPP) were purchased from Sigma Aldrich, USA. All other chemicals were used analytical grade.

Synthesis of ceftriaxone sodium CS-NP

The chitosan-TPP nanoparticles were prepared via TPP ionic crosslinking and coacervation method as previously mentioned [8]. The chitosan nanoparticle synthesis procedure was first optimized at different concentrations of chitosan and the resulting mixtures were observed to be either clear, opalescent, or consisting of aggregates.

The polymer was dissolved in 10 ml of 1% (v/v) acetic acid. The solution was continuously sonicated at room temperature and the pH of the solution was adjusted to 5.0 using 1 M NaOH. Ceftriaxone sodium at three different concentrations such as 25, 50 and 100 mg dissolved in water then added to the freshly prepared chitosan dispersion and stirred for 1 h. Finally, TPP (1 mg/ml) was added drop wise to the CS-ceftriaxone sodium solution under stirring (750rpm). 10. The resulting mixture was allowed to stir for 4 h to form ceftriaxone sodium encapsulated chitosan nanoparticles. All the process was carried out under room temperature. The synthesized nanoparticles were isolated by centrifugation. The formulations were then freeze-dried in the presence of 0.1% sucrose used as a cryoprotectant (Heto Drywinner, Thermo Scientific, USA) for 24 h at a pressure of 0.05 mmHg. The pellets were collected and stored at 4 °C for further use.

Table 1: Formulation design for the synthesis of ceftriaxone sodium loaded chitosan nanoparticles

Formulation code	Amount of drug (mg/ml)	Amount of chitosan (mg)	Amount of TPP (mg/ml)	Drug+ polymer ratio
F1	10	10	0.25	1:1
F2	10	20	0.25	1:2
F3	10	30	0.25	1:3
F4	10	40	0.25	1:4
F5	10	50	0.25	1:5

Percentage practical yield

The practical yield of chitosan nanoparticles was calculated as follows:

$$\text{Percentage Yield} = \frac{\text{weight of Lyophilized nanoparticle} \times 100}{\text{Sum of initial dry weight of starting material}}$$

Entrapment efficiency (EE) of nanoparticles

The nanoparticles suspension was centrifuged at 15000 rpm for 30 min. The supernatant solution was separated. 1 ml of supernatant was distributed in 10 ml distilled water and the absorbance was measured using a UV spectrophotometer at 240 nm using water as blank. The amount of drug untrapped in the supernatant was calculated. The amount of drug entrapped was determined by subtracting amounts of free, uninterrupted drug from the total amount of ceftriaxone sodium taken [10-12].

The drug entrapment efficiency (EE) of nanoparticles was calculated as follows:

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Total amount of drug} - \text{untrapped drug} \times 100}{\text{Total amount of drug}}$$

All measurements were performed in triplicate.

In vitro drug release study

In vitro drug release study was carried out with phosphate buffered solution (PBS) medium according to Dudhani and Kosaraju [18]. The release of ceftriaxone sodium chitosan nano formulation was determined using a modified dialysis membrane diffusion technique [13-17]. An accurately weighed amount of ceftriaxone sodium chitosan nano formulation equivalent to 50 mg of ceftriaxone sodium was transferred to a plastic cylinder having the length of 6 cm and diameter of 2.5 cm fitted at its lower end with dialysis membrane (Himedia dialysis membrane with the molecular cut off of 10 kDa) pre-soaked with distilled water for 24 h. The cylinder was then suspended in the dissolution flask of a USP dissolution apparatus using 900 ml of phosphate buffer solution (pH 7.2) [19, 20]. The cylinder was allowed to rotate at a constant speed (25 rpm) at 37 °C±0.5. Aliquots were withdrawn and replaced with dissolution media and then the drug content was determined by measuring the absorbance values at 240 nm using UV spectrophotometric method at predetermined time intervals for a total period of 24 h. All the operations were carried out in triplicate. And the release of ceftriaxone sodium was calculated from the following equations:

$$\text{Release rate (\%)} = \frac{\text{Released ceftriaxone sodium} \times 100}{\text{Total ceftriaxone sodium}}$$

UV-visible spectroscopy

The UV-visible spectra were obtained from UV-visible spectrophotometer, Shimadzu UV-1800 model, Japan. All spectra were collected by recording between 200 and 800 nm and were corrected against chitosan as a background.

Zeta sizer and zeta potential analysis

The average particle size and zeta potential of the nanoparticles was determined using zeta sizer Nano series (Malvern Instrument, MAL 1037088, USA). The measurement was carried with ultra-pure water using disposable zeta cells with the general purpose protocol at 25. The instrument was calibrated routinely with 50 mV lat ex standard. The mean zeta potential was determined using phase analysis light scattering technique [22].

Scanning electron microscope analysis [23]

The morphological features were examined by field emission-scanning electron Microscopy FE-SEM), JEOL, JSM-6700F, Japan. The samples were sprinkled onto a double-sided tape and sputter coated with a 5 nm-thick gold layer [23].

FT-IR analysis

The FT-IR spectra of the samples were analyzed using a Perkin-Elmer, FT-IR spectrophotometer, USA within the range of 4000–400 cm⁻¹. About 5 mg of sample was mixed with 100 mg of KBr and compressed into pellet using a hydraulic press [24]. All spectra were corrected against the reference spectrum of KBr pellet.

Thermo gravimetric analysis

A Perkin-Elmer Model of TGA-7 thermo gravimetric system with a microprocessor driven temperature control unit and a TA data station was used. The mass of the samples was generally in the range of 2–3 mg. The sample pan was placed in the balance system equipment and the temperature was raised from 25 to 800 °C at a heating rate of 10 °C per min with the nitrogen flow rate of 50 cm³/min. The mass of the sample pan was continuously recorded as a function of temperature [25].

RESULTS AND DISCUSSION**Synthesis of ceftriaxone sodium loaded chitosan nanoparticles**

Chitosan nanoparticles were synthesized by ionic cross linking and coacervation method with a chitosan to TPP ratio of 4:1 as reported in the literature [17]. The successful loading of ceftriaxone sodium CS-NP was optimized by varying the concentrations of ceftriaxone (10, 20, 40, 80 and 100 mg) and was further confirmed by UV-visible spectrophotometry. Ceftriaxone in acetic acid solution gives major absorption bands at 259 nm indicating the presence of hydroxyl group and the lactone ring in its structure [21]. However, the UV-visible spectrum of the synthesized ceftriaxone sodium CS-NP exhibited absorption peak at 249 nm corresponds to the wavelength absorbed in Ceftriaxone sodium. This result indicates the successful loading of ceftriaxone within chitosan nanoparticles. Following the synthesis of ceftriaxone sodium CS-NP, their encapsulation and loading efficiency was calculated based on the UV-visible absorption spectral studies. As shown in table 2 the maximum encapsulation efficiency was determined by varying the concentration of ceftriaxone sodium in CS/TPP ratio (2:1). The polymer concentration of 20 mg showed high encapsulation 86.51±50.

Table 2: Entrapment efficiency of ceftriaxone sodium into CS-NP determined by UV-visible spectrophotometry

Formulation code (drug+ polymer ratio)	Entrapment efficiency
F1 (1:1)	65.59±30
F2 (1:2)	86.51±50
F3 (1:3)	66.81±40
F4 (1:4)	49.70±90
F5 (1:5)	46.42±10

Data's are expressed as mean±SEM (n=3)

Particle size and zeta potential

Particle size and surface charge are the major parameters to be focused to get effective drug delivery. fig. 1 (A) and table 3 depicts the average size distribution of the synthesized nanoparticles using Dynamic Light Scattering zeta size analysis. The average particle size of ceftriaxone sodium loaded chitosan nanoparticle is 249 nm. As shown in fig. 1 (B), the zeta potential value of ceftriaxone sodium loaded chitosan nanoparticle was 38.5 mV which indicates the

nanoparticles to be stable. To allow efficient cell interaction and promote intracellular delivery of the chitosan nanoparticulate system, a high positive zeta potential is needed [28] Zeta potential measurement is an important index in determining the stability of the nanoparticle suspension.

High absolute zeta potential value signifies high electric surface charge on the drug loaded nanoparticles, which can cause strong repellent forces among particles to prevent it from aggregation [29].

Table 3: Particle size poly dispersibility index and zeta potential of ceftriaxone sodium loaded CS-NP

Formulation code	Particle size (nm) (mean)	Poly dispersibility index (mean)	Zeta potential (mV) (mean)
F1	271	0.021	42.6
F2	249	0.012	38.5
F3	309	0.213	46.2
F4	311.6	0.417	47.5
F5	345.3	0.434	44.7

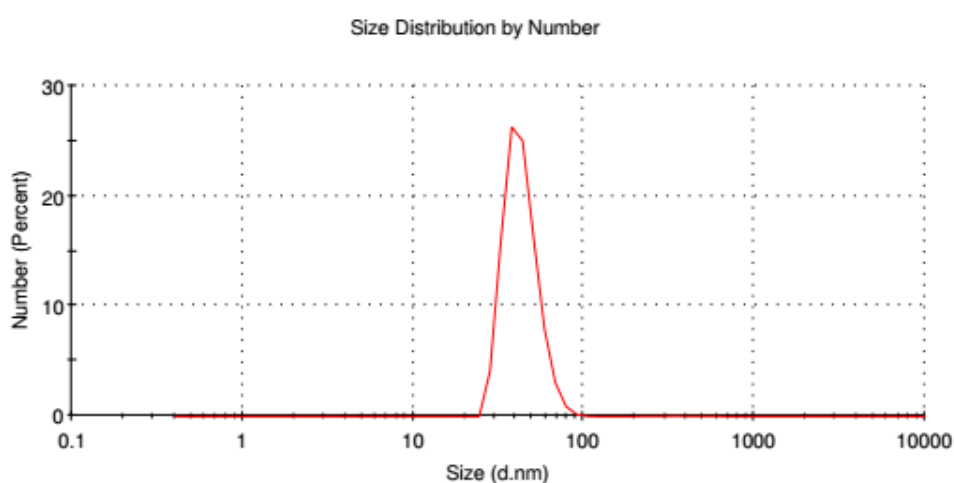


Fig. 1: (A) Particle size distribution of ceftriaxone sodium CS-NP

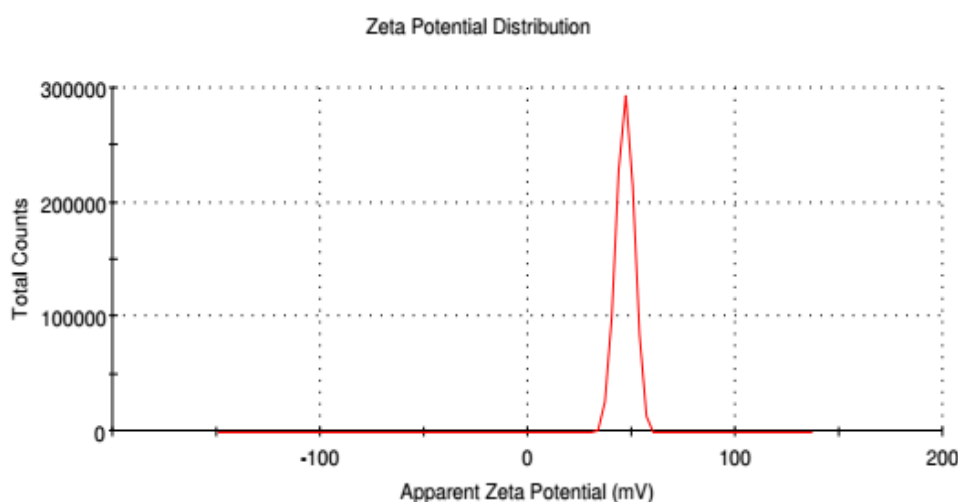


Fig. 1: (B) zeta potential of ceftriaxone sodium CS-NP

In vitro drug release study

The release profile of ceftriaxone sodium loaded nanoparticles into PBS (pH 7.4; 37 °C) are shown in table 4, fig. 2(B). The *in-vitro* drug release study was performed Drug release from nanoparticles and micro particles occur by several mechanisms including surface erosion,

disintegration, diffusion and desorption [21]. Ceftriaxone sodium loaded chitosan nanoparticles showed an initial release of 15.14% which may be accounted for the ceftriaxone adsorbed to the surface. Over 2 h, 83.03% of ceftriaxone sodium was released followed by slow release up to 24 h. The increased release in aqueous media from 15.14% to 83.03% due to the swelling property of the polymer.

Table 4: *In vitro* release profiles of ceftriaxone sodium loaded CS-NP

Time (min)	Percentage of drug release for different batches of nano formulation				
	F1	F2	F3	F4	F5
0	0	0	0	0	0
30	13.47±65	15.14±32	12.15±98	14.10±32	15.67±03
45	25.61±89	27.83±25	22.40±43	26.40±25	28.19±68
60	32.29±54	36.92±52	26.80±62	31.90±21	33.26±14
120	40.45±74	47.09±45	32.00±51	39.00±24	40.21±19
240	47.08±25	55.64±75	36.20±75	46.30±36	49.91±07
360	52.93±21	62.75±95	40.50±64	53.20±37	57.24±15
480	58.68±65	68.65±12	46.10±85	57.40±96	64.11±62
600	65.18±78	72.88±23	49.20±53	60.20±85	69.64±35
720	71.38±85	81.10±42	54.50±35	63.80±67	73.93±75
1440	72.58±25	83.08±63	57.00±43	66.80±61	77.34±42

Data's are expressed as mean±SEM (n = 3)

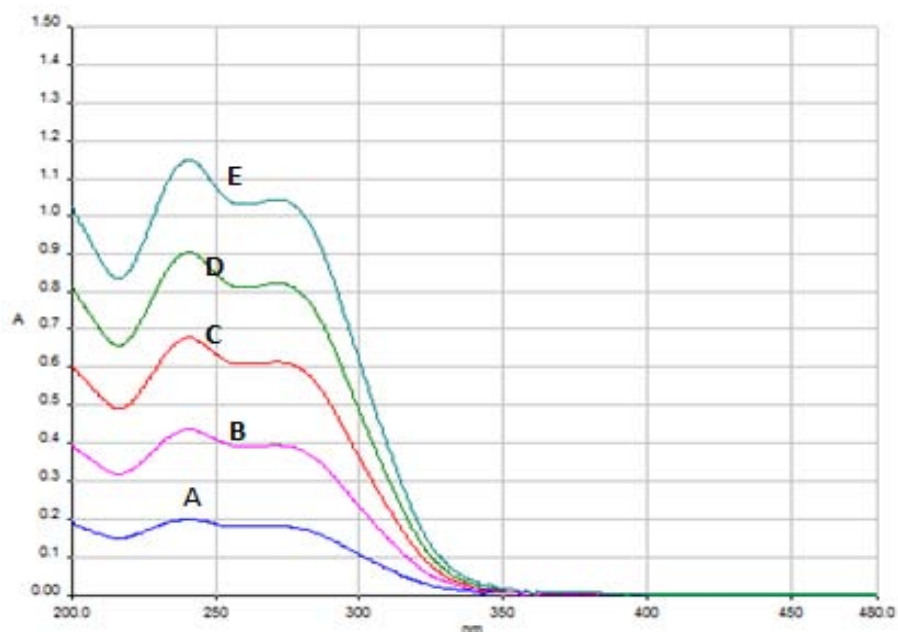


Fig. 2: (A) UV-visible spectrum of A-F1, B-F2, C-F3, D-F4, E-F5

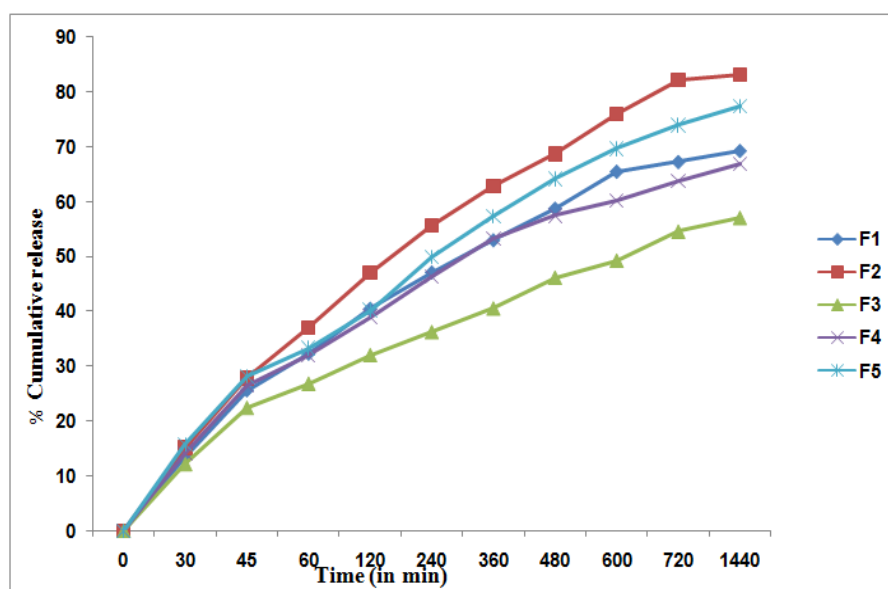


Fig. 2: (B) *In vitro* drug release profile for the formulation of ceftriaxone loaded CS-NP

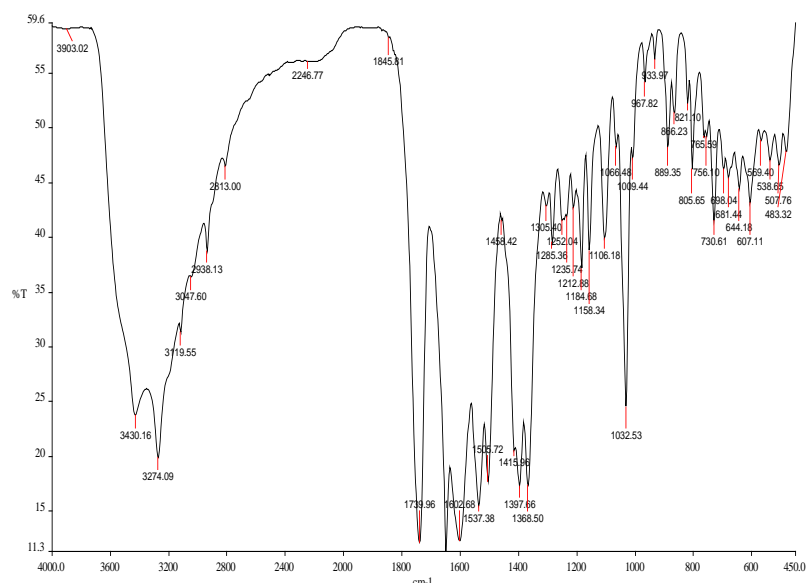


Fig. 3: FT-IR spectrum of ceftriaxone sodium loaded-CS-NP

FTIR studies for Ceftriaxone showed (fig. 3) characteristic peaks at 3432.7 cm^{-1} (N-H stretching mode of H-bonded amide group), 1741 cm^{-1} (β -lactam C=O stretching vibrations) and 1592 cm^{-1} oxime C=N stretching vibrations). The spectrum of pure chitosan exhibited an amine deformation peak at 1600 cm^{-1} and amide I carbonyl stretch at 1643 cm^{-1} (Samuels, 1981). The IR spectral

interpretation shows that the spectra obtained from the formulation matches with original spectra of drug. Similarly characteristic peaks, for the polymers were also noticed in the formulation spectrum. There was no change of any characteristic peaks which confirms that the absence of chemical interaction between the drug and polymers.

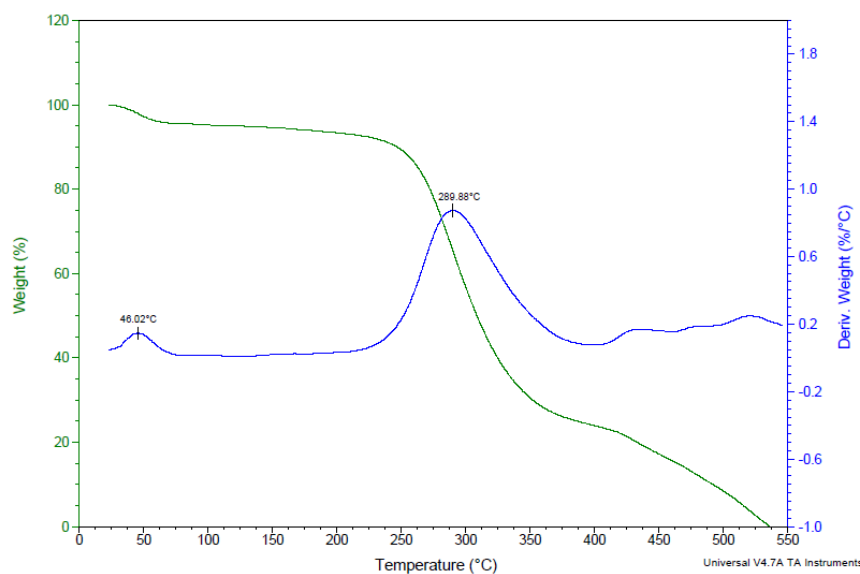


Fig. 4: Thermo gravimetric analysis of ceftriaxone loaded CS-NP

The thermogravimetric analyzer (TGA) is an essential laboratory tool used for material characterization. TGA is a technique in which, upon heating a material, its weight increases or decreases. Thermogravimetric analysis (TGA) is a simple analytical technique which is used to study the thermal stability of the sample and their weight loss at different temperatures. It also confirms the successful loading of ceftriaxone sodium within CS-NP. We observed a four step decomposition patterns on ceftriaxone sodium loaded CS-NP (fig. 4). Initially, 39% weight loss was observed at 40.02°C , due to the release of water molecules. The second step in the curve is about 75% weight loss at 289.88°C indicating the decomposition of CS and the third step was observed at 450°C with the weight loss of 19% could be due to the decomposition of both CS and ceftriaxone sodium. Finally the fourth step of decomposition was examined at 5

35°C of about 09.79% which might be due to the decomposition of pure ceftriaxone sodium. Similar decomposition pattern of ceftriaxone was also observed in solid lipid nano particle and our result correlates well with this report.

The morphology of the ceftriaxone sodium loaded chitosan nanoparticle was spherical in shape with their diameter roughly around 28 nm and was found to be aggregated. This is likely due to the fact that, as the particle size decreases they are liable to aggregate due to their high specific surface energy [24]. However, ceftriaxone sodium loaded chitosan fig. 5 shows distinct, spherical shaped particles with their average diameter around 30–250 nm This increased particle size could be due to the loading of ceftriaxone sodium chitosan nanoparticles similar results were observed in previous reports [25, 26].

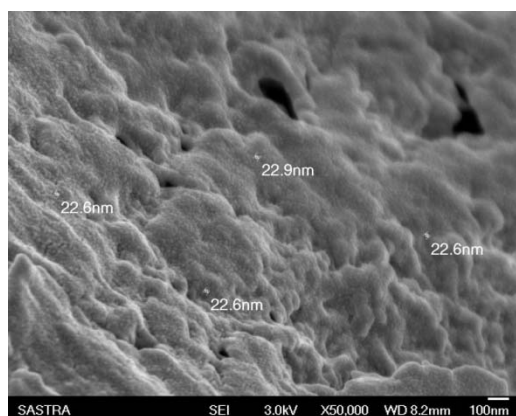


Fig. 5: SEM image of ceftriaxone sodium loaded CS-NP

CONCLUSION

We have developed a method for synthesis of ceftriaxone loaded chitosan nanoparticle using ionic cross linking method. TPP used as a cross linking agent. The final formulation ratio of drug polymer is 1;2 which act as an effective carrier with high loading efficiency. The bonding interactions between chitosan and ceftriaxone sodium were studied using FT-IR. The drug is released completely in phosphate buffer medium by 48 h at room temperature indicating sustained drug release pattern. SEM analysis revealed the spherical shaped morphology and the average particle size was 249. Finally we concluded that this method is reliable and reproducible less time consuming.

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CONFLICT OF INTERESTS

Declared none

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