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Research Article

DEVELOPMENT AND CHARACTERIZATION OF CHITOSAN NANOPARTICLES LOADED WITH AMOXYCILLIN

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ABSTRACT
This work presents result of the development and characterization of nanoparticles for entrapping Amoxicillin, an anti microbial agent. The present study was to focus on the development of amoxicillin loaded chitosan TPP nanoparticles. Chitosan concentration and drug polymer ratio in the nanoparticles influence the physicochemical characteristic such as zeta potential, poly disparity index, and average nano size diameter or percentage encapsulation efficiency of amoxicillin. An ionization method was used to entrap amoxicillin in a chitosan-matrix. The particle size analysis indicates a uniform particle size. The encapsulation efficiency decreased with increased of amoxicillin and chitosan concentration. The drug entrapment efficiency of nanoparticles having same ratio of polymer and drug was about 88%. The physical stability of nanopaticles was good as studied over a period of three weeks. The study of the release of drug from nanoparticles exhibited a prolonged release profile (79%) as studied over 28 hr. the drug release was constant showed that formulation was long term treatment. These studies showed that chitosan can complex TPP to form stable nanoparticles for amoxicillin loading, which can be useful for microbial therapy.
KEYWORDS: Chitosan, Amoxicillin, Nanoparticles, stability studies

INTRODUCTION

Chitosan has been used as a nanoparticle material owing to its versatile biodegradability, biocompatibility, and natural origin. Its hydrophilicity and solubility permit the design of nanoparticles capable of protecting the loaded drug and controlling its release[1] .Nanoparticles are defined as particles sized below 1μm and can consist out of different biodegradable materials like natural or synthetic polymer, lipid or phospholipids. The drug is dissolved, entrapped, encapsulate or attached to a nano particle matrix. Submicron particles possess very high surface volume ratios[2]. Polymer nanoparticles offer some specific advantages over liposome for instance; they increase the stability of drug and possess useful controlled release properties[3].Nanoparticulate systems for improved drug delivery are the recent advances in nano medicine. Nanoparticulate systems show their promise as a potential ideal drug delivery system for poorly soluble, poorly absorbed and labile substances[4]. In general, nano carriers may (i) protect a drug from degradation, (ii) enhance drug absorption by facilitating diffusion through epithelium, (iii) modify pharmacokinetic and drug tissue distribution profile, and/or (iv) improve intracellular penetration and distribution.

Most of nanoparticles prepared from water-insoluble polymers are involved heat, organic solvent or high shear force that can be harmful to the drug stability. Moreover, some preparation methods such as emulsion polymerization and solvent evaporation are complex and require a number of preparation steps that are more time and energy consuming. In contrast, water-soluble polymers offer mild and simple preparation methods without the use of organic solvent and high shear force. Among water-soluble polymers available, chitosan is one of the most[5]. Among water-soluble polymers available, chitosan is one of the most extensively studied. This is because chitosan possesses some ideal properties of polymeric carriers for nanoparticles such as biocompatible, biodegradable, nontoxic, and inexpensive. Furthermore, it possesses positively charge and exhibits absorption enhancing effect. These properties render chitosan a very attractive material as a drug delivery carrier. Furthermore, chitosan nanoparticles also showed to be a good adjuvant for vaccines. Therefore, the objectives of this review are to

summarize the available preparation techniques involved chitosan nanoparticles, the application of explored chitosan nanoparticles, and the mechanism of cell entry[6]. Bodmeier et al., described the mechanism of chitosan NP formation is based on electrostatic interaction between amine groups of chitosan and negatively charge group of polyamine such as tripolyphosphate[7]. Calvo et al., evaluate the size and surface charge of particles can be modified by varying the ratio of chitosan and stabilizer[8]. Maitra et al. prepared Chitosan NP by micro emulsion technique was first developed[9]. Pan et al. Prepared the insulin-loaded CS nanoparticles by ion tropic gelatin of CS with TPP anions[10]. Xu and Du studied different formulations of CS nanoparticles produced by the ionic gelatin of TPP and CS[11]. There is a growing interest in polymeric drug delivery system for efficient and potential oral administration to provide sustained release and action. Recent studies have demonstrated that the potential chitosan as a colloidal carrier of drug. Amoxicillin possesses a broad antibacterial spectrum, but exert short half-life values, which demanded frequent drug administration therefore, continuous infusion has been suggest as the most bactericidal mode of β-lactam[12], the short half- life of Amoxicillin demanded the use of controlled devices in order to reduce the number of repetitive administration. The major goal of the present work is to create a kind of new biodegradable nanoparticles for the incorporation of Amoxicillin and to evaluate their potential as a delivery system. The present study was being done to explore the potential of novel chitosan-nanoparticles as a drug delivery carrier. Therefore, amoxicillin chitosan-nanoparticles were prepared and the potential effect of encapsulation of amoxicillin was studied. An appreciable quantity of amoxicillin can be encapsulated in chitosan into stable nanoparticles.

MATERIAL AND METHODS

Amoxicillin was obtained from sunrise Pvt. Ltd., Ahmadabad, Gujarat. Chitosan was obtained from Central Institute of Fisheries Technology, Cochin. All other reagent were of analytical grade U.V.-Visible biospectrophotometer (Eli co, BL 198, Hyderabad, India), Sonicator (Sartorius AG, Lab sonic R M, Germany), Zetasizer (Malvern HAS 3000), FTIR (Shimadzu), Scanning Electron Microscope (Jeol, JSM-6480 LV, Japan) Optical microscope (Olympus, Magnus MLM, New Delhi, India), Cooling Centrifuge (Remi, C 24-BL, Vasai, India).

Preparation of chitosan nanoparticle and amoxicillin loaded nanoparticles

Chitosan nanoparticles were prepared according to the procedure first reported by calvo et. al. (1997b) based on the ionic gelatin CS with TPP cation. Chitosan was dissolved in acetic acid aqueous solution at various conc. (0.5%, 0.75%, and 1%); the concentration of acetic acid in aqueous solution was, in all case 1.5 times greater than of chitosan. Under magnetic stirrer at room temperature 20 ml. TPP aqueous solutions with various concentrations (0.5%, 0.75%, and 1.0%) was added to the 20 ml. chitosan solution respectively. Amoxicillin-chitosan nanoparticles were prepared by ionic cross-linking technique .Amoxicillin (0.5%, 0.75%, and 1%) was dispersed in distilled water and acetic acid {conc. 1.5 time greater then CS solution (20 ml.)} containing chitosan with various concentration (0.5%,0.75%,1%) stirring vigorously 30 min. at room temperature. The TPP aqueous solution with various concentrations (0.5%, 0.75%, and 1%) added drop wise to the above solution (CS Solution) under magnetic stirring for 30 min. followed by sanitation for 25 min. and the resulting chitosan nanoparticles suspension was centrifuged at 19,000rpm for 20 min. The supernatant were discarded and pellet was responded in de-ionized water followed by sanitation, centrifugation and the process was repeated three times and after drying the nanoparticles were collected. Then they were resuspended in phosphate buffer saline (PBS; pH 7.4, ionic strength 0.15 M). Finally the nanoparticles were freeze dried using 5 % glucose solution as a cry protector.

FTIR study

For the FTIR studies a specified quantity of potassium bromide and samples was blended uniformly. The resultant blend was then compressed to prepare the pellet as desired. The pellet was subjected for the analysis.

Particle size and morphological characterization:

Particle size, size distribution and zeta potential of nanoparticles were determined by scanning electron microscopy (SEM, Jeol, JSM-6480 LV, Japan). The samples were scanned, and photographs were taken.

Drug entrapment study

Yield of nanoparticles was obtained by dividing the theoretical weight of polymer and drug used by the weight of nanopaticles obtained. The encapsulation efficiency was determined as follow, an accurately weight amount of nanoparticles was dispersed in 10 ml of PBS (pH 7.4), solicited for 25 min. and centrifuged at 19000 rpm for 20 min. the absorbance of the supernatant was measured at 247 nm for amoxicillin. The amoxicillin encapsulation efficiency (A.E.E) and the amoxicillin

loading capacity (L.C.) of the nanoparticles were calculated as follows :

$$\% \text{ A.E.E} = \frac{\text{Total amoxicillin} - \text{free amoxicillin}}{\text{Total amoxicillin}} \times 100$$

$$\% \text{ L.C.} = \frac{\text{Total amoxicillin} - \text{free amoxicillin}}{\text{Nanoparticles weight}} \times 100$$

***In vitro* release of amoxicillin from nanoparticles**

In vitro release study was performed using simple diffusion cell apparatus which serve as donor and receptor compartment. After separation of free drug, the nanoparticles was transformed to a dialysis tube and subjected to dialysis with dialysis tube immersed in phosphate buffer saline pH 7.4 (100 ml). The medium was stirred by using magnetic stirrer and temperature was maintained at 35 ± 0.5 o C. At different time intervals, samples were withdrawn from receptor compartment and drug content was determined spectrophotometrically at 247 nm and equal volume of phosphate buffer saline replaced the samples that were withdrawn.

Evaluation of antimicrobial activity

For the study of the anti microbial activity the strain selected was Staphylococcus aurous. Now the strain was isolated from soil and strain appeared golden yellow colour on nutrient agar medium. The nutrient agar medium was prepared for 200ml for the culture of the strain. Now culture the strain in the sterilized nutrient agar medium in the petri plates. Each 100mg of amoxicillin loaded nanoparticle (Amo-Cs-Nano), amoxicillin (active drug) and Chitosan were placed on the pre marked area on the plates. Now the bacterial plates were incubated for 24 hours at 37°±2°C. Zone of inhibition was determined to evaluate the extent of the inhibition of the bacterial growth at the drop sites.

RESULT AND DISCUSSION

FTIR spectra of Amoxicillin Trihydrate, FTIR of physical mixture of Amoxicillin Chitosan nanoparticles and FTIR of Amoxicillin loaded Chitosan Nanoparticles were observed at 3527 cm-1, 3470 cm-1 (-OH), and amoxicillin major peak observed at 3381 cm-1 (amide NH and phenol OH stretch), 3034 cm-1 (benzene ring CH stretch), 1774 cm-1 (beta lactam CO stretch), 1685 cm-1 (amide I, CO stretch), 1519 cm-1 (benzene ring C=C stretch),1452 cm-1 (NH bend CN stretch combination band and NH3+ symmetric deformation), 1120 cm-1. When peaks were observed in amoxicillin loaded chitosan nanoparticle that 3527 cm-1,3471 cm-1,3323 cm-1, 3168 cm-1,3140 cm-1, 3141 cm-1, 1774 cm-1,1616 cm-1, 1519 cm-1, 1487 cm-1, 1452 cm-1, 1120 cm-1 comply with peak of chitosan and amoxicillin trihydrate, indicate that amoxicillin compatible with the chitosan and tripolyphosphate.

Fig. 1 represents amoxicillin-chitosan nanoparticles which were prepared according to the procedure first reported by Calvo et al. based on the ionic gelatin of CS and TPP anions. Nanoparticles as targeted carrier for amoxicillin were prepared by sodium TPP cross linking with using biocompatible and biodegradable polymer chitosan.The solubility of chitosan in acetic acid is important factor related to nanoparticles formation. The concentration of acetic acid in aqueous solution was, in all case 1.5 times that of chitosan. Suspension was observed to be milky appearance and white color. Amo-Cs-Nano have average particle size diameter of 634 nm and 816 nm with positive zeta potential at maximum and minimum chitosan concentration. In F1 (0.5%) formulation, decreases the chitosan and amoxicillin concentration decreases the nanosize particles diameter (634 nm), decrease the polydispersity index (0.070), increase decrease the zeta potential (34.2). In F2 (0.1%) formulation, higher concentration of chitosan and amoxicillin increase nanosize particles (708 nm), increase the polydispersity index (0.115), decrease the zeta potential (32.4). In F3 (1%) formulation, it was also seen that higher concentration of chitosan and amoxicillin increase nanosize particles (816 nm), increase the polydispersity index (0.119), decrease the zeta potential (29.3). From above this result observed that nanosize particles diameter, polydispersity index and zeta potential depend upon the chitosan concentration and amoxicillin concentration.

Microencapsulation efficiency of F1 formulation nanoparticles 88%, F2 formulation Nanoparticles 87%, F3 formulation nanoparticles 85%. This variation due to the chitosan concentration when increasing the chitosan concentration decrease the encapsulation efficiency due to increase the viscosity of chitosan hindered the drug encapsulation efficiency. Ionic interaction between the polymer and drug may lead to increase entrapment of the drug in nanoparticles.

The stability of the formulation during storage is of utmost important as it determines the shelf life of the formulations. The greater stability of formulation F1 over F2 and F3 may be attributed to the facts that low concentration of polymer and sodium tripolyphosphate was superior over high concentration of polymer and sodium tripolyphosphate. Table 1 shows the stability studies of nanoparticles (F1).

In vitro studies were carried with optimized formulation for their in vitro studies release pattern across cellophane membrane. The in-vitro release studies of Amoxicillin-CS-nanoparticles were shown in Table 2. Initial release of the drug is associated with those drug molecules dispersing close to the nanoparticle surface. The drug release many depend upon the chitosan concentration. Fig. 3 shown that in F1 formulation showed drug release of 79% within 28 hour give release pattern in controlled manner; F2 formulation showed drug release 66% within 28 hour give release pattern in controlled manner; F3 formulation showed drug release 62% within 28 hour give release pattern in controlled manner. In first hour F1 formulation give burst release and then give sustained release as controlled manner. One comparison of the release profile of the three formulations it was observed that release from formulation F3 was found to be slow and constant manner. From this observed data shown that increase the concentration of chitosan decreases the cumulative percentage release. It was observed that cumulative drug diffusion is in the order F1>F2>F3 Formulation gives best release pattern than other formulation.

It was observed that a time in hour is in the order Amox-CS-Nano> Amoxicillin> Chitosan. Table3 shown that within 24 hours, amoxicillin completely inhibited the Staphylococcus aureus growth. Amox-CS-Nano completely inhibited the Staphylococcus aurous growth within 36 hours. The controlled delivery of Amox-CS-Nano meant that the microorganisms were exposed to less of the drug. Thus, time required for complete inhibition was less for amoxicillin than for Amox-CS-nano because of the direct exposure of the amoxicillin to the Staphylococcus aurous

Clinical efficiency is required for any novel drug delivery system. Chitosan TPP nanoparticles herald a novel controlled targeted drug delivery, which offer several potential benefits. Chitosan nanoparticles had shown an excellent capacity for the association of amoxicillin. Amoxicillin, an antimicrobial drug, was selected as drug candidate for present study because it possesses the requisite properties necessary for formulation chitosan TPP Nanoparticles drug delivery system like rapid onset and relatively short duration of action (Half-life 1 hour), hydrophilicity. The present study was aim to develop amoxicillin loaded chitosan TPP Nanoparticles. Chitosan concentrations and drug/ polymer ratio in the nanoparticles influence the physiochemical characteristics such as zeta potential, polydispersity index, and Average nanosize diameter or percentage encapsulation efficiency of Amoxicillin. Average Nano-size diameter, Polydispersity index, zeta potential, percentage encapsulation efficiency, stability study was found to be good for optimum formulation (F1, 0.5%).

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Table-1- Stability study of Nanoparticles (F1)

Time (weeks)	% drug remaining at 4°C	% drug remaining at room temp.	% drug remaining at 45°C
Initial	100	100	100
1st week	99	99	98
2nd week	99	98	90
3rd week	98	97	83

Table-2- Cumulative % drug release from amoxillin chitosan Nanoparticles in difference formulation

Time (hours)	Cumulative % release of formulation -F1 per hour	Cumulative % release of formulation -F2 per hour	Cumulative % release of formulation -F3 per hour
1	11	8	5
2	13	10	7
3	15	12	10
4	17	14	12
5	20	17	15
6	22	20	18
16	46	40	37
18	54	45	43
20	62	51	50
24	78	60	58
26	79	63	60
28	79	66	62

Table-3- Zone of inhibition

S.No	Batch	Zone of inhibition		
		12 hours	36 hours	48 hours
1	Amo-Cs-Nano	4.65mm	9.85mm	10.75mm
2	Amoxilline(active drug)	12mm	15mm	15mm
3	Chitosan	3mm	4mm	4.5mm

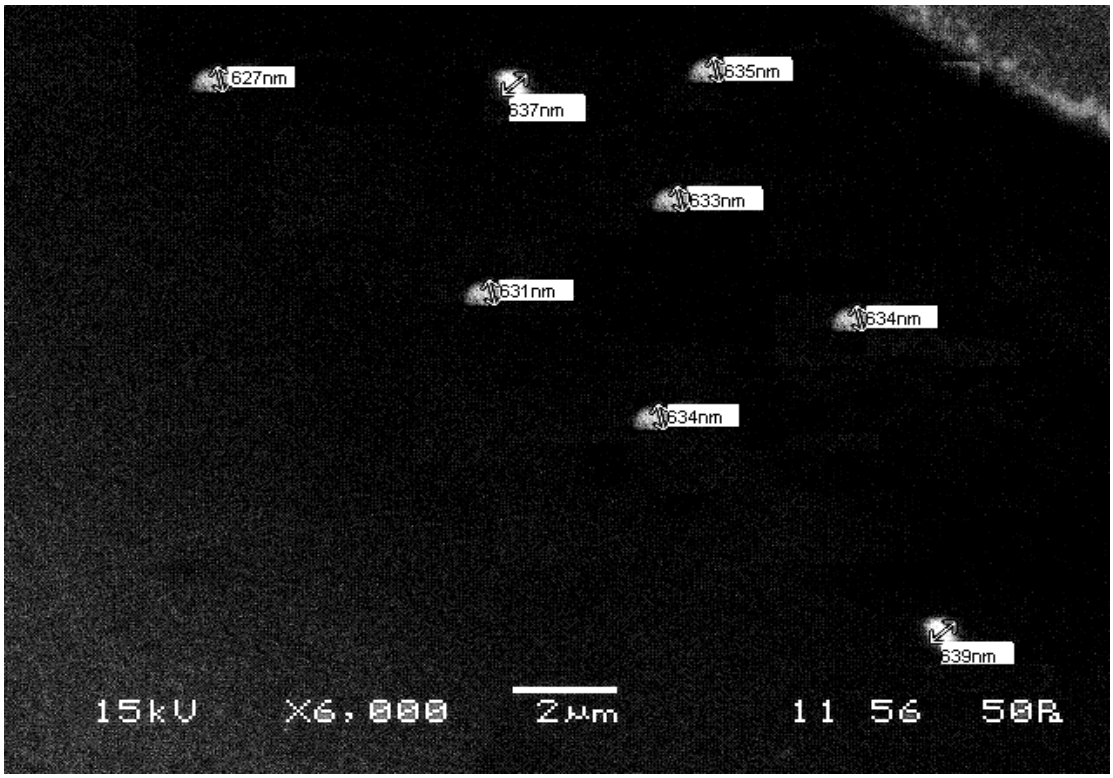
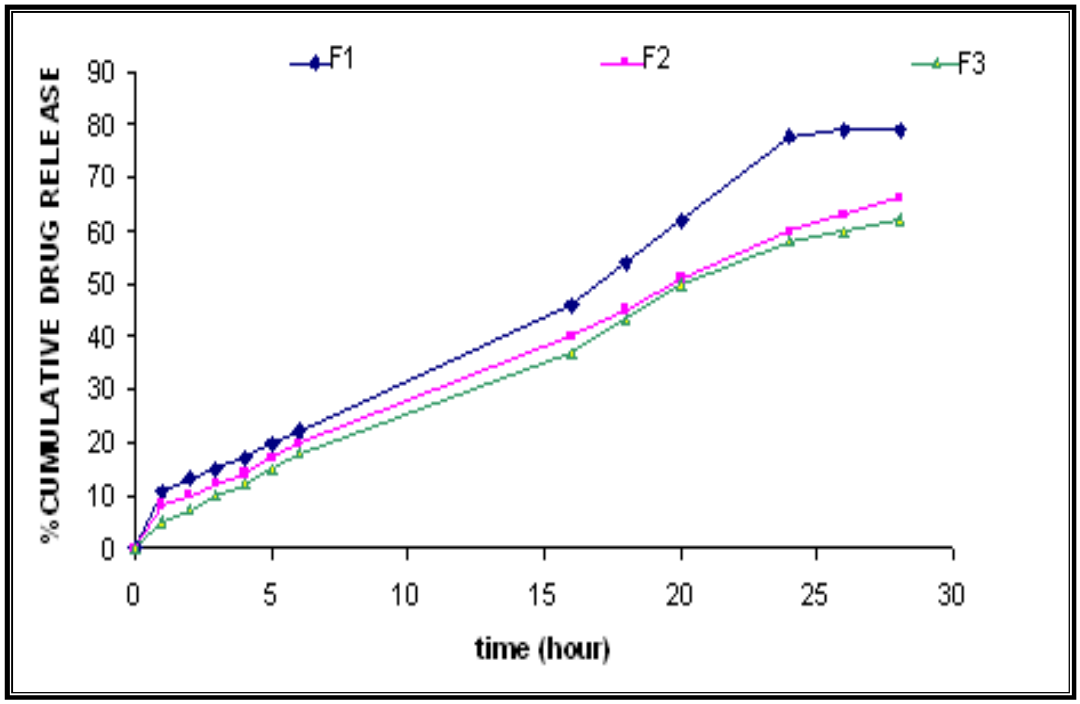


Fig.1- SEM Study of amoxicillin-chitosan nanoparticles in F1 formulation



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