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PREPARATION OF NANOPARTICLES OF CANDESARTAN CILEXETIL BY IONOTROPIC GELATION TECHNIQUE AND THEIR EVALUATION

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ABSTRACT

Candesartan cilexetil is a poorly soluble antihypertensive agent with low bioavailability. It is a prodrug which converts into active drug candesartan with hydrolysis in GIT.Ion gelation technique is utilized for delivery of protein and peptide drug and involves formation of nanoparticles by means of electrostatic interactions between the positively charged chitosan chains and polyanions employed as cross linkers. The most extensively used polyanion is the tripolyphosphate (TPP). As chitosan is soluble in acidic pH and reduction in particle size was required to enhance solubility and dissolution of candesartan cilexetil, nanoparticles of candesartan cilexetil were prepared by ionotropic gelation technique and evaluated for particle size , surface morphology, saturation solubility and multimedia dissolution. The technique was found to be effective for candesartan cilexetil with particle size in the range of 324 nm with a smooth surface. Percentage entrapment efficiency was in between 45-54%. Rise in solubility and dissolution as compared to pure drug was seen with little slow release in acidic and buffer media.

Key words: Ion gelation, candesartan, nanoparticles, solubility enhancement , increase in dissolution

INTRODUCTION

Candesartan cilexetil is a poorly soluble antihypertensive drug with low bioavailability (15%).Various methods are reported to enhance the solubility of a poorly soluble drugs including complexation[1-3] , nanoparticles[4-6] and self microemulsifying systems.[7-9] Apart from having low aqueous solubility, candesartan shows a pH dependent solubility profile (Fig.1) , so attempts were done to choose a method for preparation of nanoparticles which will release drug slowly in gastric pH in the form of nanoparticles so as to allow maximum dissolution. Chitosan is a hydrophilic polymer with positive charge that comes from weak basic groups, which give it special characteristics from the technological point of view.[10] Chitosan microspheres can be prepared by reacting chitosan with controlled amounts of multivalent anion resulting in cross linking between chitosan molecules. The cross linking may be achieved in acidic , neutral or basic environment depending on the method applied.Eventhough several techniques such as cross linking with anions, precipitation, complex coacervation, modified emulsification and ion gelation, precipitation-chemical cross linking, glutaraldehyde cross linking and thermal cross linking [11] , are reported for the formation of chitosan microparticles, principally two techniques are usually employed to obtain chitosan microparticles, in one method, chitosan chains can be chemically cross linked leading to quite stable matrixes, where the strength of covalent bonds stands out. Glutaraldehyde is broadly used as a cross linking molecule in covalent formulations. In other method chitosan hydrogels can also be obtained by ionic gelation,where micro or nanoparticles are formed by means of electrostatic interactions between the positively charged chitosan chains and polyanions employed as cross linkers.[12] The most extensively used polyanion is the tripolyphosphate (TPP). Due to the proved toxicity of glutaraldehyde and other

organic molecules used in the synthesis of gels covalently stabilized, only the second synthesis technique can be used for pharmaceutical applications. One of the most important properties of any nanogel is the extent of swelling. This means that its structure can undergo volume phase transition from swollen to collapsed state. The extent of swelling depend on several external conditions such as temperature, pH or ionic strength of the medium.
Ionic gelation method is reported to be used for the preparation of chitosan nanoparticles [13] for the delivery of proteins and peptides including insulin and also for many other drugs.[14-17]

As ion gelation method was used mainly for the delivery of proteins and peptides and no reports were available for the preparation of nanoparticles of candesartan cilexetil by ion gelation method, an attempt was tried to check the suitability of the method for the delivery of candesartan cilexetil with improved solubility and dissolution.

MATERIALS AND METHODS

Candesartan cilexetil was provided as the gift sample from Alembic research laboratory, Baroda. Chitosan was obtained as a gift sample from Marine Chemicals,Cochin. Sodium tripolyphosphate (STTP) was purchased from Otto Kemi,Mumbai. All other reagents and materials used in this research work were purchased by s.d. fine chemicals, Mumbai.

Preparation of nanoparticles using ion gelation technique: Nanoparticles were prepared using a factorial design of 2^3. Nanoparticles were prepared by the method given by Lopez leon et al.[18] Solution of chitosan in acetic acid (0.1%) was prepared in three concentrations as mentioned in Table 1 and pH of the solution was adjusted to 4 by using sodium hydroxide (10%). Solutions of STTP were also prepared in three concentrations as shown in Table 1 in distilled water. Amount of drug equivalent to 10 mg was added to 2 ml solution of STTP and drug was solubilised by adding a solution of sodium hydroxide (10%). Drug solution was added to chitosan solution dropwise with stirring on a magenetic stirrer at room temperature using a hypodermic needle. Nanoparticles were concentrated by rotating them at12000 rpm for 30 minutes and then air dried overnight. [19]
Total 9 systems were prepared.

Evaluation [20]: Prepared nanoparticles were evaluated for particle size analysis, drug content, drug loading efficiency, in-vitro release study and morphological analysis.

1. Measurement of particle size and zeta potential
Particle size was measured by using a photon correlation spectroscopy using a zetasizer.

2. Drug loading efficiency: Drug loading efficiency was calculated by analyzing amount of drug present in supernatant by UV spectroscopic analysis by using following formula-
DLE = Experimental drug content /Theoretical drug content * 100 Eq. 1

3. Percentage yield: Percentage yield was calculated using following formula-

$$\% \text{ yield} = \frac{\text{Amount in gms of nanoparticles obtained}}{\text{Total amount of drug+polymers added}} \times 100 \quad \text{Eq.2}$$

4. Drug content: Drug content was analysed by taking amount of drug equivalent to 10 mg and diluting suitably with acetonitrile and analysing the drug content by UV spectroscopic analysis at 253 nm.

5. Solubility studies: Nanoparticles containing amount of drug equivalent to 10 mg was added to vials containing 5 ml each of 0.1 N HCl, phosphate buffer pH 6.8 and water and rotated in rotary shaker for 48 hours at 37^0 C. Solutions were then centrifuged at 12000 rpm for 30 minutes and supernatant was analysed for drug content using UV spectroscopic analysis at 253 nm.

6. In vitro release study: Release was checked in all previously mentioned medias for 1 hour and subsequently for 2, 4 and 8 hours and drug content was analysed using UV spectroscopic analysis at 253 nm.

7. Morphological analysis: Surface morphology was studied by using TEM analysis.

RESULTS AND DISCUSSION

Preparation of nanoparticles using ion gelation technique: Chitosan and sodium tripolyphosphate nanoparticles were prepared by ion gelation method. The ratios of chitosan and STPP were decided based on literature survey. As it is reported that release of drug is retarded with increase in chitosan concentration, concentration of chitosan was kept below 0.1%. Total 9 systems were prepared as shown in Table 1. According to literature, with increase in drug loading particle size also increases. Amount of drug added in each batch was kept 10 mg /5 ml. Amongst all prepared systems, only systems S1, S5

and S6 were having comparative clear appearance and were chosen for further studies.

Evaluation of nanoparticles: For measurement of entrapment efficiency, supernatant was suitably diluted with methanol and analyzed at 253 nm and amount of unentrapped drug was calculated. From the value of unentraped drug , drug loading efficiency and % drug entrapment were calculated using equation 1 . Drug loading efficiency was found to vary between 24 to 35 %with system S1 showing maximum drug loading efficiency of 35.85%. Percentage yield was calculated by the formula described in experimental section in e
quation 2. Details of all evaluation parameters are summarized in Table 2. Particle size, polydispersity index and zeta potential of the given systems was calculated using photon correlation spectroscopy and electrophoretic light scattering using Delsa Nano instrument. Particle size was found to range from 209 nm-329 nm of the selected systems. It was found that lower the concentration of chitosan lower the size of particles.Details of particle size measurements and intensity distribution curves for selected systems are shown in Table 3 and figures 2,3 and 4 respectively.
From the above mentioned systems only S1 is chosen for further analysis as showing minimum particle size , acceptable polydispersity index and high drug loading efficiency. TEM image revealed smooth surfaces and formation of spherical particles as shown in figure 4.

The saturation solubility testing and multimedia dissolution study of the selected system was carried out as described in previous section of materials and methods. During saturation solubility testing at the end of 48 h , increase in solubility as compared to pure drug was found in all solvents as shown in Figure 5 and Figure 6 respectively . Solubility was found to increase more in case of phosphate buffer pH 6.8 and water as compared to 0.1 N HCl must be because of pH dependent solubility of drug as shown in Figure 1 .

During multimedia dissolution testing, it was found that dissolution was increased as compared to pure drug as shown in Figure 7 and 8 respectively. Same as that of the solubility, no marked increase was found in the dissolution of drug. In 0.1N HCl release was found to be around 8.5% at the end of 60 minutes. Even though solubility of chitosan was found to be high in acidic pH but solubility of drug in acidic pH is very low that might be the reason for low percentage cumulative release. No lag time was fond in release must be because of continuous swelling of chitosan in acidic medium. In case of water and Phosphate buffer pH 6.8 release was found to be around 14% and 5% respectively at the end of 60 minutes reason for low release in Phosphate buffer pH 6.8 must be because of slow swelling of chitosan in buffer media due to low solubility. To check maximum dissolution, study was continued up to 8 hrs. At the end of 8^{th} hour release was found to be 17%.Lag time of about 30 minutes was observed in buffer because of low solubility of chitosan. In OGD media the release was found to be around 90% at the end of one hour with not much lag time. To simulate gastrointestinal conditions and checking the effect on release of drug in Phosphate buffer pH 6.8, dissolution of drug in acidic media after one hour was continued further in phosphate buffer pH 6.8 for 8 hrs and aliquots were withdrawn at the end of 2 hrs, 4 hrs and 8 hrs and content was analyzed. Maximum release at the end of 8 hrs was found to be 36 %.

Prepared nanoparticulate systems are advantageous considerably in terms of particle size and solubility as compared to pure drug. Even though not much advantage was seen in 1 hour multimedia dissolution, in-vivo advantage might be able to be expected from extended 8 hour dissolution profile and increased saturation solubility.

CONCLUSION

Nanoparticles of candesartan cilexetil were prepared successfully by ionotropic gelation technique with decreased particle size, improved solubility and increased dissolution. The in-vivo behavior of these systems might be able to offer better advantage which is a future scope of work for this research piece.

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Table 1: Formulation design for preparation of nanoparticles by ion gelation method

Concentration of STTP ↓	Concentration of chitosan→	0.05%W/V	0.075%W/V	0.1%W/V
0.05%W/V		S1	S2	S3
0.1%W/V		S4	S5	S6
0.2%W/V		S7	S8	S9

Table 2: Evaluation of selected nanoparticle systems prepared by ion-gelation technique

Formulation code	DLE (%W/V)±SD, n=3	Particle size(nm)±SD n=50	Polydis-persity index	Zeta Pote-ntial	Percentage yield (%W/W)± SD,n=3	Drug content (%)± SD, n=3
S1	35.85	209.7	0.299	25.17	60	98.45
S5	24.62	280.8	0.261	29.34	48	99.76
S6	30.34	329.7	0.356	27.12	55	98.34

Table 3: Particle size distribution of selected nanoparticle systems prepared by ion-gelation technique

System	D(10%)	D(50%)	D(90%)
S1	24.6	75.8	209.7
S5	69.3	136.6	280.8
S6	12.8	64.4	329.7

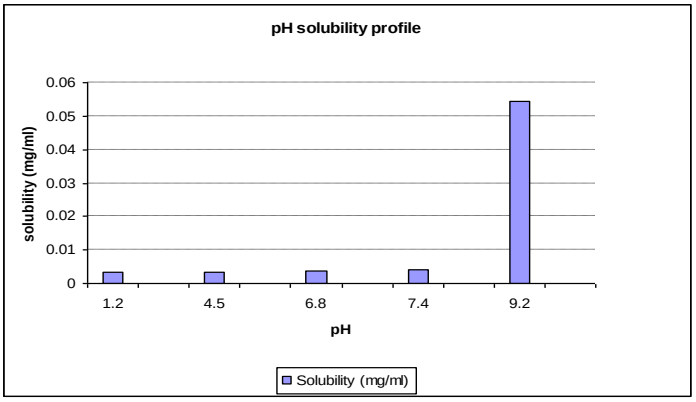

Figure 1: P^H Solubility profile of pure drug

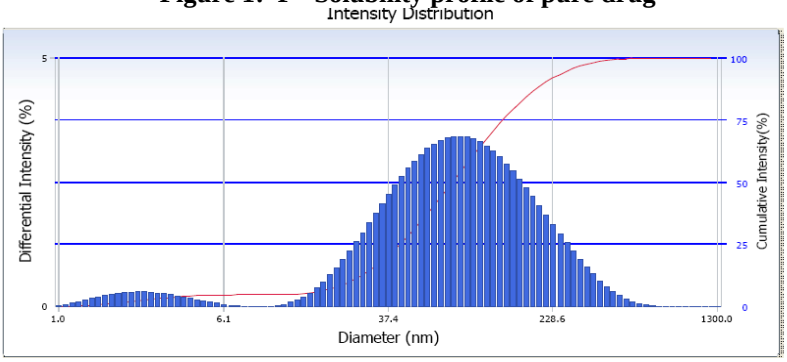

Figure2 Intensity distribution curve of S1

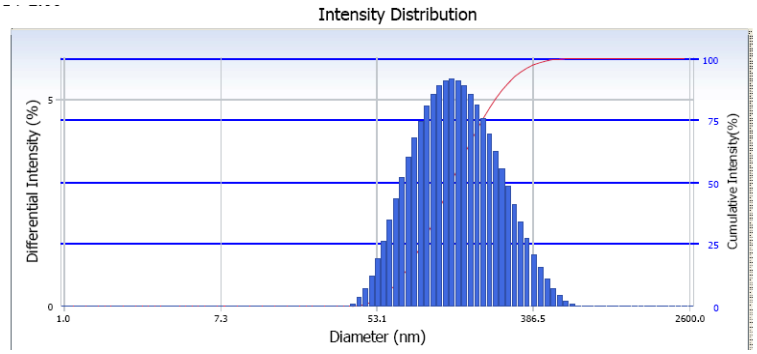

Figure 3 Intensity distribution curve of S5

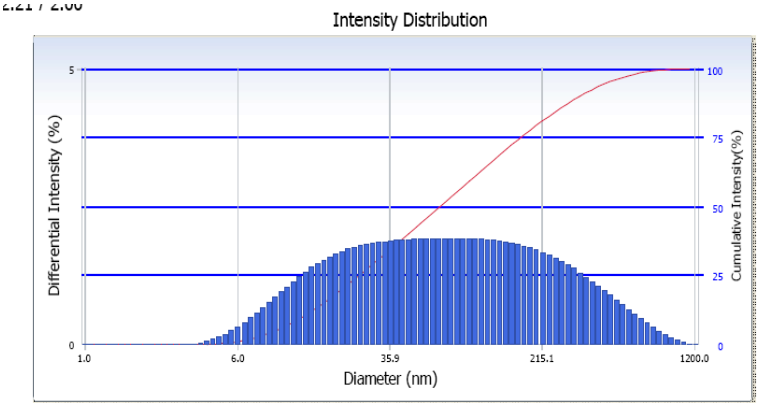

Figure 4 Intensity distribution curve of S6

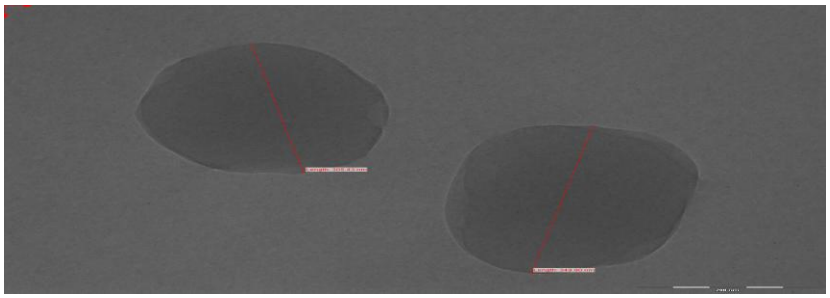

Figure 5 TEM image of Nanoparticles prepared by ion gelation technique

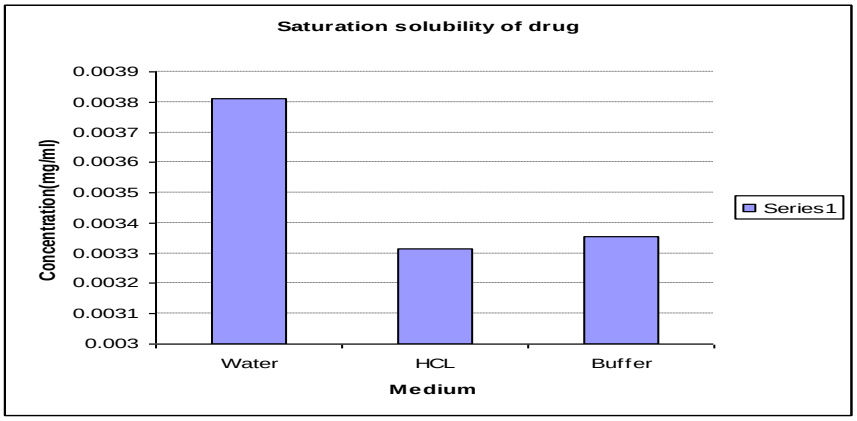

Figure 6 Saturation solubility of drug

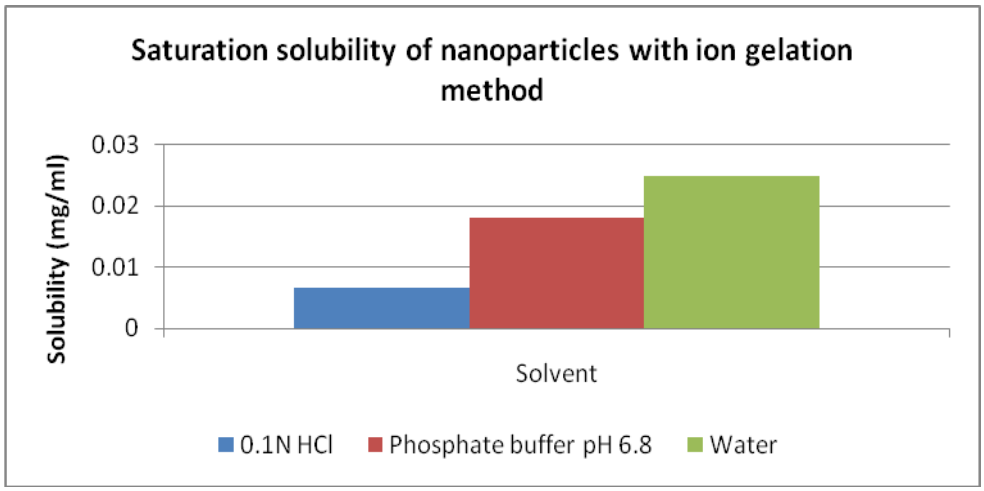

Figure 7 Saturation solubility of nanoparticles prepared by ion gelation technique

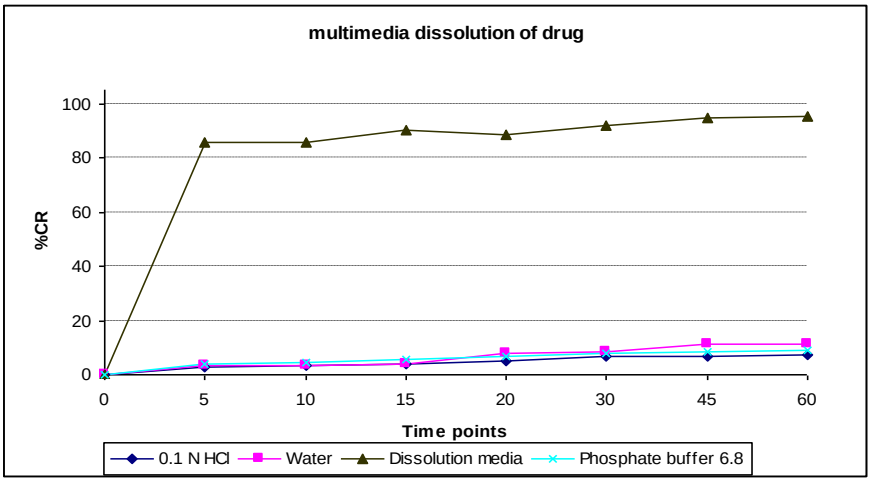

Figure 8 Multimedia dissolution of drug

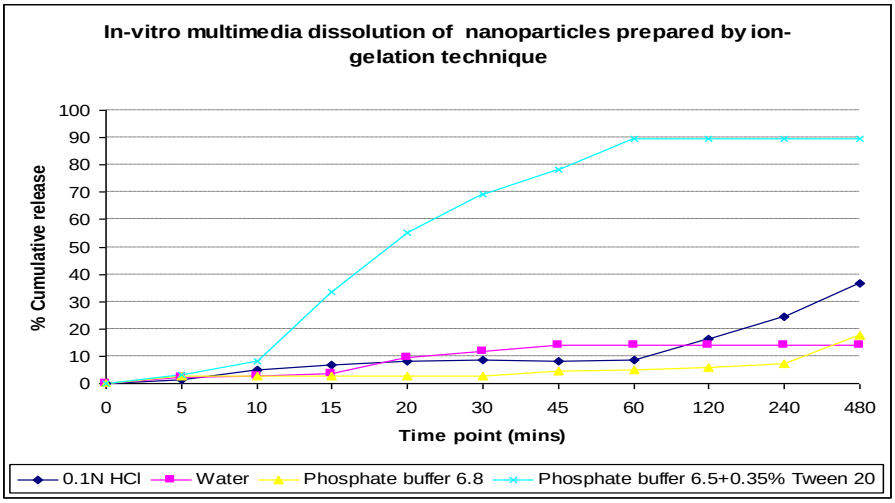

Figure 9 Multimedia dissolution of nanoparticles prepared by ion gelation technique

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