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Formulation of Natural Polymeric Nanoparticles to Overcome Barriers for the Treatment of Osteoporosis

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The present paper focuses on natural polymeric nanoparticles (NPs), described in the literature as the most effective natural polymer that have been introduced in the last decades, We have recently developed an innovative approach to this aim: a new osteotropic biomaterial was prepared, based on the conjugation of natural polymer with the bisphosphonate drug alendronate sodium (ALS). Alendronate sodium causes oesophageal side effects as ulceration. Hence, preventing the free ALS from coming in direct contact with the GI mucosa thereby reducing the possibility of side effects.
Methodology: Nanoparticles were prepared by adding drop wise aqueous solution of drug to Chitosan (CS) solutions under stirring. Above solution added to aqueous solution containing stabilizer. Mannitol as bioprotectants were used to prevent particle aggregation and to reduce mechanical stress during freezing and drying processes.
Results: The prepared ALS-loaded NPs were investigated for their physicochemical, morphological and structural characteristics by dynamic light scattering, differential scanning

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calorimetry, Entrapment efficacy and actual drug content were assessed by UV spectrophotometric method at 565 nm. *In vitro* dissolution tests performed in simulated gastro-intestinal fluids and phosphate buffer solution pH 6.8 revealed a prolonged release of ALS of 48 h. Improvement of nanoparticle stability after lyophilisation was observed in the presence of bioprotectants.
Conclusion: The ionic gelation method with low-MW chitosan was effective in achieving reproducible nanoparticles with the desired physico-chemical and safety characteristics.

Keywords: Alendronate sodium; polymeric nanoparticles; chitosan; bioprotectants; in vitro release.

1. INTRODUCTION

Osteoporosis represents a serious public health problem as all osteoporotic fractures are linked to increased morbidity and also because fractures of the hip and the vertebrae are associated with a significant mortality [1]. Conventional treatment options for the disease include the use of antiresorptive therapy or anabolic agents. Antiresorptive therapy include essentially bisphosphonates (BP), hormone replacement therapy (HRT), selective estrogen receptor modulators (SERM) and calcitonin (CT), while anabolic agents comprise parathormone (PTH) and its analogs. The goals of treatment are to prevent fracture, preserve structural bone integrity and to decrease morbidity and mortality related to fractures. All the therapies mentioned above present some bioavailability concerns. In order to overcome all of this shortcoming, many approaches were used to obtain more convenient formulations with either better oral bioavailability or providing better patient compliance such as injectable sustained drug delivery systems. Therefore, attention was paid to deliver drugs in carrier systems [2,3].

Nanoparticles represent the most appealing therapeutic nanocarrier systems by comprehensively addressing majority of the issues like stability, scalability, reproducibility, and by offering the best compromise between the efficacy and applicability. Polymeric nanoparticles have been extensively researched for their applicability as oral drug carrier systems. Factors which govern the uptake of particles from the gut include particle size, physico-chemical nature of particles, surface charge and attachment of uptake enhancers such as lectins or poloxamer [4].

The main obstacles for the oral delivery of drugs are low bioavailability and its instability problems. After oral administration of nanoparticles, they could be (i) directly eliminated in the faeces, (ii) adhering to the cells (bioadhesion) and /or, (iii) undergo oral absorption as a whole. nanoparticles results in passage across the gastrointestinal barriers and delivery of the payload into the blood, lymph and other tissues. Before this translocation can occur, the nanoparticles have to adhere to the surface of the intestine. Translocation of particles across the gastrointestinal wall can occur due to intracellular uptake by the absorptive cells of the intestine or paracellular uptake (i.e. between the cells of the intestinal wall), or phagocytic uptake by intestinal macrophages, or uptake by the M cells of the Peyer's patches [4-6].

Polymers are one of the most omnipresent classes of materials in the medical industry and in our society. Since they occur in nature, materials of both plant and animal origin are often presumed to exhibit enhanced compatibility with human hosts, the ability to exhibit bioactivity, and to undergo biodegradation. As such, natural nanomaterials and particulate materials can exhibit these characteristics in situations where synthetic materials have not met clinical expectations. In this research natural polymer chitosan used in the fabrication of drug delivery systems [7]. Chitosan (CS) based nanoparticles have attracted numerous interests because of its ability to open tight junctions (TJs) between intestinal epithelial cells and facilitate paracellular transport of drugs, TJs disruption and promote intestinal adsorption [8].

Bisphosphonates are pyrophosphate analogues able to inhibit the osteoclast resorption and are in clinical use in the treatment and prevention of bone disorders such as osteoporosis and Paget’s disease [9].

Alendronate sodium belongs to BCS class III, [10]. The most important disadvantage of ALS, however, is its low oral bioavailability (under 1%) caused by several factors such as low permeability due to its negatively charged molecules; its chelatation by Ca2+ ions resulting in non-absorbable complexes. For these reasons, recent research on ALS has been focused on increasing its bioavailability by formulation in modified release pharmaceutical

dosage forms intended for oral administration [11].

Chitosan was selected as a natural polymer for preparation of ALS loaded nanoparticles due to its literature reported high permeability and application for sustained release drug delivery systems [12]. Chitosan nanoparticles can easily be prepared by the ionic gelation method using TPP as a cross linking agent. The advantage of this method was attributed to its mild conditions achieved without applying harmful organic solvent, [13].

In the present work an attempt was made to develop Nanoparticles of ALS using chitosan by ionic gelation method. alendronate sodium suffers from the low bioavailability. Therefore, there are continued efforts to improve the pharmaceutical formulation of ALS in order to achieve an optimal therapy and ensure the desired therapeutic response of prepared nanoparticulate

2. MATERIALS AND METHODS

Alendronate sodium was a gift sample from Troikaa Pharmaceutical Pvt. Ltd, Ahmadabad, low mw Chitosan mol wt 50,000 -1,90,000 Da 75-85% purchased from leo chem. Bangalore, pluronic F 68 were obtained as gift sample from Madras Pharma, Chennai, dialysis membrane purchased from Hi-media Ltd., India vasa scientific Bangalore. Ethanol and all other reagents and chemicals used were analytical grade.

2.1 Preparation of Alendronate Sodium Loaded Chitosan Nanoparticles [14,15]

2.1.1 Nanoparticles were prepared by ionic gelation method

A Chitosan solution was obtained by dissolving low-molecular-weight Chitosan in 1% v/v acetic acid solution under stirring for 6 h. Tripolyphosphate (TPP) was dissolved in deionised water to various concentration of (0.1%, 0.2%, and 0.25%, w/v). TPP and chitosan solutions were filtered through a 0.45 µm membrane. Then, the TPP solution was added to the chitosan solution drop wise at different TPP: chitosan ratios under vigorous magnetic stirring at room temperature. The resulting suspension was then left to gellify for 30 min. Chitosan nanoparticles were prepared upon addition alendronate sodium (ALS) solution to various concentrations of Chitosan solution under gentle magnetic stirring at room temperature for 2 h. To remove the excess unreacted chitosan, the suspension was centrifuged at 15,000 rpm for 30 min, washed 3 to 4 times with deionised water and the supernatant was recovered for further analysis and finally lyophilized using mannitol as cryoprotectants. To obtain free-flowing powder. The prepared nanoparticles were stored in tightly sealed containers under refrigeration. Dried particles were redispersed and characterized in terms of size, surface charge and morphology.

2.2 Physicochemical Characterization of Nanoparticles

2.2.1 Compatibility study [16]

Compatibility of drug and polymer were analyzed using FT-IR (Fourier transform infrared) spectroscopy, Shimadzu Corporation, Japan by the potassium bromide disc method. IR spectral analysis of pure drug ALS and polymers was carried out and observation was made whether changes occurred in chemical constitution of drug after combining it with the polymers. The samples were crushed with KBr to get pellets by applying pressure on 600 Kg/cm2 and scanned with the IR instrument from 400-4000 cm-1.

DSC studies: Thermo grams were obtained by using (Netzsch Tech. DSC 200F3, Selb, Germany) at a heating rate 10℃/min over a temperature range of 35-250ºC. The sample was hermetically sealed in an aluminium crucible. Nitrogen gas was purged at a rate of 10 ml/min for maintaining inert atmosphere.

2.2.2 Entrapment efficiency (EE) [17]

The drug entrapped in the nanoparticles was estimated by dispersing the weighed amount of particles in ethanol by ultrasonication and followed by extraction of free drug into phosphate buffer pH 6.8. The extract was analysed for drug spectrophotometrically at 565 nm after suitable dilution. The drug loading efficiency (LE) and drug entrapment efficiency (EE) was calculated using Eqs. 1 and 2 respectively.

$$\text{Drug loading efficiency} = \frac{\text{Drug content in the product obtained in (mg)}}{\text{Total product weight in mg}} \times 100 \quad (1)$$

$$\text{Drug entrapment efficiency} = \frac{\text{Drug content in the product obtained in (mg)}}{\text{Total amount of drug added in mg}} \times 100 \quad (2)$$

2.2.3 Particle size and zeta potential [18]

The mean diameter of ALS nanoparticles was measured by using a laser light scattering particle size analyzer (Malvern Mastersizer Hydro 2000 SM, Malvern Instruments Ltd.,) at 25℃ with a 90° scattering angle. Milli-Q water was used as a dispersant medium. The zeta potential, average particle size and polydispersibility index were recorded for all the formulations.

2.3 Morphological Analysis

The morphology ALS -loaded nanoparticles was examined by scanning electron microscopes (Jeol JSM-840 A Tokyo, Japan).

2.4 *In vitro* Dissolution Study [19]

In vitro release was performed by using USP-I (basket type) dissolution apparatus at 37℃ ± 0.5℃ and at 50 rpm using dialysis bag (molecular weight cutoff 10-14kDa) was treated previous day prior to experiment. The dissolution media simulated gastric fluid (SGF), pH 1.2, for first two hours followed by simulated intestinal fluid (SIF), pH 6.8, for 48 h in 900 ml phosphate buffers. 10mg equivalent of drug was placed in the bag and dispersed in 1ml of medium, tied at both the ends and dipped in medium for the study and the samples were withdrawn and filtered through 0.22 µ filter and diluted, at preset time intervals and replaced by an equal volume of fresh release medium. The procedure is continued till 48 hrs. The samples are analyzed by UV spectrophotometer at 565nm. A profile showing the cumulative drug release as a function of time was plotted.

3. RESULTS AND DISCUSSION

Alendronate loaded nanoparticles were prepared by ionic gelation method, keeping amount of drug at a fixed level and varying the polymer drug ratio 1:2.5, 1:5, 1:10, 1:15, 1:20 and TPP used as a cross linking agent in different concentration 0.1, 0.2, 0.25, 0.3 to stabilize nanoparticles. TPP interact with the positive amino group of Chitosan in acetic acid solution. Different parameters influence the characters of:

3.1 Particle Size and Zeta Potential

NPs includes molecular weight of Chitosan, TPP concentration. NPs were formed at ratio 1:10:0.15 of ALS:CS:TPP NPs had the smallest particle size Table 1 shows 388 nm and PDI 0.493. Particle size is often used to characterize Nanoparticles and affects biological handling of nanoparticles. The mean size of the particle size of chitosan nanoparticles was 388 nm, Table 1 shows effect of increasing TPP concentration increase particle size, may be due to the increase in the amount of anionic groups in the preparation medium, and causes electrostatic interaction with positive amino group on chtosan. Zeta potential was found to be -18.1 which indicates high degree of stability due to inner particle repulsion but found to agglomerate after few days. This result indicates that the adhesion and transport properties of the nanoparticles can be facilitated by electrostatic attractions between the positively charged and the negatively charged cell membranes.

3.2 Entrapment Efficiency (EE) [17]

The EE slightly increased with increasing CS cross-linked TPP [i.e., 40%, 51%, and 69%, for nanoparticles (Table 1). The EE of the polar group of ALS linked to the composite might be related to hydrogen bond interactions between the NH2, COOH, OH groups of CS, TPP, and the polar groups of ALS. The Loading Capacity (LC) of the formulations was significantly influenced by the formulation parameters. The LC increased with increasing polymer concentrations and percentage of the drug.

3.3 Compatibility Study

The DSC thermogram of Alendronate sodium showed an endothermic peak at the temperature of 137.48℃. It is followed by an irregular endothermic peak with shoulders at 89.60℃ that corresponds to loss of crystal water. The DSC thermogram for the solid complex of Alendronate sodium and showed an endothermic peak at 138.34℃ associated with the formation of inclusion complex in the solid state. As such there is no interaction between ALS and chitosan. In both cases, no melting peak was observed when ALS-loaded CS nanoparticles were studied. It can be concluded that the drug is incorporated in its amorphous or disordered crystalline phase inside the nanoparticle matrix.

3.4 *In vitro* Drug Release

The drug release behavior of any polymer network depends upon the nature of the polymer, solvent compatibility, and the degree of cross-linking. However, in the case of ionic networks,

drug release behavior depends upon mass transfer limitations, ion exchange, and ionic interactions. The hydrophilic drug, the drug release of the ALS was encapsulated in the CS polymer composites at pH 1.2 and 6.8 are shown in Fig. The NPs formulations produced the release profiles with an initial burst effect in which drug release ranged between 32% and 65% within 4 h and the lowest by C3 in a time period of 48 hrs. This indicates that burst release of water soluble drug can be prevented and sustain the release of the drug. Encapsulated colloidal formulation reduces the adverse effects of alendronate sodium when administered orally. When the drug release profiles are fitted to release kinetics the slope for Peppas was in the range of 0.45 to 1.00 indicating both diffusion and erosion of polymers.

Table 1. Physicochemical characterization of nanoparticles of ALS loaded Nps

Formulation code	D:P	TPP	Particle size	PDI	% EE *
C1	1:2.5	-	493	0.681	40.76 ± 0.58
C2	1:5	0.1	409	0.467	51.65 ± 0.39
C3	1:10	0.2	388	0.493	69.87 ± 0.68
C4	1:15	0.25	556	0.769	62.35 ± 0.19
C5	1:20	0.3	879	0.831	54.35 ± 0.33

**All the tests were carried out in triplicate n=3*

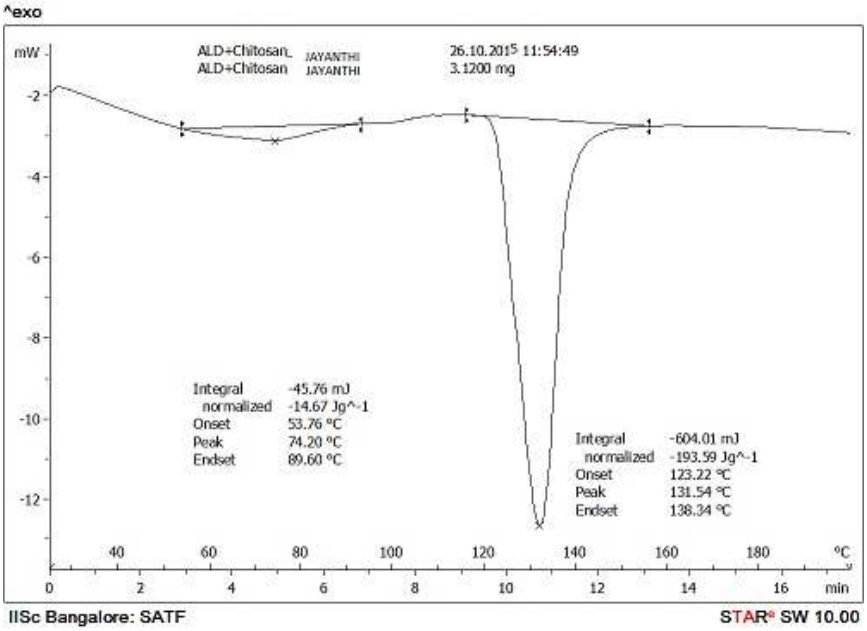

Fig. 1. DSC of physical mixture of chitosan and alendronate sodium

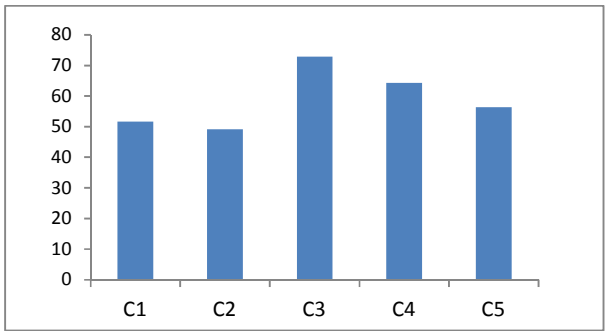

Fig. 2. % drug entrapment efficiency

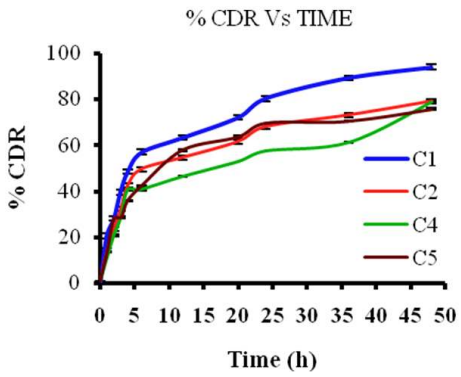

Fig. 3. *In vitro* release profile of ALS nanoparticles

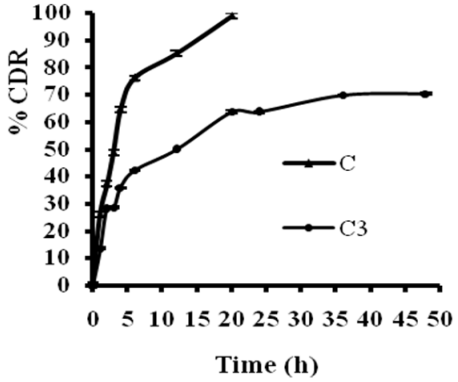

Fig. 4. *In vitro* release profile of pure drug (C) and best formula of ALS nanoparticles

It was observed that the entrapment efficiency increased with the increase in concentration of polymer in the formulations. The maximum entrapment was found in C-3 of 69.87 ± 0.68 and lowest entrapment in C1 of 40.76 ± 0.58.

4. CONCLUSION

ALS loaded nanoparticles were prepared by ionic gelation method without using organic solvents. ALS -loaded nanoparticles could be considered as a good candidate for oral delivery. It can be concluded that the formulated nanoparticulate delivery system of highly water soluble drug ALS using widely accepted and natural safe polymer chitosan was capable of exhibiting sustained release for prolonged period of time. This may improve the patient compliance by reducing adverse effects of ALS when administered orally. However other studies should be performed to improve the efficacy of these nanoparticles.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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