

DEVELOPMENT, CHARACTERIZATION AND *IN VITRO* RELEASE KINETIC STUDIES OF IBANDRONATE LOADED CHITOSAN NANOPARTICLES FOR EFFECTIVE MANAGEMENT OF OSTEOPOROSIS

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ABSTRACT

Objective: The objective of the present study was to develop, optimize, and evaluate Ibandronate-sodium loaded chitosan nanoparticles (Ib-CS NPs) to treat osteoporosis.

Methods: NPs were prepared by the Ionic gelation method and optimized for various parameters such as the effect of concentration of chitosan, sodium triphosphate (TPP), and pH effect on particle size polydispersity index (PDI), zeta potential, and entrapment efficiency. The prepared nanoparticles were characterized using particle size analyzer (DLS), transmission electron microscopy (TEM), scanning electron microscopy (SEM), and Fourier-Transform Infrared spectroscopy (FTIR).

Results: Formulated NPs were obtained in the average nano size in the range below 200 nm in TEM, SEM, and DLS studies. The particle size and encapsulation efficiency of the optimized formulation were 176.1 nm and 63.28%, respectively. The release profile of NPs was depended on the dissolution medium and followed the First-order release kinetics.

Conclusion: Bisphosphonates are the most commonly prescribed drugs for treating osteoporosis in the US and many other countries, including India. Ibandronate is a widely used anti-osteoporosis drug, exhibits a strong inhibitory effect on bone resorption performed by osteoclast cells. Our results indicated that Ibandronate sodium-loaded chitosan nanoparticles provide an effective medication for the treatment of osteoporosis.

Keywords: Bisphosphonate, Bone mineral density (BMD), Ion gelation, Nanoparticle, Osteoporosis, Release kinetics

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INTRODUCTION

Osteoporosis is a skeletal-related disease with low BMD (Bone Mineral Density). The deterioration of bone tissue can lead to bone fragility and fracture, especially of the hip, spine, shoulder, and wrist. Osteoporosis is generally caused due decreasing bone mineral density (BMD). According to World Health Organization (WHO), one-third of adult women and one in five men will sustain one or more osteoporotic fractures in their lifetime. Bones become porous and increase the high risk of fractures. The most affected bones are the hip, spine, shoulder, and wrist bones. The main cause of osteoporosis is decreasing bone density. This disease is commonly seen in old age in both genders, but women have a high risk after menopause; it is called PMO (Post-Menopausal Osteoporosis) [1, 2].

Various drugs are available to treat osteoporosis, the current treatment of osteoporosis includes bisphosphonates, Denosumab, calcitonin, selective estrogen receptor modulators (SERM), parathyroid hormone (PTH), and sufficient intake of calcium and vitamin D. Novel osteoclast targeted agents like c-src kinase and cathepsin K are under clinical development [3, 4]. Bisphosphonates are the most commonly used drugs for the treatment of osteoporosis due to the positive results of their clinical studies. The efficacy of BP's for reducing the risks of osteoporosis has been established in large clinical trials. BP's are the most potent active drugs among all available drugs for the treatment of osteoporosis. They are chemically derived from pyrophosphates that inhibit the precipitation of CaCO₃. Bisphosphonates bind the bone surface and slow down bone resorption of Osteoclast cells. The balance between osteoclast and osteoblast cells stopped the bone loss and improved bone strength, which is the basic action of Bisphosphonate class of drugs [5, 6].

Ibandronate-sodium is a BCS class III bisphosphonate, used to treat Post-Menopausal Osteoporosis (PMO) and acts as a potent, specific inhibitor of osteoclast-mediated bone resorption. It is a potent,

nitrogen-containing bisphosphonate in novel clinical development exploring its potential to be administered less frequently than at weekly intervals. It can be administered either orally or as an intravenous (i. v.) injection and the dosage interval is about two months [7, 8].

Ibandronate-sodium is nitrogen-containing bisphosphonate and its IUPAC name is 3-(N-methyl-N-pentyl) amino-1-hydroxypropane-1,1-diphosphonic acid, sodium salt, monohydrate with the molecular formula (C₉H₂₂NO₇P₂Na. H₂O) and molecular weight (319.23 g/mol). It inhibits bone resorption and is also helpful in treating Paget's disease, postmenopausal osteoporosis, and corticosteroid-induced osteoporosis metastatic bone disease. It decreases the high rate of bone mass in postmenopausal women, leading to a net gain in bone mass [9, 10].

Generally, patients suffering from bone diseases preferred oral formulations to the intravenous route. Bisphosphonate tablet causes pharyngeal and gastrointestinal tract ulceration and oral bioavailability of bisphosphonate drugs is very low and variable. The gastrointestinal tract poorly absorbs the oral ibandronate, and bioavailability in humans is around 0.63%. About 40%–60% of the absorbed dose is tightly bound to the bone surface, and the remaining absorbed Ibandronate is excreted unchanged through the kidneys. Low bioavailability and adverse effects in the gastrointestinal tract are the main limitations in the efficacy of oral formulation of any drug, so an efficient formulation is needed to overcome these limitations. Many steps have been taken to develop weekly and monthly dosages of Ibandronate to improve the therapeutic efficacy. To improve the oral bioavailability of many drugs, polymeric nanoformulation and nanoencapsulation have been proved a promising approach in previous studies [11, 12].

The most frequently applied nanotechnology-based strategies in the development of delivery systems are polymeric nanoparticles (NPs), solid lipid NPs, liposomes, nanoemulsions, nanosuspension, micelles,

etc., which provide controlled, sustained, and targeted drug delivery. The NPs based delivery systems present an effective approach for enhancing saturation solubility, absorption rate, and oral bioavailability [13-15].

Chitosan is an important polymeric carrier for many drugs because it has specific properties such as polycationic nature, biodegradability, biocompatibility, and non-toxic nature. Chitosan is a natural polysaccharide and the most important derivative of chitin. It is prepared by removing the acetate moiety from chitin is known as "Deacetylation of chitin". The electrostatic interaction between the amine group of chitosan and a negatively charged group of polyanion such as sodium tripolyphosphate (TPP) is the basic mechanism of the formulation of CS NPs. In many previous studies, it is reported that different drugs loaded CS nanoparticulate formulations are stable, permeable, and therapeutically active [16-18].

Many natural and synthetic polymers were used for the formulation of drug-loaded nanoparticles, i.e., alginate, cyclodextrin, chitosan, PLGA, etc. However, chitosan has been used in both the medical and pharmaceutical fields because of its specific properties like natural carbohydrate, biodegradable, biocompatible, non-toxic, and easily abundant in nature [19, 20].

Many cross-linking agents like glutaraldehyde, glyoxal, and ethylene glycol, etc., have been used for the preparation of CS nanoparticles, but TPP is preferred to all of them due to its non-toxic effect [21]. The interaction of chitosan with sodium tripolyphosphate (TPP) forms biocompatible cross-linked chitosan nanoparticles, which are efficiently used in drug delivery. The hydrophilicity of cross-linked chitosan, cross-linking density, its antioxidant and antimicrobial activities can allow its use for drug release studies [22-24].

The authors have developed Ibandronate sodium-loaded Chitosan NPs for better bioavailability and acceptability of drug to the biological systems in the present study. Drug delivery systems are designed for promoting the therapeutic effect of a drug and minimizing its toxic side effects, which is achieved by different process variables. The drug-loaded NPs were prepared by the ionotropic gelation method using sodium tripolyphosphate (TPP) as crosslinking agent [25-27]. The effect of Chitosan (CS) concentration, TPP concentration, and stirring speed on the drug entrapment efficiency and particle size was evaluated. Different mathematical models were used for drug release kinetics study of formulated drug-loaded NP [28-30].

MATERIALS AND METHODS

Materials

The drug Ibandronate-sodium (M. W. 319.23 g/mol) was purchased from Sigma Aldrich. CS with medium molecular weight (M. W. =750 000 Da) was purchased from Himedia (India). Methanol and sodium hydroxide were purchased from Merck Limited (Mumbai, India). Dialysis membrane was purchased from Himedia (India). High purity water was used for all experiments, prepared by using (Millipore). All other chemicals and reagents were of analytical grade.

Methods

Preparation of Ibandronate sodium-loaded chitosan nanoparticles (Ib-CS NPs)

The Ib-CS NPs were prepared by the Ionotropic gelation method [31-35]. CS-NPs were prepared by the previously reported method with some modifications. The formation of NPs resulted from the ionic interaction between the positively charged amino groups of CS and negatively charged TPP. For this purpose, CS was dissolved in (2% w/v) acetic acid solution and stirred well up to complete dissolution, then filter with 0.2 μ filter paper. PH was adjusted up to 4.8-5.0 by adding 0.1 M NaOH solution. The CS-NPs were prepared by the drop-wise addition of TPP solution to chitosan solution at room temperature on magnetic stirring for 3-4 h. A fixed amount of drug (10 mg) was added to the CS solution after adjusting the pH, and Pluronic F-68 was added as a surfactant. The prepared NPs were analyzed by transmission electron microscopy (TEM), SEM, and Dynamic light scattering (DLS) for particle size and surface morphology. The optimized nanoparticulate suspension was

centrifuged and freeze-dried using a lyophilizer (Decibel Digital Technologies, India). The supernatant was analyzed by UV spectrometer to calculate the entrapment efficiency (%).

Entrapment efficiency (EE) %

The Entrapment efficiency (EE) of Ib-CS NPs was determined by the centrifugation method [36]. The nanoparticles were centrifuged, and the pellet of NPs was collected, and the supernatant was separated. The amount of untrapped drug in the supernatant was determined at 238 nm using a UV-Visible spectrophotometer (U-1800, Hitachi) after proper dilution. The percentage entrapment efficiency (% EE) was calculated by using the following formula.

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount of drug in nanoparticles}}{\text{Amount of drug used initially}} \times 100$$

Characterization of Ib-CS NPs

Particle size, poly dispersive index (PDI), and zeta potential

Particle size, Poly dispersive index (PDI) and Zeta Potential (ZP) of formulated Ibandronate loaded CS NPs were determined through dynamic light scattering analysis (DLS) with Malvern Zetasizer Nano S (Malvern, UK).

Surface morphology

The surface morphology of the prepared formulation was carried out using Scanning Electron Microscope (SEM), Nanosem, Quantum 200E Instrument. The prepared sample was examined by Transmission Electron Microscope (TEM), Morgagni 268D TEM, Boston, MA.

Fourier transform infrared (FTIR) studies

The interaction between drug and polymer was identified from the Fourier transform-infrared, Attenuated total reflection FTIR (ATR-FTIR, Bruker Tensor-37) studies. The FTIR spectrum of pure drug Ibandronate, polymer (chitosan), and Ibandronate loaded CS NPs were obtained. The samples were prepared by grinding with anhydrous KBr powder and compressed into pellets. The FTIR spectra of drug and drug-loaded NPs were measured over the range of 4000-400 cm^{-1} .

Drug release kinetic studies

The drug release of NPs was studied using the dialysis bag method [37, 38]. The membrane with a pore size of 2.4 nm and molecular weight cut-off between 12,000 and 14,000 in phosphate buffer saline (PBS) pH 6.8 at 37 \pm 2 $^{\circ}$ C was used. The drug-loaded NPs were placed into a dialysis membrane, tied at both ends, and placed in a beaker containing 100 ml of diffusion medium (PBS pH 6.8). Temperature and speed were maintained at 37 \pm 2 $^{\circ}$ C and 100 rpm, respectively, using a magnetic stirrer. Aliquot samples were withdrawn at predetermined time intervals, and the same volume was replaced with fresh buffer to maintain the sink condition. The amount of drug released was analyzed spectrophotometrically at 238 nm for the Ibandronate drug. Cumulative percentage release was calculated from the amount of drug release. The release kinetics was determined by some mathematical kinetic equations such as zero order, first order, Higuchi's model, and Korsmeyer-Peppas model. Values of R² and K were calculated from the linear curve obtained by regression analysis of the plots.

RESULTS AND DISCUSSION

Preparation of Ibandronate loaded chitosan nanoparticles (Ib-CS NPs)

In this study, Ib-CS NPs were prepared by ionotropic gelation method using Chitosan (CS), TPP, and surfactant (poloxamer). Different ratio of chitosan and TPP (CS: TPP) were used to prepare the optimized formulation i.e. 2:1, 1:1, 0.5:1, 1:2 and 1:0.5. The results of particle size analysis and the potential study revealed the best result of the 1:0.5 ratios of chitosan and TPP. Change in the concentration of CS has shown significant change in the entrapment efficiency. It is due to the increasing viscosity and ionic gel formation at high CS levels that resist the diffusion of the drug into the external phase.

The optimized formulation of Ib-CS NPs was selected based on the minimum value of particle size and the maximum value of entrapment efficiency. The Optimized formulation has shown a minimum particle size of 176.1 nm and maximum drug entrapment

efficiency of 63.28%. These results showed the best condition for preparing the optimized formulation of Ib-CS NPs. The above-optimized formulation was considered for further studies i.e., characterization and *in vitro* drug release kinetic studies.

Characterization

Fourier transform infrared spectroscopy (FTIR) measurements

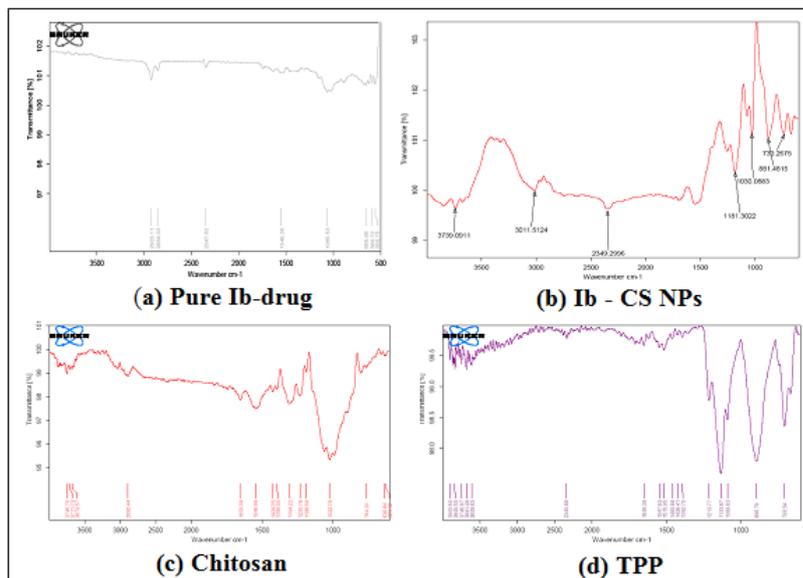


Fig. 1: (a-d) Elaborating FTIR analysis of individual drug, polymer, and drug along with synthesized Ib-CSNs, (mean±SD, n=3)

The FTIR spectra of the Ibandronate drug, polymer (chitosan), TPP, and Ibandronate loaded chitosan nanoparticles (Ib-CS NPs) are shown in fig. 1 (a-d). The Ibandronate showed peaks at 2923.11 cm^{-1} (N-H group stretching), 1546.36 cm^{-1} (-CH group stretching), and 1065.53 cm^{-1} due to C-C group stretching, respectively [39, 40]. Chitosan showed a well-defined peak at 2890 cm^{-1} due to C-H

stretching, 1546 cm^{-1} for N-H bending, and 1304.22 for C-N stretching. TPP showed the featured peaks at 1213.77 cm^{-1} for P=O Stretching, 1133.87 cm^{-1} due to O-P=O Vibrations, and 896.79 cm^{-1} for Stretching vibration of the P-O-P bridge. The FTIR spectra of Ib-CS NPs showed separately identified peaks. It reveals that there was no chemical interaction between the drug and polymers.

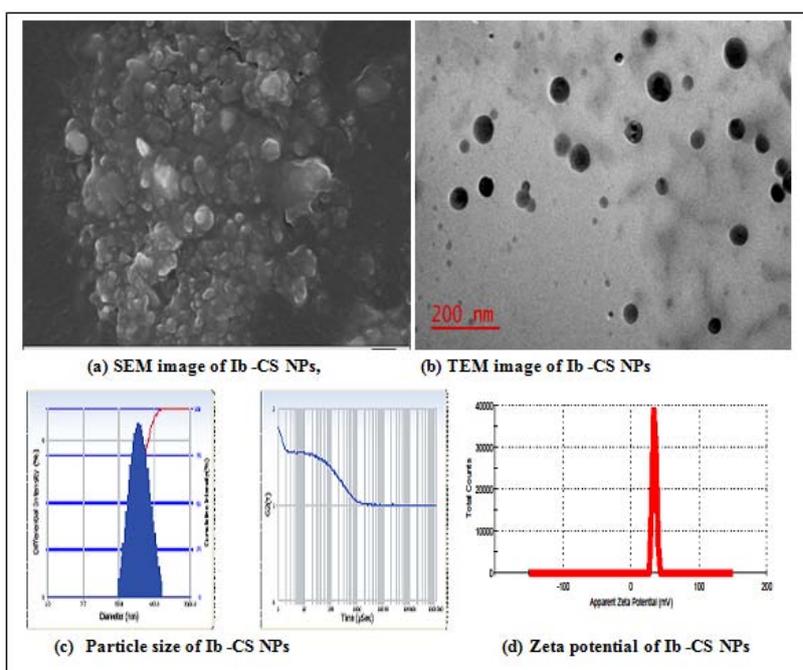


Fig. 2: Results of physico chemical characterizations (a) SEM image (b) TEM image (c) Particle size (d) Zeta potential

Nanoparticle size, PDI, and zeta potential

Ib-CS NPs were prepared by the Ionotropic gelation method. This method was used to prepare the desired NPs and is considered to be adequate nanocarriers for the encapsulation of drugs due to its easy methodology, cost-effectiveness, and non-toxic nature, and formulated NPs were characterized by SEM, TEM and DLS studies for particle size, shape and morphology determination [41]. The prepared Ib-CS NPs were found in the nano range, and their spherical shapes were confirmed using SEM and TEM in fig. 2 (a and b). The particle size and Zeta potential were analyzed by Malvern Nano Zetasizer as shown in fig. 2 (c and d).

The morphology of nanoparticles was analyzed by using SEM. The Scanning Electron Microscopic studies show the particle size in the nano range. The particle size of optimized CS-NPs was also characterized with TEM, and the result was found to be in the range below 200 nm. TEM image showed completely spherical and symmetrical nanoparticles were formed in the optimized formulation.

Based on the results of particle size and entrapment efficiency, the optimized formulation showed the lesser particle size (176.1 nm) and entrapment efficiency (63.28%), and the Poly dispersive index (PDI) was 0.269. The zeta potential of the optimized formulation was found to be +34.2 mV. Based on results found in the optimization studies, the best ratio between CS and TPP (1:0.5) was found optimum in which the particle size was found in the nano range (below 200 nm).

Drug release kinetic study

When any new solid dosage drug form is formulated, it is necessary to ensure that the new dosage form is showing dissolution in the appropriate right manner or not. Drug dissolution/release study is

the main focused parameter of the Pharmaceutical industries for any novel drug. At present, it is easy to obtain the correct values for the quantitative analysis of drug release of any dosage form by using suitable mathematical formulas [42, 43]. Kinetic models correlate the amount of dissolved drug (C) from a solid dosage form as a function of test time "t" or

$$C=f(t)$$

The quantitative analysis of dissolution/release rates becomes easier by using some specific mathematical models. When mathematical formulae are used to describe the dissolution process known as "Drug release kinetics" [44]. These mathematical models can help to optimize the new dosage of nano-drug formulations to yield information on the efficacy of various release models.

In vitro drug release studies were carried out using the dialysis bag method for different mathematical kinetic models. For the kinetic study of formulated drug NPs, the plots were made in the following way:

- (i) Zero-order kinetic model (cumulative % drug release vs time)
- (ii) First-order kinetic model (log of cumulative % drug remaining vs time)
- (iii) Higuchi model cumulative (% drug release vs square root of time) and
- (iv) Korsmeyer–Peppas model (log cumulative % drug release vs log time)

Plots of the above-mentioned models are shown in fig. (3), and the results are summarized in table (1), where "R²" is the correlation value, "K" is the rate constant and "n" is the release exponent.

Table 1: Interpretation of R² values and rate constants (K) of release kinetics of Ib-CS NPs

Kinetic models	Correlation value (R ²)	Rate constant (K)	Release exponent (n)
Zero-order	0.997	4.013	-----
First-order	0.9488	0.914×10 ⁻¹	-----
Higuchi model	0.9656	0.127×10 ⁻¹	-----
Korsmeyer–Peppas	0.946	1.269×10 ⁻¹	0.52

Based on the above values of (R²), the best fit kinetic model with the highest correlation value is the "Zero-order model". It is concluded that the optimized formulated NPs follow Zero-order kinetics. In the

Korsmeyer-Peppas model, release exponent value "n" is 0.52. The magnitude is in the range (0.45<n<0.89) indicates the release mechanism is non-Fickian diffusion.

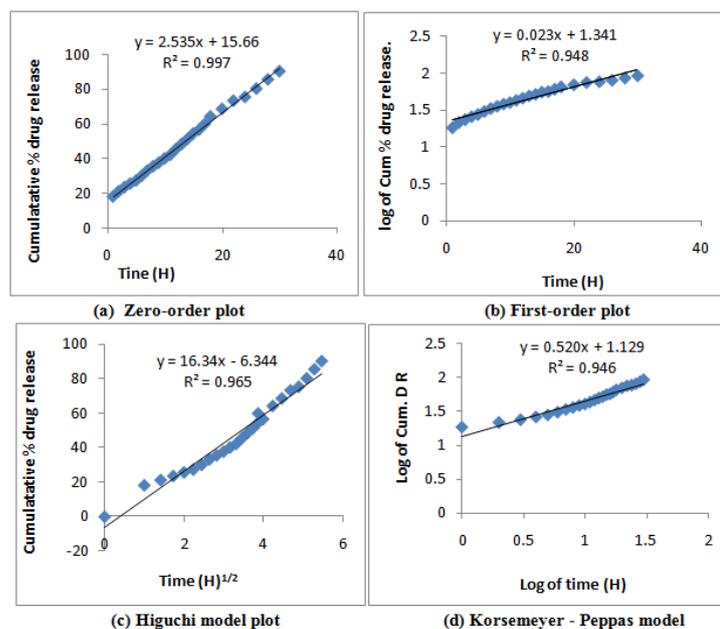


Fig. 3: Plot showing the *in vitro* drug release kinetics as per the various mathematical models (a) Zero-order plot (b) First-order plot (c) Higuchi plot (d) Korsmeyer–peppas plot. Data represents mean±SD (n=3)

CONCLUSION

Osteoporosis is the second common worldwide disease after cardiovascular disease. Bisphosphonates are the most effective treatment option for Osteoporosis, but there is increasing concern about their long-term safety. The major challenge in the formulation development is the poor aqueous solubility of existing drug molecules. The conventional approaches of the formulation are difficult because of many pharmacological or therapeutically performance issues. Medications with novel mechanisms and novel drugs like drug-loaded polymeric NPs can be expected to treat Osteoporosis in the future. The NPs provide a promising approach for enhancing solubility and oral bioavailability of drugs molecules. The results of the current study revealed that stable NPs were formulated using ionic gelation technique, and optimized formulation has shown minimal particle size and maximum entrapment efficiency. The formulation of Ib-CS NPs could be an effective strategy for enhancing the oral bioavailability of Ibandronate and would help us to find a new approach for pharmacokinetics and pharmacodynamics studies. Nanoformulations are an ideal platform to design combinatorial drug therapies that stimulate or inhibit specific pathways in targeted tissues. Nanotechnology is useful in enhancing a more specific target delivery, preventing drug absorption in other organs as well as for a continuous slow release of orthopaedic drug concentration for a long-term effect of bone regeneration.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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