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ORIGINAL ARTICLE

Risedronate functionalized Chitosan based Polymeric Nanoparticles Formulation to in-vitro release kinetic study

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Abstract

The current study was focused on development and characterization of novel formulation of Risedronate-Sodium (RS) loaded chitosan (CS) nanoparticles (NPs) for the effective management of a common bone disease Osteoporosis.

In the present study the RS-CS NPs were prepared by the **Ionic gelation** method with some modifications and optimized for various process variables and characterized for particle size distribution, zeta potential (ZP), entrapment efficiency (EE %) and release kinetics study. The results indicated the NPs with the value of ZP to be **+12.9 mV**; average particle size was **190.4 nm.** The EE % was **68.53 ±1.05 %**. SEM study showed agglomeration of NPs due to the gelation. The in vitro release study of drug for 48 hours indicated the sustained release with **74.63%** cumulative drug release. Formulated RS-CS NPs showed the promising approach to increase oral bioavailability and can reduces the GI tract side effects.

Key words: Bone Mineral Density (BMD), Bisphosphonates, Drug nanoparticles, Osteoporosis, Release kinetics, Risedronate- sodium

INTRODUCTION

Osteoporosis is a Progressive skeletal related disease diagnosed by low bone density and deterioration of bone tissues, with a consequent increase in bone fragility and susceptibility to fracture. Osteoporosis is called a "**Silent disease**" because its symptoms can not identify at early stage and fracture occurs. The fractures caused by osteoporosis have a great impact on public health [1, 2]. Currently most drugs available in the markets decrease bone loss by inhibiting bone resorption, but the novel therapies may increase bone mass by directly increasing bone mass as is the case of parathyroid hormone. Present treatment of osteoporosis includes bisphosphonates, calcitonin, selective estrogen receptor modulators and sufficient

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intake of calcium and vitamin D. Newer osteoclast targeted agents like cathepsin K and c-src kinase are under clinical development [3,4].

Bisphosphonates are the most commonly used drugs for the treatment of osteoporosis; the efficacy of this drug class for reducing the risks of osteoporosis has been well established in large clinical trials. Bisphosphonates are used to treat osteoporosis in the US and many other countries including India. Osteoclasts are the target cells of bisphosphonates, though the most drug-sensitive steps of their formation and activity have not been determined. Bisphosphonates (BPs) are highly hydrophilic compounds with low oral bioavailability having the property of most effective bone resorption inhibitors. The mechanism of action of BPs is induction of apoptosis in osteoclasts [5, 6]. .

Risedronate monosodium salt (Sodium 1-hydroxy-1-phosphono-2- (pyridin-3-yl ethyl) phosphate) is a member of Class BCS (III), the third-generation BPs. Risedronate is an efficient anti- osteoporosis drug among other BPs, which reduces the gastro-intestinal (GI) side effects. It is more potent drug having good solubility in water, but poor oral bioavailability (less than 1%, about 0.63%) [7,8].

There are a number of drugs whose clinical development failed due to poor solubility, inadequate bioavailability, and other poor biopharmaceutical properties, so at present research work is going on to solve this problem. The major task in the development of these drugs is the improvement in solubility, thereby enhancing oral bioavailability. The most frequently applied nanotechnology-based strategies in the development of delivery systems are polymeric nanoparticles (NPs), solid lipid NPs, liposomes, nanoemulsions, nanosuspension, and micelles etc., which provide controlled, sustained, and targeted drug delivery. The NPs based delivery systems present a significant approach for enhancing solubility and oral bioavailability of drugs [9-11].

Chitosan is an important polymeric carrier for many drugs because of its specific properties such as polycationic nature, biodegradability, biocompatibility and non-toxic nature. Chitosan is a natural polysaccharide derived by the process of, “Deacetylation of chitin”. Ion gelation is the commonly used method for the formulation of CS NPs. 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 this method. In many previous studies, it is reported that different drugs loaded CS nanoparticulate formulations are stable, permeable and therapeutically active **[12, 13].**

In the current study the authors have developed Risedronate loaded CS NPs for better bioavailability and acceptability of this drug to the biological systems. 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 NPs were prepared by by Ionotropic gelation method and were characterized for average particle size, surface charge, size distribution, drug entrapment efficiency and In vitro drug release. The effect of CS concentration, TPP concentration and stirring speed on the drug entrapment efficiency and particle size was evaluated. Drug delivery systems are designed for promoting the therapeutic effect of Risedronate drug and minimizing its toxic side effects, which is achieved by different process variables. In vitro release kinetic study was performed with the help of Dialysis membrane and different mathematical models were used for drug release kinetics study of formulated drug loaded NP **[14-17].**

MATERIALS AND METHODS

Materials

The drug Risedronate-sodium (M.W. of 305.09 g/ moL) was procured from Sigma Aldrich. (Mumbai, India). CS with medium molecular weight (M.W. =750 000 Da) was purchased from Himedia (India). Dialysis membrane (Mol. wt. cut-off: 12 000 Dalton, flat with 25 mm, diameter of 16 mm) 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.

Preparation of Risedronate loaded CS- NPs

Risedronate loaded CS NPs were prepared by the ionic gelation method **[18-23]**. In this method, CS-NPs were obtained upon the addition of aqueous sodium-tripolyphosphate (TPP) solution to acidic CS solution stirred at room temperature. NPs formation was the result of ionic interaction between positively charged amino groups of CS and negatively charged TPP. In this method, CS was dissolved in (2% w/v) acetic acid solution and stirred well up to complete dissolution then filters it with 0.2 µ filter paper. PH was adjusted up to 4.8-5.0 by adding 0.1 M NaOH solution. A fix amount of drug (20 mg) was added in CS solution after

adjusting the pH and Pluronic F-68 was added as surfactant 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 hours. The process variables such as CS concentration, TPP concentration, stirring speed and pH for nanoformulations were optimized. The prepared NPs suspension was analyzed by transmission electron microscopy (TEM) and Dynamic light scattering (DLS) for particle size. The optimized NPs suspension was centrifuged at 15000 rpm for 30 min using cooling centrifuge (C_{24}, Remi Centrifuge, and Mumbai (India). The pellets were freeze-dried and stored at 5 ± 3°C. The weights of freeze-dried nanoparticles were also measured. The entrapment efficiency (%) was analyzed by UV spectrometer for the supernatant. Centrifuged NPs were lyophilized by using lyophilizer (LABCONCO, GNCIIM) for 36 h.

Entrapment efficiency (EE)% of Risedronate loaded CS- NPs

The Entrapment efficiency (EE) of Risedronate loaded CS NPs was determined by the indirect method. The nanoparticles were centrifuged at 15,000 rpm for 30 min and the pellet of NPs was collected and the supernatant was separated. The amount of unentrapped drug in the supernatant was determined by using the method developed by Ostovic et al.and used by (Cohen-Sela et al **[24, 25].** EE% was determined at **262 nm** wavelength spectrophotometrically. Encapsulated drug amount was obtained by using UV-Visible spectrophotometer (UV-1800 Shimadzu, Japan)) after proper dilution. The percentage entrapment efficiency (% EE) was calculated by using the following formulae:-

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

CHARACTERIZATION

Particle Size, PDI, and Zeta Potential

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

Surface Morphology

Surface morphology of the best formulation was carried out using Scanning Electron Microscope (SEM) using **(Nanosem, Quantum 200E Instrument).** Formulated NPs were also confirmed using **Transmission Electron Microscope (TEM)** for surface morphology. The prepared sample was examined by **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 Risedronate, and drug 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 dialysis bag method **[26, 27]**. 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 ± 2°C was used. The drug-loaded NPs were placed into a dialysis membrane, tied at both the ends and placed in a beaker containing 100mL of diffusion medium (PBS pH 6.8). Temperature and speed were maintained at 37 ± 2°C and 100 rpm, respectively, using 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 **262 nm** (λ_{max} value) for Risedronate drug. Cumulative percentage release was calculated from the amount of drug release. The release kinetics were determined by some mathematical kinetic equations such as zero order, first order, Higuchi's model and Korsmeyer-Peppas model. Values of R^2 and K were calculated from the linear curve obtained by regression analysis of the plots [28].

RESULTS AND DISCUSSION

Preparation of Risedronate dronate loaded Chitosan Nanoparticles (RS-CS NPs)

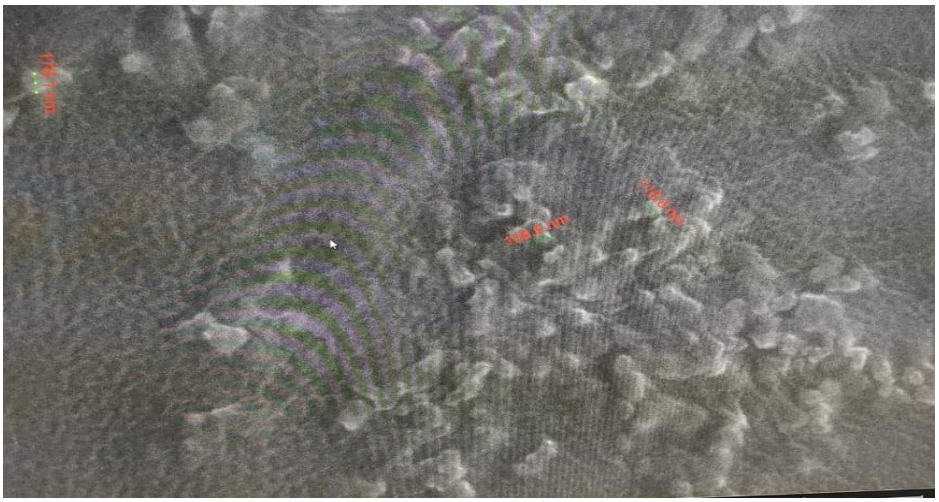
In this study, RS-CS NPs were prepared by ionotropic gelation method using Chitosan (CS), TPP, and surfactant (poloxamer). Different ratio of chitosan and TPP (CS: TPP) were used to prepare the optimized formulation i.e. 2:1, 1:1, 0.5:1, 1:2 and 1:0.5. The results of particle size analysis and the potential 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 RS-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 **190.4 nm,** PDI **0.267** and maximum drug entrapment efficiency of **68.53 ±1.05 %.** These results showed the best condition for preparing the optimized formulation of RS-CS NPs. The above-optimized formulation was considered for further studies i.e., characterization and in-vitro drug release kinetic studies.

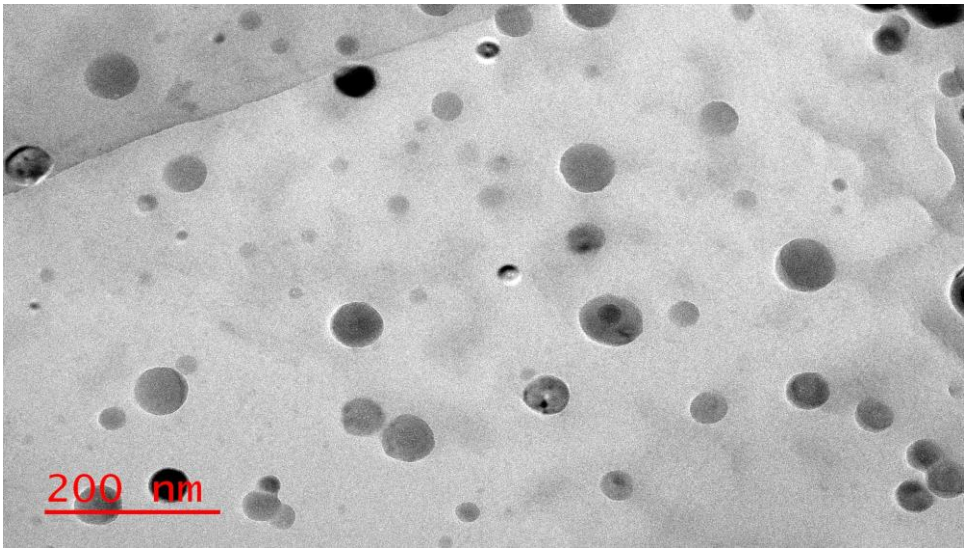
CHARACTERIZATION

Particle Size, PDI, and Zeta Potential

RS-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 [29]. SEM, TEM and AFM images are shown in fig 1(a -c). The particle size and Zeta potential were analyzed by Malvern Nano Zeta sizer as shown in fig.1 (d & e).



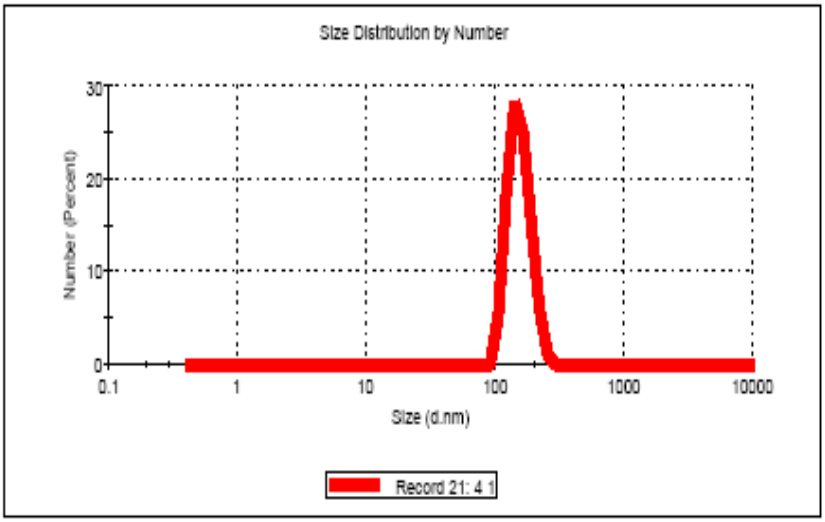
(a)

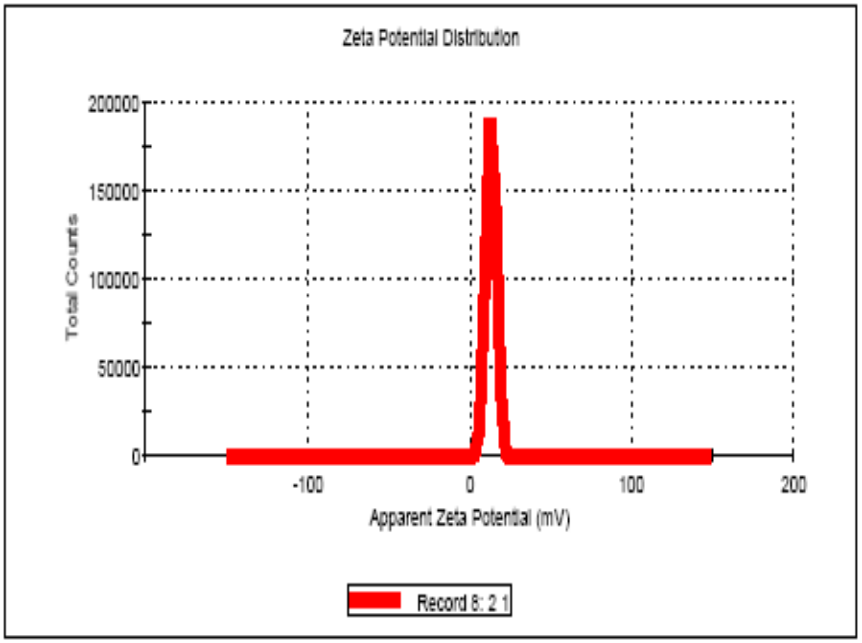


(b)

(c)

Figure 1 (a) SEM image of of RS- CS NPs (b) TEM image of of RS- CS NPs (c) AFM image of of RS- CS NPs





(d) Average Particle size of RS- CS NPs (e) Zeta Potential of RS- CS NPs

Based on the results of particle size and entrapment efficiency, the optimized formulation showed the lesser particle size (**190.4 nm**) than the other batches with higher entrapment efficiency (**68.53 ±1.05 %)** and Poly dispersive index (PDI) was **0.267**. The zeta potential of the optimized formulation was found to **be +12.9 mV.**

Surface Morphology

The morphology of nanoparticles was analysed by using to SEM ,the scanning Electron Microscopic studies shows the particle size range in 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.

The prepared RS-CS NPs were found in the nano range, and their spherical shapes were confirmed using SEM and TEM in fig.1 (a & b). SEM study showed spherical particles with agglomeration because of the gel formation.AFM studies also confirmed the nano size of formulated NPs.

Fourier Transform Infra Red (FTIR)

The FTIR spectra of the Risedronate drug (API), polymer (chitosan), TPP, and Risedronate loaded chitosan nanoparticles (RS-CS NPs) are shown in fig.2 (a-d). The drug risedronate sodium showed the specific peak at 1028.56 cm^{-1}, which indicates aliphatic P = O stretching. . The peaks at 2923.91 cm^{-1}. and 1028.56 cm-1.is because of C–H stretch and aromatic P= O stretch, respectively. C=C and C=N stretch was characterised by the peaks between 1400 and 1600 cm-1**.** 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 cm^{-1} 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 **[30,31]**. The FTIR spectra of RS-CS NPs showed separately identified peaks. It reveals that there was no chemical interaction between the drug and polymers.

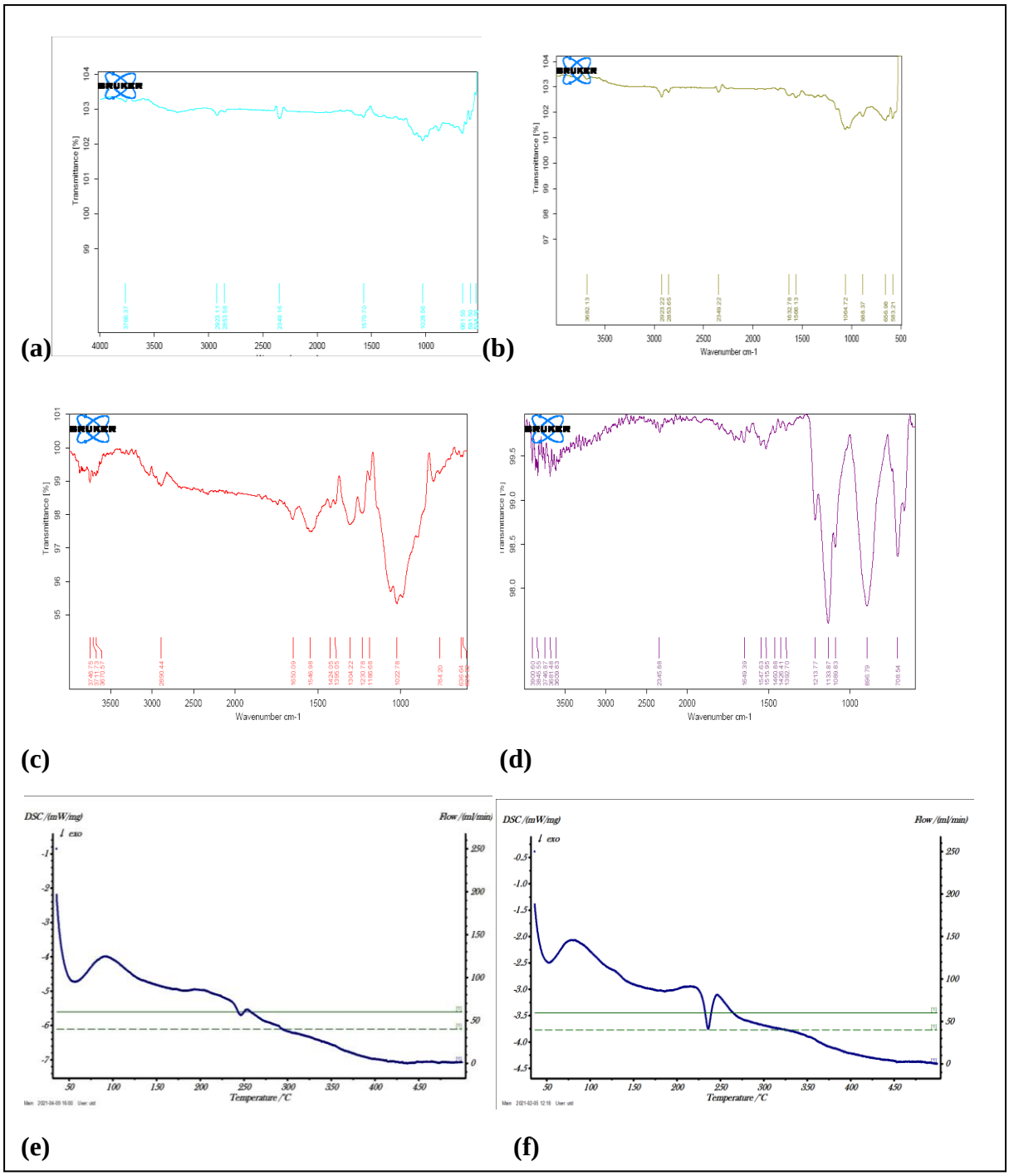


Figure 2 (a) FTIR spectra of Risedronate (API) (b) FTIR spectra of RS- CS NPs
(c) FTIR spectra of Chitosan (d) FTIR spectra of TPP
(e) DSC curve of RS (API) (e) DSC curve of RS- CS NPs

Differential scanning calorimetric (DSC) study

Differential scanning calorimetry (DSC) thermogram of risedronate sodium is shown in the fig. 2 (e & f). Differential scanning calorimetric (DSC) studies indicate a sharp endothermic

peak at 250˚C (approx.) corresponding to the melting point of the sample (262˚C) which matches with the melting point of risedronate sodium indicating the identification of the drug. From the DSC thermogram of pure drug and drug loaded NPs it is clearily shown that, the drug did not form a complex with the excipients as the endothermic peaks remained unchanged in position.

Drug Release kinetic study

In Drug release study, ADME process is involved, in which drug molecules migrate from the initial position and available for the biological action in to the systemic circulation. In vitro drug release of formulated Alendronate loaded CS NPs was compared with pure drug solution shown in (Figure 3). Results showed that release of pure drug was fast, about 85% drug release within 8-10 hours, while CS NPs formulation showed the sustained release of drug up to 48 hours. Initially, CS NPs formulation characterized an initial burst release (50% drug release in 10h), followed by sustained releases up to 48 hours. Initial fast release may be due to the presence of the adsorbed drug on the surface of CS NPs. Subsequently, the chitosan undergoes swelling which leads to sustained release of Risedronate drug.

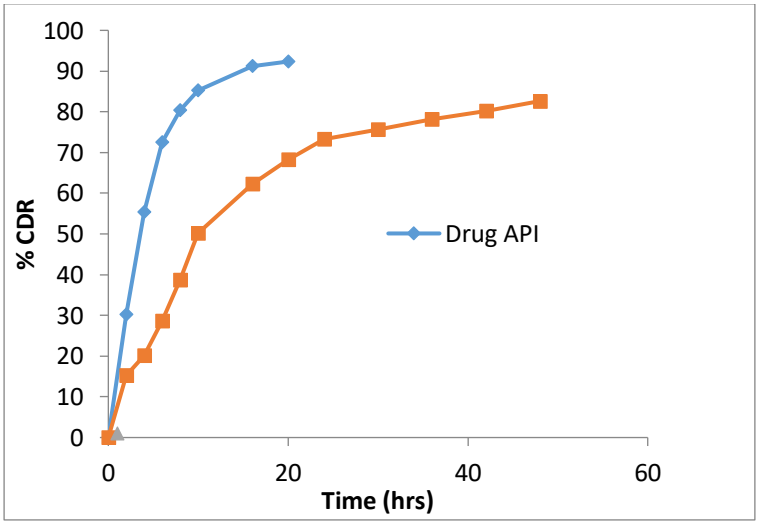


Figure 3: In –vitro drug release profile of Risedronate loaded CS NPs and Pure drug in PBS (P^H 6.8)

In-vitro drug release studies were carried out using dialysis bag method for different mathematical kinetic models. For kinetic study of formulated drug NPs, the plots were made for Zero order kinetic model (cumulative % drug release vs time), First order kinetic model (log of cumulative % drug remaining vs time), Higuchi model cumulative (% drug release vs

square root of time) and Korsmeyer– Peppas model (log cumulative % drug release vs log time). Plots of above mentioned models are shown in **Fig. (4)** and results are summarized in **Table (1).** In the above table "**R^2"** is correlation value and "**n**" is release exponent.

Different Mathematical Models

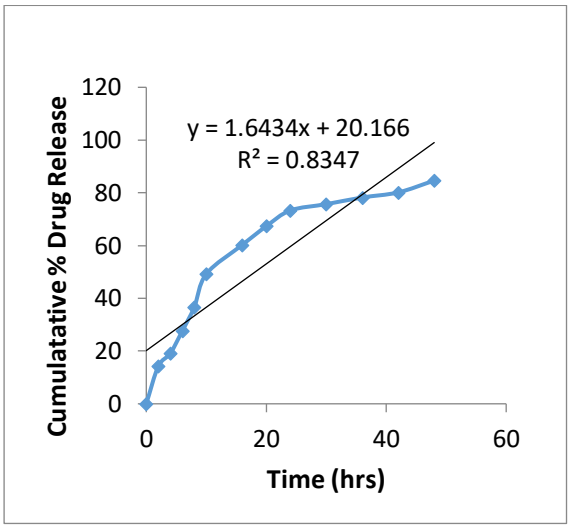
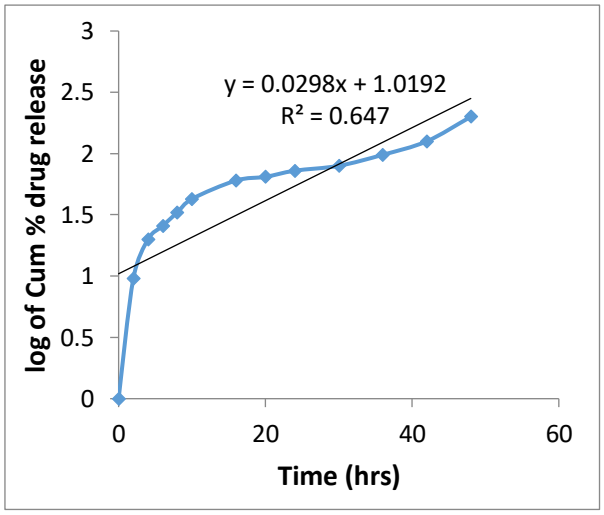
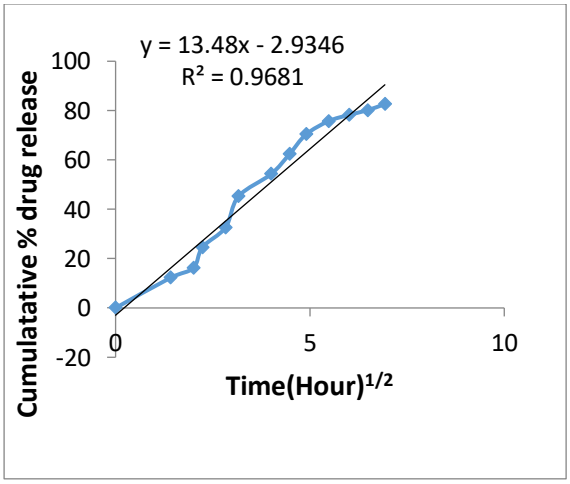


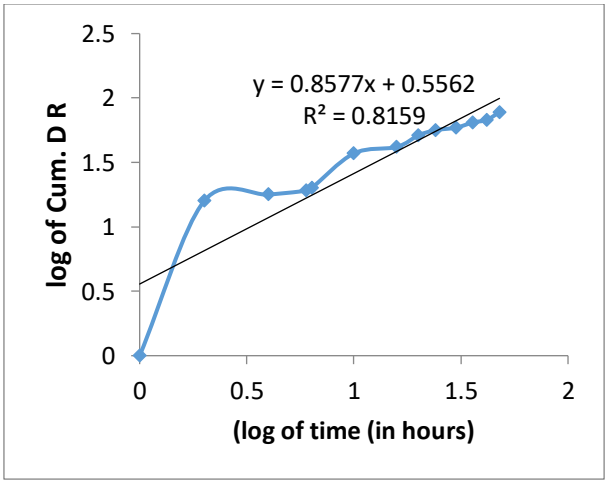
Fig.(4) (a) Zero order plot



(b) First order plot



(c) Higuchi model plot



(d) Korsemeyer - Peppas model

On the basis of above values of (R^2), the best fit kinetic model with the highest correlation value (0.9681) is shown in Higuchi model. It is concluded that in the optimized formulated NPs follow Higuchi model kinetics. In the Korsmeyer-Peppas model, release exponent value

"n" is 0.85. The magnitude is in the range (0.45 < n < 0.89) indicates the release mechanism is non-Fickian diffusion.

Table (1) Interpretation of R^2 values and rate constants (K) of release kinetics of NPs

Kinetic Models	Correlation value (R^2)	Release exponent (n)
Zero order model	0.8347	-----
First order model	0.647	-----
Higuchi model	0.9681	------
Korsmeyer– Peppas model	0.8159	0.85

CONCLUSION

Risedronate the potent bisphosphonate drug for the treatment of Osteoporosis, but there is increasing concern about their long-term safety. Medications with novel mechanisms and novel drugs like drug loaded polymeric NPs can be expected to treat osteoporosis in future. The results of the current study would help us to find a new approach for drug discovery and drug delivery by preparing the anti-osteoporotic drug Risedronate in the Nano-range. Drug loaded CS NPs prepared via ionic gelation method presented the significant results of drug release profile and increased the therapeutic efficacy of Risedronate drug for the effective treatment of osteoporosis.

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