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Bioavailability Enhancement of Risedronate Sodium by Formulation of Nanoparticles for Treatment of Osteoporosis

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ABSTRACT

The present research work focuses on improving the bioavailability of the anti osteoporotic drug Risedronate Sodium. This drug belongs to BCS class III which implies that it is permeability rate limited. Hence an attempt was made to reduce the particle size to nano dimensions using ionotropic gelation technique. In this technique, chitosan was used as the polymer and sodium Tri poly Phosphate was used as the cross linking agent. The resulting nanoparticles were optimized using 3^2 full factorial design and characterized for their entrapment efficiency, percent yield, in vitro diffusion studies. The particle size and zeta potential was found out and surface morphology was studied using Scanning electron microscopy. The in vivo studies clearly showed a marked improvement in the bioavailability of the nanoparticles as compared to the plain drug suspension.

Keywords: Risedronate Sodium, Nanoparticles, Bioavailability.

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INTRODUCTION

The underlying mechanism in all cases of osteoporosis is an imbalance between bone resorption and bone formation. The former refers to the process by which osteoclasts break down bone and release the minerals, resulting in a transfer of calcium from bone fluid to the blood. Hormonal factors strongly determine the rate of bone resorption; lack of estrogen (e.g. as a result of menopause) increases bone resorption as well as decreasing the deposition of new bone that normally takes place in weight-bearing bones[1]. In postmenopausal women, reduced levels of endogenous bioavailable estrogen are associated with lower bone mineral density (BMD) and higher risk for fragility fractures. Hormonal replacement therapy (HRT) had in the past been widely used in the prevention and treatment of postmenopausal osteoporosis. While the benefit of HRT on the prevention and treatment of postmenopausal osteoporosis and related fracture has been demonstrated, its effects on the health of other estrogen sensitive tissues such as the uterus and breast must be carefully considered. In many circumstances, estrogen induces proliferation of the uterine and breast tissues. In addition, postmenopausal women are at a higher risk for coronary heart disease (CHD) and breast cancer than their premenopausal counterparts, and the effects of HT may modify these risks. Therefore these side effects prompt for a better therapy for the use of specific category of drugs to selectively target the bone[2]. Risedronate comes under bisphosphonates which are a recent class of drugs found to have the greatest efficacy in the treatement of osteoporosis[3]. They exhibit a powerful binding affinity to bones and are routinely used for treatment in bone resorption. This class of drug decreases the elevated rate of bone turnover that is typically seen in postmenopausal osteoporosis. They are called bisphosphonates because they have two phosphonate (PO_3) groups[4]. Despite its advantages Risedronate suffers from very poor bioavalibility, serious interferences of absorption by foods and beverages other than water and side effects that consist of irritation to the upper gastrointestinal mucosa. To overcome these limitations Risedronate is given in a relatively large dose in a fasting condition while maintaining an upright position for at least half an hour after dosing. The standard treatment with Risedronate is chronic and daily so the inconvenience to the patient can lead to non compliance with the dosage regimen. Since Risedronate is not metabolized, dosing can be reduced to once a week by administering very large sustained release doses of the drug. But while large dosing helps improve patient compliance, it has the potential of excraberating the upper GI side effects of the drug[5,6]. Risedronate is categorized under BCS Class III which means that they are freely soluble but are permeability rate limited. Therefore, poor membrane permeation can be

identified as a cause of low oral bioavailability. One of the prime strategies adopted by a formulation scientist for resolving a membrane permeation problem includes changing the dosing vehicle. A vehicle such as nanoparticles has been reported to enhance the permeability of many drugs[7,8,9].

MATERIALS AND METHOD

Formulation of Nanoparticles

Risedronate loaded chitosan nanoparticles were prepared by ionotropic gelation technique. This technique involved the cross linking of chitosan solution with Sod. Tripolyphosphate(Sod. TPP). Initially chitosan was dissolved in 1% acetic acid solution. Subsequently, Sod TPP solution was added to 10 ml of the chitosan solution containing 10mg drug. pH of the solution was adjusted to 6 by adding 0.1 N NaOH solution. Stirring was continued for 1 hour. The resulting nanoparticles were separated by centrifuge at 15000 RPM[10]. The formulation was optimized by using 3^2 factorial design. Concentration of chitosan and sod. TPP were taken as the independent variables and their upper and lower limits were found out by performing systematic experiments(Table 1) . Entrapment efficiency of the nanoparticles was selected as the independent variable.

Table 1: Actual and coded values of independent variables for Risedronate NP Formulation

Factor	Level		
	Low	**Medium**	**High**
X_1	1 mg/ml	3 mg/ml	5 mg/ml
X_2	0.5	1.0	1.5
Coded Values	-1	0	+1
X_1 = Concentration of Chitosan in 1% v/v aqueous acetic acid		X_2 = Concentration of TPP in water. (% w/v)	

Characterization of Nanoparticles

- Percentage Yield (%)

The % percentage yield was calculated as per the formula mentioned below.

$$\%\ \text{Percentage Yield} = \frac{\text{Weight of dried SLN}}{\text{Weight of solid used (excipients + drug)}} \times 100$$

- % Drug Entrapment Efficiency (%EE)

% EE was determined by using following equation:

$$\%\ \text{EE} = \frac{\text{Weight of total drug} - \text{Weight of free drug}}{\text{Weight of total drug}} \times 100$$

- % Drug content

Drug content in the NPs was determined by using following equation:

$$\% \text{ Drug content} = \frac{\text{Practical drug content}}{\text{Weight of NPs taken}} \times 100$$

***In-Vitro* drug release study**

In vitro release studies were carried out by using dialysis tubes with dialysis membrane having molecular weight of 12000—14000 Da. Sample from receptor compartment was taken at various intervals of time over a period of 24 h and each time it was replaced with 5 ml of fresh phosphate buffer pH 6.8. The amount of drug released was determined spectrophotometrically at 262 nm.

Optimization of formulation

The optimization of prepared batches was done by considering % drug entrapment by studying interaction between factors which are considered as follows.

- Interaction between the factors

The statistical evaluation of all the obtained results data was carried out by analysis of variance (ANOVA) using Microsoft Excel Version-2007. The significant factors in the equations were selected for the calculation of regression analysis. The terms of full model having non-significant p value have negligible contribution in obtaining dependent variables and thus neglected. The equations represent the quantitative effect of the formulation variables on responses.

- Construction of contour plots

Two dimensional contour plots were established using reduced polynomial equation. Contour plots were constructed by using sigma plot version 11.0 (Systat software Inc.).

Particle Size and Zeta Potential

The average diameter (Z-AVE), and zeta potential of optimized Risedronate nanoparticles was determined by photon correlation spectroscopy (PCS) (Zeta- sizer Nano ZS, Malvern Instruments, UK) at room temperature.

Differential scanning calorimetry

Thermograms of pure drug Risedronate and excipient used were obtained by using Differential Scanning Calorimeter (Shimadzu). Samples were weighed directly in pierced DSC aluminum pan and scanned in the temperature range of 50-300°C under an atmosphere of dry nitrogen. Heating rate of 10°C/min was used and thermogram obtained was observed for interaction between drug and excipient.

Scanning Electron Microscopy (SEM)

Surface morphology of optimized formulation was obtained by using scanning electron microscope.

Stability study

The optimized formulation of Risedronate loaded nanoparticles were placed in screw capped glass container and stored at various ICH storage conditions i.e. 25ºC ± 2^0C/60 ± 5% RH and 40ºC ± 2^0C/75 ± 5% RH for a period of 60 days. The samples were analyzed for physical appearance, drug content and in-vitro drug release study at regular interval of 15 days.

***In Vivo* Pharmacokinetic study in rats**

The experiment was carried out on healthy female Wistar rats weighing 200-250 g. Rats were housed in polypropylene cages, maintained under standardized condition (12 h light/dark cycle, 24°C, 35-60 % humidity) and allowed free access to diet. Chromatographic separation was carried out on a Shimadzu UFLC prominance liquid chromatographic system, controlled by LC solution software. It was equipped with LC 20AD Binary pump, a manual injector, a column and a photo diode array (PDA) detector (SPD 20A). The mobile phase consisted of a mixture of Phosphate Buffer pH 6.8, Acetonitrile, methanol in the ratio 450:480:70 respectively. The mobile phase was prepared daily and degassed by sonication and filtered through a 0.45µm membrane filter before use. The column was maintained at room temperature. The mobile phase was delivered isocratically with a flow rate of 1 mL min^{-1}, the injection volume was 20 µL and the wavelength for UV detection was 262 nm. For chromatographic separation, Enable C18 250mm × 4.6 mm column, 5 µm was used. All the chromatograms were analyzed by LCs solution. The animals were fasted at least 12 h prior to dose administrations and for 4 h after dosing with free access to water. Animals were divided into two groups each consisting of six animals. All animals were given different formulation group wise as described underneath.

Group I: Control group (Plain RIS suspension in 0.5% w/v sodium CMC , 3mg/kg, p.o.)

Group II: Formulation 1 (optimized Risedronate Nanoparticles equivalent to 3mg/kg, p.o.)

Serial blood samples (0.5ml) were withdrawn through capillary inserted in to retro orbital plexus under mild ether anesthesia at a time interval of predose 1, 2, 4, 8, 12 and 24 h post dose. Blood samples were collected in micro centrifuge tubes containing anticoagulant (3.8% w/v sodium citrate). The plasma samples were collected immediately from aforementioned samples after centrifugation at 5,000 rpm at 4°C for 10 minutes and stored immediately at -20°C until further analysis. Samples were analyzed by standard HPLC method after sample extraction procedure. Pharmacokinetic parameters were estimated by using Microsoft Excel 2007 add in PK solver. Various parameters like maximum plasma concentration (C_{max}), time for achieving maximum plasma concentration (T_{max}), mean residence time (MRT) and relative bioavailability (F) were calculated.

Statistical analysis of the obtained data was carried out by using data analysis feature of Microsoft Excel 2007. The student's *t* – test was calculated with the level of significance, P<0.05.

RESULTS AND DISCUSSION

Characterization of Nanoparticles

- Percentage yield (% yield), Percentage entrapment efficiency (% EE) and Percentage drug content (% DC)

The percentage yield for each formulated batches was found to be in the range of 79.44 ± 2.22 to 59.03 ± 3.46. It was observed that production yield was varied with change in concentration of chitosan or sodium TPP. RIS/02/12, RIS/02/15, RIS/02/16 batch gave a yield of above 75% (Figure 1) The percentage entrapment for each formulation was found to be in the range of 77.80 ± 1.37 to 44.55 ± 0.39. The maximum percentage drug entrapment was obtained for the formulation RIS/02/16. It was observed that increase in concentration of chitosan and sodium TPP significantly increases percentage entrapment. However, further increase in chitosan concentration from 5 mg/ml and sodium TPP concentration from above 0.5%w/v caused a significant decrease in percentage entrapment. This can be attributed to the fact that chitosan and TPP react at a slower rate at higher concentration (Figure 2). Percentage drug content was determined for all formulated batches by using UV visible spectrophotometric method at λ_{max} of 262 nm. It was found in the range of 3.60 ± 0.21 to 9.72 ± 0.08 (Figure 3).

In-vitro drug release study

In-vitro drug release study was performed for each formulation by using dialysis sac method. It was observed that the in vitro drug release was characterized by an initial burst release and subsequent controlled release in dissolution media of phosphate buffer pH 6.8. The release of all the batches continued upto a time period of 24 hours after which the concentration showed a decrease at 48 hours. Batch RIS/02/16 showed the maximum release of 84.03 ± 5.07 over a period of 24 hours. The initial burst drug release can be attributed to the phenomenon of diffusion of the drug at the surface and the desorption of the drug at the surface of the nanoparticles. Subsequently, the chitosan undergoes swelling which leads to sustained release of Risedronate. Thus the initial burst release can be considered a drawback of nanoparticles as it decreases sustained release effect of the formulation.(Figure 4)

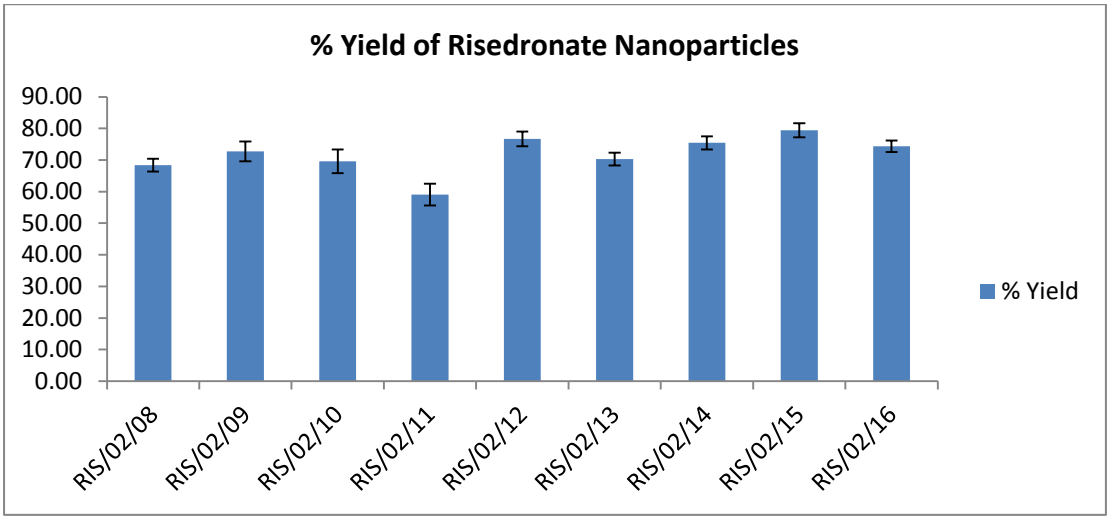

Figure 1: % Yield of Risedronate Nanoparticles

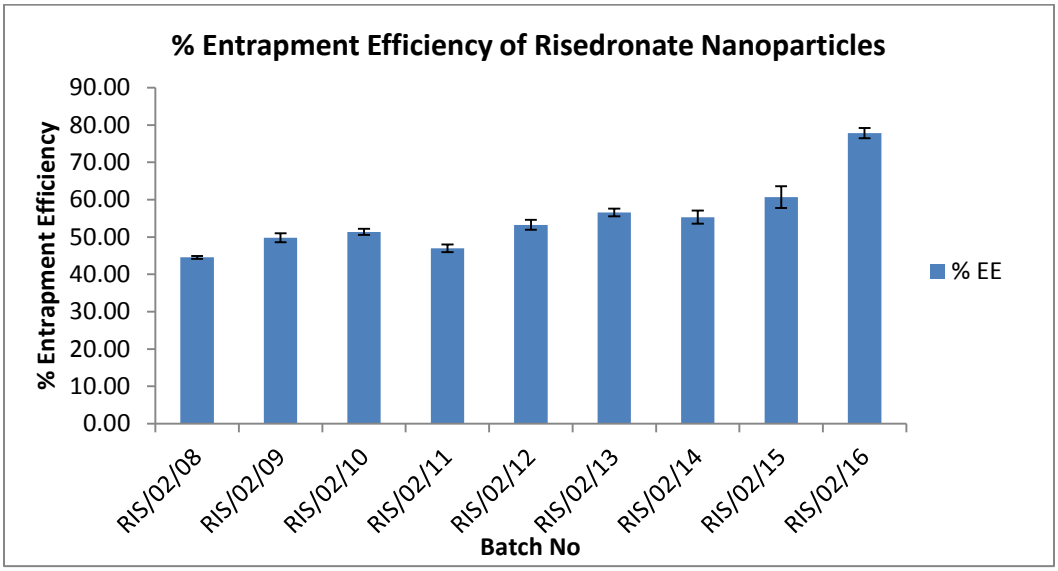

Figure 2: % Entrapment Efficiency of Risedronate Nanoparticles

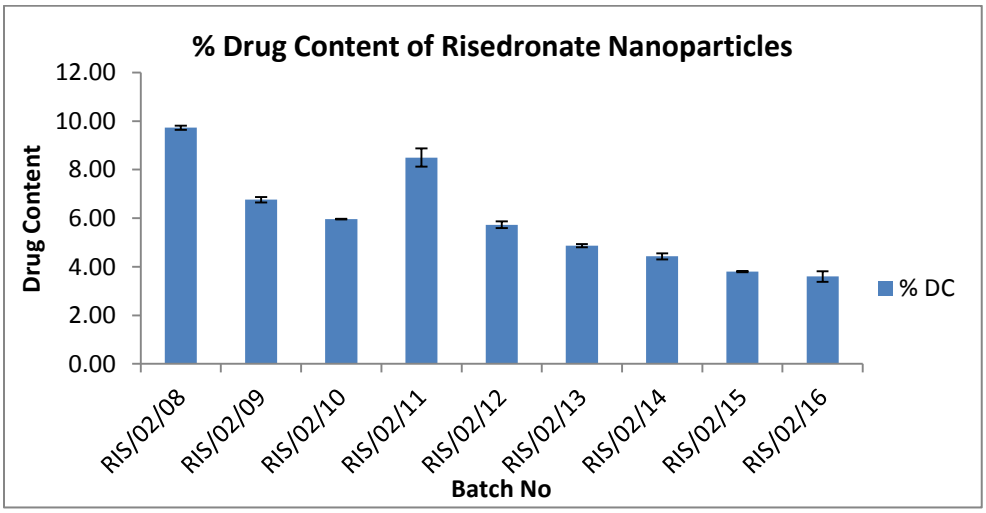

Figure 3: % Drug Content of Risedronate Nanoparticles

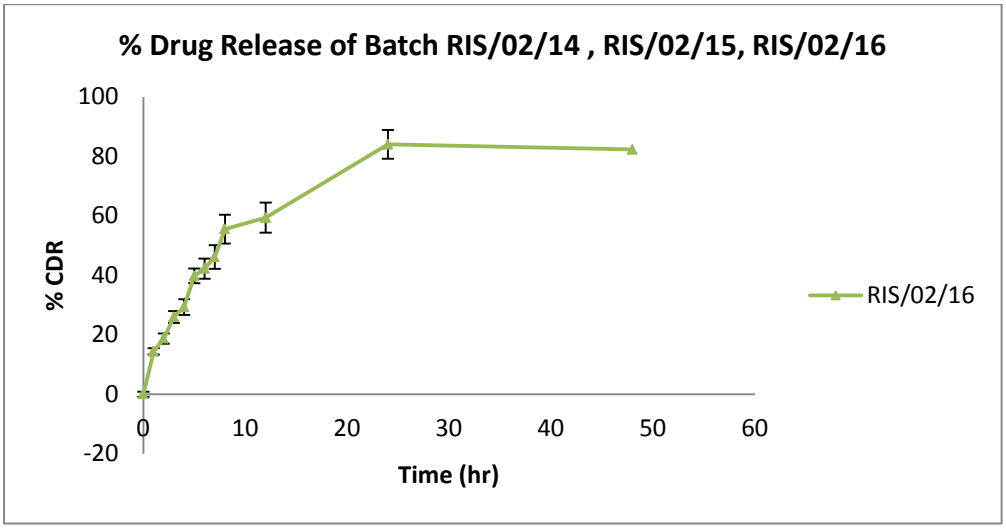

Figure 4: % Drug Release of Batch RIS/02/16

Optimization of formulation

Mathematical modeling

Coded value and actual values of varying two independent variables, polymer concentration (X_1) and Stabilizer concentration (X_2) are represented in table 2

Table 2: Various batches of Risedronate nanoparticles with obtained entrapment efficiency along with interaction factors

Batch No	X_1	X_2	X_{11}	X_{22}	X_1X_2	% Entrapment
RIS/02/08	-1	-1	1	1	1	43.32
RIS/02/09	-1	0	1	0	0	48.91
RIS/02/10	-1	1	1	1	-1	50.73
RIS/02/11	0	-1	0	1	0	48.22
RIS/02/12	0	0	0	0	0	52.77
RIS/02/13	0	1	0	1	0	57.13
RIS/02/14	1	-1	1	1	-1	55.19
RIS/02/15	1	0	1	0	0	60.93
RIS/02/16	1	1	1	1	1	77.48

From the above information, results of analysis of variance (ANOVA) of full model and reduced model were calculated and the F statistics was applied to check whether the non-significant terms can be omitted or not from the full model.

Thus, a full model equation was generated which is as follows:

$Y = 51.94 + 8.44X_1 + 6.43X_2 + 3.38X_{11} + 1.14X_{22} + 3.72X_1X_2$

By considering very significant terms from full model, reduced model was established

$Y = 51.94 + 8.44X_1 + 6.43X_2$

As the calculated F value (0.7548) was found to be less than the tabular F value ($\propto$=0.05, 3), it can

be concluded that the neglected terms do not significantly contribute in the prediction of the entrapment efficiency. The value of the coefficient of X_1 was found to be greatest. From this it was concluded that X_1 is the factor which affects the entrapment efficiency maximum. The value of the adjusted coefficient R^2 for the full model and the reduced model was found to be 0.8950 and 0.8040. As these values are above 0.8, a high significance of the model is achieved. The determination coefficient R^2 gives an excellent idea about the goodness of the fit of the model. The R^2 values for the full and the reduced model ware found to be 0.9606 and 0.9530 respectively. From this we can conclude that above 90% of the variations are explained by the model. Lastly, high values of the correlation coefficient for the full model and the reduced model implies a very good correlation between the selected independent variables.

Contour plots and response surface analysis

Two dimensional contour plots and three dimensional response surface plots were prepared for the response percentage entrapment efficiency. It was found that as the values of chitosan and sod TPP were low, the entrapment was also low. But an increase in the concentrations also increased the entrapment efficiency. This was further confirmed by plotting response surface plots which depicted the same results. Thus from the plots a range was established for the independent variables. It was found that when chitosan is taken in the range of 4.5 – 5.0 mg/ml and sod TPP was taken in the range of 1.2 – 1.5, satisfactory entrapment can be achieved. However maximum entrapment could be achieved at 1.5% w/v sod TPP solution and 5 mg/ml chitosan concentration. (Figures 5 and 6)

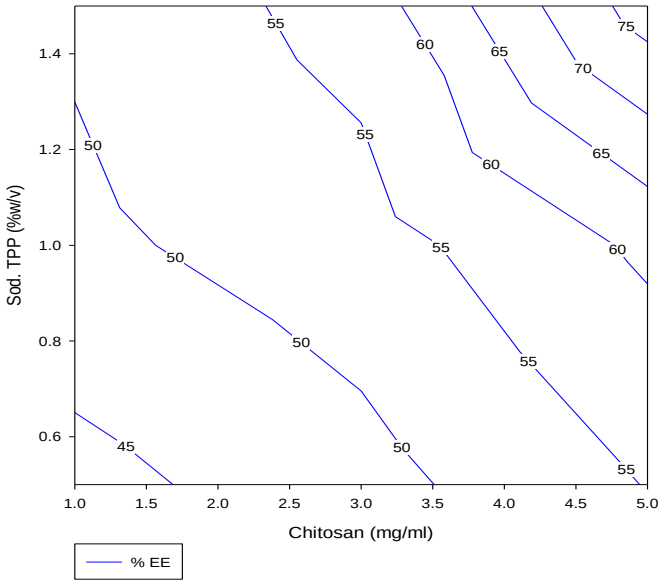

Figure 5: Contour Plot of Risedronate Nanoparticles

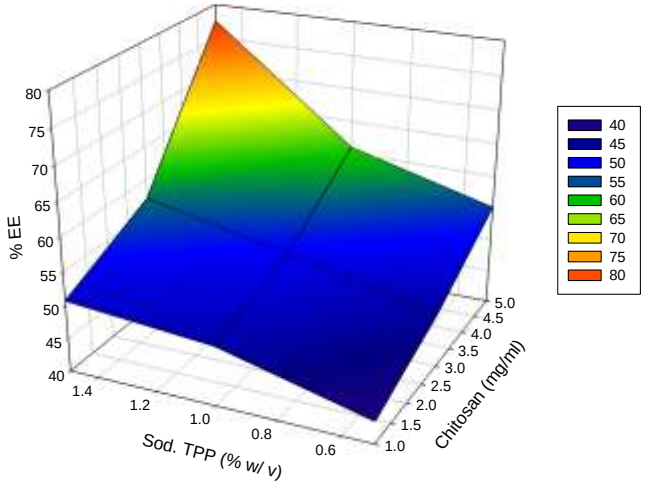

Figure 6: 3 D plot of Risedronate nanoparticles

Particle Size

The particle size of optimized formulation was found to be mean average of 729nm. Zeta potential was found to be -7.46 mV for optimized formulation, thus it suggests the possible aggregation of particles.

Differential scanning calorimetry (DSC)

The thermogram of the drug showed a sharp peak at 262 °C which is the reported melting point of the drug. The thermogram of the formulation blend also did not show any significant change in the melting point of the drug further indicating that there does not exist any incompatibility between the drug and the polymers.

Scanning Electron Microscopy (SEM)

The particle shape was found to be fairly spherical structure with possibility of aggregation as considerable number of large particles were observed (Figure 7).

Stability study

The optimized formulation was subjected to stability studies at various ICH storage conditions i.e. 25 ºC ± 2 ºC/60 ± 5 % RH and 40 ºC ± 2ºC/75 ± 5 % RH for a period of 60 days. The formulation was evaluated for physical appearance, drug content and in-vitro drug release study at regular interval of 15 days. No major changes were observed in physical appearance, drug content and *in – vitro* drug release profile when stored at room temperature. It indicates that the formulation were stable at various ICH storage condition for longer period.

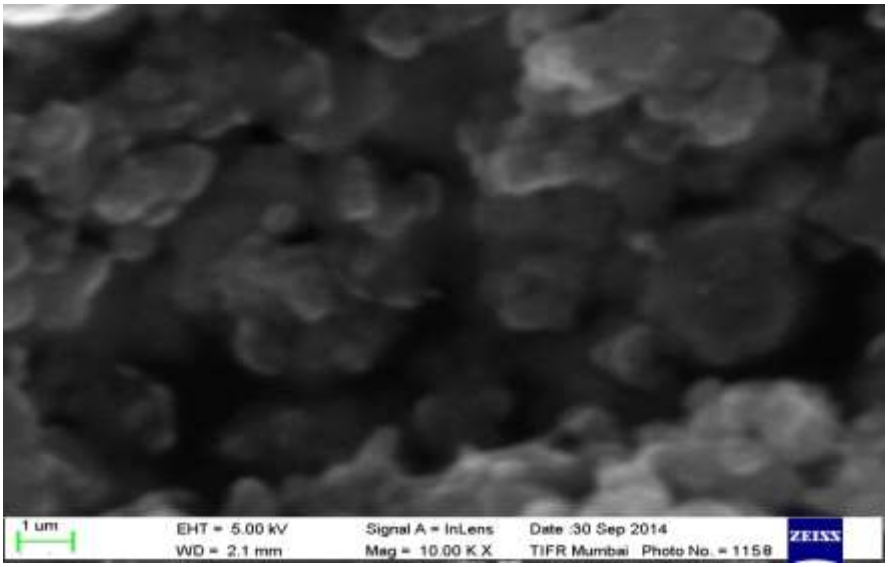

Figure 7: SEM image of Risedronate nanoparticles

***In Vivo* Pharmacokinetic studies**

In vivo pharmacokinetic studies were conducted on rats and the results showed a significant difference in the various parameters like c_{max}, t_{max} and AUC. The c_{max}, t_{max} and AUC values for the nanoparticles were found to be 260.26 ± 7.47ng/ml,6 hrs and 1647.36 ± 15.64 respectively. This was found to be significantly higher as compared to the original drug suspension. (Figures 8,9,10)

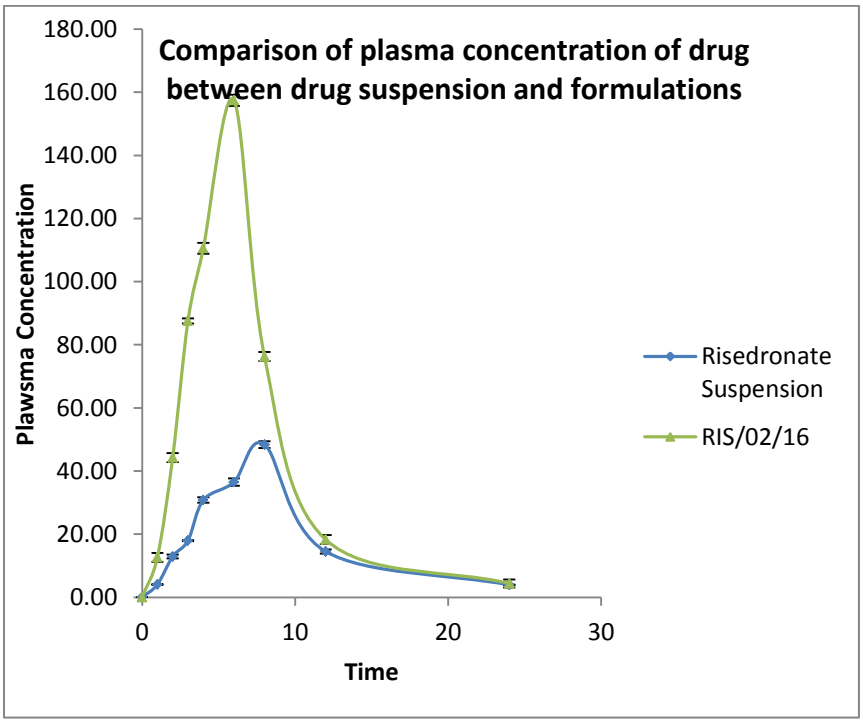

Figure 8: Comparison of plasma concentration of drug between drug suspension and nanoparticles

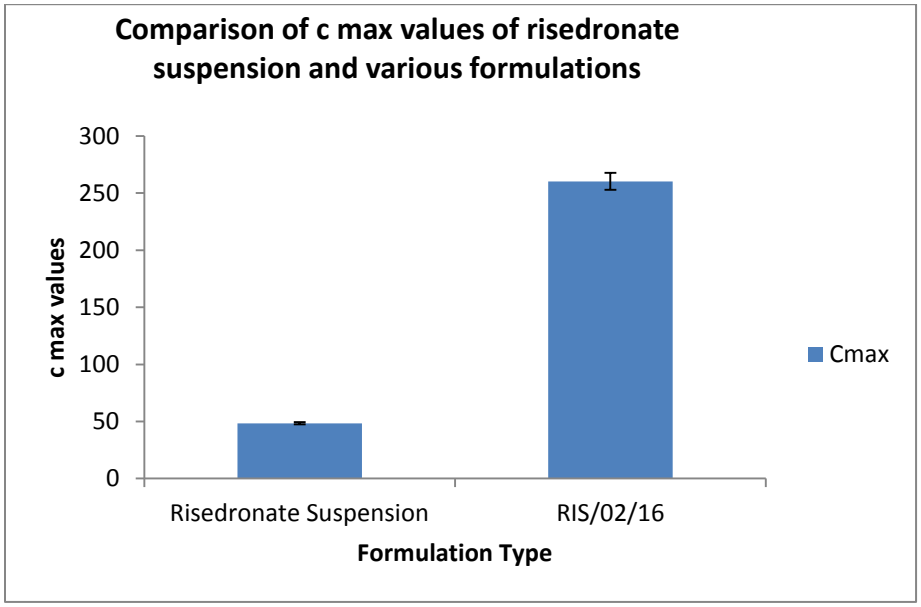

Figure 9: Comparison of c $_{max}$ values of risedronate suspension and various formulations

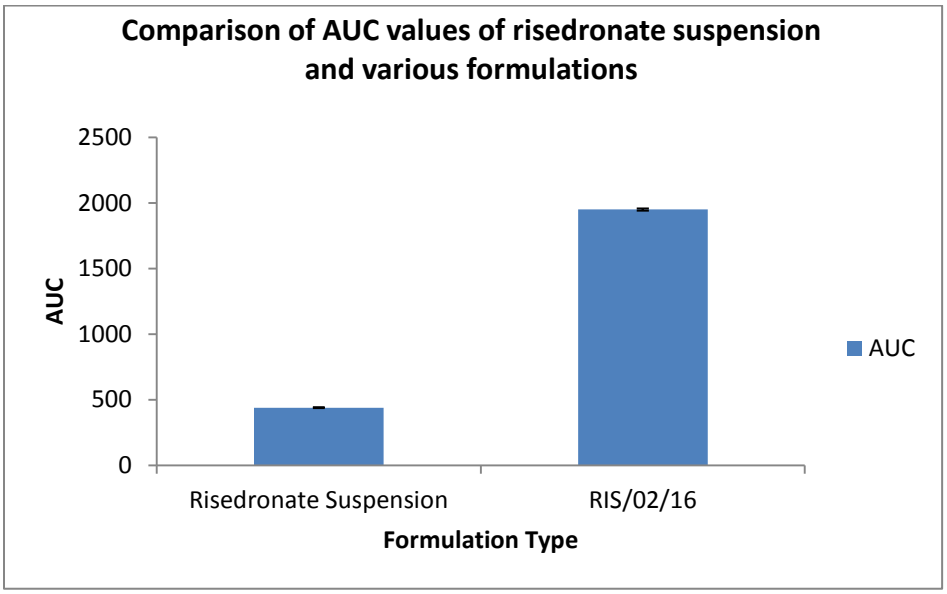

Figure 10: Comparison of AUC values of risedronate suspension and various formulations

CONCLUSION

The present research work concludes that the bioavailability of BCS class III drugs can be improved if delivered through nanocarrier system. The research work concludes that the concentration of chitosan and Sodium Tri polyphosphate showed to be a key factor to optimize the nanoparticles The work demonstrated that drug entrapment and particle size vary with the different concentration of polymer chitosan and TPP in nanoparticles The factorial design of optimization of formulation showed that nanoparticles of risedronate showed high drug entrapment (77.80±1.37) at optimum concentration of 5mg/ml chitosan and 1.5%w/v of TPP. Thus, it can be concluded based

on the result obtained that nanocarriers of the selected drugs can provide higher bioavailability than the other dosage forms of BCS class III drugs in order to get potentially more rapid onset of action.

REFERENCES

1. LG R. Pathogenesis of osteoporosis: concepts, conflicts, and prospects. J Clin Invest. 2005;115(12):3318–3325
2. Biller BM SV, Herzog DB, Rosenthal DI, Holzman S, Klibanski A. Mechanisms of osteoporosis in adult and adolescent women with anorexia nervosa. J Clin Endocrinol Metab. 1989 68(3):548-54
3. Roy Yuen-chi Lau XG. A Review on Current Osteoporosis Research:With Special Focus on Disuse Bone Loss. Journal of Osteoporosis. 2011;2011:1-6.
4. Rottman RA. Applied Therapeutics: The Clinical Use of Drugs. 9 ed. Troy DB, editor. Baltimore: Lippincott Williams & Wilkins; 2009
5. hajuria DK RR, Mahapatra DR. Drugs for the management of osteoporosis: a review. Rev Bras Reumatol. 2011;51(4):365-82
6. Cauley JA RJ, Chen Z, Cummings SR, Jackson RD, LaCroix AZ et al. Effects of estrogen plus progestin on risk of fracture and bone mineral density: the Women's Health Initiative randomized trial. JAMA. 2003;290:1729-38
7. Waalen. J. Current and emerging therapies for the treatment of osteoporosis. J Exp Pharmacol. 2010;2:121-34
8. Bischoff HA SH, Dick W, Akos R, Knecht M, Salis C et al. Effects of vitamin D and calcium supplementation on falls: a randomized controlled tria. J Bone Miner Res. 2003; 18:343-51.
9. Trivedi DP DR, Khaw KT. Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. BMJ 2003(326-369).
10. Nesalin JAJ, Smith AA. Preparation and evaluation of chitosan nanoparticles containing zidovudine. Asian J Pharm Sci. 2012;7:80-4.