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Research article

Bioavailability Enhancement of Alendronate by Nanoparticle Formulation for Treatment of Osteoporosis

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ABSTRACT
Alendronate was selected for this study to improve the bioavailability to treat osteoporosis efficiently by developing nanocarrier system like nanoparticles. This drug belong to the BCS class III in which permeability of drug through gastrointestinal mucosa is the limiting factor to show high bioavailability. Despite their advantages bisphosphonates suffer from very poor bioavailability, 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 bisphosphonates are 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 bisphosphonates is chronic and daily so the inconvenience to the patient can lead to non compliance with the dosage regimen. Since bisphosphonates are 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 exacerbating the upper GI side effects of the drug. An effort was done to formulate and evaluate the nanocarriers of bisphosphonates for improving the permeability and hence the bioavailability for the better and efficient treatment of osteoporosis. Ionotropic gelation method was used to develop the polymeric nanoparticles. The work demonstrated that drug entrapment and particle size vary with the different concentration of polymer chitosan and Sodium Tri polyphosphate in nanoparticles.

Keywords: Osteoporosis, Alendronate, nanoparticles, chitosan, Sodium Tri polyphosphate.

INTRODUCTION

The term osteoporosis is derived from the Greek words osteon (bone) and poros (pore).Osteoporosis is a progressive bone disease that is characterized by a decrease in bone mass and density which can lead to an increased risk of fracture[1]. Osteoporosis is called a "silent disease" because it progresses without symptoms until a fracture occurs[2].In osteoporosis, the bone mineral density (BMD) is reduced, bone microarchitecture deteriorates, and the amount and variety of proteins in bone are altered. Osteoporosis is defined by the World Health Organization (WHO) as "a bone mineral density of 2.5 standard deviations or more below the mean peak bone mass (average of young, healthy adults) as measured by dual-energy X-ray absorptiometry; the term established osteoporosis includes the presence of a fragility fracture." [3]The fundamental system in all instances of osteoporosis is an awkwardness between bone resorption and bone arrangement. In ordinary bone, network rebuilding of bone is consistent; up to 10% of all bone mass may be experiencing redesigning anytime. The three primary instruments by which osteoporosis creates are a deficient top bone mass (the skeleton creates lacking mass and quality amid development), over the top bone resorption, and insufficient arrangement of new bone amid redesigning.[4]interchange of these three systems underlies the advancement of delicate bone tissue. Hormonal variables unequivocally focus the rate of bone resorption; absence of estrogen (e.g. as a consequence of menopause) expands bone resorption, and in addition diminishing the effect of new bone that typically happens in weight-bearing bones[5]. Along these lines the limit of issue that remains to be worked out is that mechanical fractures and cracks depends on the amount of bone tissue as well as on its quality[2].At present there are numerous treatments accessible for the treatment of osteoporosis, yet the current treatments have notable issues including viability and safety issues in the long run[2]. Alendronate hinders osteoclast-interceded bone-resorption. Like all bisphosphonates, it is synthetically identified with inorganic pyrophosphate, the endogenous controller of bone turnover. However, while pyrophosphate represses both osteoclastic bone

resorption and the mineralization of the bone recently shaped by osteoblasts, Alendronate particularly hinders bone resorption with no impact on mineralization at pharmacologically achievable measurements. Its hindrance of bone-resorption is more or less 1,000 times more grounded than the equimolar impact of the first bisphosphonate drug, etidronate. Under treatment, typical bone tissue creates, and Alendronate is stored in the bone-network in pharmacologically latent structure. Bisphosphonates are 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 stratergies adopted by a formulation scientist for resolving a membrane permeation problem includes changing the dosing vehicle. A vehicle such as a nanocarrier system has been reported to enhance the permeability of the above drug class[6, 7]. Thus, the best stratergy which can be used to increase patient compliance, to overcome the potential disadvantages as well as increase the bioavailability issues related to bisphosphonates is formulation of nanocarrier system of this particular class of drugs.

MATERIALS & METHODOLOGY

Materials

The drug (Alendronate) and excipients like Chitosan and Sodium Tripolyphosphate were purchased from local vendors

Formulation development of nanoparticles by Ionotropic gelation method

The concentration of TPP and chitosan were considered as the independent variables and their concentration was varied to fix the upper and the lower limits for the factorial design. Based on the limits selected, a 3^2 full factorial design was set up and various batches of Alendronate loaded nanoparticles were prepared accordingly. Table 1 shows the upper and lower limits along with the coded values.

In the above factorial design, the amount of drug was kept constant (10mg) and the ratio proportion for volume of chitosan solution to sodium TPP solution was also kept constant.

Chitosan was dissolved in aqueous glacial acetic acid (1%v/v) to obtain different concentrations (1 mg/ml, 3 mg/ml and 5 mg/ml). Under magnetic stirring at room temperature, 10 ml of TPP solution was added drop wise using syringe needle to 20 ml of chitosan solution containing 10 mg drug. The stirring was continued for about 30 minutes. The resultant nanosuspension was centrifuged at 15000 rpm using cooling centrifuge for 30 minutes at 4°C. The particles obtained after centrifugation were finally freeze dried and stored in glass vials for further study[8, 9].

CHARACTERIZATION OF NANOPARTICLES

Percentage Yield (%)

The percentage yield of nanoparticles of various batches were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of nanoparticles[10].

The % percentage yield was calculated as per equation

$$\%\ \text{Percentage Yield} = \frac{\text{Weight of dried nanoparticles}}{\text{Weight of solid used (excipients+drug)}} \times 100 \ldots\ldots\ldots\ldots (1)$$

% Drug Entrapment Efficiency (%EE)

Analysis of Alendronate from nanoparticles was done by separating free drug from the nanoparticles dispersion. The separation was done by centrifugation of nanoparticulate dispersion at 15000 rpm for 30 minutes at 4°C (cooling centrifuge).

The entrapment efficiency of the drug in nanoparticles was estimated by indirect method. In this method, analysis of Alendronate from nanoparticles was done by appropriately diluting supernatant with aqueous glacial acetic acid (1 % w/v) and absorbance was taken against aqueous glacial acetic acid (1 % w/v) as a blank on UV-Visible Spectrophotometer after reaction with ninhydron reagent (at 570 nm wavelength of absorption maxima)[10].

% EE was determined as per equation 2.

$$\%\ \text{EE} = \frac{\text{Weight of total drug} - \text{Weight of free drug}}{\text{Weight of total drug}} \times 100 \ldots\ldots\ldots (2)$$

% Drug content

The drug content in the nanoparticles was determined by dissolving 10 mg of lyophilized NPs in few ml of methanol and make up the volume up to 10 ml with distilled water. Absorbance of the resulting solution was then measured spectrophotometrically at 570 nm after filtration and appropriate dilution with distilled water and reaction with ninhydrin reagent. Drug content in the NPs was determined as per equation 3[10].

$$\%\ \text{Drug content} = \frac{\text{Practical drug content}}{\text{Weight of NPs taken}} \times 100 \ldots\ldots\ldots\ldots (3)$$

In-Vitro Drug Release Studies

In vitro release studies were carried out by using dialysis tubes with dialysis membrane having molecular weight of 12000—14000 Da. The

prepared Alendronate nanoparticles were re-dispersed in 5 ml of phosphate buffer pH 6.8 (strength 10 mg/ml) and subjected to dialysis by immersing the dialysis tube to the receptor compartment containing 50 ml of phosphate buffer pH 6.8. The medium in the receptor was agitated continuously using a magnetic stirrer and the temperature was maintained at 37 ± 0.5°C. 5 ml of 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[11].

The amount of drug released was determined spectrophotometrically at 570 nm after appropriate reaction with ninhydrin reagent.

Table 1: Actual and coded values of independent variables for Alendronate Nanoparticle Formulation

Factor	Level		
	Low	Medium	High
X_1	2 mg/ml	4 mg/ml	6 mg/ml
X_2	0.5	1.0	1.5
Coded Values	-1	0	+1
X_1 = Concentration of Chitosan in 1% w/v aqueous glacial acetic acid		X_2 = Concentration of TPP in water. (% w/v)	

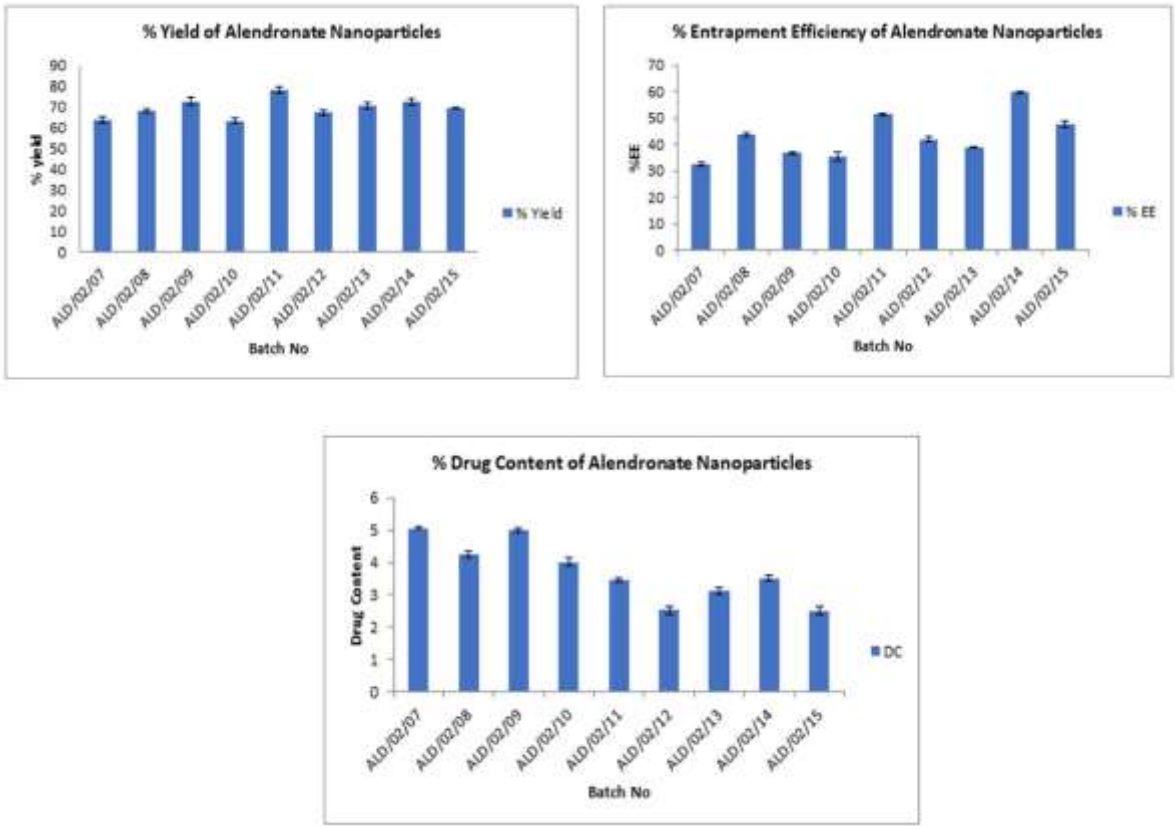

Figure 1: Characterization of Alendronate Nanoparticles

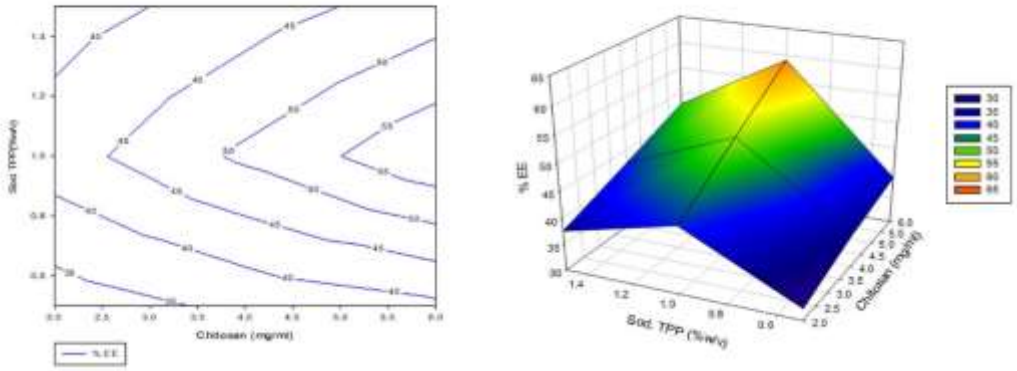

Figure 2: Contour graph and Response Surface Plot of Alendronate Nanoparticles

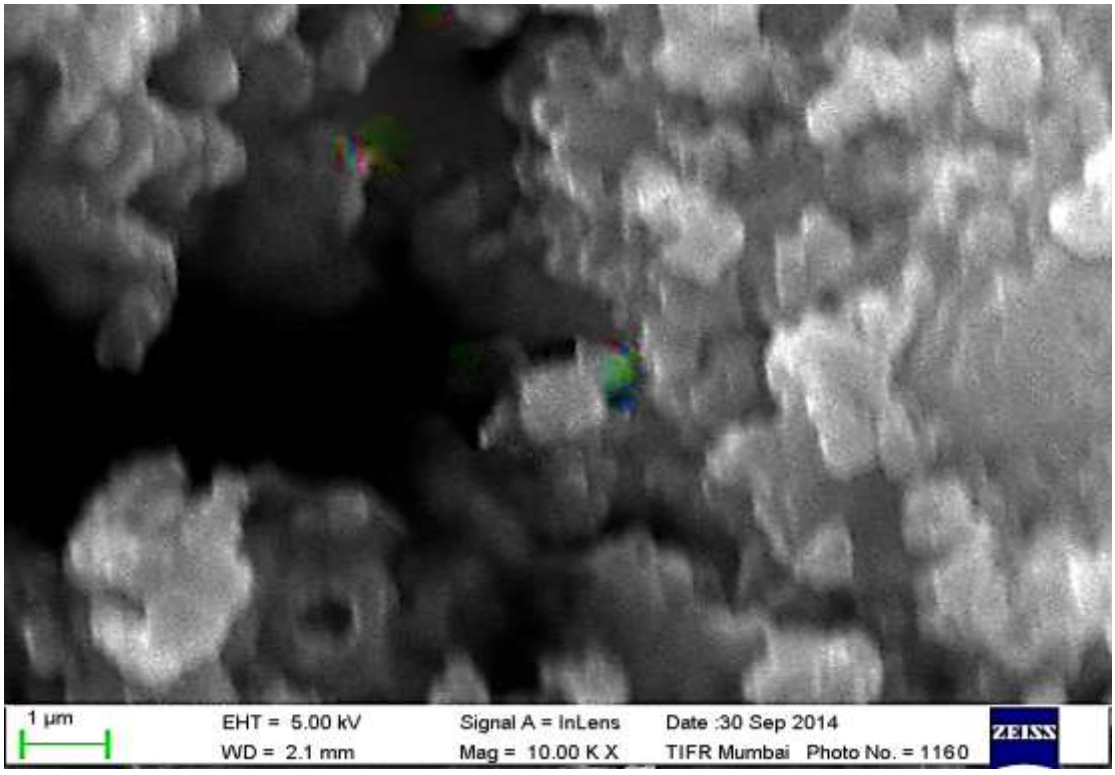

Figure3 : SEM image of Alendronate Nanoparticles

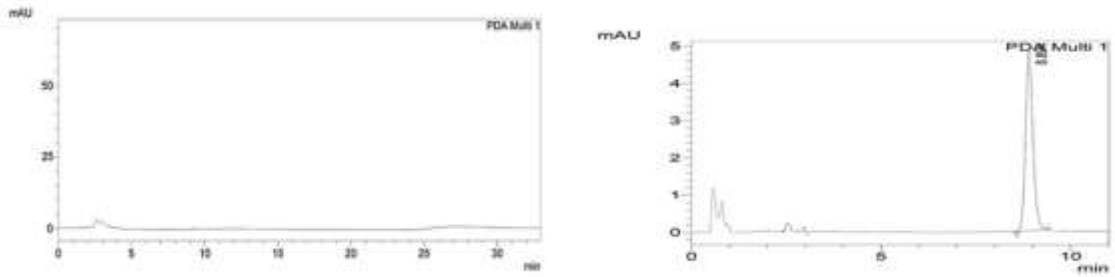

Figure 4: Chromatogram of Rat Plasma and Alendronate in rat plasma

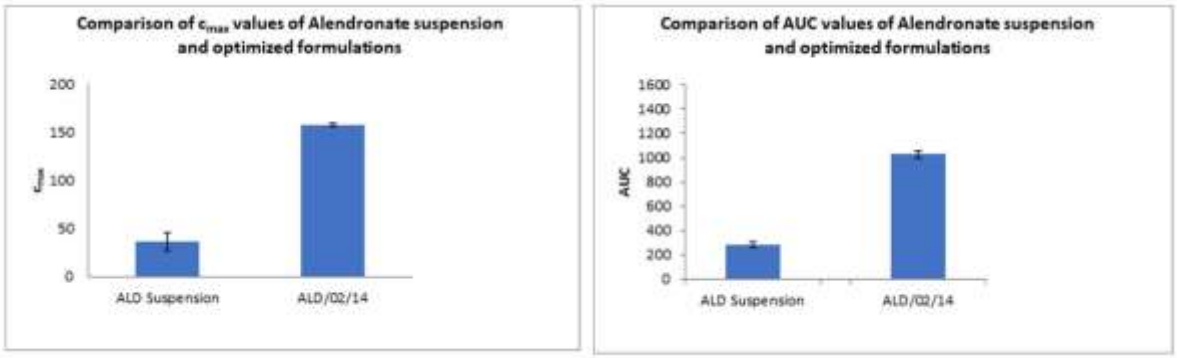

Figure 5: Comparison of AUC and C_{max} values of Alendronate suspension and Nanoparticles.

OPTIMIZATION OF FORMULATION

The optimization of prepared batches was done by considering % drug entrapment and 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. Regression coefficients are statistically significant if $P < 0.05$. 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.).

Evaluation of model / Check point analysis

In order to assess the reliability of the model, a checkpoint analysis was done to confirm the effect of the independent variables on the dependent variables. Any two values of the independent variables were selected from the contour graph the responses were estimated by using the equations and experimental procedure.

Kinetics of Drug Release

In order to investigate the mechanism of drug release from nanoparticles of an optimized batch (ALD/02/14), the release data obtained from in-vitro release studies were fitted to various kinetic equations. The kinetic models used were a Zero order equation ($Qt = Q_0 - K_0t$), first order equation ($\ln Q_t = \ln Q_0 - Kt$), Higuchi's equation ($Qt = Kht_{1/2}$). In these plots, Qt was taken as the percent of drug released at time t, $Q_0$was considered as the initial amount of drug percent in nanoparticles and K_0, K and K_h were constant of the equation of zero order, first order and Higuchi model respectively[10].

Particle Size and Zeta Potential

The average diameter and zeta potential of an optimized formulation (ALD/02/14) was determined by photon correlation spectroscopy (PCS). Nanosuspension in distilled water was added to the sample dispersion unit (deionized water) and stirred at 2000 rpm with magnet in order to reduce the inter-particulate aggregation. The samples were adequately diluted with deionized water and placed in an electrophoretic cell. The average particle size was measured after performing the experiment in triplicate[10].

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry is widely used in thermal analysis to monitor endothermic processes (melting, solid-solid phase transitions and chemical degradation) as well as exothermic processes (crystallization and oxidative decomposition). It is extremely useful since it indicates the existence of possible drug-excipients or excipient-excipient interactions in formulation. Thermograms of pure drug Alendronate and excipients used in the formulation of nanoparticles 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[10].

Scanning Electron Microscopy (SEM)

Surface morphology of optimized formulation (ALD/02/14) was obtained by scanning electron microscope. Electron gun produces a beam of electrons, which follows the vertical path through the microscope between electromagnetic fields and lenses towards the sample due to which electrons, and X-rays are ejected from sample.

Prior to examination, the samples were mounted on to metal stubs using a double sided adhesive tape under vacuum. The scanning electron microscope was operated at an acceleration voltage of 25 kV[10].

Stability Studies

The optimized formulation of Alendronate loaded nanoparticles were placed in screw capped glass container and stored at various ICH storage conditions i.e. 25ºC ± 2ºC/60% RH ± 5% RH and 40ºC ± 2ºC/75% RH ± 5% RH for a period of 60 days.

The samples were analysed for physical appearance, drug content andin vitro drug release study at regular interval of 15 days[10].

IN VIVO PHARMACOKINETIC STUDY IN RATS

Experimental Animals

The experimental protocol in the present study was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the Institutional Animal Ethics Committee (IAEC). 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 (Nav Maharashtra oil mills ltd, Pune) and purified drinking water[12].

Bioanalytical Method

In the present work, 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 0.05 M sodium citrate and sodium phosphate buffer (pH 8) acetonitrile methanol (75:20:5 v/v/v). 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[13].

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 266 nm. For chromatographic separation, Enable C18 250mm × 4.6 mm column, 5 µm was used. All the chromatograms were analyzed by LCs solution.

Experimental Design

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 four groups each consisting of six animals. All animals were given different formulations group wise as described underneath.

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

Group II: Formulation (optimized Alendronate 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 Data Analysis

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

Statistical analysis of the obtained data was carried out by using data analysis feature of Microsoft Excel 2007. The t – test was calculated with the level of significance, $P<0.05$.

RESULTS AND DISCUSSION

Ionic gelation method was selected for the formulation of Nanoparticles. The percent yield, percent Entrapment efficiency and percent drug content of all the factorial batches are shown graphically in Figure 1. The percentage yield for each formulated batches was found to be in the range of 63.39 ± 1.13to 72.87 ± 1.68. It was observed that production yield was varied with change in concentration of chitosan or sodium TPP. ALD/02/09, ALD/02/13, and ALD/02/14 batch gave a yield of above 70%. It was also found that, the formulation containing similar concentration of chitosan and sodium TPP were gave higher yield as compared to other formulation batches.

The percentage entrapment for each formulation was found to be in the range of 32.50 ± 0.50 to 59.83± 0.44. The maximum percentage drug entrapment was obtained for the formulation ALD/02/14. It was observed that increase in concentration of chitosan and sodium TPP significantly increases percentage entrapment. However, further increase in chitosan concentration from 6 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.

Percentage drug content was determined for all formulated batches by using UV visible spectrophotometric method at λ_{max} of 570 nm. It was found in the range of 2.50 ± 0.13 to 5.07 ± 0.05. It is essential to determine drug content for performing the in-vitro drug release study as an equivalent amount of drug can be calculated from percentage drug content.

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 ALD/02/07 showed the maximum release of 88.08± 0.76 over a period of 24 hours

Two-dimensional contour plots were established using reduced polynomial equation for % entrapment efficiency. Contour plots were established between X1 and X2 at fixed level of -1, 0 and 1.

On the basis of obtained result for characterization of nanoparticle, it was found that change in the concentration of chitosan and sodium TPP has higher influence on entrapment efficiency. Thus a 3^2 full factorial design was employed in optimizing the formula. The concentration of chitosan (X_1) and concentration of sod TPP (X_2) were taken as the independent variables whereas the entrapment efficiency was taken as the dependent variable. The plots are represented in Figure 2.

Check point analysis was carried out to confirm the reproducibility of the factorial design experiments. Here any two random values of the independent variables were taken and was compared with the values obtained earlier. The percent error was calculated. The difference was found to be insignificant.

The square of the regression coefficients of zero order, first order and Higuchi's model was calculated. It was found that the square of the regression coefficient was the maximum in Higuchi's model.

From the analysis of particle size, the mean particle size was found to be 722 nm and zeta potential was found to be -11.9 mv.

The Thermograms of the pure drug, Alendronate sodium and the formulation blend did not show any significant difference in the melting point of the drug indicating that there is no interaction between the drug and the polymer. The SEM image of the optimized batch is showed in Figure 3.

The stability study was carried out for optimized formulation ALD/02/14 indicated that there was no significant deviation in the physical properties and release profile of the formulation.

In vivo pharmacokinetic studies were conducted on rats and the results showed a significant difference in the various paramentres like c_{max}, t_{max} and AUC. The c_{max}, t_{max} and AUC values for the nanoparticles were found to be 157.45 ± 1.80 ,6 hrs and 1026.88 ± 32.11 respectively. However, it was found that the relative bioavailbility value in case of Polymeric nanoparticles was more than conventional pure drug. Thus, nanoparticles of Alendronate can be considered a superior formulation in the present work. The chromatogram of blank rat plasma and chromatogram of the drug are shown in Figure 4. Comparison of c_{max} and AUC values of Alendronate suspension and nanoparticles are shown in Figure 5.

CONCLUSION

The present research work concludes that the bioavailability of BCS class III drugs can be improved if delivered through nanocarrier system. The concentration of chitosan and Sodium Tri polyphosphate showed to be a key factor to optimize the nanoparticles. Alendronate can provide higher bioavailability than the other dosage forms of BCS class III drugs in order to get potentially more rapid onset of action.

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