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Formulation and Evaluation of Solid Lipid Nanoparticles of Alendronate Sodium using Different Lipids

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Abstract

Solid lipid nanoparticles are the rapidly developing field of nanotechnology with several potential applications in drug delivery, clinical medicine and research. The purpose of this research was to develop novel particulate carriers such as Solid Lipid Nanoparticles (SLN) of Alendronate Sodium as an anti-osteoporotic agent which was prepared by hot homogenization method to sustained release of drug and to improve bioavailability and to study its effects. The factorial design was used to design the formulation of Solid Lipid Nanoparticles of Alendronate Sodium. Glyceryl Monostearate (GMS), Compritol, Dynasan as lipid, Poloxamer as surfactant and Stabilizer are used for Solid Lipid Nanoparticle preparation. The entrapment efficiency and drug content indicated that, as the concentration of drug to polymer ratio increases the entrapment efficiency and drug content also increases. Based on entrapment efficiency and *in-vitro* behavior, formulation code F7 was considered as the optimized formula. The particle size was found to be 202 nm. The compatibility study, DSC is performed on optimized formulation. *In-vitro* study showed the controlled release of SLN formulation on Pure Alendronate Sodium drug over a period of 36 hrs. At the end of accelerated stability studies for the selected formulation F7 showed almost same drug content, entrapment efficiency, *in vitro* drug release and no changes observed when compared to the initial product.

Keywords

Nanoparticles, Alendronate sodium, Osteoporosis, optimized formulation.

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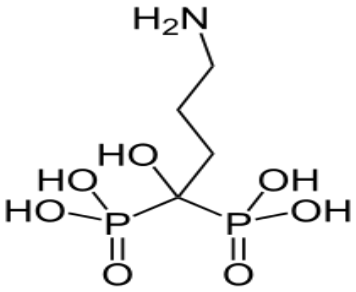
INTRODUCTION

Solid lipid nanoparticles (SLNs) introduced in 1991 and are worthy carriers for oral delivery of lipophilic and to some extent for hydrophilic drug candidates (1). SLNs an alternative and better carrier system to traditional colloidal carriers such as emulsions, liposomes and polymeric micro and nanoparticles. SLNs are colloidal carrier system composed of a high

melting point lipid as a solid core coated by aqueous surfactant and the drugs used are of Biopharmaceutical classification system (BCS) Class II and IV. Previously reported works showed enhanced bioavailability of poorly water-soluble drugs when encapsulated in lipid-base vehicles (2,3). The use of solid lipid instead of liquid lipid is beneficial as it has been shown to increase control over the release kinetics of encapsulated compounds and to improve the stability of incorporated chemically sensitive lipophilic ingredients. In SLNs the lipid matrix is made from physiological lipid which decreases the danger of acute and chronic toxicity, have very high long-term stability (4). SLN offer unique properties such as small size, large surface area, high drug loading and the interaction of phases at the interface and are attractive for their potential to improve performance of pharmaceuticals (5). SLN not only has combine advantages of colloidal drug carrier systems such as liposomes, polymeric nanoparticles and emulsions but also avoid the drawbacks associated with respective carrier system (6). Possibilities of controlled drug release, increased drug stability, high drug pay load, no bio-toxicity of the carrier, avoidance of organic solvents, incorporation of lipophilic and hydrophilic drugs show it's positive over water soluble drug (7).

Figure 1: Structure of Alendronate



Alendronate (Fig-1), a second-generation bisphosphonate is the first member of a group of drugs which strengthens bone. Alendronate is used to reduce hypercalcemia in tumor-induced bone disease, to treat corticosteroid-induced osteoporosis and Paget's disease and to prevent osteoporosis in postmenopausal women (8). The action of Alendronate on bone tissue is based partly on its affinity for hydroxyapatite, which is part of the mineral matrix of bone. Nitrogen-containing bisphosphonates (such as pamidronate, alendronate, risedronate, ibandronate and zoledronate) appear to act as analogues of isoprenoid diphosphate lipids, thereby inhibiting farnesyl pyrophosphate (FPP) synthase, an enzyme in the mevalonate pathway (9). Inhibition of this enzyme in osteoclasts prevents the biosynthesis of isoprenoid lipids (FPP and GGPP) that are essential for the posttranslational farnesylation and geranylgeranylation of small GTPase signalling proteins. This activity inhibits osteoclast activity and reduces bone resorption and turnover. Relative to an intravenous (IV) reference dose, the mean oral bioavailability of alendronate in women was 0.7% for doses ranging from 5 to 40mg when administered after an overnight fast and two hours before a standardized breakfast (10). Oral bioavailability of the 10mg tablet in men (0.59%) was similar to that in women (0.78%) when administered after an overnight fast and 2 hours before breakfast (10). Alendronate can damage the esophagus both by toxicity from the medication itself and by nonspecific irritation secondary to contact between the pill and the esophageal mucosa, similar to other cases of "pill esophagitis" (11). On the other hand, oral bioavailability of alendronate sodium is very low. Intake together with meals and beverages other than water reduces its bioavailability even more (12). Poor absorption is attributed to its high hydrophilicity and complexation with divalent cations, like Ca^{2+} (12). Thus, the aim of this study is preparation of solid lipid nanoparticles of Alendronate Sodium is to sustain the drug release and enhances bio availability of drug.

MATERIALS AND METHODS:

Alendronate sodium was obtained by Troikaa Pharmaceutical Pyt. Ltd, Ahmedabad, India , Compritol, Dynasan and Glycerol Monosterate by Mohini Organic Pvt. Ltd, Mumbai, India, Dialysis Membrane from Hi - Media Ltd., India, Disodium hydrogen phosphate by Priya Research Labs, Bangalore, India, Mannitol, Ninhydrin and Potassium dihydrogen phosphate from S.D Fine Chemicals Ltd, Mumbai, India, Poloxamers by Madras Pharma ,Chennai, India, Pyridine obtained from Karnataka Fine Chem, Bangalore, India and Lipoid from Thomas baker chemicals Ltd, Mumbai, India respectively as a gift sample for research use only.

Experimental Methodology is divided into three sections as: a) Pre-formulation Studies b) formulation development c) evaluation.

Pre-formulation studies:

Pre-formulation studies were carried out to investigate the salient physicochemical properties of the drug molecule and to evaluate the possible drug excipient interactions. Initially standard curve of drug was obtained by UV method, followed by Solubility studies, differential scanning calorimetry

(DSC), TEM, Lyophilizer, Zetasizer Ver. 7.0 and X-ray diffraction studies. Studies were carried out for the pure drug and excipients. Solubility of the Alendronate Sodium was carried out in dissimilar solvents like ethanol, alcohol and water but solubility was found to be more in water. Estimation of alendronate sodium is done by using the Milli Q water. 1000µg/ml of standard stock solution is prepared by weighing 10 mg of accurately weighed drug and dissolving in Milli Q water. Working stock of 100µg/ml is prepared from the above standard stock solution. The working stock solution is scanned in UV visible spectrophotometer at λ max of 565 nm. Differential scanning calorimetry studies were conducted using DSC Q 2000. Sample was weighed (10.0 ± 0.5 mg) and placed in sealed aluminium pans. The coolant was liquid nitrogen. The samples were scanned at 100˚c/min from 50 to 300˚c. DSC thermograms of pure Alendronate Sodium, physical mixtures and polymer were taken.

Formulation Studies:

Nine formulations (F1-F9) were prepared in which F1, F2 and F3 contains 10mg Sodium Alendronate with 100, 150and 200mg GMS respectively; F4, F5 and F6 contains 10mg Sodium Alendronate with 100, 150and 200mg Dynasan respectively and F6, F7 and F8 contains 10mg Sodium Alendronate with 100, 150and 200mg Compritol 888 ATO respectively. Alendronate sodium loaded SLN formulations were prepared by hot homogenization Solid lipid (GMS, Dynasan and Compritol 888 ATO) with 100µl Tween 20 as a surfactant was heated at 70˚C in a boiling water bath to be melt under continuous stirring (oil phase). In a separate container, surfactant Poloxamer 407 was dissolved in ultra-pure water and heated to the same temperature of the oil phase (aqueous phase). The drug was dissolved in 2ml of aqueous phase and added into the oil phase under homogenization at 20,000 rpm to form the initial water-in-oil emulsion. Then the hot w/o emulsion was added to drop by drop into aqueous phase containing surfactant under stirring at 20000 rpm to maintain temperature.

Lyophilization:

The various cryoprotectants can be used to limit freezing and thawing damage to SLN such as sucrose, and mannitol. In the present study, all the lipid formulations were freeze temperature - 40˚C for one hour 30 min and sample condense at - 60˚ C under reduced pressure for 24 hours to get a free-flowing powder. The formulations were refrigerated until further uses have been used as cryoprotectants.

Factorial design and desirability function - Optimization responses:

In the numerical optimization techniques, the desirability approach was used to generate the optimum settings for the formulation. For the optimized formulation, Drug: Lipid was kept in range, drug release at 0.5 to 24hrs was kept minimum and particle size, entrapment efficiency was kept maximum. The composition of optimized formula is Alendronate Sodium, Glyceryl Monostearate, Dynasen, Compritol-888 ATO Poloxomer-407 and RPM. The optimized formulation was prepared according to predicted model and evaluated for responses.

Evaluation of nanoparticles:

The size of Nanoparticles was determined by dynamic light scattering (Nano ZS3600, Malvern Instruments, Malvern, UK), with varying duration greater than 20s. The dispersant used was water having RI (1.59), viscosity (0.8872 cP).

Determination of total drug content:

Accurately 1ml of prepared SLN dispersion was taken and diluted to 10 ml with Water, further dilutions were made using 6.8ph Phosphate buffer and absorbance was measured at 565nm using drug unloaded SLM'S as blank. Percentage drug content in the SLM was calculated from the equation:

$$\% \text{ Drug content} = \frac{\text{Analyzed content of drug in SLM} \times 100}{\text{Quantity of Drug taken}}$$

Determination of drug entrapment efficiency:

SLN dispersion was centrifuged at 10,000 rpm for 30 min. The amount of free Alendronate in the supernatant was determined at 565 nm using UV–Vis spectrophotometer. Entrapment efficiency was calculated using the formula:

$$\text{Entrapment Efficiency} = \frac{\text{Total Drug-Free Drug}}{\text{Total Drug}}$$

X-Ray Powder Diffraction (XRPD) studies:

Vacuum grease was applied over a glass slide to stick the sample. About 50mg of sample was sprinkled over it to make a layer thickness of about 0.5 mm. All the experiments were performed on an X-ray diffract meter (Philips PW, 1729) having sensitivity of 0.1mg. The sample slide was placed vertically at angle of zero degree in the sample chamber. An X-ray beam (Philips Cu target x-ray tube) of 2 kW was allowed to fall over the sample. As slide moves at an angle of theta degree, a proportional detector detects diffracted x-rays at angle of 2θ degrees ranging from 2-800. XRD patterns were recorded using Philips JPCD software for powder diffractometry.

In vitro drug release:

In vitro drug release in Phosphate buffer pH 6.8 was determined. 900 ml of the medium was placed in 900 ml beaker. Nanoparticles containing 10 mg equivalent of drug was placed in dialysis bag (10,000 - 12,000 Daltons) previously soaked in 6.8 Phosphate buffer and was placed in medium. The assembly was stirred at speed of 50 rpm at 37 ± 5 ˚C. The aliquots of 1 ml were removed at specific time intervals 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 12, 24, and 48 hrs replacing by fresh medium every time and were analysed by using UV method.

Stability Study:

The alendronate nanoparticles were subjected to stability analysis over a period of 3 months. Stability studies were carried out for the formulation F7 as per ICH guidelines for a period of three months under the storage conditions of 5 ± 3°C.

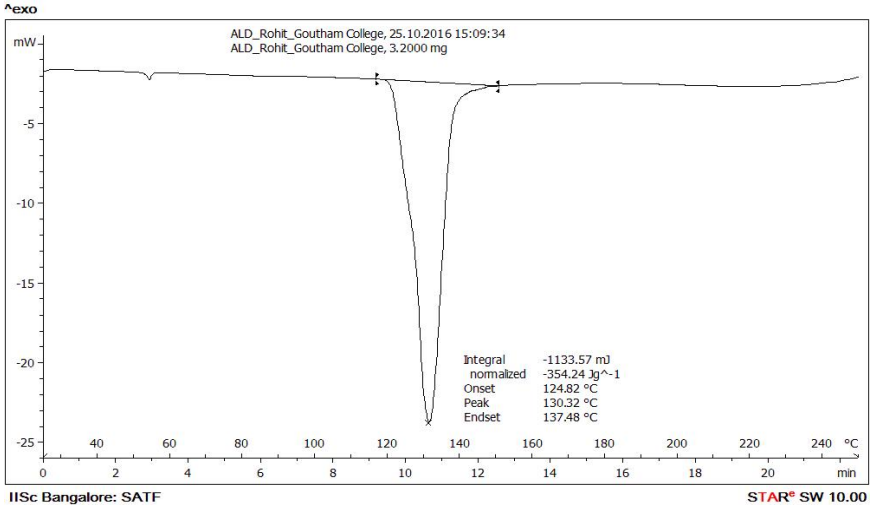
RESULTS AND DISCUSSION

The melting point of the obtained drug sample was found to be 234ºC which is within the reported range of 230 to 270ºC as Dolatabadi et al., 2014 (13). It complies with the standards thus indicating the purity of the drug Alendronate Sodium can be used for SLN preparation.

The solubility profile of Alendronate Sodium in various media was carried out and found that it was highly soluble in water and sparingly soluble in organic solvent like methanol, ethanol, DMSO as reported by Sweetman, 2009 (14).

In the development of SLNs the confirmation of desired physical state of matrix lipid is of crucial importance which can be determined by the DSC. DSC analysis was performed in order to investigate the melting and recrystallization behaviour of crystalline materials like SLNs. When the DSC thermo grams of the bulk lipids and corresponding SLNs are compared the difference in the position and shape of the signals are usually observed. The DSC curve of the pure drug ALD shows that it is in crystalline anhydrous state, exhibiting a sharp endothermic peak at 130.32 °C with peak onset at 124.82°C and end set at 137.48°C (Figure 2).

Figure 2: DSC of pure drug sample of Alendronate



The partition coefficient of Alendronate Sodium conducted by shake-flask method was found to be 0.493 as reported by Miladi et al., 2015 (15). The partition coefficient value indicates that it was more soluble in aqueous phase and hence drug is hydrophilic in nature.

In this method, different lipids such as Glyceryl Monostearate, Dynasan, Compritol was used as a suitable controlled delivery component; poloxamer was used as a surfactant for the formulation of Solid Lipid Nanoparticles. Conventional hot homogenization gave best result in SLN formulation

hence this method was finalized and used. During the formulation the temperature was maintained at (60-80) °C, mixing speed was 1000- 3000rpm for 30 minutes and centrifugation speed was 6000 rpm and the formed nanoparticles were dried by lyophilization process. In the present work, all the nine formulations (F1-F9) were evaluated for entrapment efficiency, drug content, particle size and *In vitro* studies (Table 1). Based on all the evaluation, F7 was found to best formulation. Further, evaluation and characterization were carried out for entrapment efficiency, drug content, *In vitro* study, thermal studies, XRD studies, particle size, TEM and stability studies.

Particle size analysis for optimized formulation was carried out using Optical microscope. It showed that the average diameter of the nanoparticles was found to be 202 nm as also reported by Alarfaj et al., (16). And the single peak obtained from analysis indicates uniformity of the particle size which is the result of the use of conventional hot homogenization technique. Decreasing of the particle size with the increasing of stirring rate can be explained by the intensification of the micro mixing (i.e. mixing on the molecular level) between the multi-phases. High micro mixing efficiency enhanced the mass transfer and the rate of diffusion between the multiphase, which induced high homogenous supersaturating in short time and thus rapid nucleation to produce smaller drug particles (17). Hence, higher stirring rate favoured the formation of the smaller and more uniform drug particles. But there is a slight increment in particle size with the increase of the stirring rate. May be the high stirring rate might result in the formation of the small particles and then the small particles could aggregate to form large nanoparticles because of the absence of enough surfactants. As the lipid concentration was increased, more particles were aggregated resulting in an increased particle size. When the concentration of the lipid exceeded with a fixed concentration of surfactants, there were insufficient surfactants available to coat the surface of all the lipid droplets, resulting in particle aggregation and an increase.

It is also reported by Yeole et al, (18) that high micro mixing efficiency increased mass transfer and the rate of diffusion between the multi-phases, which induced high homogeneous super saturation in short time thus rapid nucleation to produced smaller drug particle. In the addition high stirring speed prevent the particle growth by preventing their aggregation. Hence formation of smaller and more uniform drug particles with high stirring speed.

Hot homogenization technique was employed; this gave satisfactory product after lyophilization. Entrapment efficiency of these formulations is given in Table 1. Entrapment efficiency in these formulations ranged from (51.70-61.82). Among the nine formulations, F4 and F8 have the highest % entrapment i.e.51.70% and 61.82% respectively while other formulations also gave satisfactory drug entrapment efficiency.

Drug content of these formulations is given in Table 1. The drug content of the formulations ranged from 44.15 to 80.46 among the nine formulations, F8 was found to be the highest drug content of 80.46 while other formulations also gave satisfactory drug content. Drug release from solid lipid nanoparticles and subsequent biodegradation are important for developing successful formulations. The release rate of nanoparticles depends upon i) Desorption of the surface-bound/adsorbed drug; ii) Diffusion through the nanoparticle matrix; iii) Diffusion (in case of nano-capsules) through the polymer wall; iv) Nanoparticle matrix erosion: and iv) A combined erosion/diffusion process (19, 20).

Thus, diffusion and biodegradation govern the process of drug release. SLN of Alendronate Sodium formulations F1 to F9 were subjected to *in vitro* dissolution studies using USP apparatus type II in phosphate buffer pH 6.8, in order to compare the release of drug from different lipid formulations and of which is shown in Table 2. The highest release of the drug took from the formulation F7 i.e., 91.115% at 48 hrs. Data obtained from *in-vitro* release study showed that F8 (74.617%) were found to control the release of the drug for longer period of time i.e. for 48 hours.

Figure 3: Diffraction spectra of formulation F7

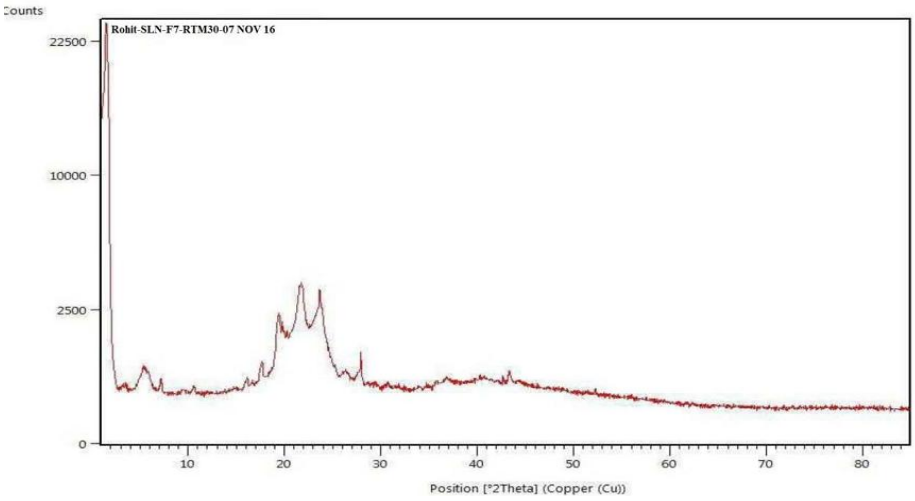
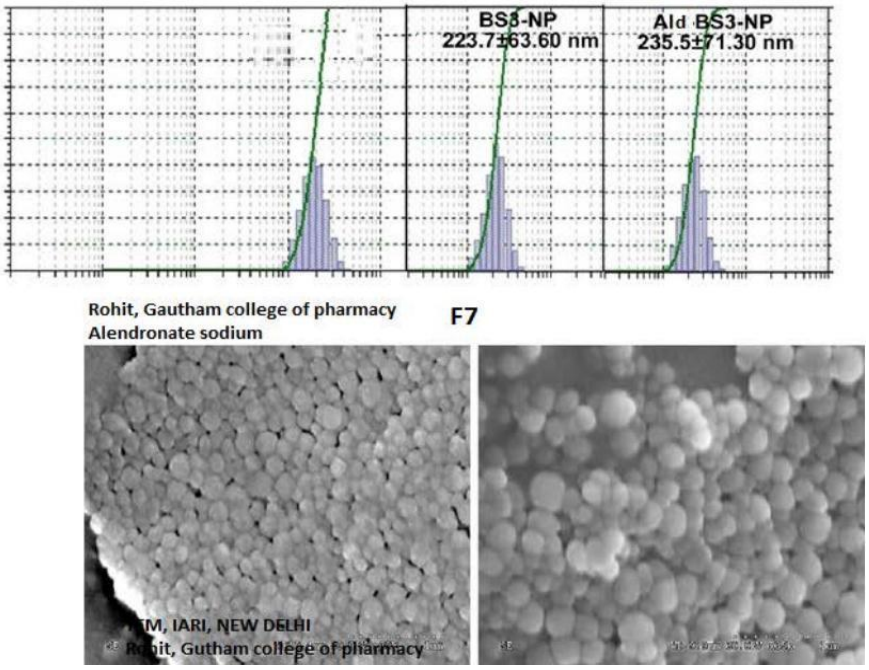


Figure 4: TEM photographic of formulation F7



Diffraction spectra formulation F7 was obtained in the Figure 3. The XRD of formulation F7 shows less numbers of peaks than pure dug which less are sharp in nature, diffused with reduced intensities in the range of 0-80 (degrees 2θ). This indicates that SLN of Alendronate Sodium i.e. F7 is less crystalline.

The TEM photographic data of SLN indicated that Nanoparticles prepared by conventional by hot homogenization method were found to be discrete, fully spherical, and slightly smooth in surface. The results showed that wall structure of the spheres was porous and contains inter-granular spaces (Figure 4). The alendronate nanoparticles were subjected to stability analysis over a period of three months (90 days). The obtained results of alendronate content in nanoparticles after 30, 60 and 90 days of stability studies for each formulation are tabulated in Table 3. Drug loading data of freshly prepared formulations stored at 5±3°C were considered for the control and compared against samples. Stability studies of F7 were carried out for the formulation F7 as per ICH guidelines for a period of three months under the storage conditions of 5±3°C. Thus, according to this finding, we can conclude that for better stability, the formulation should be stored under refrigerated conditions.

Table 1: Data showing Evaluation of % yield, % drug content, % entrapment efficiency and Particle Size of formulations F1-F9.

Formulation %	Drug Content %	Entrapment Efficiency %	Particle Size (nm)
F1	50.4	45.87	144.9
F2	44.15	37.96	238.9
F3	44.74	45.91	245.3
F4	57.19	51.70	2204
F5	48.86	42.13	157.8
F6	45.86	39.07	1552
F7	56.76	51.24	202.2
F8	80.46	61.82	556.0
F9	58.21	48.97	879.5

Table 2: Comparative data of percentage drug release from the formulations F1 to F9.

Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0.5	6.197	6.674	7.638	4.843	5.367	5.527	7.471	6.384	7.790
1	15.69	14.95	15.553	14.73	17.07	16.83	22.828	18.979	23.573
1.5	17.28	17.49	16.605	15.25	17.80	17.22	23.864	19.241	24.078
2	20.55	22.15	21.601	17.51	20.60	18.12	24.693	20.115	25.897
3	21.89	23.75	23.442	20.18	22.67	19.78	26.558	21.338	27.109
4	23.99	26.15	25.808	23.06	24.25	24.02	27.801	22.648	30.141
5	28.08	28.28	27.386	26.15	25.1	27.61	30.288	25.530	33.172
6	35.81	34.78	32.382	34.17	33.99	36.20	32.875	27.626	50.551
7	45.20	41.92	40.796	43.01	40.56	41.07	55.365	46.056	60.454
12	64.69	64.78	65.382	65.64	65.27	66.21	74.743	60.992	62.475
24	78.93	77.83	78.004	77.67	77.81	79.55	85.416	65.708	65.506
48	85.70	85.29	82.343	85.49	85.97	81.34	91.115	74.617	85.109

Table 3: Stability studies of formulation F7

Sl. No.	Sampling Interval	Particle size (nm)	PDI	Drug content (%)
1	Initial	202±1.38	0.275	75±0.54
2	30 Days	208±1.47	0.3	73±0.40
3	60 Days	214±0.45	0.41	70±0.3
4	90 Days	220±1.54	0.49	65±0.5

CONCLUSION

The present study was carried out to overcome the low bioavailability problem of an Alendronate Sodium BCS class III drug due to its high aqueous solubility with high permeability. In order to enhance the bioavailability, Solid Lipid nanoparticles (SLN) of Alendronate Sodium were prepared and various excipients were optimized. The studies have shown combination of drug, different lipids such as Dynasan, Poloxamer-407 shows better permeability in compare with other lipids and also shows better entrapment efficiency and drug content, lipid for preparation of SLN. The effect of excipients on the thermal behavior of Alendronate Sodium was studied through DSC which has shown the intactness of drug in formulation. The SLN observed through the TEM were found to be oval with a smooth surface the in-vitro studies have shown that the developed solid lipid nanoparticles of Alendronate Sodium have improved controlled drug release over 48 hrs which may result in increased bioavailability of Alendronate Sodium. Finally, the developed SLN of Alendronate Sodium were kept for accelerated stability study under condition of 2-3°C, in humidity chamber for 3 months and at regular interval samples were evaluated for drug content and any physical change, which has proved the formulation is stable under refrigerated conditions.

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