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Efficacy of Alendronate Functionalized Solid Lipid Nanoparticles for Osteoporosis Treatment-Development and Release Kinetics Study

Sandhya Pathak, Satyendra Kumar Tripathi and Archna Pandey
Department of Chemistry
Dr. H. S. Gour Central University, Sagar, MP, India
sandhyapathak935@gmail.com

Abstract: *Osteoporosis means "Porous bone" is a disease characterized by progressive bone thinning. The deterioration of bone tissue can lead to bone fragility and fracture, especially of the hip, spine, shoulder and wrist. Osteoporosis is caused generally due the decreasing bone mineral density (BMD). Osteoporosis affects 30-40% women after menopause all around the world. Bisphosphonates are the most commonly prescribed drugs for the treatment of osteoporosis in the US and many other countries including India. Alendronate- sodium (AS) is a widely used anti-osteoporosis drug, exhibits strong inhibitory effect on bone resorption performed by osteoclast cells and acts as a potent, specific inhibitor of osteoclast-mediated bone resorption. AS was the first FDA approved bisphosphonate for treatment of osteoporosis in the US in 1995. The objective of the present study was to develop, optimize, and evaluate Solid Lipid Nanoparticles (SLN) of Alendronate-sodium drug which improve the solubility, dissolution rate and enhance the bioavailability of the drug. AS loaded Solid Lipid Nanoparticles have been developed using Glyceral Monosterate (GMS) as lipid and poloxamer 407 as the emulsifier by Emulsion -Solvent evaporation method. Different process variables i.e. concentration of surfactant, homogenization speed and time have been optimized. Formulated SLNs with GMS showed low particle size and high entrapement efficiency. The SLNs were characterized using Zeta sizer, transmission electron microscopy (TEM) and scanning electron microscopy (SEM). In-vitro drug release study was performed by dialysis bag diffusion method and different mathematical models were applied for the release study.*

Keywords: Bisphosphonates, Bone Mineral Density (BMD), Drug release, Osteoporosis, Solid Lipid Nanoparticles (SLNs)

I. INTRODUCTION

Osteoporosis is one of the most common bone diseases diagnosed by low bone density (BMD) and fragility of bone tissues with a consequent increase in susceptibility to fracture. It is called a "Silent disease" because its symptoms cannot identify at an early stage and fracture occurs. Osteoporotic fractures often increase mortality, reduce life quality, long hospital stays and high economic costs. It is commonly seen in old age, but after menopause, women have a high risk of post-menopausal osteoporosis (PMO) [1-3].

Common drugs, which are available in the market, inhibit bone resorption and decrease bone loss, but novel therapies may increase bone mass (BMD). "Current treatment of osteoporosis includes calcitonin, bisphosphonates, Denosumab, selective estrogen receptor modulators (SERM), i.e. Raloxifene and sufficient intake of calcium and vitamin D. Various drugs are used to treat Osteoporosis; Alendronate was the first FDA approved bisphosphonate drug for treatment of osteoporosis in the US in 1995. The osteoclast cells are responsible for bone resoption and caused osteoporosis, so the bisphophonate drugs target to these cells and inhibits the the activity of osteoclast cells. Alendronate is the most potent and recommended drug for the treatment of osteoporosis in medical trials [4-6].

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Alendronate is highly efficient but presents low absorption after oral administration, due to high water solubility fast release occurs. Low systemic bioavailability and burst release of the drug inside the body are main challenges in Alendronate drug delivery systems. Drug nanocarries help in reducing toxicity, improving solubility and bioavailability, enhancing release and provide better formulation opportunities for drugs. In pharmaceutics, there are different drug carrier systems like polymeric nanoparticles (NPs), solid lipid NPs (SLNs), liposome, nanoemulsions, nanosuspension, and micelles etc. can be used at a lower concentration and can lead to early onset of bioactivity., which provide sustained and controlled targeted drug delivery [7-11].

At present, lipids and polysaccharides are commonly used for the formulation of NPs because both materials are natural and more biocompatible than other synthetic materials. Lipids used in these nanoparticles are biocompatible and completely tolerated by the body, like triglycerides, fatty acids, steroids, and waxes. The SLNs formulation can be efficiently stabilized by the use of emulsifiers. SLNs have many advantages in comparison to other nano-carrier systems, such as the easy method of formulation, biocompatible and biodegradable nature of the materials, less toxic, enhanced drug solubility, possibility of controlled drug release and applicable to both hydrophilic and lipophilic drug incorporation. SLNs are thermodynamically stable dispersion of oil and water, stabilized by surfactants and co-surfactants [12-16].

Solid lipid nanoparticles (SLNs) are the first generation of lipid-based nanocarriers, which are stabilized by emulsifiers and solid at the body temperature. The main advantages of SLNs Drug over the conventional drug therapies is biocompatibility, biodegradability, protection against unfavourable environmental situations and easy large scale production. Solid lipid nanoparticles (SLNs) are the most recently used nanocarriers in drug delivery systems after the use of liposomes, emulsions, and polymeric nanoparticles. The low toxic effect, better physical stability and drug loading, easy production at the commercial level and cost-effectiveness are the main features of SLNs [17-19].

In current studies, lipid-based formulations have been given more attention than traditional drug delivery systems to improve the oral bioavailability of poorly water-soluble drugs. In this drug delivery system, the drug is incorporated into lipid carriers (Matrixes) which may be triglycerides, fatty acids, steroids, and waxes. Biocompatible surfactants, i.e. polysorbate, poloxamer and soybean lecithin, are used as stabilizing agents. The SLNs size may be in the range between 50 to 1000 nm and provide a larger surface area, sustained release of the drug and fast uptake by cells, which is helpful in enhancing the solubility and of the drug [20- 25].

In the present study, the authors have developed the Solid Lipid Nanoparticles (SLNs) of Alendronate to enhance the drug bioavailability. This novel drug delivery system is designed for a more efficient therapeutic effect of this drug and minimizing its toxic effects, which is achieved by different process variables. Emulsion -Solvent evaporation method was applied for the formulation of Alendronate loaded SLNs using Glycerol Monostearate (GMS) as lipid carrier and poloxamer 407 as the emulsifier. The prepared SLNs were evaluated for particle size, distribution(PDI), zeta potential, entrapment efficiency, surface morphology, and in vitro drug release study by using different mathematical models [26-29].

II. Materials and Methods

2.1 Materials

The drug Alendronate-sodium (M.W. of 249.09 g/moL) was procured from Sigma Aldrich. Glycerol Monostearate (GMS) (M.W. 358.87) was purchased from CDH (India). Poloxamer 407 (P407) was purchased from Signet Chemicals, Mumbai, India. Dialysis membrane was purchased from Himedia (Mumbai, India). All other chemicals and reagents were of analytical grade. High purity water was used for all experiments, prepared by using (Millipore).

2.2 Preparation of Alendronate Loaded SLNs

Emulsion -Solvent evaporation method was used for the preparation of Alendronate loaded SLNs [30, 31]. In this method, the lipid is dissolved in an organic solvent such as acetone or chloroform to prepare the organic phase, and the surfactant solution in water formed the aqueous phase. The organic phase is added to the aqueous phase under continuous stirring at a fixed temperature (70-80 °C). The stirring will be continued till the complete evaporation of the

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organic phase. In the present work, a fixed amount of drug Alendronate (20 mg) was dissolved in methanol, and 100 mg of lipid (GMS) was dissolved in chloroform separately. Both solutions of lipid and drug were mixed slowly. A rotatory evaporator was used to complete the evaporation of organic solvent at 700C by purging N_2 gas. This drug contained lipid layer was added into the aqueous solution containing surfactant poloxamer 407 (1% w/v) at 70^0C using a hot plate and homogenized for 20 minutes. Now, the formulated SLNs suspension was allowed to cool at room temperature.

The prepared SLNs were analyzed by Dynamic light scattering (DLS), transmission electron microscopy (TEM) for particle size and distribution. The formulated SLNs suspension was then allowed to cool at room temperature and lyophilized using a lyophilizer (LAMBCONCO, GNCIIM) for future preservation.

2.3 Entrapment Efficiency (EE%)

The Entrapment efficiency (EE%) of Alendronate loaded SLNs was determined by centrifugation at 15000 RPM for 20 minutes. The NPs were centrifuged, and the pellet of NPs was collected. 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 [32]. EE% was determined at 240 nm wavelength following the addition of copper (II) reagent (5 mM copper sulfate in1.5 × 10^{-3}M HNO_3) by complexation between Alendronate and copper ions. 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:-

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

III. Characterization

The most important parameters in solid lipid nanoparticles (SLNs) characterization are particle size and size distribution (PDI), zeta potential, entrapment efficiency, surface morphology, and drug release study.

3.1 Particle Size, PDI, and Zeta Potential

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

3.2 Surface Morphology Study

The prepared RL-HCL loaded SLNs were evaluated by Scanning Electron Microscope (SEM) for Surface morphology using (NOVA, NANO FESEM 450). Formulated NPs were also confirmed using Transmission Electron Microscope (TEM) for surface morphology and size. The prepared sample was examined by TEM (TECNA).

3.3 In Vitro Drug Release Studies

The dialysis bag method was used for in -vitro drug release study of prepared SLNs using pH 6.8 phosphate buffer (PBS) as diffusion medium and dialysis membrane (M.W. 12,000–14,000 Daltons) [33]. The drug-loaded SLNs were placed into a dialysis membrane, tied at both ends and placed in a beaker containing 100 mL of diffusion medium PBS (PH 6.8). A magnetic stirrer (REMI, India) was used to maintain temperature and stirring speed, 37 ± two °C and 100 rpm, respectively. Dialysis membrane was immersed in a beaker of diffusion medium, 5 ml of aliquots withdrawn from the beaker at fixed time intervals and the same volume was filled with fresh buffer (PBS) into beaker to maintain the sink condition. The drug release at fixed time intervals was analyzed spectrophotometrically at 240 nm for Alendronate. The cumulative % drug release was calculated from the amount of drug release. Some mathematical kinetic equations were used for determination of release kinetics, such as zero order, first order, Higuchi's model and Korsmeyer-Peppas model. Values of *R2* (and K (rate constant) were calculated from the linear curve obtained by regression analysis of the plots [34].

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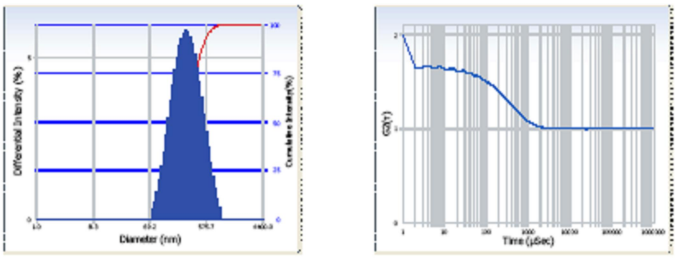
IV. Results and Discussion

4.1 Experimental Design

Solid lipid nanoparticles are novel potential drug carrier systems having many advantages such as more biocompatibility and less toxicity than other drug nanocarrier systems. Several methods are available for the preparation of SLNs, i.e. hot and cold high-pressure homogenization, solvent emulsification/evaporation, microemulsion formation technique and ultrasonic solvent emulsification method. In the current study, SLNs were prepared by solvent emulsification/evaporation method because it is a simple, reliable and reproducible method used in many previous studies.

In this study, Alendronate loaded SLNs were prepared by using Glycerol Monostearate (GMS) as lipid carrier and poloxamer 407 as the emulsifier. Prepared SLN dispersion was found to be uniform and homogenous in appearance. This formulation of RL-HCL loaded SLNs was considered for further studies, i.e. characterization and in-vitro drug release kinetic studies.

4.2 Particle Size, PDI, and Zeta Potential (ZP)



(a) Particle size AS loaded SLNs

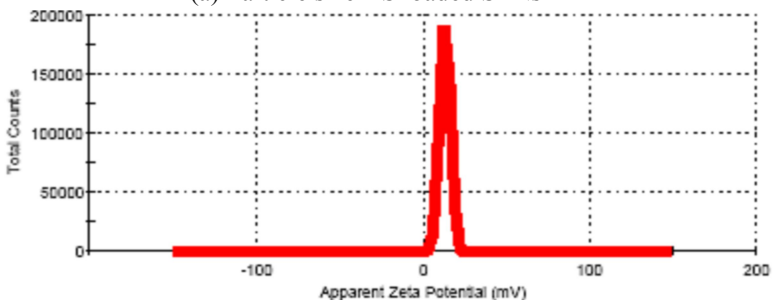


Figure 1: (b) Zeta Potential of AS loaded SLNs

The results of DLS study of Alendronate loaded SLNs by Malvern Nano Zetasizer are shown (Figure 1, a & b). Based on the results of DLS studies of prepared SLNs formulation, particle size was in nano range (248.8 nm) and entrapment efficiency was 67.21± 1.5 %. The ratio of Drug / lipid (1:4) helped in increasing drug solubility and entrapment efficiency %. Poly dispersive index (PDI) was 0.241, represents narrow distribution of nanoparticles within the system. Zeta potential is very important factor for the stability of SLNs colloidal dispersion during storage. Zeta potential of the formulation was found to be +12.9 mV; this positive value might be due to the presence of one basic nitrogen atom on the surface of SLNs.

4.3 Surface Morphology of SLNs

The morphology of nanoparticles was analyzed by using the Scanning Electron Microscopic (SEM) and Transmission Electron Microscopic(TEM) was used to confirm their spherical shapes. SEM and TEM images of the prepared SLNs formulation in (Figure 2 a & b). The result were found in the nano range. TEM image showed completely spherical and symmetrical nanoparticles were formed in the SLNs formulation.

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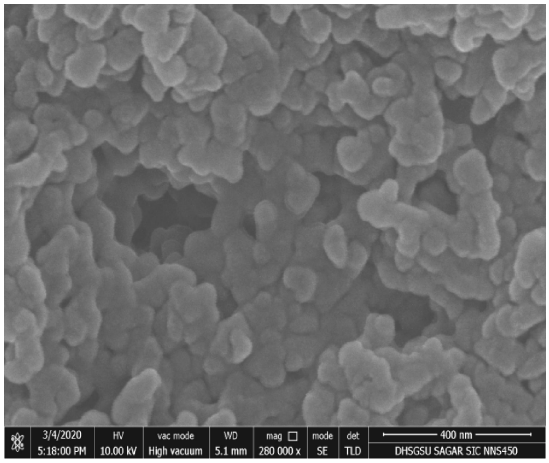
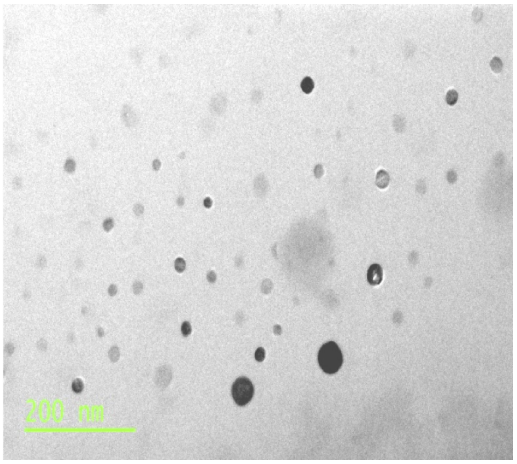


Figure 2: (a) SEM image AS loaded SLNs



(b) TEM image of AS loaded SLNs

4.4 Drug Release Kinetic Study

The dialysis bag method was applied for the In-vitro drug release study of formulated RL-HCL loaded SLNs. Different mathematical kinetic models were used for the kinetic study of formulated drug-loaded SLNs. The plots were made (cumulative % drug release vs time) for Zero order kinetic model, (log of cumulative % drug remaining vs time) for the First-order kinetic model, (% drug release vs square root of time) for Higuchi model and (cumulative and log cumulative % drug release vs log time) for Korsmeyer– Peppas model.

In vitro drug release of formulated RL-HCL loaded SLNs was compared with pure drug solution shown in (Figure 3). Results showed that release of pure drug was fast, about 85% drug release within 7-8 hours, while SLNs formulation showed the sustained release of drug up to 30h. Initially, SLNs formulation showed the burst release (45% drug release in 7h), followed by sustained (76.23 % drug release at 30h). Initial fast release may be due to the presence of the adsorbed drug on the surface of SLNs.

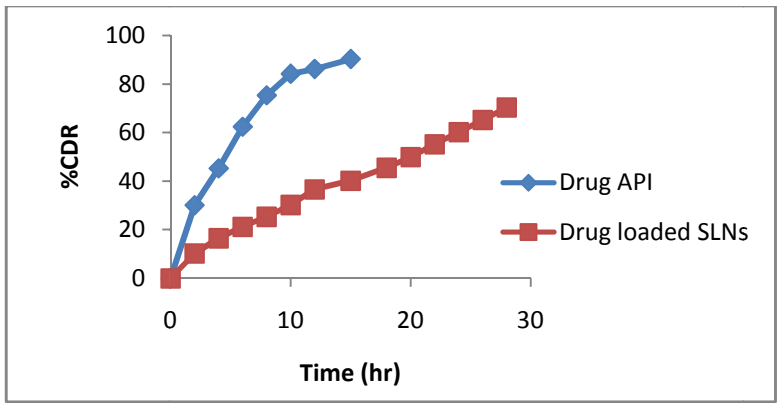


Figure 3: In –vitro drug release profile of Alendronate loaded SLNs and Pure drug in PBS (P^H 6.8)

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, "K" is rate constant and "n" is release exponent.

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

Kinetic Models	Mathematical Expression	Correlation value (R^2)	Release exponent (n)
Zero order	$Q_t = k_0 t$	0.9916	-----
First order	$\log Q_0 - \log Q_t = k_1 t/2.303$	0.9030	-----
Higuchi model	$Q_t = k_H t^{1/2}$	0.9627	------
Korsmeyer– Peppas	$Q_t = k_{KP} t^n$	0.9940	0.56

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Where, Q_0 is the initial concentration of drug, Q_t is the concentration of drug at time "t". k_0, k_1, k_H,, k_{KP} are the rate constants for zero order, first order, Higuchi and Korsmeyer– Peppas model respectively. On the basis of above values of (R^2), the best fit kinetic model with the highest linearity for Korsmeyer– Peppas model. It is concluded that in formulated SLNs follow Korsmeyer– Peppas model kinetics. In the Korsmeyer-Peppas model, release exponent value "n" is 0.56. The magnitude is in the range ($0.45 < n < 0.89$) indicates the release mechanism is non-Fickian diffusion.

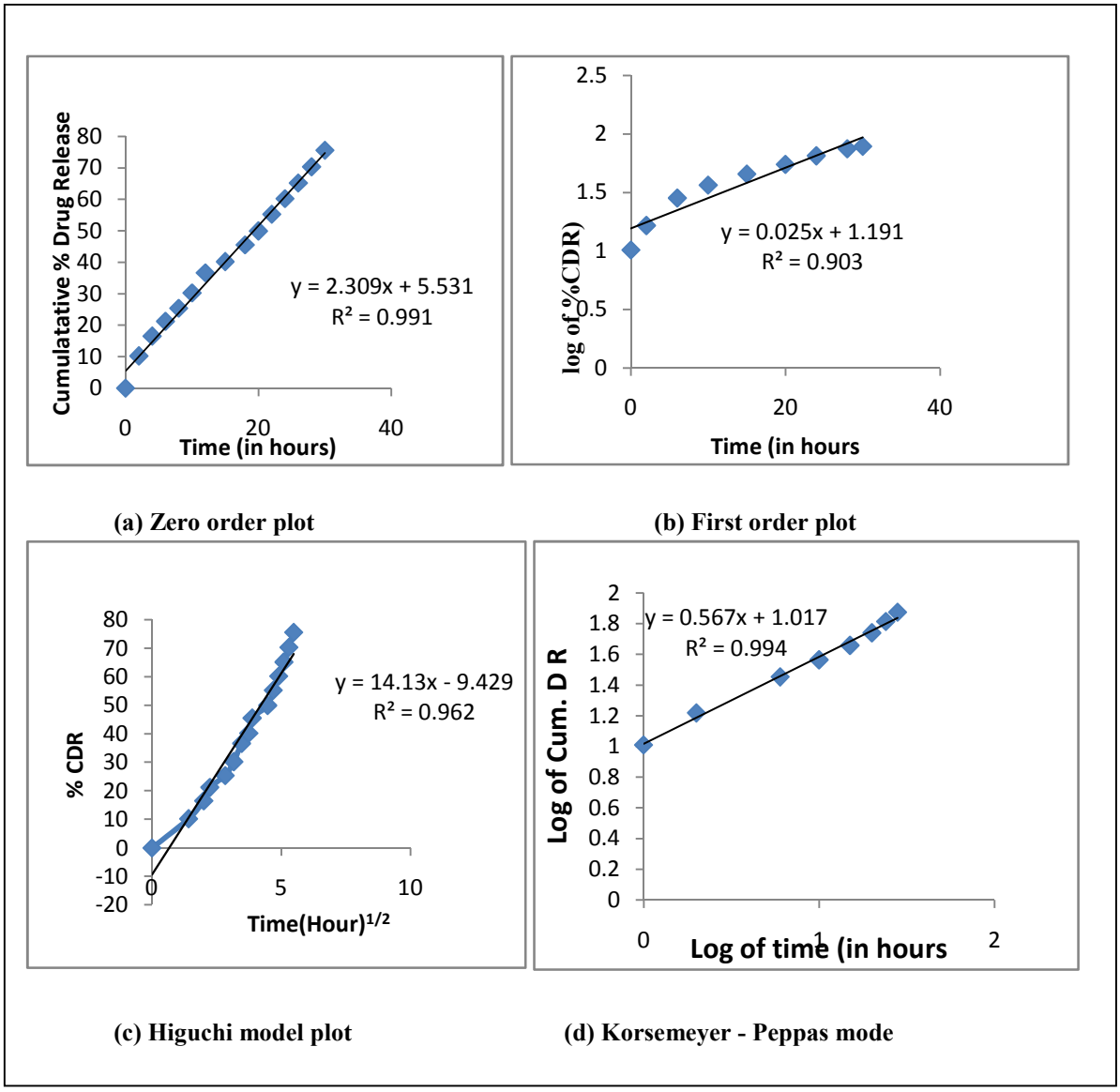

Figure 4 (a) Zero order Plot (b) First order (c) Higuchi Plot (d) Korsemeyer – Peppas Plot

V. CONCLUSION

Osteoporosis is a world-wide disease, caused severity for people generally in old age and women after menopause. Alendronate is the potent bisphosphonate drug for Osteoporosis treatment, but there is increasing concern about their long-term safety, insufficient therapeutic level and uncontrollable release kinetics. Medications with novel mechanisms and novel drugs like drug-loaded solid lipid nanoparticles (SLNs) can be expected to treat Osteoporosis in future. The results of the current study would help us to find a novel and promising approach for drug delivery by preparing the anti-osteoporotic drug Alendronate in the Nano range. The dialysis bag method was applied for the In-vitro drug

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release study of formulated RL-HCL loaded SLNs. Different mathematical kinetic models were used for the kinetic study of formulated drug-loaded SLNs.

Abbreviations

- BMD: bone mineral density;
- SLN: Solid Lipid Nanoparticles;
- GMS: Glyceral Monosterate;
- FDA: Food and Drug Administration.

Conflict of Interest

The authors declare that they have no competing interests.

Data Availability

Not declared.

Funding Source

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