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CHAPTER 2

Raloxifene Hydrochloride as Osteoporosis Treatment

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ABSTRACT : Osteoporosis is a bone related disease in which bones become porous and deterioration of bone tissue can lead to bone fragility and fracture, especially of the hip, spine, shoulder and wrist. Osteoporosis is caused generally due to decreasing bone mineral density (BMD). The most common Postmenopausal Osteoporosis (PMO) affects 30-40% of women after menopause. Raloxifene hydrochloride (RL-HCL) is a selective estrogen receptor modulator (SERM) drug that has been approved for the prevention and treatment of Postmenopausal osteoporosis and reduces the risk of vertebral and increases BMD after treatment. The objective of the present study was to develop, optimize, and evaluate Solid Lipid Nanoparticles (SLNs) of Raloxifene hydrochloride (RL-HCL). It belongs to the benzothiophene group of compounds with poor water-soluble (0.23 mg/ml) and high permeability. Its oral bioavailability is only 2%, hence improving the solubility, dissolution rate and enhance the bioavailability is very important to pharmaceutical researchers. It can be enhanced by reducing the particle size to the nano range. In present work, RL-HCL loaded SLNs have been developed by Emulsion -Solvent evaporation method, using Glycerol Monostearate (GMS) as lipid carrier and poloxamer 407 as the emulsifier. Formulated SLNs with GMS showed small particle size and high entrapment efficiency. The SLNs were characterized using Zeta sizer, transmission electron microscopy, and scanning electron microscopy. In-vitro drug release studies were performed by dialysis bag diffusion method. The results indicated that formulated SLNs are in the nano range confirmed by SEM, TEM and DLS studies. The release profile of SLNs was followed the Higuchi model release kinetics.

Keywords: Bone Mineral Density, Drug release, PMO, SERM, SLNs

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 [4, 5]. Various drugs are used to treat Osteoporosis; Raloxifene hydrochloride (RL-HCL) is one of them. It is mainly used for the treatment of Post-menopausal Osteoporosis (PMO). RL-HCL is a selective estrogen receptor modulator (SERM) drug that belongs to the second-generation non-steroidal benzothiophene group. It was approved by the Food and Drug Administration (FDA) in 1997 for the prevention and treatment of PMO, and 60 mg/day dose was fixed. Its oral bioavailability is about 2%, and it is characterized as a highly permeable, low soluble and low bioavailable drug [6-8].

Clinical development of many drugs failed due to poor solubility, low bioavailability, and other poor biopharmaceutical properties. Currently, research work is going on to solve these problems. Improvement in the solubility and enhancing the oral bioavailability of these drugs are the main aim of the new formulations. 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. The NPs based delivery systems present a significant approach for enhancing solubility and oral bioavailability [9-11].

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 [17-20]. 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 [21, 22].

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 [23, 24].

RL-HCL is a non-steroidal selective estrogen receptor modulator (SERM) of the benzothiophene group, which has been approved and marketed for the prevention and treatment of PMO. It shows an antiresorptive effect and works as an estrogen agonist in bone. It has been shown in many clinical trials that RL-HCL reduces the rate of bone loss and helps in increasing bone mass (BMD) at specific sites. Its oral bioavailability is only 2%. To improve the oral bioavailability of this drug, several research works are being carried out by formulating it as microspheres, microemulsions, tablets, transdermal patch, and polymeric nanoparticles [25-28], still it is required to design the new formulation, Solid Lipid Nanoparticles (SLNs), having the properties like controlled drug release and avoiding the first-pass metabolism of RL-HCL. SLNs are assumed as an effective strategy to enhance the oral bioavailability of lipophilic drugs such as Raloxifene [29, 30].

In the present study, the authors have developed the Solid Lipid Nanoparticles (SLNs) of RL-HCL to enhance the drug bioavailability. This novel drug delivery system is designed for a more efficient therapeutic effect of RL-HCL drug and minimizing its toxic effects, which is achieved by different process variables. Emulsion -Solvent evaporation method was applied for the formulation of RL-HCL 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.

MATERIALS AND METHODS

Materials

The drug Raloxifene-hydrochloride (M.W. of 510.05 g/mol) was received as a gift sample from Sunpharma Ltd. (Gurgaon, India). 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).

Preparation of RL-HCL Loaded SLNs

Emulsion -Solvent evaporation method was used for the preparation of RL-HCL loaded SLNs [31-34]. 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 organic phase.

In the present work, a fixed amount of drug RL-HCL (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 700C 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, Freezing at -43^{0}C and 0.2-1.0 m Pa) for future preservation.

Entrapment efficiency (EE)

The Entrapment efficiency (EE) of RL-HCL loaded SLNs was determined by centrifugation at 20000 RPM for 20 minutes. The nanoparticles were centrifuged, and the pellet of NPs was collected. The supernatant was analysed for determination of unentrapped drug amount, using a UV-Visible spectrophotometer (Labindia)) determined at 285 nm (λ-max for Raloxifene). The percentage entrapment efficiency (% EE) was calculated by using the following formula:-

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

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.

Particle Size, PDI, and Zeta Potential

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

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).

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) [35, 36]. 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 285 nm for RL-HCL. 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 [37, 38].

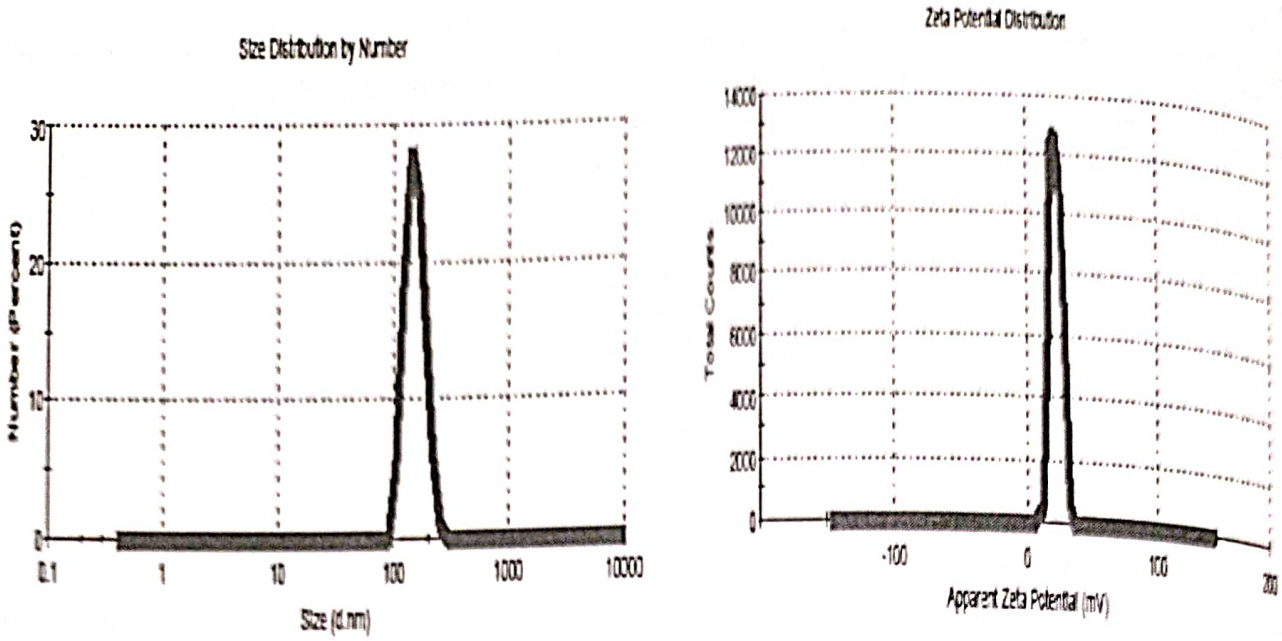
RESULTS AND DISCUSSION

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 are better delivered by solid lipid nanoparticles, and a physically stable system for delivery of Lipophilic drugs like RL-HCL. 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, RL-HCL 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.

Particle Size, PDI, and Zeta Potential (ZP)

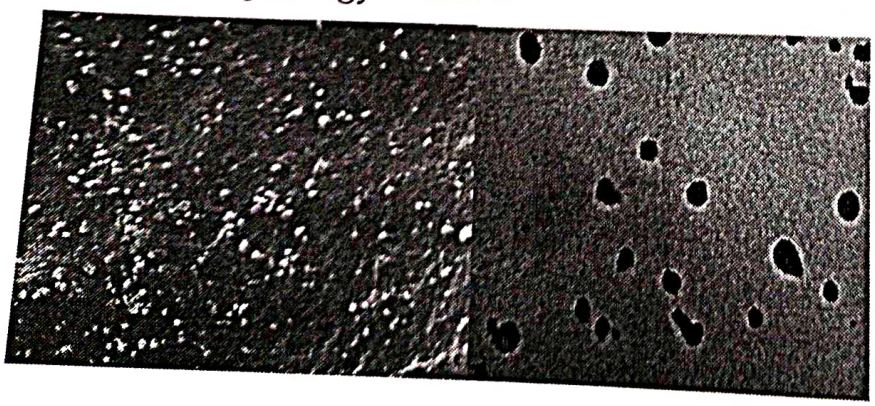


(a) (b)

Figure 1: (a) Particle size of RL-HCL loaded SLNs
(a) Zeta Potential of RL-HCL loaded SLNs

The results of DLS study of RL-HCL 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 (190.4 nm) and entrapment efficiency was 61.32± 1.5 %. The ratio of Drug / lipid (1:5) helped in increasing drug solubility and entrapment efficiency %. Poly dispersive index (PDI) was 0.267, 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 +21.6 mV, this positive value might be due to the presence of one basic nitrogen atom on the surface of SLNs. (having one basic nitrogen atom in the structure of (RL-HCL).

Surface Morphology of SLNs



(a) (b)

Figure 2: (a) SEM image of RL-HCL loaded SLNs (a) TEM image of RL-HCL loaded 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 range below 200 nm. TEM image showed completely spherical and symmetrical nanoparticles were formed in the SLNs formulation.

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.

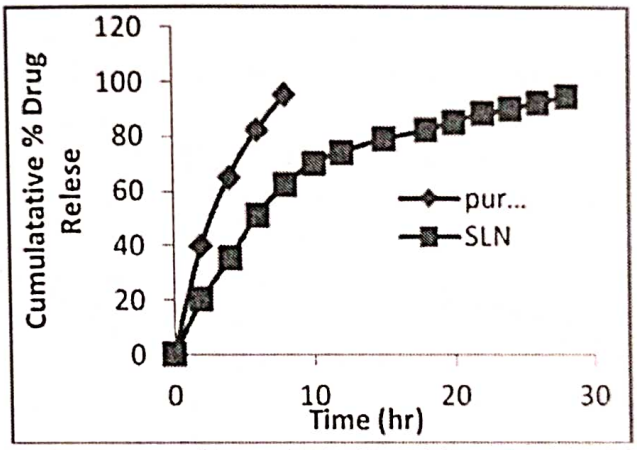


Figure 3: In –vitro drug release profile of RL-HCL loaded SLNs and Pure drug in PBS (P^{H} 6.8)

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 95% 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 (65% drug release in 7h), followed by sustained (96.23 % drug release at 30h). Initial fast release may be due to the presence of the adsorbed drug on the surface of SLNs.

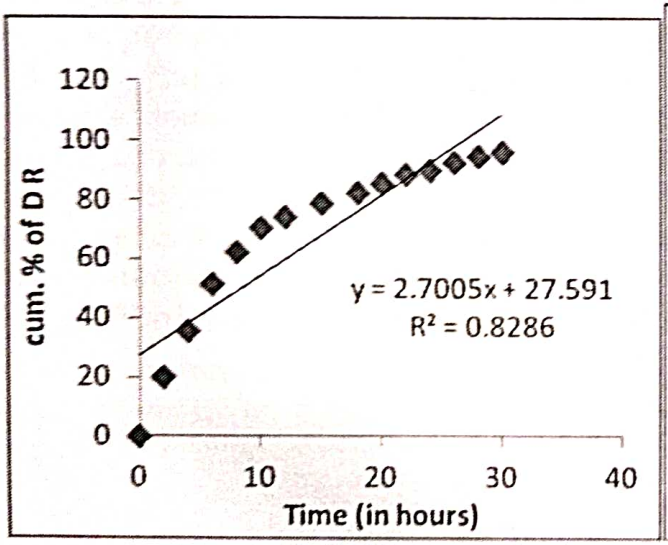
Plots of above mentioned models are shown in Fig. (4) And results are summarized in Table (1). In the above table "R^2" is correlation value, "K" is rate constant and "n" is release exponent.

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

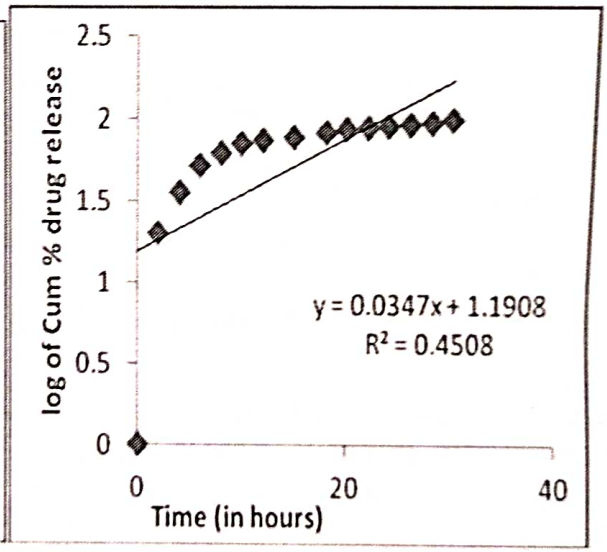
Kinetic Models	Mathematical Expression	Correlation value (R^2)	Rate Constant (K)	Release exponent (n)
Zero order	$Q_t = k_0 t$	0.8286	0.703×10^{-1}	-----
First order	$\log Q_0 - \log Q_t = k_1 t / 2.303$	0.4548	3.45×10^{-2}	-----
Higuchi model	$Q_t = k_H t^{1/2}$	0.9622	2.2×10^{-1}	------
Korsmeyer-Peppas	$Q_t = k_{KP} t^n$	0.7645	1.626×10^{-1}	1.036

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.

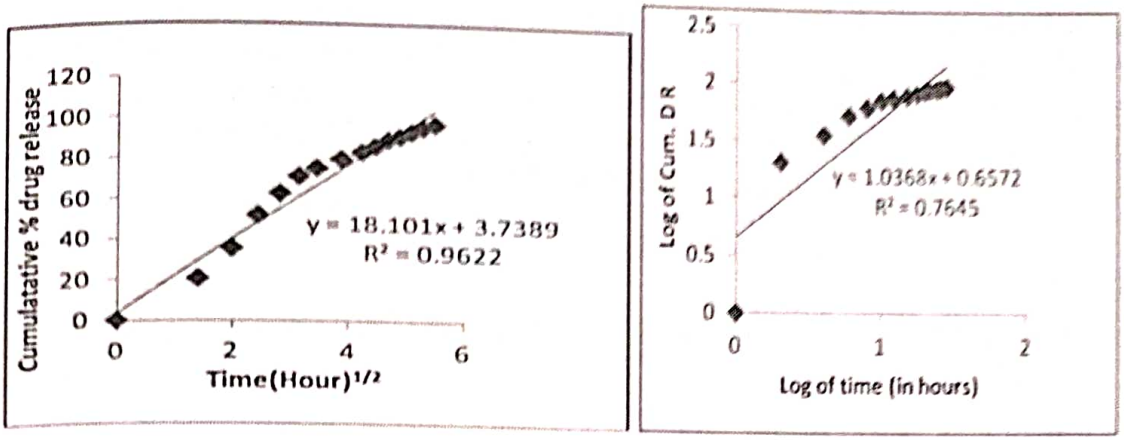
On the basis of above values of (R^2), the best fit kinetic model with the highest linearity for Higuchi model of release kinetics. It is concluded that in formulated SLNs follow Higuchi model kinetics. In the Korsmeyer-Peppas model, release exponent value "n" is 1.03. The magnitude is in the range (n > 0.89), indicates the release mechanism is super case II transport.



(a) Zero order plot



(b) First order plot



(c) Higuchi model plot

(d) Korsemeyer - Peppas model

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

CONCLUSION

Osteoporosis is a world-wide disease, caused severity for people generally in old age and women after menopause. Raloxifene is the most effective treatment option for Post Menopausal Osteoporosis (PMO), 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 PMO 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 Raloxifene in the Nano range. 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.

ABBREVIATIONS

BMD: bone mineral density; PMO: Postmenopausal Osteoporosis; SERM: selective estrogen receptor modulator; 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.

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