Research Article

Formulation Preparation and Evaluation of Raloxifene Nanoparticles

Nikita Nagar, Rajesh Singh Pawar*

Truba Institute of Pharmacy, Karond, Bypass Road, Bhopal, 462038, Madhya Pradesh, India

Received: 28 June 2023 Revised: 24 August 2023 Accepted: 30 August 2023

Abstract

Background: Nanotechnology is process for preparation of nanoparticle having size range of 1-100 nm. The technology enables the delivery of poorly water-soluble Raloxifene (RLX) drugs and preventing the first pass metabolism of drug and increases oral bioavailability of drugs. **Objective:** In this research work we prepared nanoparticle of poorly water-soluble drug raloxifene, to improve the bioavailability of drug. **Material and methods**: Prepared formulations were characterized by FTIR, particle size and zeta potential, differential scanning calorimetry, X-Ray diffraction, surface morphology and stability study. **Results and conclusion:** Particle size and TEM study of formulation confirms nano sized discrete spherical globules with smooth surface area. Formulation shows extremely stability at room condition for six months that supports the fact that dried lyophilized nanocarriers may remain stable for longer period. Pharmacokinetic study shows pronounced improvement in pharmacokinetic parameters (Cmax, Tmax and [AUC] 0-24) which are responsible for enhanced absorption and bioavailability of drug from NLCs. The pharmacokinetic study of RLX loaded NLCs showed 3.75-fold significant improvement in bioavailability of poorly soluble RLX than plain drug the treatment of osteoporosis. For the commercial purpose, this dried NLCs product can be used orally either by incorporating into capsule or by making dispersion of powder in distilled water.

***Keywords*:** Raloxifene, pharmacokinetic, nanoparticle, osteoporosis, bioavailability

Introduction

Nanotechnology employs knowledge from the fields of physics, chemistry, biology, materials science, health sciences, and engineering. In recent years, scientists concentrated their focus on nanoparticles due to their different magnetic, optoelectronic, and physicochemical properties that are regulated by their size and shape distribution. It has immense applications in almost all the fields of science and human life. Nanoparticles can be defined as particulate dispersions or solid particles with a size in the range of 10- 1000 nm. The drug is dissolved, entrapped, encapsulated, or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. Nanodrug delivery have several advantages like it improve the bioavailability of poorly water-soluble drug, protect drugs from the degradation in the gastrointestinal tract and deliver the drug molecule at specific site of organ (Sathali and Gopinath, 2013).

In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly ethylene glycol (PEG) known as long-circulating particles, have been used as potential drug delivery devices. They have ability to circulate for a prolonged period time and target a particular organ, as carrier of DNA in gene therapy, and their ability to deliver proteins, peptides, and genes.

In most cases, either polymers or lipids are used as carriers for the drug, and the delivery systems have particle size distribution from few nano-meters to few hundred nano-meters. Due to their small sizes, the nanostructures exhibit unique physicochemical and biological properties (Enhanced reactive area as well as an ability to cross cell and tissue barriers) that make them a favourable material for

Address for Corresponding Author:
Dr. Rajesh Singh Pawar
Professor and Principal
Truba Institute of Pharmacy, Karond, Bypass Road, Bhopal, 462038, Madhya Pradesh, India
Email: drrajesh.pawar@trubainstitute.ac.in

DOI: https://doi.org/10.31024/ajpp.2023.9.5.3

biomedical applications. The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. Polymeric nanoparticles offer some specific advantages over liposomes. For instance, they help to increase the stability of drugs/proteins and possess useful controlled release properties.

In this research work we are preparing nanoparticle of Raloxifene (RLX) to decrease drug resistance, decrease toxicity, enhance oral bioavailability, enhance rate of dissolution, enhance solubility, Increase the stability of drug and formulation, increase drug targeting ability and patient compliance, increase surface area, Reduce the dose needed (Balfour and Goa, 1998).

Material and Methods

Selection of solid lipid

Selection of solid lipid based on the solubility of the drug in solid lipid and partition behaviour of RLX drug. In this study we used Dynasan 114, Dynasan 118, stearic acid and GMS solid lipid. To check the solubility of drug in solid lipid, we take 50 mg of solid lipid in 10 ml vials and melted it in water bath, then observe the solubility of RLX in each lipid separately (Bandela and Anupama, 2009).

Partition behaviour of RLX in various solid lipids are measured by mixing the 25 mg of drug into 5 gm of melted solid lipid with 5 gm hot water. This Mixture was shaken on an isothermal orbital shaker (MSW-132, Macro Scientific Work Pvt Ltd, Delhi, India) at 70 ± 2.0˚C for 24 hr to reach equilibrium followed by separation of aqueous phase through centrifugation at 5000 rpm for 5 min using cooling centrifuge (C-24 BL, Remi Instrument Pvt Ltd, Mumbai, India). Drug content was analyzed spectroscopically at 288 nm using UV visible spectrophotometer (UV-1800, Shimadzu, Japan).

Selection of liquid lipid

Liquid lipid was selected based on the maximum solubility of the drug in different liquid lipids. Lipids used for this study were Capmul MCM C8, Isopropyl myristate, oleic acid, Labrafil ILM 1944 CS and Lebrafec CC. Excess amount of drug was taken in stopper vials containing 5 g of liquid lipids and mixing was carried out on a vortex mixer for 10 min. Thereafter, vials were kept in an isothermal orbital shaker at 25 ± 2.0˚C for 24 h to reach equilibrium. Supernatant was separated by centrifugation at 5000 rpm for 15 min and analyzed drug content spectroscopically at 289 nm (Shah et al., 2016).

Formulation of RLX loaded NLCs

Design of the experiment

A complete 32 factorial design was utilized to study the effect of two independent variables namely solid lipid to liquid lipid concentration and stabilizer concentration on entrapment loaded NLCs. Based on preformulation studies GMS, Capmul MCM C8 and PVA were selected as solid lipid, liquid lipid, and stabilizer, respectively. Preparation of NLCs loaded with RLX were developed using solvent diffusion method in aqueous system with some modification (Duong et al., 2020). Drug (5% w/w to the total weight of drug and lipids) and Capmul MCM C8 were mixed in a 10 mL solvent mixture of ethanol and acetone (1:1 v/v) followed by bath sonication (SW- 4, Toshniwal Instruments Pvt Ltd, Ajmer, India) for 10 min. The obtained mixture was kept on a water bath maintained at 60°C followed by addition of GMS to make clear solution of lipids and drug in organic solvent system. The resultant organic mixture was hastily added into 100 mL of an aqueous phase comprising of PVA as stabilizer kept on water bath maintained at 70°C under mechanical agitation of 500 rpm for 10 min using mechanical stirrer (RQ-121/D, Remi Instrument Pvt Ltd, Mumbai, India). The obtained RLX loaded NLCs dispersion was cooled at room temperature for 20 min on magnetic stirrer for the liberation of organic solvent (Hu et al., 2006; Sanad et al., 2010). The prepared NLCs dispersion was transferred to centrifuge tubes equipped with cooling centrifuge and centrifugation was carried out for 17,000 rpm and 1 hr at -10°C to separate precipitated NLCs. NLCs were collected and lyophilized using freeze dryer (MSW-137, Macro Scientific Work Pvt Ltd, Delhi, India) (Shete and Patravale, 2013).

Evaluation of RLX loaded NLCs

Percentage yield

The percentage yield was determined by dividing the weight of recovered nanoparticles with the weight of drug and lipids used for the preparation of nanoparticles.

Drug loading and entrapment efficiency

Prepared NLC was centrifuged and supernatant was separated followed by dilution, RLX content was determined spectroscopically at 288 nm (Joshi et al., 2008). Entrapment efficiency of drug was calculated as follows:

$$\%\ Entrapment\ efficiency = \frac{[RLX]total - [RLX]supernatant}{[RLX]total} \times 100$$

Where "[RLX] total" is the weight of total incorporated drug; "RLX supernatant" is the weight of free drug analyzed in supernatant layer (Subedi et al., 2009).

Loading capacity of drug was calculated as follows:

$$\%\ Drug\ loading = \frac{Amount\ of\ RLX\ entrapped\ in\ NLCs}{Amount\ of\ RLX\ and\ lipid\ added} \times 100$$

Optimization of formulation

The formulations were optimized by measuring percentage of drug entrapped and studying interaction between factors as discussed underneath. The statistical evaluation of all the obtained results data was carried out by analysis of variance (ANOVA) using Microsoft excel version 2007. The ANOVA results (P value) showed the effect of various independent variables on dependent parameter like percentage drug entrapment. After regression analysis of all formulations, full polynomial model was obtained followed by equation represents effect of independent formulation variables on entrapment efficiency. Make contour and response surface plots Both plots were constructed from reduced polynomial equation using sigma plot version 11.0 by keeping one parameter stationary and varying others. Evaluation of model / check point analysis. Checkpoint analysis was carried out to evaluate the dependability of the model through comparison between experimental and predicted values of the responses.

In vitro drug release studies

In vitro drug release of plain drug suspension and prepared NLCs was carried out using the dialysis sac method (Kushwaha et al., 2013). (Himedia-Dialysis membrane 135, Mol. cut off 12000-14000 Da, Mumbai, India). An accurately measured amount of plain drug suspension and NLCs formulations equivalent to 5 mg of RLX were introduced into sac and both ends of the sac were tied with the help of thread. The sac was hanged with the assistance of thread in beaker comprising of 200 mL of Citro phosphate buffer pH 7.6 with 1% of polysorbate 80 kept on magnetic stirrer (Ravi et al., 2014). The temperature of the receptor compartment was maintained at 37 ± 1°C. Aliquots of 5 mL were withdrawn at predefined time interval with a pipette and replaced with fresh buffer at each time. The filtered samples (0.45 µm membrane filter) were analyzed spectroscopically at 288 nm. Blank formulations were prepared and treated in same manner as discussed above. Blank formulations were taken for base correction by suitable dilution with buffer system in UV-Visible spectrophotometer to nullify any effect of ingredients used in formulation other than drug. Each test was carried out in triplicate.

Characterization of optimized RLX loaded NLCs

Fourier transform infrared (FTIR) spectroscopy

The FTIR spectroscopy was done for study of interaction between drug and excipients. Samples were mixed with KBr in a ratio of 1:300 and spectrum were recorded in the range of 4000–400 cm^{-1}.

Characterization of particle size and zeta potential

The particle size and zeta potential of optimize formulation NLC-8 were measured by Malvern zeta sizer (Nano ZS, Malvern Instruments, Worcestershire, UK) after suitable dilution with distilled water.

Differential scanning calorimetry (DSC) analysis

Thermogram of samples was recorded by Differential scanning calorimeter (DSC TA –60, Shimadzu, Japan). Samples were weighed directly in aluminium pan and scanned at 50-300°C temperature under dry nitrogen atmosphere at the heating rate of 10°C/min.

X-Ray diffraction (XRD) study

XRD study of samples was performed by Panalytical X pert PRO X-Ray Diffractometer (XpertPro MPD, Panalytical, Netherlands) where Cu Kα radiation wavelength of 1.5405 Å was used as X-ray source. For the measurements, samples were kept in the glass sample holders followed by scanning from 2° to 60° with scan angular speed (2θ/min) of 2°/min, 40 kv working voltage and 30 mA current.

Surface morphology study

Surface morphology of optimized formulation NLC-8 was studied by Transmission Electron Microscope (TEM) (Philips Tecnai – 20, USA). NLCs were dispersed in distilled water and a drop of dispersion was placed on carbon coated copper grid followed by drying. This grid was mounted in the instrument and photographs were taken at various magnifications.

Stability study

The samples were placed in vials and kept at 25 ± 2°C/60 ± 5% RH and 40 ± 2°C/75 ± 5% RH atmospheric conditions using stability chamber (Macro scientific work Pvt Ltd, Delhi, India) over period of six months. The samples were analyzed for entrapment efficiency and physical appearance at specified time intervals (0, 15, 30, 60, 120 and 180 days of storage). Cumulative drug release study was also carried out at the end of stability study for both storage conditions.

Results and Discussion

Selection of solid lipid

To keep the drug in solubilization form, it is of prime important that drug have higher solubility in solid lipid. It was found from the study that drug solubility in Dynasan 114, Dynasan 118 and stearic acid was indistinct but found fairly visible in GMS.

Partition behavior of RLX in various solid lipids

Determination of partition behaviour of drug in lipid is important criteria in controlling two parameters namely drug entrapment efficiency and drug release profile. Therefore, the success of development of NLCs is depending on selection of proper lipid for formulation of

nanoparticles. Result was found that RLX had higher partitioning in GMS compared to other lipids (Table 1). This finding also supported the high solubility of drug in GMS as discussed earlier. Therefore, GMS was chosen as solid lipid for development of NLCs owing to its high potential for solubilization and thereby entrapment of more amount of drug in NLCs formulation.

Selection of liquid lipid

As discussed earlier for solid lipid, a variety of short chain liquid lipids are also playing major role in entrapment of more amount of drug in case of NLCs formulation. It was found from the result that Capmul MCM C8 has maximum drug solubility (2.55 ± 0.96 mg/g) than Isopropyl myristate (1.14 ± 0.14 mg/g), Oleic acid (2.08 ± 0.24 mg/g), Labrafil IC M 1944 CS (1.24 ±0.18 mg/g) and Lebrafec CC (0.74 ± 0.07 mg/g). Therefore, Capmul MCM C8 was selected as liquid lipid to make a matrix with solid lipid GMS for the development of NLCs.

Evaluation of RLX loaded NLCs

Percentage yield, drug loading and entrapment efficiency

The Percentage yield of NLCs formulations was found with significant differences ranging from concentration. The percentage yield of NLCs was increasing significantly with stabilizer concentration increased from 0.5% to 1.0% w/v ($P < 0.05$) but non- significant increment observed with concentration from 1.0% to 1.5% w/v **(Figure 1)**. This study revealed that formulation with optimum 1.0% w/v PVA concentration may achieve maximum nanoparticles yield with good stability.

The drug entrapment efficiency and loading capability of NLCs were remarkably increased from 30.83 ± 2.39 to 74.78 ± 3.34% and from 1.92 ± 0.12 to 4.02 ± 0.17%, respectively with increasing the proportion of Capmul MCM C8 from 5 to 15% w/w. Furthermore, it was reported that Capmul MCM C8 being a Mono-glycerides of caprylic acid form unstructured matrix with many imperfections providing a space to incorporate more amount of drug (Li et al., 2010). As shown in **Figure 2,** it was observed that 15% w/w liquid lipid content in formulation improves drug entrapment significantly ($P < 0.05$) compare to 5% w/w and 10% w/w liquid lipid content. High proportion of liquid lipid may help in increasing drug solubility in lipids matrix followed by high entrapment efficiency.

Optimization of formulation Interaction between the factors

A 32 full factorial design was employed in optimizing the formula. The concentration of GMS: Capmul MCM C8 (X1) and concentration of PVA solution (X2) were taken as the independent variables and the entrapment efficiency as the dependent variable. The maximum percent entrapment (74.78%) was found at 1 level of X1 and 0 level of X2 as shown in **Figure 3A.** The entrapment efficiency was obtained by conducting systematic experiments at various levels and was subjected to regression analysis to obtain a polynomial equation of the full model as follows:

$Y = 58.39 + 17.07\ X1 + 5.21\ X2 - 1.11\ X12 - 6.52\ X2 + 0.56\ X1X2$

Non-significant terms were rejected ($P > 0.05$) to obtain reduced model as follows:

$Y = 58.39 + 17.07\ X1 + 5.21\ X2 - 6.52\ X2$

Based on the P value, X1, X2 and X22 factors were found to

Table 1. Partition coefficient of RLX in various solid lipids

Name of lipid system	Apparent partition coefficient ± SD
Water/Dynasan 114	58.58 ± 3.69
Water/Dynasan 118	72.89 ± 10.47
Water/Stearic acid	66.34 ± 5.41
Water/GMS	85.12 ± 9.48

Value are expressed as mean ± SD, n=3

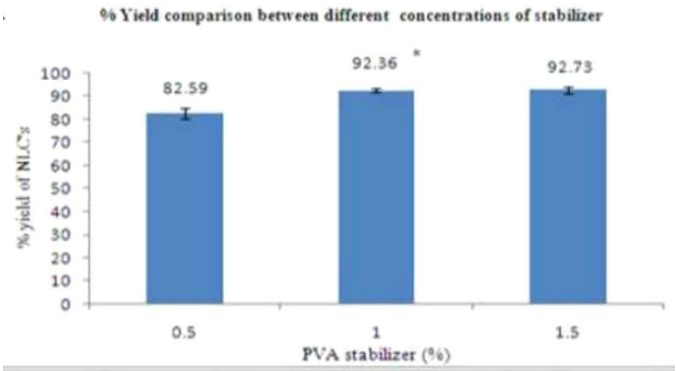

Figure 1. Percentage yield of NLCs with reference to stabilizer

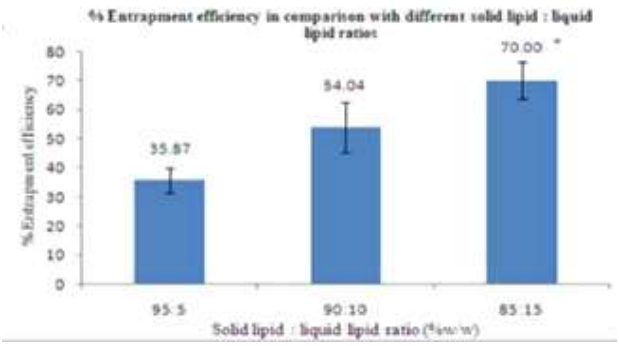

Figure 2. Percentage of drug entrapment in liquid lipid formulation

be significant and all other factors were found to be insignificant. For the given model, calculated F value was found very low than the tabular F value (0.05, 2) so it can be confirmed that the omitted terms do not significantly contribute in the prediction of the entrapment efficiency. High coefficient value of X1 reveals that it can affect the entrapment efficiency maximum. However, at the same time the value of X22 was also found to be significant. Therefore, the concentration of surfactant can also be considered as critical factor in formulation along with concentration of solid lipid to liquid lipid.

Contour and response surface plot were drawn at the selected values of the independent variables. The plots shown in **Figure 3B** were found to be nonlinear and having curved segment for each prefixed values that signify nonlinear relationship between the selected variables.

Check point analysis

Check point analysis was performed to verify the effectiveness of established contour plot and reduced polynomial equation in development of drug loaded NLCs. The percent error for entrapment efficiency in the check point analysis was found very less between theoretical value and experimental value. This finding signifies the role of the reduced model, contour plots and the check point analysis in the mathematical modelling. By studying full 32 factorial design, it was notified that formulation NLC-8 showing maximum entrapment efficiency of 74.78 ± 3.34% and may be optimized for further characterization but that can be confirmed only after performing in vitro release study of all prepared NLCs formulations.

In vitro drug release

In vitro release profile of RLX from all NLCs formulations showed burst drug release for initial 8 h followed by slow and sustained release up to 36 hr. However, from the data, it was found that drug release profile of RLX was improving from formulations NLC-1 to NLC-9 as the concentration of liquid lipid in formulations increases. The formulation containing 15% w/w Capmul MCM (NLC-8) showed considerable improvement in release profile (90.82 ± 2.4 %) compared to other NLCs formulations. Therefore, NLC-8 formulation was selected for further characterization based on its improved drug release profile and maximum drug entrapment efficiency optimized by 32 factorial designs. The optimized formulation (NLC-8) also showed significant enhancement ($P < 0.05$) in drug release profile compared with plain drug suspension as shown in **Figure 4.** Such type of drug release pattern in NLCs was most likely related with allotment of liquid lipid in nanoparticles. It was reported in earlier study (Hu et al., 2005) that when NLCs were prepared by solvent diffusion method at 70°C, liquid lipid was not allotted equivalently with solid lipid matrix. In such cases, more amounts of liquid lipid remain at the external shell of nanoparticles and very less liquid lipid incorporated into the centre during cool process. Therefore, the external part of particles become soft and exhibited significantly more solubility for hydrophobic drugs which imparts initial burst effect in release profile (Muhlen et al., 1996). Various release kinetic models were fitted to determine release pattern of optimized formulation. The release kinetics of optimized formulation calculated by the regression analysis (R2 value) had higher linearity for zero order and Higuchi model. Therefore, it can be concluded mechanism as per Higuchi model.

Characterization of optimized RLX loaded NLCs

FTIR spectroscopy

As shown in **Figure 5** of NLC-8, it was observed that

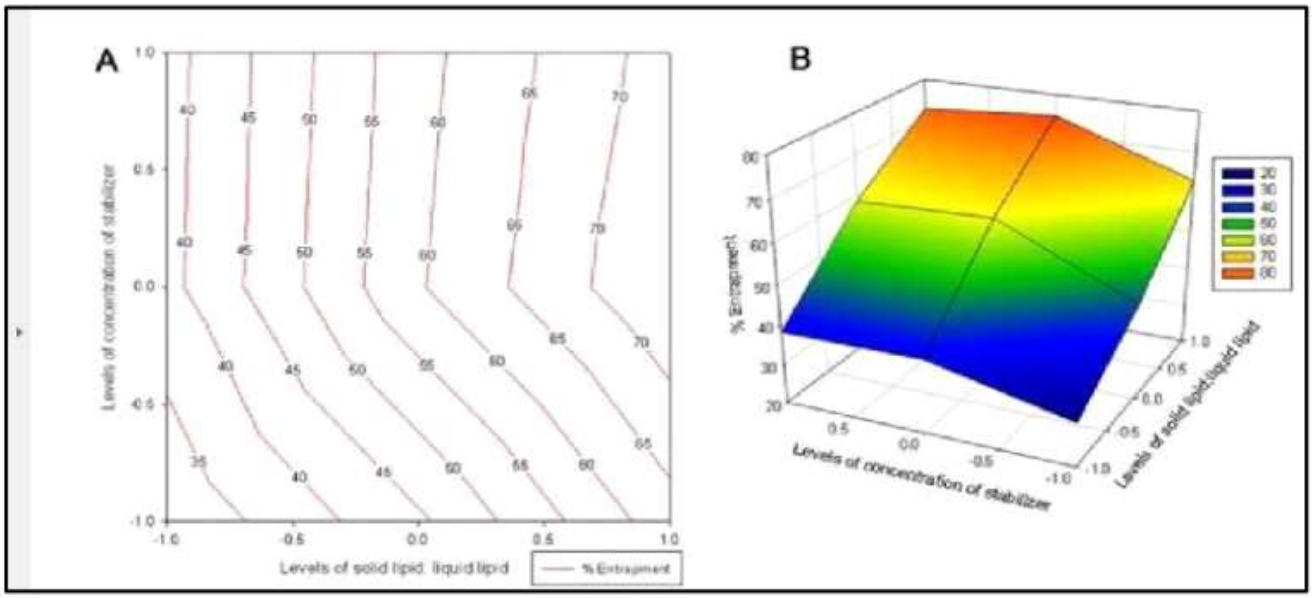

Figure 3. (A) Entrapment efficiency of drug in liquid lipid; **(B)** Contour map of selected entrapment efficiency

characteristic peaks of drug 947.00 cm-1 (Benzene ring), 1458.18 cm-1 (-S- benzothiophene) and 1600.92 cm-1(-C-O-C-stretching) were found to be similar with pure drug spectra. This reveals no physicochemical interaction between drug and excipients in NLCs formulation.

Particle size and zeta potential

The particle size of NLC-8 was found to be 32.50 ± 5.12 nm (mean size) with less polydispersive index that represents narrow distribution of nanoparticles within the system. The zeta potential of optimized formulation was found -12.8 ± 3.2 mV, which impart good stability of NLCs dispersion.

Differential scanning calorimetry

Thermogram of RLX and GMS showed endothermic peaks at 272.92°C and 62.89°C corresponding to their melting points as depicted in Figs. 5A and 5B, respectively. DSC plot of physical mixture showed showed sharp peaks at 272.18°C and 61.94°C representing melting points of drug and GMS, respectively. Thermogram of NLC-8 showed endothermic peak at 63.42 °C representing melting point of GMS but absence of endothermic peak within the melting range of RLX indicates either solubilization or conversion of drug from crystalline to amorphous form in the solid and liquid matrix.

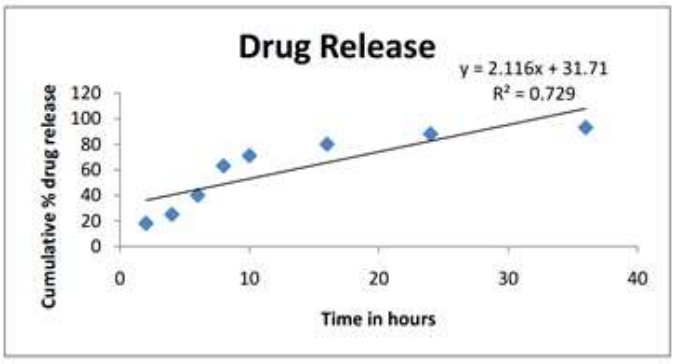

Figure 4. Drug release profile of RLX

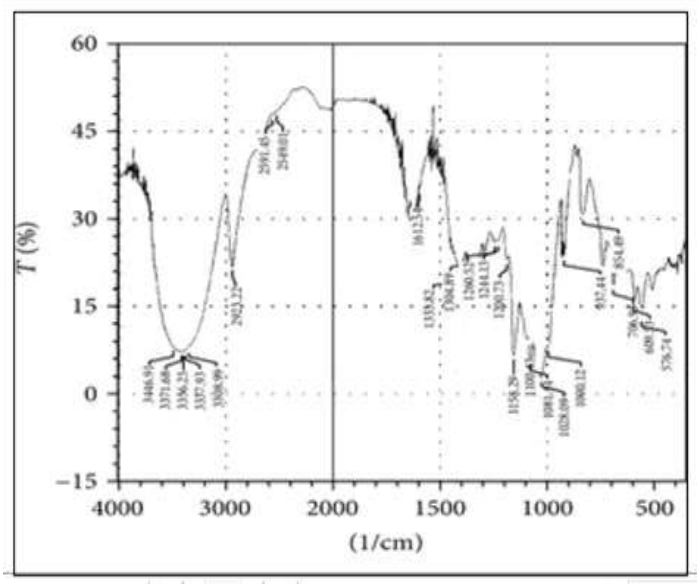

Figure 5. FTIR of Raloxifen

X-Ray diffraction study

The XRD spectrums of drug in and physical mixture showed distinct and intense peaks at 2θ scale indicate crystalline nature of drug. In contrast, there was a considerable decline in intensity of all peaks in XRD pattern of NLC-8. Therefore, it can be revealed that RLX drug is completely in amorphous state in optimized NLCs formulation with solid lipid and liquid lipid.

Stability study

The result of stability study showed no change was observed in physical appearance of formulation in both stability conditions but significant reduction was notified in entrapment efficiency at accelerated condition. The release rate for the formulation kept at room condition was satisfactory. The result shown for accelerated condition may attribute small degradation of drug at this condition which supports the fact that accelerated temperature is not a suitable storage condition for lipid-based formulation. Therefore, it can be concluded that the room condition (25±2°C/60 ±5% RH) is a more favourable storage condition than the accelerated condition for NLCs formulation for a longer period.

Conclusion

In the present work we improve bioavailability of poorly soluble RLX by preparing nanostructured lipid carrier. NLCs were prepared by solvent diffusion method at 70°C which exhibit high entrapment efficiency with sustained release of drug up to the period of 36 h. DSC and XRD confirms the transformation of crystal nature of drug into amorphous nature that plays an important role in enhancement of absorption rate followed by bioavailability. Particle size and TEM study confirms nano sized discrete spherical globules with smooth surface area. Stability study of optimized formulation at room condition shows extremely stable formulation for the period of six months that supports the fact that dried lyophilized nanocarriers may remain stable for longer period. Result of pharmacokinetic study shows pronounced improvement in pharmacokinetic parameters (Cmax, Tmax and [AUC]0-24) which are responsible for enhanced absorption and bioavailability of drug from NLCs. The pharmacokinetic study of RLX loaded NLCs showed 3.75-fold significant improvement in bioavailability of poorly soluble RLX than plain drug the treatment of osteoporosis. For the commercial purpose, this dried NLCs product can be used orally either by incorporating into capsule or by making dispersion of powder in distilled water.

Acknowledgment

The authors gratefully acknowledge to Truba Institute of Pharmacy Bhopal, India and PBRI research lab Bhopal, India for providing facilities and platform for the research work.

References

Balfour JA, Goa KL. 1998. Raloxifene. Drugs Aging. 12(4):335-41.

Bandela JJ, Anupama CH. 2009. Advanced PEGylation for the Development of Raloxifene Hydrochloride, BCS Class II Drug. Journal of Young Pharmacists, 1(4): 295-300.

Duong VA, Nguyen TTL, Maeng HJ. 2020. Preparation of Solid Lipid Nanoparticles and Nanostructured Lipid Carriers for Drug Delivery and the Effects of Preparation Parameters of Solvent Injection Method. Molecules. 25: 4781.

Hu FQ, Jiang SP, Du Yuan H, Ye YQ, Zeng S. 2005. Preparation and characterization of stearic acid nanostructured lipid carriers by solvent diffusion method in an aqueous system. Colloids Surf Biointerfaces 45:167–73.

Hu FQ, Jiang SP, Du YZ, Yuan H, Ye YQ, Zeng S. Preparation and characteristic of monostearin nanostructured lipid carriers. Int J Pharm 2006; 314, 83-89.

Hu FQ, Jiang SP, Du YZ, Yuan H, Ye YQ, Zeng S. 2006. Preparation and characteristic of monostearin nanostructured lipid carriers. International Journal of Pharmaceutics 314:83-9.

Joshi M, Pathak S, Sharma S, Patravale V. 2008. Design and invivo pharmacodynamics evaluation of nanostructured lipid carriers for parenteral delivery of artemether: nanoject. International Journal of Pharmaceutics 364:119-26.

Kushwaha AK, Vuddanda PR, Karunanidhi P, Singh SK, Singh S. 2013. Development and evaluation of solid lipid nanoparticles of raloxifene hydrochloride for enhanced bioavailability. BioMed Research International 2013:1–9 article ID 584549.

Li F, Wang Y, Liu Z, Lin X, He H, Tang X. 2010. Formulation and characterization of bufadienolides-loaded nanostructured lipid carriers. Drug Development and Industrial Pharmacy 36:508-17.

Muhlen AZ, Muhlen EZ, Niehus H, Mehnert W. 1996. Atomic force microscopy studies of solid lipid nanoparticles. Pharmaceutical Research 13:1411-6.

Ravi PR, Aditya N, Kathuria H, Malekar S, Vats R. 2014. Lipid nanoparticles for oral delivery of raloxifene: optimization, stability, in vivo evaluation and uptake mechanism. European Journal of Pharmaceutics and Biopharmaceutics 87: 114-24.

Sanad RA, AbdelMalak NS, elBayoomy TS, Badawi AA. 2010. Formulation of a novel oxybenzone-loaded nanostructured lipid carriers (NLCs). AAPS PharmSciTech 11(4):1684–94.

Sathali AH, Gopinath M. 2013. Formulation and evaluation of Paliperidone Nanoparticles. BioMedRx. 1(5): 422-438.

Shah NV, Seth AK, Balaraman R, Aundhia CJ, Maheshwari RA, Parmar GR. 2016. Nanostructured lipid carriers for oral bioavailability enhancement of raloxifene: Design and in vivo study. Journal of Advanced Research 7(3): 423-434.

Shete H, Patravale V. 2013. Long chain lipid based tamoxifen NLC. Part I: preformulation studies, formulation development and physicochemical characterization. International Journal of Pharmaceutics 454:573-83.

Subedi RK, Kang KW, Choi HK. 2009. Preparation and characterization of solid lipid nanoparticles loaded with doxorubicin. European Journal of Pharmaceutical Sciences 37:508-13.