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ORIGINAL ARTICLE **OPEN ACCESS**

Formulation and Evaluation of Nanoparticulate Lipid Carriers (NLC) Drug Delivery of Raloxifene Hcl

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ABSTRACT
The purpose of this research is to formulate and assessthe Raloxifene hydrochloride nanoparticulate lipidcarriers(RH-NLC) drug delivery systems and comparing with the marketed product. RH NLC formulations were made using stearic acid as a solid lipid, medium chain triglyceride as a liquid lipid, and polysorbate 80 as a hydrophilic surfactant, and homogenization followed by ultrasonication.. Based on the experiments, the composition of optimized formulation was RH-NLC12 consisting of 60 mg of RH, 210 mg stearic acid, 90mgMCT 55/45, 33.6 mg polysorbate 80 and 20 mg soya lecithin with 15 min of ultrasonication time. The optimized formulation of RH-NLC12 was found to be with mean particle size of 146 ± 0.59 nm,When compared to the marketed product, drug release was raised by 5-9 percent in the initial time points up to 3 hours, and the release was sustained for 12 hours, compared to 7 hours for the marketed product.. The drug release kinetics of RH NLC 12 was best explained by Korsmeyer Peppas plot which demonstrates Diffusion and erosion are used to release drugs.This research is an attempt was made to design Raloxifene hydrochloride nanoparticulate lipidcarriers drug delivery systems for sustained drug release in treatment of postmenopausal osteoporosis.
Keywords: *Raloxifene hydrochloride, Nanostructured lipid carriers, Sustained release.*

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Introduction

Exploration of innovative lipid-based formulations is attracting researchers from all over the world who want to improve the in vivo performance of highly lipophilic medications. Poor water solubility and intrinsic dissolution rate have a significant impact on medication delivery. The rate-limiting stage in the absorption of hydrophobic medicines is undoubtedly dissolution. Such medications are usually classified as Class II or IV by the BCS, and they have a limited oral bioavailability due to their weak water solubility. Aside from that, the lipophilic medicines' unstable metabolism makes oral bioavailability a difficult task.
All of these factors are taken into consideration by the nanocarrier system, which then closes the gaps in lipophilic drug delivery. Lipid-based nanocarriers are a viable formulation method in this regard, as they have the ability to solve these problems by enhancing and normalizing medication absorption (1). Solid lipid nanoparticles (SLNs) are colloidal drug carrier systems that are similar to nanoemulsions but differ in that they are lipid-based. At room temperature, solid lipids such as glycerides or waxes with a high melting point replace the liquid lipid element of emulsions. Because of its potential as an alternative carrier system to outdated colloidal carriers such as emulsions, liposomes, and polymeric micro and nanoparticles, as well as their potential to be used in various routes of drug delivery, interest in SLN as a novel particle technology has recently increased.(2).
NLCs have unique properties and are made from a combination of solid and liquid lipids, resulting in less complex structures that allow for the safer incorporation of pharmacological molecules, better payload capacity, and long shelf store stability. As a result, NLCs are a superior carrier compared to other lipid-based systems, as well as having a greater entrapment efficiency due to the higher solubility of medicines in liquid lipids over solid lipids. Furthermore, NLCs have the potential to include both hydrophilic and lipophilic medicines, as well as offer sustained drug release and target them to the site of action by improving drug solubility in the intestinal milieu, decreasing gastric emptying rate, and boosting mucosal permeability.(3, 4).

Raloxifene HCl (USP) is a selective estrogen receptor modulator (SERM) used to prevent and treat osteoporosis in postmenopausal women. The medication has a very poor solubility in water (less than 0.25 mg/l) and is classified as BCS Class II (low solubility and high permeability). RH is absorbed 60% orally, has substantial presystemic glucuronide conjugation, and has a 2% absolute bioavailability. Tmax is approximately 6 hours, and plasma elimination half-life is 27.7 hours. The current study used Nanostructured Lipid Carriers (NLCs) and suitable lipids in an unique drug delivery system technique to optimize the dissolving rate, maintain drug release, and increase RH bioavailability. As a result, the goal of this study was to develop and assess Raloxifene hydrochloride nanoparticulate lipid carriers (RH-NLC) drug delivery systems, as well as compare them to commercially available products.

MATERIAL AND METHODS

Drug and chemicals

Raloxifene HCl(Cadila Pharmaceiticals, India) as a drug candidate; Solid lipid consists of Stearic acid / Stellipress micro 50®; liquid lipid consists of Medium chain triglycerides /StelliestersMCT65/35®(Strearinerie Dubois, France).The lipophilic surfactantSoya lecithin/Lipoid S-20® (Lipoid, Germany); aqueous phase consisted of Polysorbates 80 (Croda, USA) as the hydrophilic emulsifier.All the above were materials were obtained as gift samplesfrom Cadila Pharmaceuticals Ltd., Ahmedabad.

Instruments

Tablet compression machine (Karnavati Compression, Ahmedabad), disintegration test apparatus (Electro lab, Mumbai), electronic balance (AW-220, Shimadzu), high-vacuum evaporator (JFC-1100 fine coat ion sputter; Tokyo, Japan), high performance liquid chromatography (HPLC) (prominence-i LC-2030, Shimadzu, Japan), friability tester (Toshiba, India), micrometer (Moore and Wright Ltd. Britain Tool, Factory Sheffield, UK),ultracentrifuge (Beckman Optima TM XL, Indianapolis, IN),Roche friabilator (Electrolab, Mumbai), scanning electron microscope (Zeiss, TIFR, Mumbai),spectrophotometer (Shimadzu, the model UV-1800 PC, Kyoto, Japan)and zeta sizer (Malvern, Model: Nano ZS90)are the instruments used in this study.

Formulation of RH NLCs blend

The preparation Raloxifene HCl into RH NLCs by using high-speed homogenization followed by ultrasonication and their composition of DoE trials were discussed in the earlier research article (5).The 2^3 full factorial designs were utilized to assess the connection between the critical material attributes and critical process parameters variable. The most basic quality characteristics were studied with the risk assessment matrix. (6) The prototype and optimized formulationRH NLC 12 was prepared based on DoE trials evaluated in this research article.

In *vitro* evaluation of RH NLC blend (7)

The flow properties of RH NLC blend were characterized by measuring angle of repose, bulk density and tapped density, Carr's compressibility index and Hausner's ratio and the results are tabulated in Table 1.

Angle of repose

Angle of repose in the surface of a pile of blend and the horizontal plane were used to measure the frictional forces of the liquisolid blend of RH NLC s. The funnel method was used to determine the angle of repose (). In a funnel, 20 gm of the liquisolid RH NLC blend was placed, the height of the funnel was adjusted, and the tip of the funnel just touched the apex of the granules blend heap (a distance of 10 cm from the flat surface). By removing the cotton plug from the 8 mm funnel orifice, the RH NLC blend was allowed to flow through, and the height of the heap (h) and radius of the heap (r) were measured. The RH NLC blend heap's diameter was measured, and the angle of repose was calculated using the following equation:

$$\theta = \tan (h/r)$$

Where, θ = Angle of repose, h = height of RH NLC blend and r = radius of RH NLC blend heap.

Bulk density and tapped bulk density

The following approach was used to determine the loose bulk density (LBD) and tapped bulk density (TBD) of the liquid solid mix of RH NLC blend. On a chemical balance, 20 gram of RH NLC blend was weighed and placed into a 100 ml measuring cylinder. The cylinder was dropped three times at two-second intervals from a height of 2.5 cm onto a wooden platform. The bulk volume was defined as the volume occupied by the blend. After that, the cylinder was tapped on the wooden platform until the RH NLC blend's volume stayed steady. The tapping was then continued until there was no further change in volume. The following formulae were used to calculate LBD and TBD:

$$\text{LBD} = \frac{\text{Weight of the blend}}{\text{volume of the packing}}$$

$$TBD = \frac{\text{Weight of the blend}}{\text{tapped volume of the packing}}$$

The data generated were used in calculating the Carr's compressibility index and Hausner's ratio.

Carr's compressibility index

Carr's compressibility index has been proposed as an indirect measure of bulk density, size and shape, surface area, moisture content, and cohesiveness of RH NLC blend because all of these factors can influence the observed compressibility index. The optimized RH NLC blend was evaluated for this study which influences the flow properties of the RH NLC blend. Compressibility Index (Carr's Index) was determined by using the following equation:

$$\text{Carr's compressibility Index (\%)} = \frac{\text{Tapped density} - \text{bulk density}}{\text{Tapped density}} \times 100$$

Hausner's ratio

The Hausner's ratio is a measure of the ease with which powder flows. The following formula is used to calculate it.

$$\text{Hausner's Ratio} = TD / BD$$

Where, TD=Tapped Density, BD = Tapped density.

Characterization of the Optimized RH NLC

Particle size analysis

The particle sizes of RH- NLCs were measured by Malvern laser light scattering Zetasizer equipped with an Argon laser was used for evaluating the particle size and particle size distribution. For the measurement, 100 µl of the RH- NLCs was diluted ten-fold with double distilled water to a suitable scattering intensity prior to measurements diluted with an appropriate volume of distilled water and the diameter of the vesicle was determined. The average particle size was measured after performing the experiment in triplicate and shown in FIG 1. (8, 9)

Entrapment efficiency

The drug entrapment efficiency (EE) was determined by centrifuging an aliquot (2 ml) of RH- NLCs at 10,000 RPM for 2 hours in an ultracentrifuge. A spectrophotometer set to 288 nm was used to compare the proportion of free drug (RH) in supernatant fluid to a blank. (10) After that, the entrapment efficiency was calculated using the equation below:

$$\text{\% Entrapment Efficiency} = \frac{Da - Df}{Da} \text{ X } 100$$

Where, EE% is the percentage of entrapment efficiency, Da is the amount of drug added during preparation of NLC's and Df is the amount of free drug present in supernatant fluid after centrifugation and shown in Table 2.

Measurement of zeta potential

A zeta sizer was used to determine the zeta potential of the optimized RH-NLC. The charge was measured in the electric field strength was around 23.2 V/cm at 25°C and the average size of NLCs was expressed in diameter (nm). Before measuring zeta potential, the sample's conductivity was adjusted to 50 mS/cm using a 0.1 mmol/l sodium chloride solution (11). The zeta potential result is shown in FIG: 2

Scanning electron microscopy (SEM)

RH-NLCs were examined under a scanning electron microscope for surface morphology. Prior to analysis, 100 l of NLC suspension was vacuum-dried overnight on an aluminum stub. This was then sputter-coated with a thin gold-palladium layer using a gold sputter module in a high-vacuum evaporator under an argon atmosphere. After that, the coated samples were scanned and photomicrographs were taken using a 15 kV acceleration voltage (12). FIG: 3 shows a photomicrograph of a SEM.

Formulation of RH NLC tablets

Liquisolid method, a novel technique for adsorbing a liquid or oil onto a suitable solid carrier by converting the liquid formulation into free-flowing powder, was used to optimize RH NLC stable, solid powders suitable for tabletting. The oily liquid of the NLC preparation was adsorbed on to the mesoporous silica gel and cooled at room temperature.The NLC blend was prepared by liquisolid technique from the optimized RH NLC denoted RH-NLC12 consisting of 60 mg of RH, 210 mg Stearic acid, 90mg MCT 55/45, 33.6 mg Polysorbate 80 and 20 mg Soya lecithin was transferred to a mortar. The mesoporous silica gel has adsorption capacity of about three time of it weight;(12)hence, as the total quantity of the RH NLC is 413.6 mg the quantity of mesoporous silica gel taken was 137.9 mg. The calculated amount of the mesoporous silica gel was added to the RH NLC dispersion and blended in a porcelain mortarto maintain acceptable flow. (8, 13)

Using a multistation rotary punching machine, the NLC adsorbed blend was manually compressed into tablets using 12 mm round shape punches. Depending on the weight of the tablet and the ingredients in the formulation, the compression force was adjusted to achieve the desired hardness of 6–8 kg/cm2. FIG 4 depicts a photograph of compressed tablets that was taken in order to record their description and surface physical properties.

Evaluation of tablet properties

For the prepared RH NLCs tablets, different quality control parameters for all the batches were investigated by adopting the method described in Indian Pharmacopeia 2010. (14)

Tablet thickness

A micrometer was used to measure the thickness of 10 RH NLCs tablets that were randomly selected from the prepared tablets. The mean thickness was measured in millimeters, and the standard deviation (SD) was calculated for the diameters.

Weight variation test

Tablets were designed to contain a specific amount of drug as specified in manufacturing formula. To study weight variation, twenty RH NLCs tablets of the prepared formulation were weighed using an electronic balance, and the test was performed according to the official method and their average weight was calculated.

Hardness test

To withstand mechanical shock during manufacturing, packing, and shipping, RH NLCs tablets require a high level of hardness and resistance to friability. RH NLCs tablets were placed between two anvils for this test, and the force applied to the anvils and the crushing strength that caused the RH NLCs tablets to break were manually recorded. The hardness of RH NLCs tablets was measured using a Monsanto hardness tester. For the hardness tests, twenty RH NLCs tablets were used, and the results were expressed in kg/cm2.

Disintegration time

Disintegration test apparatus was used to determine the in-vitro disintegration time. In each of the apparatus's six tubes, six RH NLCs tablets were placed. The basket was immersed in a water bath at 37 2°C with the bottom surface made of a stainless-steel screen (mesh no.10). The time in minutes taken for complete disintegration of the RH NLCs tablets with no palpable mass remaining in the apparatus was measured in minutes.

Friability test

The friabilator was used to test the friability of RH NLC tablets, which is a measure of their strength. The RH NLCs are subjected to abrasions and shock in a plastic chamber that rotates at 25 rpm and drops the tablets from a height of 6 inches with each revolution. Twenty RH NLCs tablets were pre-weighed and subjected to 100 revolutions in the friabilator. RH NLCs tablets were dedusted and reweighed using a soft muslin cloth. The following formula was used to calculate percent friability (percent F):

$$\% F = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Drug content estimation by HPLC

Twenty RH NLCs tablets were weighed and powdered individually. The powder equivalent to the average weight of RH NLCs tablets was weighed, and RH was extracted with 0.1 N HCl by sonication for 10 minutes, followed by filtration through a 0.45 membrane filter and dilution with 0.1N hydrochloric acid to a suitable concentration. HPLC was used to determine the RH content of these solutions; the analytical method was carried out in the HPLC using a Hypersil BDS C8 (250 mm x.6 mm), 5 m or equivalent column, and UV-Visible detector. Buffer: Acetonitrile was used as the mobile phase (67:33).

Transfer about 25.0 mg of RH into a 50 mL volumetric flask, add 20 mL of mobile phase, sonicate to dissolve, and dilute to volume with mobile phase and mix well. This solution was also diluted with mobile phase from 5.0 mL to 50.0 mL. The chromatograms were recorded after injecting the prepared sample into the HPLC. The peak responses for all peaks were measured, and any peaks due to diluents were ignored. The results of RH absorbance values at 288 nm wavelength were calculated, and the drug content was calculated as shown in table 4.

***In vitro* drug release**

Using two open ended dialysis tubes with an artificial membrane and an optimized formulation of RH-NLC, in vitro RH NLCs release studies were carried out. The prepared RH NLC tablet was re-dispersed in 5 mL of the USFDA's OGD (Office of Generic Drugs) recommended media, 0.1 percent polysorbate 80 in purified water, and dialysis was performed by immersing the dialysis tube in a receptor compartment containing 50 mL of 0.1 percent polysorbate 80 in water (15). The temperature was kept at 37 0.5 °C and the receptor medium was agitated continuously with a magnetic bead under a magnetic stirrer. Over the

course of 12 hours, 1 ml of receptor compartment was taken at predetermined intervals and replaced with 1 ml of fresh media. A spectrophotometer set to 288 nm was used to determine the amount of drug released.

Release kinetics studies

The results of dissolution studies were computed in different kinetics models of zero order, first order, Higuchi, and Korsmeyer pappas equation to study the release kinetics of the RH NLCs tablets from the test formulation (RH NLC-12). The regression coefficient values were calculated using data from the optimized matrix formulation's release profiles. The computed RH NLCs tablet release kinetics for the marketed product and optimized RH NLC tablets are shown in Figures 7 and 8, and the data is summarized in Table 7. The slope of the dissolution profiles was used to calculate the value of K (release rate constant). The R2 value indicates how close the data are to the fitted regression line. The value that was closest to 1 was deemed the most desirable.

Stability studies

The RH NLCs tablets stability study was carried out by keeping the samples at 251°C and 60% RH for six months, then checking for changes in the potential, particle size, and drug content according to the ICH Guideline for the stability testing of new drug substances and products (Q1A (R2) (ICH 2003) as well as the times when the samples were tested for physicochemical properties like particle size, zeta po, and drug content. Tables 8 and 9 show the stability data for 251˚C and 60% RH and 52˚C conditions, respectively.

RESULTS AND DISCUSSION:

Evaluation of RH NLC12 blend

The angle of repose, bulk density, tapped density, Hausner's ratio, and Carr's compressibility index were used to characterize the flow properties of the RH NLC blend. By combining RH NLC12 with self-adsorbent mesoporous silica gel, the RH NLC12 was created. The flow properties of the granules obtained were evaluated as shown in table 1. The angle of repose of the RH NLC12 blend was observed to be 38.50.18, and the RH NLC12 blend prepared by adsorption proved to be flowable. The loose bulk density and tapped density of the prepared RH NLC blend are 0.48 0.02 gm/cm3 and 0.62 0.01 gm/cm3, respectively. The RH NLC12 blend's Hausner's ratio was 1.23, and the RH NLC blend's Carr's compressibility index was 22.3. The prepared blend had good flow properties based on these results.

Entrapment efficiency of optimized RH NLC12

As shown in table 2, the observed response for the optimized RH NLC12 entrapment efficiency was found to be 89 percent, which was well matched with the predicted response of 86 percent. The percentage error was found to be +3.488, which is less than 5% and falls within acceptable limits.

SEM of optimized RH NLC12

High-energy electrons are generated by an electron gun and focused into a fine beam that is scanned across the surface of the RH NLC-12 specimen. FIG: 3 shows the SEM image of the RH NLC12, which revealed the presence of spherical vesicles with a diameter of 146 nm. The scanning electron microscopic images of RH NLC 12 shown above match the findings of previous research. (16)

Zeta potential of RH NLC12

Photon correlation spectroscopy (PCS) was used to determine the zeta potential of RH NLC 12 at room temperature. The optimized formulation's zeta potential was found to be -37.7 mV, indicating a stable RH NLC 12 formulation as shown in FIG:2 along with values. The stability of a value greater than +25 mV or less than –25 mV is high.

Composition of optimized formulation of RH-NLC 12

Based on the optimized (5) CMAs of solid lipid to liquid lipid ratio and surfactant concentration and CPP of ultrasonication time (as mentioned in the earlier work), an optimized batch was prepared and about three times the quantity of NLC, the mesoporous silica gel was used as solid carrier to convert the liquid NLC into the free flowing powder to compress into tablets (17). The final optimized formulation of RH-NLC 12 and composition with its image are shown table 3 and FIG:4 To compare the predicted results with experimental values additional experiment was performed and characterized.

Physico-chemical characterization of the RH NLC12 tablets

The weight variation test revealed a variation of less than 5%, which was within pharmacopoeial specifications. The maximum thickness variation allowed is 5% of the tablet's total size. Friability of the formulated RH NLC12 tablets is less than 1%, indicating that the tablet has good mechanical resistance. The hardness of RH NLC tablets was found to be 7.1 0.165kg/cm2 for various formulations, indicating satisfactory mechanical strength and uniformity. RH NLC tablets' hardness and friability are within acceptable limits.

The drug content is an important metric for ensuring not only the quantity but also the consistency of the drug substance. The drug content in (RH NLC tablets) had a low coefficient of variation of 59.65 0.48 mg, indicating content uniformity in the prepared batches. Table 6.23 shows the results of the evaluation for RH NLC12 tablets, which showed a disintegration time of 4:35 0.32.

Table: 1 Flow properties of RH NLC12 blend

Formulation Code	Angle of repose (θ)	Bulk density (gm/cm^3)	Tapped density (gm/cm^3)	Hausner's ratio	Carr's compressibility index
RH NLC12	38.5 ±0.18	0.48 ± 0.02	0.62 ± 0.01	1.23	22.3

Table: 2 Entrapment efficiency of optimized formulation of RH NLC12

Variables	Predicted response	Observed response*	% Predicted error	Acceptance criteria for % PE
Entrapment efficiency	86 ± 0.39	89± 0.42	+3.488	Less than 5.0 %

*The results obtained were average of three optimized RH NLC batches

Table: 3 Composition of optimized formulation of RH-NLC 12

Sr. No	Ingredients	Quantity (mg)	% w/w
1	Raloxifene HCl	60.0	10.91
2	Stearic acid (Stearic 50)	210.0	38.2
3	Medium Chain Triglycerides (MCT 55/45)	90.0	16.4
4	Polysorbate 80	33.6	6.1
5	Soya Lecithin (Lipoid S-20)	20.0	3.6
6	Mesoporous silica gel (Syloid XDP 3150)*	136.4	24.8
	Total Weight =	550.0	100

*Used as adsorbent to convert the liquid NLC into the free flowing powder for tabletting

Table: 4 Results of physico-chemical characterization of RH NLC tablets

Formulation Code	Thickness (mm)	Uniformity of weight (mg)	Hardness (kg/cm^2)	Friability (%)	Drug content (mg)	Disintegration time (min)
RH NLC12	4.10 ± 0.084	550 ± 7.15	7.0±0.165	0.65±0.15	59.65± 0.48	4:35 ± 0.32

Table: 5 *In- vitro* dissolution studies data

Time (hrs)	Finalized Formulation RH NLC12
0	0
1	12.4
2	24.8
3	36.3
4	48.0
5	55.4
6	60.9
7	66.4
8	72.6
9	80.9
10	86.8
11	93.0
12	98.6

Table: 6 Comparative *in-vitro* release of RH NLC12 with the marketed product.

Time (hrs)	Marketed Tablet	Finalized Formulation RH -NLC12
0	0	0
1	7.4	12.4
2	15.7	24.8
3	29.1	36.3
4	61.6	52.0
5	75.5	55.4
6	84.7	60.9
7	98.3	66.4
8	-	72.6
9	-	80.9
10	-	86.8
11	-	93.0
12	-	98.6

Table: 7 Release kinetics and correlation coefficients of optimized RH NLC12

S. No	Zero order	First order	Higuchi	Korsmeyer Peppas	"n" value	Remark
Marketed formulation	0.9702	0.7862	0.8389	0.9795	0.8292	Korsmeyer Peppas
Final formulation	0.9697	0.8235	0.9734	0.9830	0.8674	Korsmeyer Peppas

Table: 8 Stability studies for RH NLC12 tablets on Storage at (25°C ± 2°C and 60% ± 5% RH)

Parameters	Storage conditions (25°C ± 2°C and 60% ± 5% RH)				
	Initial	After 1 month	After 2 month	After 3 month	After 6 month
Physical appearance	White to off white Tablets	White to off white Tablets	White to off white Tablets	White to off white Tablets	White to off white Tablets
Particle Size (nm)	146± 0.34	151 ±0.47	165 ±0.53	162 ±0.39	195±1.05
Drug Content (mg)	59.40 ±0.41	57.50 ±0.38	56.59 ±0.72	57.10±0.67	54.85±0.43
Zeta Potential (mV)	-37.70 ±0.41	-36.54 ±0.41	-36.12±0.17	-36.34 ±0.63	-35.48 ±0.88

Table: 9 Results of the stability studies for RH NLC12 tablets on Storage at (5°C ± °C)

Parameters	Storage conditions (5°C ± 3°C)				
	Initial	After 1 month	After 2 month	After 3 month	After 6 month
Physical appearance	White to off white Tablets	White to off white Tablets	White to off white Tablets	White to off white Tablets	White to off white Tablets
Particle Size (nm)	146± 0.34	159 ± 0.49	156 ± 0.15	167 ± 0.37	181 ± 0.91
Drug Content (mg)	59.40 ±0.41	58.79 ±0.25	58.34 ±0.91	57.25 ±0.54	56.19 ± 0.68
Zeta Potential (mV)	-37.70 ±0.41	-37.45±0.57	-36.29 ±0.18	-35.34 ±0.72	-35.98 ± 0.45

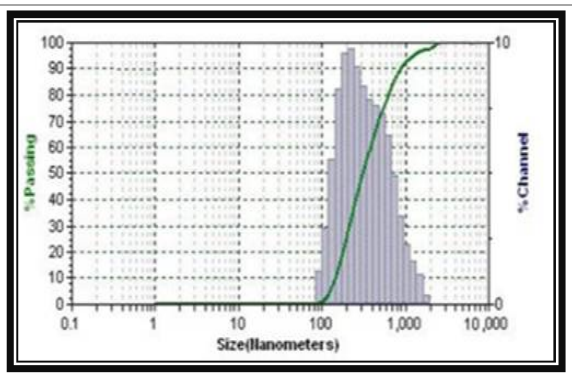


FIG: 1 Histogram of particle size distribution analysis of RH NLC12

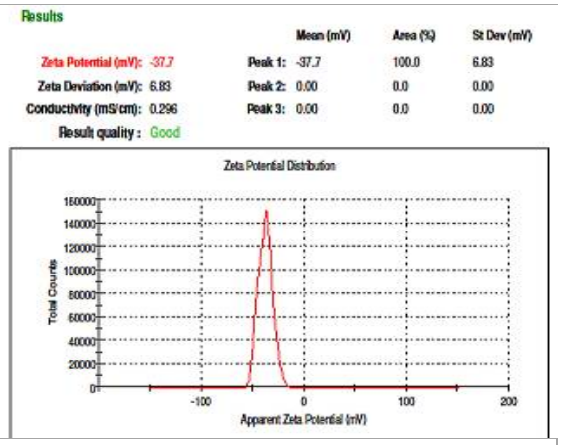


FIG: 2 Histogram of Zeta potential analysis of RH NLC12

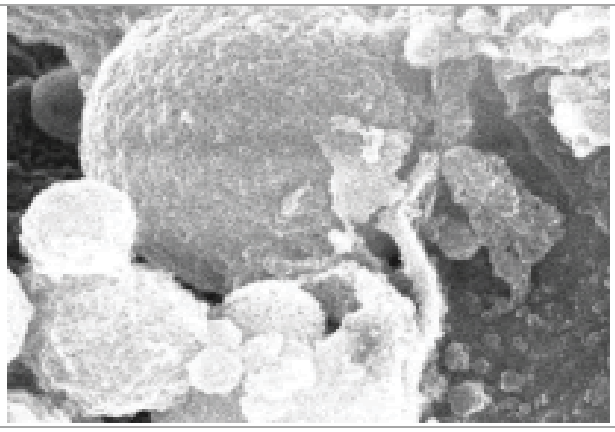


FIG: 3SEM images of RH NLC12

FIG: 4 Image of RH NLC12 Tablet

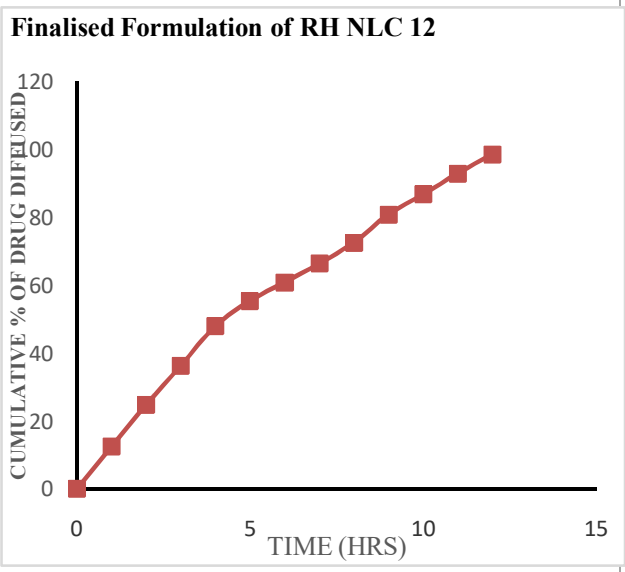


FIG: 5*In-vitro* dissolution profile of RH NLC12 tablet

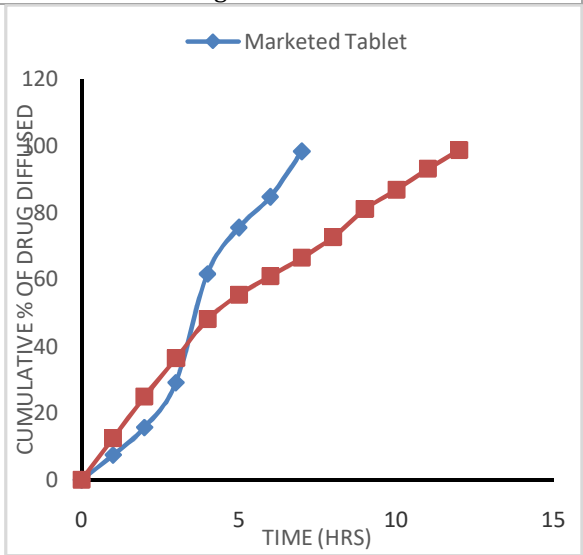
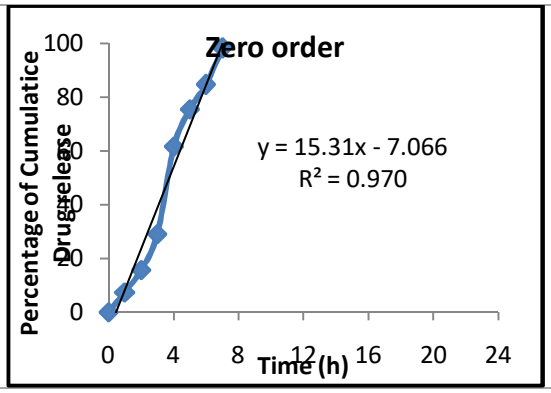
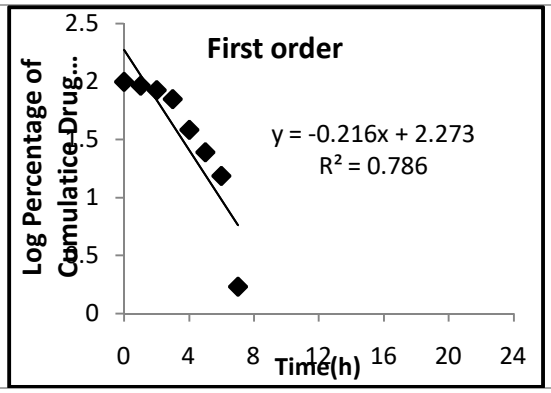


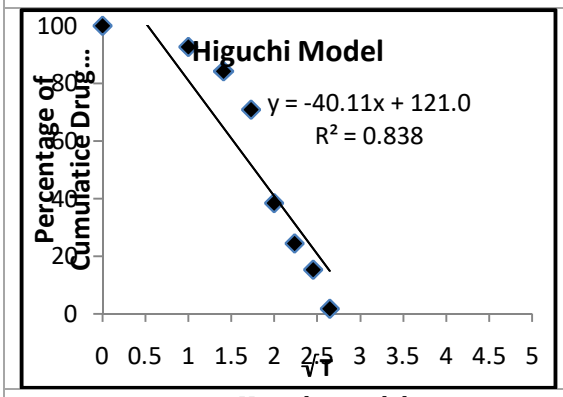
FIG: 6 *In-vitro* dissolution profile of marketed tablet and RH NLC12 tablet.



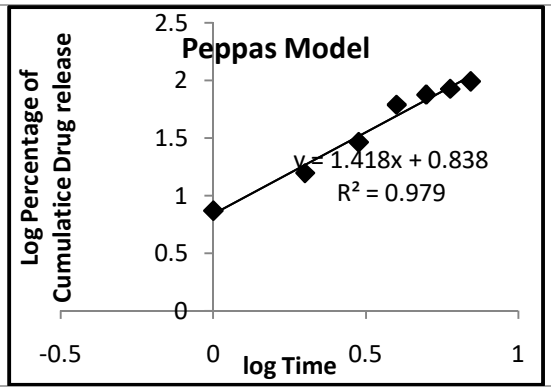
First order model



First order model

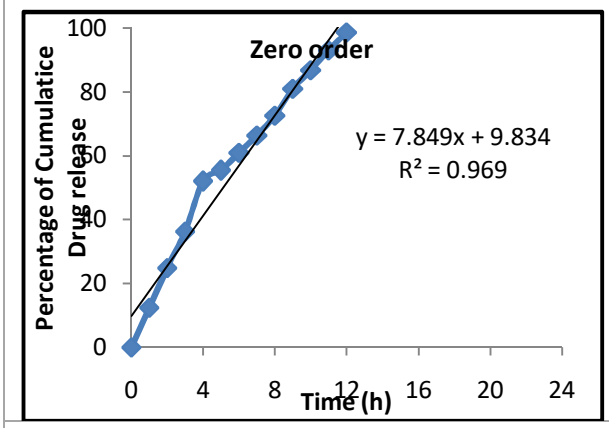


Higuchi model

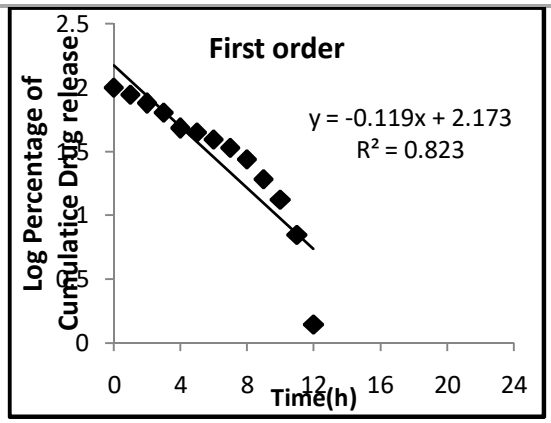


Korsmeyer Peppas model

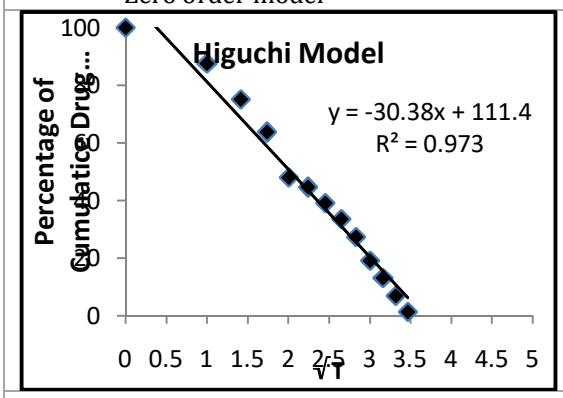
FIG: 7 The release kinetics models of marketed formulation



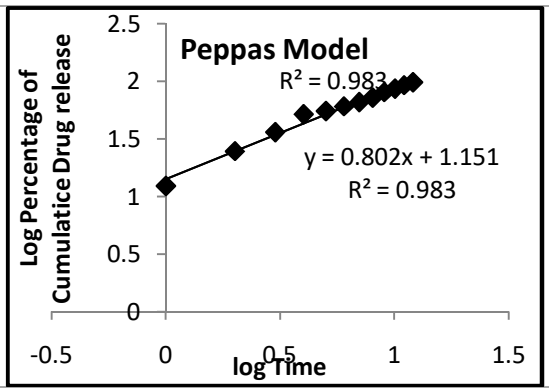
Zero order model



First order model



Higuchi model



Korsmeyer Peppas model

FIG: 8 The release kinetics models of optimized formulation of RH NLC12

In-vitro release of optimized RH NLC12

In-vitro release studies were performed in 0.1% polysorbate 80 in purified water and a sustained drug release of RH up to 12 hrs was observed. The RH NLC12 was subjected to *in vitro* dissolution studies and the dissolution data obtained are furnished in table 5.The results of percentage drug release from RH NLC12 formulations were graphically represented in FIG: 5, the above *in vitro* dissolution studies of RH NLC supports earlier authors work. (18)

Comparative *In-vitro* release of optimized RH NLC12 with the marketed product

The *in-vitro* dissolution of the marketed product was performed and compared with the optimized RH NLC12 Tablets. The comparative dissolution results are shown in table6 and graph in FIG 6. The dissolution data demonstrates that in 1hr, 2hrs and 3 hrs the drug release is 7.4 %, 15,7 % and 29.1 % respectively for marketed product where as 12.4 %,24.8 % and 36.3 % respectively for optimized RH NLC 12. This shows that the drug release in RH NLC12 formulation is about 5-9 % increase in initial time points upto 3hrs as compared to the marketed product and the release is sustained up to 12 hrs as compared to the 7hrs for the marketed product. This demonstrates that the biphasic drug release pattern of the optimized RH NLC formulation. The drug is released in a burst followed by a continuous release at a constant rate. The presence of liquid lipid in the outer layers of the nanoparticles of drug-enriched casing may be responsible for the initial drug release, which results in a burst release of the drug. Unlike SLN, these oil-enriched outer layers have significantly higher lipophilic drug solubility; (19) as a result, a larger amount of drug could be loaded and released via drug diffusion or matrix erosion (20) Slow drug release from the solid lipid core follows the initial faster drug release phase. Surprisingly, in NLC, the release profiles can be tweaked as a function of the lipid matrix composition, for example, by varying the amount of liquid lipid content in relation to solid lipid content (21)

In-vitro release kinetics of the optimized RH NLC12 and marketed formulation

By computing the data of release profiles of the optimized formulation of RH NLC 12 and marketed product, the results of the release data obtained with the regression coefficient values of different release kinetics equations were evaluated. It was found that both the marketed product and optimized RH NLC12 were best explained by Korsmeyer Peppas plot showed highest linearity with R^2 of 0.9705 and 0.9830 respectively. The Korsmeyer Peppas model indicated good linearity (R^2 = 0.9830) the release component n was found to be 0.8674 and follows non-Fickian diffusion mechanism as shown in table 7 and the kinetic model graphs are presented in FIG 7 and 8 for marketed and optimized formulation. The Korsmeyer-Peppas model confirms drug release from a polymeric system and describes several release mechanisms at the same time, including water diffusion into the matrix, matrix swelling, and matrix dissolution by a combination of diffusion and erosion (22, 23)

Stability studies for RH NLC12 Tablets at 25°C ± 2°C and 60% ± 5% RH for a period of 6 months

The physical stability studies for the RH NLC12 tablets maintained at storage conditions (25°C ± 2°C and 60% ± 5% RH) from 1st month to 6 months showed that there is no change in description of the tablets on storage. RH NLC12 Tablets was white to off white tablets. The particle size and drug content varied between 151 ±0.47 to 195±1.05 nm and 57.50 ±0.38 to 54.85±0.43 mg respectively. Likewise the zeta potential varied between -36.54 ±0.41 mV to -35.48 ±0.88. The results showed that there was no significant changes on storage at 25°C ± 2°C and 60% ± 5% RH for a period of 6 months for the RH NLC12 Tablets and does not undergo degradation on storage.

The particle size (nm), the drug content (mg) and zeta potential (mV) were not significantly altered. The results of the stability studies for RH NLC12 Tablets on storage at 25°C ± 2°C and 60% ± 5% RH for a period of 6 months are shown in Table: 6.27.

Stability studies for RH NLC12 Tablets on storage at 5°C ± 3°C for a period of 6 months

The physical stability studies for the RH NLC12 Tablets maintained at storage conditions (5°C ± 3°C) from 1st month to 6 months showed that there is no change in description of the tablets on storage. RH NLC12 Tablets was white to off white tablets. The particle size and drug content varied between 159 ± 0.49 to 181 ± 0.91 nm and 58.79 ±0.25 to 56.19 ±0.68 mg respectively. Likewise the zeta potential varied between -37.45±0.57 mV to -35.98 ±0.45 mV. The results showed that there were no significant changes on storage at 5°C ± 3°C for a period of 6 months for the RH NLC12 Tablets (F12) and do not undergo degradation on storage.

The particle size (nm), the drug content (mg) and zeta potential (mV) were not significantly altered. The results of the stability studies for RH NLC12 Tablets on storage at 25°C ± 2°C and 60% ± 5% RH for a period of 6 months are shown in Table: ---.

CONCLUSION

The goal of this study was to show how NLCs can be used as a novel drug delivery system for a variety of drugs. In this study, an attempt was made to design NLC of Raloxifene Hydrochloride for sustained drug

release using stearic acid and medium chain triglycerides as solid and liquid lipids, respectively, which could be a useful novel drug delivery system in the treatment of postmenopausal osteoporosis. In comparison to other lipid-based formulations with uniform lipid matrices, NLC lipid matrices result in higher drug loading, higher drug entrapment, modulated drug release, and ultimately enhanced drug absorption. These unique characteristics of NLC can only be attributed to their composition, which is made up of a mix of compatible solid and liquid lipids. The blood/lymphatic transport system is thought to work in three ways: a) through M-cells and gut-associated lymphoid tissue, b) by stimulating chylomicron production and transport via triglyceride-rich lipoproteins, and c) by lipid digestion by lipases and the formation of vesicles/micelles.

FUTURE SCOPE

Future development of RH NLC would be facilitated by an *in-vivo* performance with enhanced practical understanding. Establishing the scale-up process of prepared RH NLC formulationand the modulation of drug release by optimizing in the formulation variables.

DECLARATION OF INTERESTS' STATEMENT

The authors declare no conflict of interest.

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