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Research Article

FORMULATION DEVELOPMENT, CHARACTERIZATION, AND *IN VITRO-IN VIVO* STUDY OF ANTIHYPERLIPIDEMIC DRUG ROSUVASTATIN CALCIUM - SOLID LIPID NANOPARTICLES

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ABSTRACT

Objective: The aim of this study was to formulate and optimize solid lipid nanoparticles (SLNs) for the enhancement of solubility and bioavailability of the poorly aqueous soluble drug rosuvastatin calcium.

Methods: SLNs were prepared by slight modification of solvent emulsification-diffusion technique and analyzed for particle size, zeta potential, drug entrapment efficiency, *in vitro* drug release, stability, and pharmacokinetic studies. Rosuvastatin calcium SLNs were formulated using stearic acid as main lipid, poloxamer 407 as surfactant, and Tween 80 as cosurfactant.

Results: All parameters were found to be in an acceptable range. Optimized formulation OR2 SLNs have shown mean particle size 115.49±2.97 nm with polydispersity index value of 0.456, zeta potential - 18.40 mV, 60.34% drug loading, and 97.16% drug entrapment efficiency. *In vitro* drug release was found to be 88.70±3.59% after 12 h with sustained release and was fitted with Higuchi model with a very high correlation coefficient (R^2=0.9905). Transmission electron microscopy confirms that the SLNs of selected optimized formulation are circular in shape. Differential scanning calorimetry and X-ray diffraction confirm the formation of amorphous product. ^{1}H nuclear magnetic resonance studies confirm the intermolecular hydrogen bonding between drug and lipid. Pharmacokinetic studies showed an optimized formulation OR2 SLNs enhanced bioavailability with 4.44-fold as compare to plain drug suspension. Optimized formulation OR2 SLNs have shown good stability at 25±2°C and 60±5°C relative humidity for 180 days.

Conclusion: Thus, the current study can be useful for the successful development of optimized SLNs and able to enhance the bioavailability of poorly soluble drug rosuvastatin calcium.

Key words: Rosuvastatin, Stearic acid, Solid lipid nanoparticles, Pharmacokinetic study.

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INTRODUCTION

Oral route of medication is the most common and preferred route among all methods of administering medication. Solid dosage forms have gained popularity because of their ease of administration, accurate dosages, self-medication, pain avoidance, and, most importantly, patient compliance, but the oral route depends on the bioavailability of effective pharmaceutical ingredients. The speed and degree of medication reach the blood circulation, and thus, metabolism of the drug in the system [1]. Solubility of non-dissolving drugs in improving their pharmacological and biological level remains one of the major technological problems. Water solubility is one of the major determinants of the development of new chemical entities as effective drugs. Nearly 40% of all new drug candidates are classified as poorly soluble, preventing the development of pharmaceuticals [2]. Lipid-based drug delivery systems are introduced to overcome the limitations associated with traditional formulations. These systems offer large variety of options such as solutions, suspensions, emulsions, microemulsions, self-emulsifying drug delivery systems (SEDDSs), dry emulsions, and solid lipid nanoparticles (SLNs) [3]. SLNs are a newer approach gaining popularity; nowadays, as it is the most desirable and easy to manufacture formulation for the enhancement of solubility of poorly water-soluble drugs. The formulation of nanoparticles is giving higher efficacy and lesser toxicity for the cure of numerous diseases [4]. SLNs were introduced in 1991 and provide delivery systems for attractive, less toxic drugs compared to polymer systems that combine the advantages of polymeric nanoparticles, lipid and liposomal emulsions [5]. SLNs are attractive submicron colloidal carriers (50–1000 nm) used for both water-loving and fat-loving drugs. Drugs are entrapped in the heart of the biocompatible lipid and the surface agent in the outer layer. SLN can be used to improve biological availability and achieve continuous release of the drug. They provide benefits such as the lack of acute and chronic toxicity of carrier, good tolerability, and biodegradability, as well as widespread expansion in production. In addition, the method can be modified to achieve the release of the desired drug and the drug can be protected against chemical/enzymatic degradation [6]. Hence, SLNs are, therefore, a better alternative to polymeric nanoparticles, lipoproteins, microemulsions, nanoparticles, and self-emulsifying delivery systems. Rosuvastatin calcium is a fully synthetic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor and has dose-linear pharmacokinetics. Rosuvastatin calcium is BCS Class-II drug (low solubility and high permeability). Rosuvastatin is used as an adjunct to dietary therapy to treat primary hyperlipidemia, mixed dyslipidemia, and hypertriglyceridemia. The absolute oral bioavailability of rosuvastatin calcium is approximately 20% and a half-life of 19 h [7]. Rosuvastatin is subjected to metabolism for the first passage and a low rate of rosuvastatin (10%) is recovered as metabolites, mainly N-desmethyl rosuvastatin, which have about one-sixth to half the inhibitory activity of rosuvastatin HMG-CoA reductase. Cytochrome P450 (CYP) 2C9 is the primary enzyme responsible for the metabolism of rosuvastatin, with minimal effect of CYP2C19 [8]. The solubility of the drug can be improved by reducing the particle size of the drug or by increasing the drug's moisturizing properties of drugs through the use of surfactant. Thus, the objective of this study is prepared and optimize SEDDS (SLNs) to improve the solubility, and hence the bioavailability of rosuvastatin calcium, a poorly water-soluble drug.

MATERIALS AND METHODS

Materials

Rosuvastatin calcium was provided as a gift sample by Sun Pharmaceuticals Ltd. (Gurgaon, Haryana, India). Stearic acid (Acros Organics, U.S.A), Poloxamer 407 (BASF, USA), palmitic acid, and Tween 80 LR were supplied by CDH Ltd., New Delhi, India. Precirol ATO 5, Glyceryl monostearate, and Compritol ATO 888 were obtained as a gift from Asoj Soft Caps, Baroda, India. Double distilled water was used throughout the studies. All other solvents and chemicals were of analytical grade.

Methods

Excipients selection

Lipid and surfactants are the main components of SLNs. The most important factors determining the loading capacity of the drug in the lipid are the solubility of the drug in the dissolved lipid. However, studies of equilibrium solubility cannot be done in this case. Therefore, we used a slightly modified method to determine the solid lipid that had the best dissolving or melting potential of the drug [9]. Stearic acid, Compritol ATO, 888, Precirol ATO 5, Glyceryl monostearate, and palmitic acid were tested for their ability to solubilize rosuvastatin. At first, solid lipids are heated above their melting points. Excess rosuvastatin (20 mg) was placed in the threaded tubes. These lipid melts were gradually added in doses to the drug-containing tubes with continuous agitation using a vortex mixer and maintaining the same temperature (above the lipid melting point). The last point was the formation of a faint pale yellow solution of molten fat. The amount of molten fat required to dissolve visual rosuvastatin has been observed. The experiment was conducted in triplicate. The compatibility between the lipids and drug was identified by Fourier transform-infrared spectrophotometer (FTIR). Infrared spectra were obtained using a Shimadzu FTIR-8400 spectrometer (Shimadzu, Japan). The sample of pure drug, lipid, and physical mixture (PM) was previously ground and mixed thoroughly with KBr, an infrared transparent matrix. The KBr disks were prepared by compressing the powder. The four scans were executed at a resolution of 1/cm (from 400 to 4000/cm) [10].

Preparation of rosuvastatin-loaded SLNs

In an introductory laboratory study, several factors such as lipid concentration (stearic acid, 20–100 mg), surfactant concentration (Poloxamer 407 and Tween 80 in 1:1 [% w/v], 0.5–2.5%), stirring speed (2000–4000 rpm), drug amount (rosuvastatin, 10–50 mg) chloroform: ethanol ratio (1:1, 2.5% v/v) as the solvent of drug and lipids and sonication time 5 min were fixed and their effect on particle size, entrapment efficiency was determined. These factors were optimized in the study [11]. In the current study, all of the experiments were performed in triplicate and the averages were considered as the response. Table 1 summarizes the composition of various batches. Rosuvastatin calcium-loaded SLNs were prepared by slight modification of the previously reported solvent emulsification-diffusion technique [12-15].

Accurately weighed amount of lipid (20–100 mg) was dissolved in a 2.5 mL (2.5%v/v) mixture of methanol and chloroform (1:1) as the internal oil phase. Drug (10–50 mg, ratio of drug to lipid 1:2) was dispersed in the above solution. This organic phase was added drop by drop into a homogenizer tube containing 22.5 mL of an aqueous solution of surfactant and cosurfactant Poloxamer 407 and Tween 80 in 1:1% w/v (0.5–2.5% w/v) as external aqueous phase and homogenized for 30 min at stirring speed (2000–4000 rpm) (Remi Instruments Pvt., Ltd., India) to form a primary emulsion (o/w). The above primary emulsion was then poured into 75 mL of ice-cold water (2–3°C) containing surfactant (0.5–2.5% w/v) and stirred to extract the organic solvent into the continuous phase and for proper solidification of SLNs. The stirring was continued for 2 h at (2000–4000 rpm) to disperse the SLNs. The SLN dispersion was sonicated for 5 min (1 cycle, 100% amplitude, Bandelin Sonopuls, Germany) to produce SLN dispersions of uniform size. The dispersion was then centrifuged at 18,000 rpm for 20 min (Remi Instruments Pvt., Ltd., India) to separate the solid lipid material containing the drug and washed with deionized water time to ensure the complete removal of organic solvent. This was then redispersed in (0.5–2.5% w/v) aqueous surfactant mixture of surfactant and cosurfactant Poloxamer 407 and Tween 80 in 1:1% w/v and sonicated for 5 min to obtain the SLNs. The SLN dispersions were lyophilized in the presence of 5% (w/v) mannitol as cryoprotectant.

Particle size, polydispersity index (PDI), and zeta potential

Average particle size, the PDI (to determine the width of particle size distribution), the zeta potential (to characterize the particle's surface charge and the physical stability of colloidal pigments) from the rosuvastatin calcium-induced SLN dispersion measured by photon correlation spectroscopy (PCS) using the Malvern Instruments, HAS 3000: Malvern, United Kingdom. The analysis was performed at 25°C with a detection angle of 90°. The dilute nanoparticle dispersion was poured into the disposable sizing cuvette and then placed in the cuvette holder of instrument and analyzed. Air bubbles, if any, are removed from the capillaries before measurement [16].

Drug loading and drug entrapment efficiency

A fixed quantity of SLNs dispersions (10 mL) was ultracentrifuged for 1 h at 20°C and 18,000 rpm (REMI International, Mumbai, India), and the amount of drug presents in the clear supernatant after centrifugation was determined (w) by UV spectrophotometer (Shimadzu ultraviolet [UV]-1700, Kyoto, Japan) at 243 nm [17]. The drug loading (%) and drug entrapment efficiency (%) were calculated by following equations:

$$\text{Drug loading (\%)} = (Wt\text{-}Ws)/(Wt\text{-}Ws+W_L)\times 100 \quad (1)$$

$$\text{Drug entrapment efficiency (\%)} = (Wt\text{-}Ws)/Wt\times 100 \quad (2)$$

Where, Ws is the weight of drug in the supernatant after centrifugation, Wt is the total weight of drug used, and W_L is the weight of the lipid used in preparing the SLNs.

In vitro drug release and release kinetics study

A ROS-SLN release study was performed using a dialysis membrane (Hi-Media, 12,000–14,000 D molecular weight of the cut). An appropriate amount of ROS-SLN was taken containing a drug equivalent to 5 mg in the dialysis bag and sealed at both ends. A dialysis bag is placed in the receptor chamber containing 100 ml of phosphate buffer pH 6.8. The temperature was maintained at 37°C±2°C and was magnetically stirred at 100 rpm. Samples were taken from the receptor chamber at regular intervals of 60 min–12 h from the receiving chamber (flask) and replaced with an equal amount of the fresh phosphate buffer solution to maintain the sink condition. Sampling was determined by a UV spectrophotometer (Shimadzu UV-1700, Kyoto, Japan) at 243 nm. All

Table 1: Composition of various batches of rosuvastatin-loaded SLNs

S. No.	Formulation code	Variables			
		Lipid (mg)	Surfactant (%w/v)	Stirring speed (rpm)	Drug (mg)
1.	OR1	80	2	3500	40
2.	OR2	80	2.25	4000	40
3.	OR3	90	1.5	3500	40
4.	OR4	90	2	4000	40

SLNs: Solid lipid nanoparticles

experiments were conducted in 3 times. The graph was drawn between the percentage cumulative of drug release versus time (hours) [18,19].

The results obtained from the *in vitro* release studies in nanoparticles have been fit into many kinetic equations such as zero order (percentage cumulative release vs. time), the first order (log percentage remaining vs. time), Higuchi (cumulative release of drugs vs. square), and Korsmeyer–Peppas (log cumulative percentage drug release vs. log time). The correlation coefficients (R^2) and K were determined for the linear curve obtained by regression analysis of the plots. Initial equations for various models are given below:

Zero-order model: $X=Kt$ (3)

First-order model: $\log X=Kt/2.303$ (4)

Higuchi release model: $X=K(t)^{1/2}$ (5)

Korsmeyer–Peppas equation: $\log X=\log K+n\log t$ (6)

Where, X is the amount of drug released, K is the release rate constant, and t is time. To construct the plot for zero-order and the Higuchi model, percent cumulative drug release determined, for the first model, log percent drug remaining was determined, and for Korsmeyer–Peppas equation, log cumulative percent drug release was determined [20-23].

Surface morphology by transmission electron microscopy (TEM)

TEM was used for microscopic evaluation of the optimized formulation ROS-SLNs. Surface morphology was determined from SLNs designed using TEM (Philips CM 10, Netherlands). To evaluate, one drop (about 10 µl) of SLN dispersions was diluted with distilled water (1:100), filtered (0.22 µm), and applied to a carbon-coated grid with dye solution phosphotungstic acid 2% (PTA) for 30 s. The dried coated mesh was taken onto a slide and covered with a coverslip and was placed on 400 mesh copper grids with films to monitor the surface morphology [24].

X-ray diffraction (XRD) analysis

Diffraction patterns of pure drug, lipid, and optimized ROS-SLNs were improved with the D-8 advance SRD-Bruker (Germany). A 40 kV and 30 mA power were used for the generator, with copper being used as anode in the tube. The solids of the Cu-Kα radiation (α1=1.54060 Å and α2=1.54439 Å with α1/α2 of 0.5) were exposed in the range of 2θ angles from 10°C to 30°C, at the angular velocity of 1° (2θ) min. Percentage crystallinity index was calculated by following equation No. 7 [25].

Where, % crystallinity index $=I_{020}-I\,am/I_{020}\times100$ (7)

I_{020} is intensity at 20°

I am is lowest 2θ near 8°.

Differential scanning calorimetry (DSC)

The improved thermal images of pure drug, lipid, PM, and optimized formulation ROS-SLNs were recorded in the Perkin-Elmer (Pyris Diamond) model differential scanning calorimeter. About 10 mg samples were sealed in aluminum trays and an empty aluminum tray used as reference. The experiment was conducted under a stream of nitrogen (20 ml/min) at a rate of 10°C/min from 30 to 300°C [26].

^{1}H nuclear magnetic resonance (NMR) spectroscopy

To determine the nature of the proton or the proton group in the pure drug, lipids, and optimized formulation ROS-SLNs, the ^{1}HNMR spectrum in dimethyl sulfoxide was recorded in the Bruker Advance II FT-NMR (DRX-400, JAPAN) 300 MHz spectroscopy, using tetramethylsilane as an internal standard, chemical shift (δ) was recorded in ppm [27].

Pharmacokinetic studies

The pharmacokinetic study of optimized ROS-SLNs was determined in comparison with pure drug in male albino Wistar rats (adult/weighing 180–250gm). The animals were procured from animal house Devsthali Vidyapeeth College of Pharmacy, Kichha Road, Lalpur, Rudrapur, U.S. Nagar, UK-263148, India. General and environmental conditions were strictly monitored. Animal handling routines were performed according to good laboratory practice. The temperature of 22±3°C and relative humidity (RH) 30–70% was maintained. Animals had free access to food and water was made available *ad libitum*. A study protocol for animal studies was approved by the Institutional Animal Ethics Committee of Devsthali Vidyapeeth College of Pharmacy, Kichha Road, Lalpur, Rudrapur, U.S. Nagar, UK-263148, India (Registration No:1452/PO/Re/S/11/CPCSEA).

The male Wistar rats were divided into three groups - control, reference, and test each consisting of six animals. In Group I - control (distilled water 10 mL/kg,); Group II - (reference) plain drug suspension in 700 ng/kg, and Group III - (test) optimized ROS-SLNs (700 ng/kg) were taken and in Group II and III additionally CMC-Na (0.5% w/v) as a suspending agent was received, respectively. The animals were kept on fasting for 10–12 h before the study. Under light ether anesthesia, blood samples (about 0.3 mL) were withdrawn into heparinized tubes 0, 0.5, 1, 2, 4, 8, and 24 h through retro-orbital puncture or from tail vein route. Plasma was separated by centrifugation at 3000 rpm, 15 min at 4°C. About 100 µL of plasma sample along with 100 ng/mL of simvastatin, as internal standard, was mixed with 1 mL ethyl acetate as extracting solvent (liquid-liquid extraction technique). The samples were vortexed for 5 min and then centrifuged at 4000 rpm for 10 min. The remaining supernatant (900 µL) was evaporated under a gentle stream of nitrogen at 40°C using nitrogen gas at room temperature. The dried samples were reconstituted with 0.3 mL of mobile phase and evaluated by high-performance liquid chromatography for the presence of ROS. Plasma drug concentration-time profile of samples for each group was evaluated with pharmacokinetic software (PK functions for Microsoft Excel, Pharsight Corporation, Mountain View, CA, USA). Various pharmacokinetic parameters such as C_{max}, T_{max}, AUC_{0-t}, K_E, $T_{1/2}$, MRT, and $AUMC_{0-t}$ were calculated [28,29].

Stability studies

A stable product is one, which retains its chemical safety and energy mark (chemical properties) within specified limits, retains its appearance, uniformity, palatability, its physical viability, solubility, resists microbial growth (microbiological stability), and the therapeutic effect remains unchanged without significant increase in toxicity over the course of life. ICH guidelines determine storage conditions to evaluate the stability of pharmaceutical products. The stability study was conducted to determine the effect of formulation additives on the stability of the drug and also to determine the physical stability of the prepared preparation under storage temperature and RH. The optimized formulation ROS-SLNs were analyzed for stability studies and these studies were performed in triplicate. The storage conditions used for stability testing were 4±2°C (refrigerator), 25±2°C/60±5°C RH, and 40±2°C/75±5°C RH, in stability chamber (Hicon Instruments Ltd., New Delhi). The samples were withdrawn after the period of 0, 1, 3, and 6 months, and the effect on particle size, PDI, zeta potential, entrapment efficiency, and loading capacity was evaluated [24,30].

Statistical analysis

All the data are reported as mean ± standard deviation (SD). Differences between the groups were tested using t-test. $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Excipients selection

Excipients play a key role in formulation development of dosage forms. It should be pharmaceutically inert, acceptable, non-sensitive, and non-irritating in nature. They must be regarded as safe. For the preparation of SLNs, selection of a suitable lipid and surfactant is important. The drug solubility in lipid is an important determinant of its encapsulation efficiency. High encapsulation efficiency is due to high lipid solubility of drug and vice versa [31].

Measurement of the solubility of drug in the lipids was determined and the data are presented in Table 2. Solubility studies result shows that the drug was maximum soluble in stearic acid as compare to Compritol ATO 888, Precirol ATO %, Glyceryl monostearate, and palmitic acid. The solubilizing efficiency of drug in lipid, already reported biocompatibility and its acceptability for SLNs approve its selection for the current research work [32].

The FTIR spectra scanning range was 400–4000/cm for ROS, stearic acid and PM are shown in Fig. 1. Results of FTIR spectra show the characteristic peaks at 3452.12/cm (O–H stretch vibration), 2850.19/cm (C–H stretch vibration), 1724.12/cm (stretch vibration – C=O carbonyl functional group), 2968.52/cm (-N–H stretch vibration), 1547.88/cm (–C=C stretch vibration), 2850.19/cm (=C-H stretch vibration), 1509.81/cm (-N-H bending), 1381.13/cm (-CH3 symmetric), and 1335.60/cm (-S=O symmetric). The FTIR spectra of PM seemed to be only a summation of drug and lipid. This result suggested that there were no interactions between drug and lipid in PM, and also, rosuvastatin maintained its crystallinity as observed in thermal analysis [33].

On the basis of drug-lipid solubility and drug-lipid compatibility study, stearic acid was selected as the lipid. The surfactant for preparing SLNs was selected on the basis of hydrophilic-lipophilic balance (HLB) value that can emulsify lipid and form the stable microemulsion in an acceptable concentration. Poloxamer 407 with HLB of 18 was selected as the surfactant and Tween 80 with HLB of 15 was selected as the cosurfactant.

Particle size, PDI, and zeta potential

The surfactant concentration was optimizing as in respect to get a smaller size of SLNs with greater entrapment efficiency. The optimized surfactant concentration was 2.25% w/v. The average particle size, PDI, and zeta potential of optimized formulation (OR2) were found to 115.49 nm, 0.456, and - 18.40 mV, respectively. The mean particle size of different batches of SLNs ranges from 115.49±2.97 to 141.65±3.89. The particle size of optimized formulation (OR2) was appreciably lower 115.49±2.97 nm compared to other batches. This is due to increase in concentration of surfactant in OR2 formulation in comparison of other batches. Furthermore, by the addition of surfactant to SLNs that cause the interfacial film to condense and stabilize [34]. The particles of all batches are in the nano range which is proved from the PDI values. PDI is essentially the ratio of SD to mean particle size. The value of PDI is shown in Table 3 and was acceptable for all batches. Percent entrapment efficiency and drug loading of all batches are shown in Table 3 and value of the optimized batch (OR2) was 97.16±3.73 and 60.34±2.51, respectively. The surface carried negative charge with zeta potential of optimized batch was found to be -18.40 mV.

Surface morphology by TEM

TEM results show that the selected optimized formulation (OR2) SLNs are circular in shape and particles are nanometric having size less than 125 nm. The SLNs are smooth and perfectly separated on the surface. TEM image also confirms that SLNs have no drug crystal, i.e., irregular crystallization or rod crystal was visible (Fig. 2).

XRD analysis

The XRD pattern of pure drug showed various distinctive peaks in the region of 8–25° (2θ) (6.24, 9.26, 10.88, 15.57, 16.51, 17.10, 18.75, 19.54, 22.75, and 23.38) which indicated the highly crystalline nature of rosuvastatin. In case of lipid, the predominant peaks were observed at 19.08° and 23.21° that indicated the crystalline nature of lipid. The summation of distinctive diffraction peaks of rosuvastatin and lipid in the PM as shown in Fig. 3. Optimized formulation (OR2) exhibited considerable diminution of diffraction peaks than PM. Crystallinity index was found to be 72.35%, 58%, 64%, and 52.03% for pure drug, lipid, PM, and optimized formulation (OR2) SLNs. This indicates that the drug was completely converted from crystalline to amorphous form. Hence, increased dissolution of the drug was observed since an amorphous form will dissolve at the faster rate owing to its higher internal energy and thermodynamic properties relative to crystalline materials.

DSC

DSC is productive technique for evaluating the thermal properties of formulation, giving data about the physiochemical condition of drug in the system [35]. The DSC thermogram shows that a sharp endothermic peak corresponding to the melting point of crystalline pure drug was found at 128°C and DSC thermogram of the stearic acid showed endothermic peak at 69°C. The thermogram of the PM was merely a combination of thermogram of pure drug and lipid as shown in Fig. 4.

Table 2: Solubility study of drug in various lipids

S. No.	Lipid name	Melting point of lipid in (°C)	Amount of lipid required*
1.	Stearic acid	69	48.66±0.87
2.	Compritol ATO 888	70	51.21±0.56
3.	Precirol ATO 5	56	53.34±0.86
4.	Glyceryl monostearate	59	84.87±0.78
5.	Palmitic acid	63	127.46±0.42

*All the values are expressed as mean±SD, n=3. SD: Standard deviation

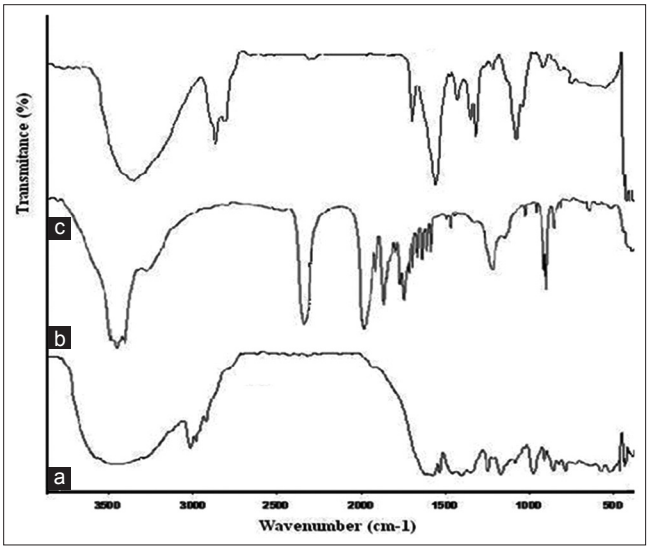

Fig. 1: Fourier transform-infrared spectra of (a) rosuvastatin calcium, (b) stearic acid, and (c) physical mixture

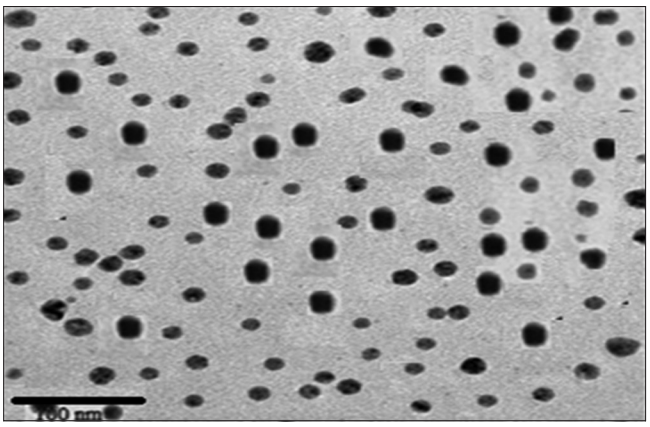

Fig. 2: Transmission electron microscopy image of rosuvastatin calcium-loaded optimized formulation (OR2) solid lipid nanoparticles

Table 3: Observed response of different selected batches

Formulation code	Response				
	Entrapment efficiency*	Drug loading*	Particle size* (nm)	PDI	Zeta potential
OR1	96.14±7.67	58.71±5.65	130.34±17.32	0.403	−12.47
OR2	97.16±3.73	60.34±2.51	115.49±2.97	0.456	−18.40
OR3	95.25±5.65	57.53±2.56	141.65±3.89	0.434	−16.14
OR4	96.77±3.67	59.47±1.78	124.12±4.67	0.442	−12.47

*All the values are expressed as mean±SD, n=3. SD: Standard deviation, PDI: Polydispersity index

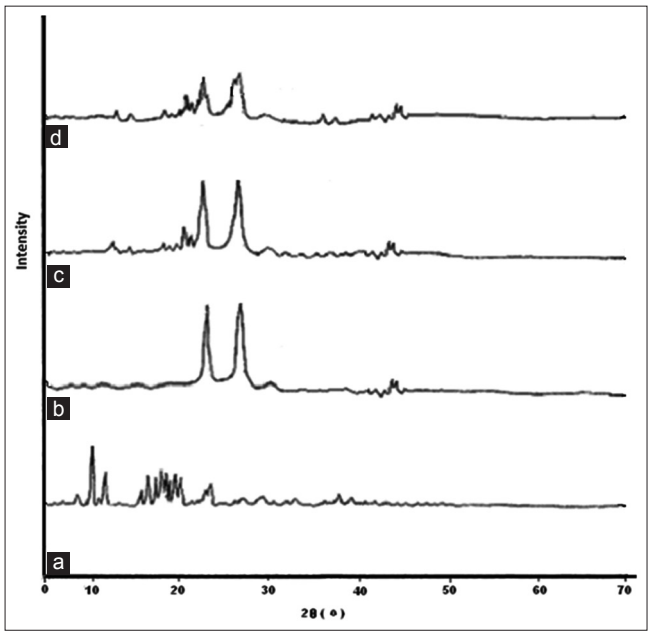

Fig. 3: X-ray diffraction spectra of (a) rosuvastatin calcium, (b) lipid (stearic acid), (c) physical mixture, and (d) optimized formulation (OR2) solid lipid nanoparticles

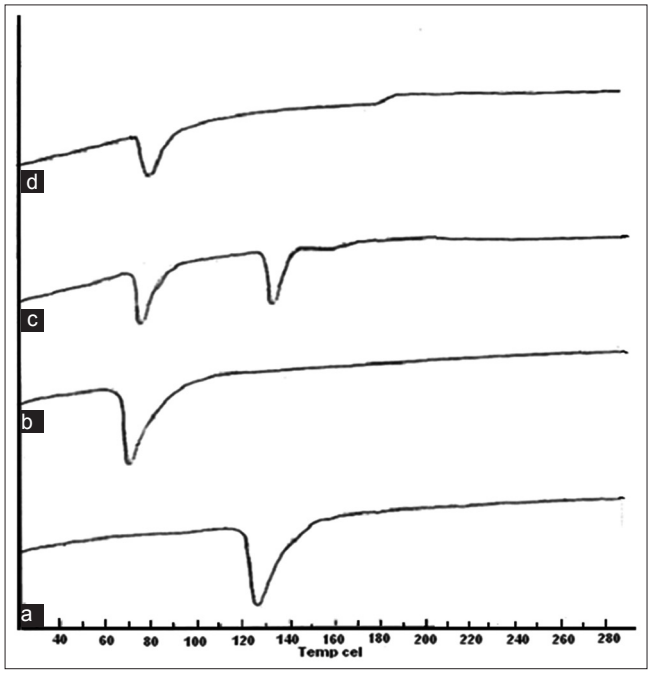

Fig. 4: Differential scanning calorimetry thermograms of (a) rosuvastatin calcium, (b) lipid (stearic acid), (c) physical mixture, and (d) optimized formulation (OR2) solid lipid nanoparticles

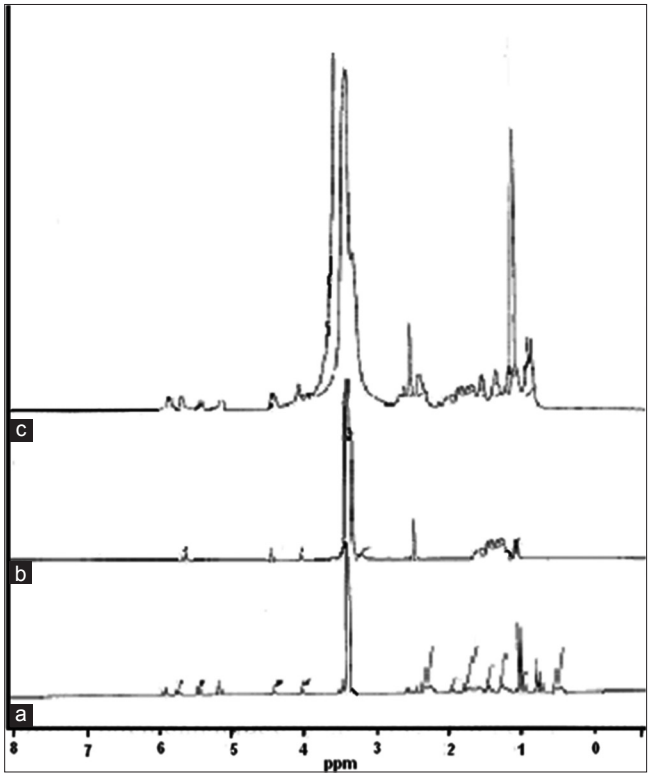

Fig. 5: ^{1}H nuclear magnetic resonance spectra of (a) rosuvastatin calcium, (b) lipid (stearic acid), and (c) optimized formulation (OR2) solid lipid nanoparticles

In the DSC thermogram of SLNs of optimized formulation OR2, the endothermic peak corresponding to melting of pure dug was absent. This might be due to the presence of the amorphous form of pure drug in the SLNs, and also, due to complete drug entrapment in the lipid matrix [36].

^{1}HNMR spectroscopy

FT-NMR (^{1}HNMR) spectrum of rosuvastatin, lipid (stearic acid), and SLNs of optimized formulation OR2 was shown in Fig. 5. The ^{1}HNMR spectrum of pure drug showed chemical shift from 5.927 to 5.958 ppm (d, 1H, C_5H), 5.729 to 5.791 ppm (t, 1H, C_4H), 5.478 ppm (s, 1H, C_6H), 2.248 to 2.376 ppm (m, 1H, OH), 3.464 ppm (s, 10H, C_3H, C_7, C_2, C_3, $C_{3''}$, C_8, C_9, C_{10}, $C_{2''}$, $[CH_3]_2$), 1.475 to 1.81 ppm (m, 6H, $C_{3''}$, $C_{7''}$), 0.981 to 1.031 ppm (m, 6H, $C_{4''}$, $C_{5''}$, $C_{4'}$), 1.423 to 1.470 ppm (m, 2H, $C_{5'}$), 3.304 to 3.710 ppm (m, 9H, CH_3), and 2.494 ppm (s, 1H, OH). Lipid showed chemical shift from 3.521 to 3.620 ppm (m, 89H, CH_2), 1.011 ppm (s, 4H, CH_3), and 3.294 to 3.428 ppm (s, 2H, CH) confirmed all protons of rosuvastatin calcium and stearic acid, thus identity and purity of sample. FT-NMR (^{1}HNMR) spectrum of optimized formulation OR2 showed similar peaks of drug and lipid, 5.928 ppm (d, 1H, C_5H), 5.590–5.759 ppm (t, 1H, C_4H), 5.434 ppm (s, 1H, C_6H), 2.321–2.364 ppm (m, 1H, OH), 3.459–3.718 ppm (m, 89H, CH_2), 3.445 ppm (s, 10H, C_3H, C_7, C_2, C_3, $C_{3''}$, C_8, C_9, C_{10}, $C_{2''}$, $[CH_3]_2$), 1.521–1.825 ppm (m, 6H, $C_{3''}$, $C_{7''}$), 0.970–1.024 ppm (m, 6H,

$C_{4'}$, $C_{5'}$, $C_{4'}$), 1.026 ppm (s, 4H, CH_3), 1.454–1.479 ppm (m, 2H, $C_{5'}$), 3.312–3.582 ppm (m, 9H, CH_3), and 3.325 ppm (s, 2H, CH) which confirms the intermolecular hydrogen bonding between drug and lipid [27].

In vitro drug release and release kinetics study

The optimized rosuvastatin calcium-loaded SLNs (OR2) showed much faster *in vitro* dissolution rate than pure drug suspension as shown in Fig. 6. The optimized SLNs show drug release from 21.70±3.23% to 88.70±3.59%. It was apparent that optimized formulation showed a rapid initial release followed by slow drug release. The rapid release of drug at initial phase may be due to release of drug takes place from the surface of nanoparticles, while at the later stage sustained release of drug was observed from nanoparticles core as a consequence of lipid hydration and swelling that is responsible for the prolonged release, which is desired for controlled release [37]. The drug release data were fitted into zero-order, first-order, and Higuchi's model [9]. For the optimized batch, the highest value of correlation coefficient (R^2=0.9905) was observed for Higuchi' model, followed by the Korsmeyer–Peppas (R^2=0.9754), first-order (R^2=0.9612), and zero-order (R^2=0.9291) models, as shown in Fig. 6.

Pharmacokinetics studies

The different pharmacokinetic parameters of optimized rosuvastatin-loaded SLNs (OR2) when administered through oral route were determined, and concentrations of drug in blood plasma were calculated as shown in Table 4. Results of the study showed that optimized rosuvastatin-loaded SLNs (OR2) remarkably increased the rosuvastatin concentration in plasma as compared to concentration found with plain drug suspension after oral administration. The two-tailed unpaired *t*-test showed statistically significant differences in the drug plasma concentration ($p<0.05$) between the optimized rosuvastatin-loaded SLNs (OR2) and plain drug suspension after oral administration at T_{max}. The optimized loaded SLNs (OR2) exhibited 4.44-fold increase in bioavailability as compared to plain drug suspension as shown in Fig. 7 and able to provide sustained release for prolong period of time. The rate and extent of absorption of drug form SLNs suggested excellence in the oral bioavailability of drug from the system.

Stability studies

As per the ICH guidelines, for optimized formulation ROS-SLNs (OR2) as intended to be stored at 4±2°C (refrigerator), 25±2°C/60±5°C RH, and 40±2°C/75±5°C RH and samples withdrawn at 0, 1, 3, and 6 months. There were no significant changes observed in particle size and PDI when they are stored at 4±2°C (refrigerator), 25±2°C/60±5°C RH, but particle size was increased due to zeta potential dropped at 40±2°C/75±5°C RH. This type of effect might be due to dissolution of

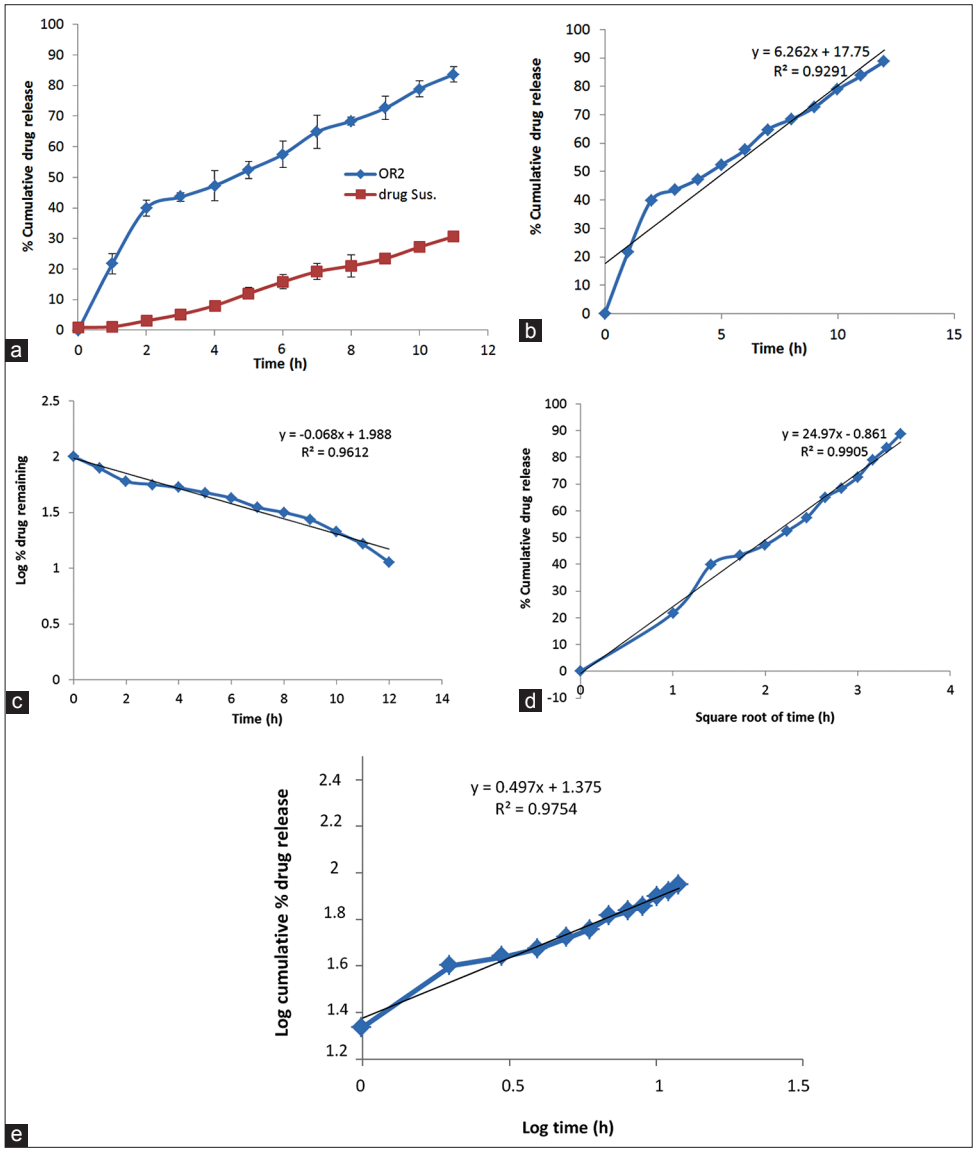

Fig. 6: *In vitro* drug release from (a) optimized formulation (OR2) solid lipid nanoparticles, (b) zero-order release model, (c) first-order release model, (d) Higuchi release model, and Korsmeyer peppers release model

the coating of lipid at accelerated temperature and humidity which leads to cluster or aggregation of particles ($p<0.05$, Table 5) [24].

CONCLUSION

In the present study, rosuvastatin-loaded SLNs were successfully prepared by the slightly modified emulsification-diffusion technique and evaluated for different parameters such as particle size, PDI, zeta potential, *in vitro* release, pharmacokinetic, and stability studies. The mean particle size of SLNs decreased with increase in surfactant concentration. All the measurements were found to be in acceptable range. *In vitro* drug release was found to be 88.70±3.59 % over 12 h showing initial rapid and then sustained drug release. Pharmacokinetic studies were performed on male Wistar rats, and it was found that optimized SLNs were able to improve bioavailability with 4-fold as compared to plain drug suspension and able to provide sustained release for prolong period of time.

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Table 4: Plasma pharmacokinetic parameters after oral administration of plain drug suspension and optimized formulation (OR2) SLNs

S. No.	Pharmacokinetic parameters	Plain drug suspension	Optimized ROS SLNs (OR2)
1.	C_{max} (µg/mL)	48.62±4.63	72.74±7.63
2.	T_{max} (h)	2	4
3.	AUC_{0-t} (µg.h/mL)	439.81±43.72	958.585±77.7356
4.	K_E (h^{-1})	0.034478±0.00652	0.039576±0.0083
5.	$T_{1/2}$(h)	7.735±0.942	12.73±1.02
6.	MRT	11.08765±1.5234	6.7995±0.7423
7.	$AUMC_{0-t}$ (µg.h^2/mL)	4876.46±273.74	6517.899±392.7456

*All the values are expressed as n=6, mean±SD. SD: Standard deviation, SLNs: Solid lipid nanoparticles, ROS: Rosuvastatin calcium

Table 5: Characteristics of optimized SLNs after 6 month stability studies at different conditions

Temp (°C)/RH(%)	Time (month)	Characteristics parameter				
		Particle size (nm)	PDI	Zeta potential (mV)	Entrapment efficiency (%)	Drug loading (%)
4±2°C (refrigerator)	0	112.56±3.35	0.453	– 18.5±1.3	95.12±2.32	59.54±1.54
	1	116.74±2.43	0.432	– 17.5±1.5	93.89±3.20	60.25±2.34
	3	115.32±2.76	0.342	– 17.4±1.7	96.93±2.87	60.37±2.45
	6	124.65±3.23	0.478	– 18.2±1.9	97.65±1.76	58.89±1.96
25±2°C/60±5°C RH	0	112.56±3.35	0.453	– 18.5±1.3	95.12±1.36	59.54±1.21
	1	112.74±1.04	0.552	– 17.3±1.2	92.53±2.27	60.15±2.75
	3	119.72±4.76	0.459	– 16.2±1.2	94.93±2.34	58.37±1.46
	6	128.43±5.46	0.598	– 15.9±3.2	95.65±2.14	59.64±2.58
40±2°C/75±5°C RH	0	112.56±3.35	0.453	–18.5±1.3	95.12±2.32	59.54±1.23
	1	121.74±2.43	0.432	–14.5±1.8	93.89±2.57	60.63±2.87
	3	315.32±2.76	0.542	–12.4±2.8	94.85±1.58	57.37±1.36
	6	1124.65±3.23	0.678	–7.54±3.5	91.37±2.52	54.89±0.96

*All the values are expressed as n=3, mean±SD; $P<0.05$. SD: Standard deviation

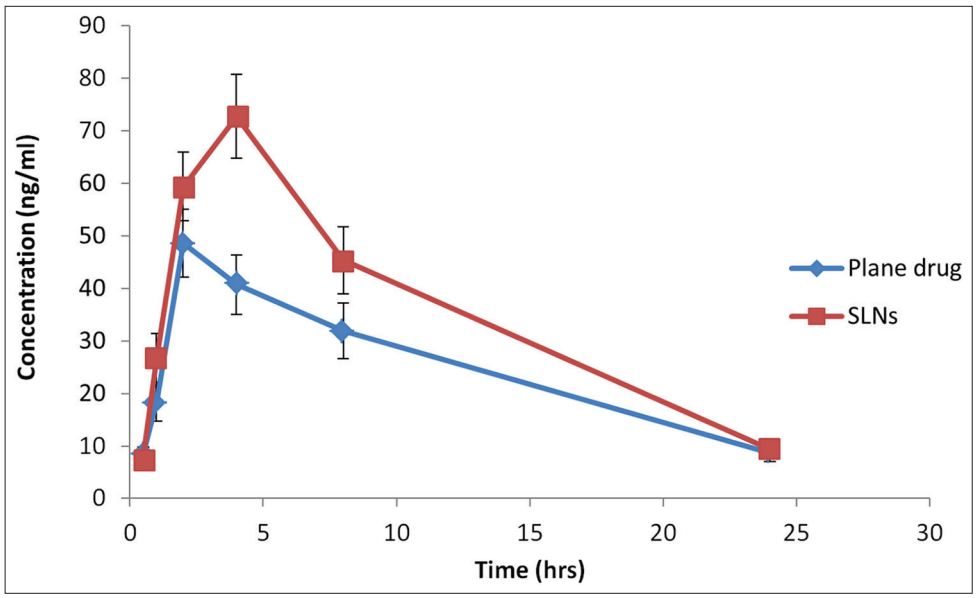

Fig. 7: Plasma concentration versus time profile of rosuvastatin after single-dose oral administration of plain drug suspension and optimized formulation (OR2) solid lipid nanoparticles

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AUTHOR'S CONTRIBUTION

The first author has carried out all the research work. The second and third authors have provided project concept, work design, manuscript preparation, and critical revision.

CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest.

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