ORIGINAL ARTICLE

Formulation and Evaluation of Self-Emulsifying Drug Delivery Systems of Rosuvastatin Calcium

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Abstract

Aim: The objective of this study was to develop a novel self-nanoemulsifying drug delivery system (SNEDDS) which produced very small and uniform emulsion droplets, resulting in enhanced solubility, dissolution, and oral bioavailability of poorly water-soluble rosuvastatin (RST) calcium. **Materials and Methods:** The effects of oil, surfactant, and cosurfactant on the drug solubility were assessed, and pseudoternary phase diagrams were plotted. Among the liquid SNEDDS formulations tested, the liquid SNEDDS composed of cinnamon oil (oil), Cremophor EL (surfactant), and transcutol P (cosurfactant) at a ratio of 2:1 (S_{mix}) and 1:5 (oil:Smix) ratio, produced the smallest emulsion droplet size. The RST-loaded liquid SNEDDS formulation was assessed for the emulsion droplet size, solubility, and dissolution of the emulsified SNEDDS and compared to the pure drug. Different SNEDDS formulations of RST calcium were prepared by aqueous phase titration method. Selected formulations were characterized in terms of self-emulsification time, cloud point temperature, drug content, and particle size. Finally, selected SNEDDS (F1–F8) was subjected to *in vitro* dissolution/drug release studies. **Results and Discussion:** Droplet size of formulation F5 was found to be lowest as compared to other formulations. *In vitro* drug release studies showed 98.3% release of drug from optimized formulation, which was found to be much faster than marketed RST calcium. **Conclusion:** Thus, this novel SNEDDS developed represents a potentially powerful oral delivery system for RST calcium to enhance solubility and thereby bioavailability.

Key words: Cinnamon oil, Cremophor EL, rosuvastatin calcium, self-emulsifying drug delivery systems, solubility

INTRODUCTION

Rosuvastatin (RST) calcium is a most effective statin, antihyperlipidemic drug commonly used to treat hypercholesterolemia that produces a considerable dose-dependent reduction in low-density lipoprotein cholesterol[1] and raising high-density lipoprotein cholesterol levels thereby. It is the latest synthetic drug in the statin group, which acts as an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (the rate-limiting enzyme in cholesterol biosynthesis).[2] It was also reported to treat benign prostatic hyperplasia, osteoporosis, and Alzheimer's disease. It is a Class II drug in the Biopharmaceutics Classification System that shows low dissolution, thus poor oral bioavailability of 20%. It extensively metabolized by the liver[3] through oxidation, lactonization, and glucuronidation. The major factor in tailoring oral pharmaceutical delivery systems is enhancing the solubility and bypassing the hepatic metabolism is a desirable approach for improving RST calcium therapeutic performance. Hence, self-nanoemulsifying drug delivery system (SNEDDS) was proposed to simultaneously improve solubility, avoid the first pass metabolism, and facilitate the lymphatic absorption. It also improves the bioavailability of the drug through improving its solubility and increasing the membrane permeability of the gastrointestinal tract.[4,5]

Lipid-based formulation approaches, particularly the self-emulsifying drug delivery system (SEDDS), are well known

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for their potential as alternative approach for delivery of hydrophobic drugs,[6] which are associated with poor water solubility and low oral bioavailability.[7] SEDDSs are isotropic and thermodynamically stable solutions consisting of oil, surfactant, cosurfactant, and drug mixtures that spontaneously form oil-in-water (O/W) emulsion when mixed with water under gentle stirring. The motility of stomach and intestine provides the agitation required for self-emulsification *in vivo*.[8] This spontaneous formation of an emulsion in the gastrointestinal tract presents the drug in a solubilized form, and the small size of the formed droplet provides a large interfacial surface area for drug absorption. Apart from solubilization, the presence of lipid in the formulation further helps to improve bioavailability by enhancing the drug absorption.

The aim of this study is to develop and characterize SNEDDS formulations containing RST calcium using different oils, surfactants, and cosurfactants. The main objective was to increase the therapeutic efficacy of RST calcium.

MATERIALS AND METHODS

RST calcium was obtained as gift sample from Dr. Reddy's Laboratories (Hyderabad). All other chemicals, Tween 80 (polyoxyethylene sorbitan monooleate), Tween 20 (polyoxyethylene sorbitan monolaurate), and PEG 400 (polyethylene glycol), were obtained from Merck, Mumbai. Cremophor EL, Transcutol P, Propylene glycol, Span 80, and oils (Capmul MCM oil, cinnamon oil, neem oil, and oleic acid) were obtained from Sigma (St. Louis, MO), Fine-Chemicals Limited, Mumbai. Madhuca oil was obtained from KS Essentials, New Delhi. Corn oil and castor oil were obtained from Deve Herbes, Delhi. Other chemicals were in analytical grade.

Solubility studies of drug in various excipients

The solubility study was used to identify and select the suitable oil, surfactant, and cosurfactant that possess good solubilizing capacity for RST for the formulation of liquid SEDDS. Two milliliters of each of the selected vehicle (oil/surfactant/cosurfactant) were added to each capped vial containing an excess of drug.[9] After sealing, the mixture was shaken well and vortexed for 2 min for proper mixing of drug with the vehicle until suspension was formed. Formed suspensions were then shaken using orbital shaking incubator at 25°C for 48 h. Supernatant was collected and centrifuged at 3000 rpm for 15 min to sediment undissolved drug present if any. Post-centrifugation supernatant was collected and was evaluated by UV–Visible Spectroscopic method at 248 nm. Based on the result of drug solubility, solvents from each of three categories (oil, surfactant, and cosurfactant) with superior solubility and emulsifying ability were selected for further study.

Pseudoternary phase diagrams

The pseudoternary phase diagram was constructed from Smix ratio and oil which was obtained from preliminary tests, was thoroughly mixed in varying ratios from 1:9 to 9:1 in stopper glass vials. The homogenous and transparent mixture of oil and S/CoS which was formed after vortexing was visually observed for phase clarity. The phase diagrams were plotted using Chemix software for the identification of self-emulsifying systems[10] of various ratios of oil, surfactant, and cosurfactant with drug. The system with more area of micro emulsification was optimized.

Preparation of RST SNEDDS

Once the self-emulsifying region was identified that the desired component ratios of SNEDDS were selected for drug incorporation and further optimization. Ten milligrams of drug and mixed surfactant and cosurfactant were incorporated in their determined ratios into oil phase containing drug. Finally, homogenous mixture was obtained by vortex mixing. The prepared RST calcium SNEDDS was subjected to further studies, that is, self-emulsification time, dispersibility study, average globule size analysis, percent transmittance and cloud point determination, and percent drug release studies.[11]

Characterization of SEDDS

Infrared spectroscopy

Drug-excipient compatibility was studied by utilizing ATR-FTIR spectroscopy (PerkinElmer). The FTIR overlay of RST calcium and optimized formulation, samples were scanned for IR spectra from 4000 to 400 cm^{-1}

Self-emulsification time

The undiluted SNEDDSs were used to perform self-emulsification time. In this test, dissolution apparatus Type II was used. Dissolution vessel was filled with 500 mL of distill water at temperature of 37°C and maintains agitation at 50 rpm. The time required to form nanoemulsion was noted. According to time and appearance of solution, they are classified into five grades from Grade A to Grade E.

A: Rapidly forming (within 1 min) nanoemulsion, having a clear or bluish appearance.
B: Rapidly forming slightly less clear emulsion having a bluish-white appearance.
C: Fine milky emulsion that formed within 2 min.
D: Dull, grayish-white emulsion having slightly oily appearance that is slow to emulsify (longer than 2 min).
E: Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface. Grades A and B formulation will remain as nanoemulsion when dispersed in GIT, while formulation falling in Grade C could be recommended for SEDDS formulation.[12]

Percentage transmittance

The percentage transmittance of prepared SEDDS was determined at 638.2 nm using UV spectrophotometer (UV1601, Shimadzu Corporation, Japan) keeping distilled water as blank.[13]

Cloud point measurement

It is examined by visual perception. 0.5 ml of pre-concentrate is diluted to 50 ml with distilled water in glass receptacle. The specimen is warmed at the rate of 0.5°C/min. A nearby perception is shown up of scattering with increment in the temperature. The temperature at which dispersion becomes cloudy is taken as Tc.[14] After the temperature exceeds the cloud point, the sample is cooled below Tc, and then, it is heated again to check the reproducibility of the measurements.

Drug content

The drug content of prepared formulations was found out by accurately weighing sample and dissolving it in 10 ml of methanol. Further, the solution was filtered using Whatman filter paper, and the amount was estimated at 248 nm by the UV spectrophotometer.[15]

Particle size and polydispersibility index (PDI)

The average droplet size and PDI of SNEDDSs were measured by photon correlation spectroscopy using a Malvern Zetasizer (Nano ZS90, Malvern Instruments Ltd., UK) with a 50 mV laser. The formulation (0.1 mL) was dispersed in 100 mL of water under gentle stirring in a glass beaker. A 1 mL aliquot was withdrawn and added into a sample cell for droplet size measurement.[16,17]

Dissolution test

Dissolution studies were carried out for plain drug RST calcium and for optimized formulations in phosphate buffer media pH 6.8 to compare release pattern employing USP paddle type apparatus (DS 8000, LABINDIA, Mumbai, India) at a stirring rate of 50 rpm and at constant temperature (37 ± 0.5°C). At prefixed time points, 5 mL aliquots were taken and replenished with fresh media. Samples were filtered (0.45 µm) and analyzed spectrophotometrically at 248 nm.[18].The *in vitro* dissolution data were analyzed and cumulative percentage drug release was plotted against time.

RESULTS AND DISCUSSION

Solubility

The solubility of RST in various oils, surfactants, and cosurfactants was studied. According to solubility results, it is apparent that transcutol P (89.56 ± 0.67 mg/mL) showed the highest solubility as a cosurfactant. Cinnamon oil was chosen as oil phase because the solubility of RST in cinnamon oil is found to be higher than the other oils. Finally, Cremophor EL (41.5 ± 0.93 mg/mL) was chosen as surfactant considering their high solubilizing capacity of RST [Table 1]. Cinnamon oil and Cremophor EL, which served high solubility for RST, are medium length alkyl chain surfactants. Furthermore, it was reported that they enhanced intestinal absorption of drugs.[11,19,20] Furthermore, the surfactant had hydrophilicity which assisted the immediate formation of O/W droplets and rapid spreading of the formulation in the aqueous media.[21] This increases the water penetration of oil droplets, resulting in disruption of the interface and thereby decreasing the droplet size and eventually increasing the release rate.

From the solubility data and emulsifying ability, cinnamon oil was selected for formulation, Cremophor EL and transcutol P were selected as surfactant and cosurfactant. The oil is titrated with different Smix ratio from 1:1 to 1:9 and 2:1 to 9:1. The cinnamon oil has ability to form clear nanoemulsion when mixed with S_{mix} of Cremophor EL and transcutol P forms nanoemulsion at 1:1, 2:1, and 3:1 and subjected to ternary diagrams. From the ternary phase diagrams, eight formulation ratios were selected and presented in Table 2.

ATR-FTIR spectroscopy

No significant chemical incompatibility was present between drug, carrier, and other constituents, thereby suggesting the compatibility of excipients.

Table 1: The solubility of RST in various oil/surfactant/cosurfactant components

S. No.	Excipients	Solubility (mg/ml)
1.	Oils	
	Cinnamon oil	97.3±0.91
	Capmul MCM oil	79.37±0.85
	Neem oil	09.43±0.64
	Corn oil	05.87±0.71
	Oleic acid	1.87±0.11
	Madhuca oil	1.54±0.43
	Castor oil	11.21±0.21
2.	Surfactants	
	Tween 80	20.07±1.20
	Tween 20	17.2±0.25
	Cremophor EL	41.5±0.93
	Caprol 3GO	14.28±2.15
	Miglyol 812	0.13±0.3
3.	Cosurfactants	
	Transcutol P	89.56±0.67
	Span 80	60.45±0.46
	PEG 400	18.34±0.95

RST: Rosuvastatin

Percent transmittance

All selected formulations are subjected to percent transmittance and values are presented in Table 3. The value which is >98% shows that the particles present in formulation are in nanometric scale. These formulations have more efficiency to form SNEDDS.

Self-emulsification time

Assessment of the emulsification time will also ensures no further precipitation in the dispersed O/W emulsion. Formulation F5 has shown less emulsification time of 16 s.

Table 2: Formulation chart containing cinnamon oil, Cremophor EL, and transcutol P

S. No.	Composition code	Oil %	Surfactant %	Cosurfactant %
1.	F1	20	40	40
2.	F2	16.6	41.6	41.6
3.	F3	25	50	25
4.	F4	20	53.3	26.6
5.	F5	16.66	55.6	27.8
6.	F6	14.2	57.2	28.6
7.	F7	50	37.5	12.5
8.	F8	33.3	49.95	16.5

Cloud point temperature

After the formation of emulsion, there must be no phase separation. We observed that there are no phase separation and no precipitation in formulations. The cloud point is measurement at which temperature the clear solution turns into cloudiness. The cloud points of all formulations are very high which is in the range of 70.5–84.0, thus sufficiently stable when after administration into body.

Particle size

From the particle size, analysis value shows that all formulations were present with in nano region and PDI values were also shows homogeneity in particle size. Formulation F5 has obtained a particle size of 16.90 nm and PDI value of 0.209 is shown in Figure 1. A lower droplet size of the nanoemulsion results in less emulsification time attributed to greater surface is resulting in enhanced absorption by lymphatic uptake and helps in intensifying efficacy of the drug.

Drug content

Drug content of all the formulations was found to be in the range of 98.25–99.50%

Table 3: Characterization data of all formulations

S. No.	Formulation	Percentage transmission	Self-emulsification time (s)	Cloud point temperature	Drug content	Dissolution test
1.	F1	98.6±0.5	19±0.5	78.5±0.5	98.25±0.2	90.9±0.5
2.	F2	98.9±1.1	20±0.4	76.5±0.5	99.0±0.5	91.2±0.6
3.	F3	99.2±0.4	18±0.6	75±1.2	98.75±0.9	92.8±0.4
4.	F4	99.0±0.7	18±0.1	75±0.9	99.21±1.2	94.4±0.8
5.	F5	99.6±0.6	16±0.9	79.5±1.1	99.50±0.4	98.3±0.7
6.	F6	99.1±0.4	20±0.4	70.5±0.5	99.45±0.8	95.8±0.4
7.	F7	98.5±1.2	21±0.6	84±0.9	98.36±0.6	96.1±0.8
8.	F8	99.3±1.1	24±0.2	81±1.2	99.0±1.1	96.3±0.1

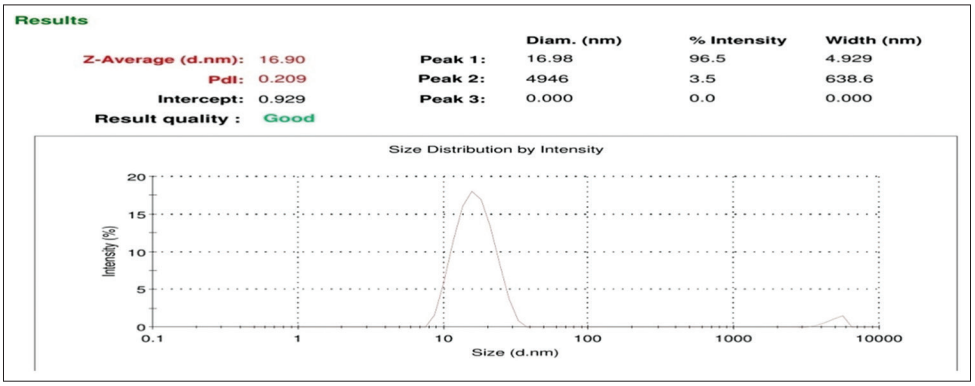

Figure 1: Particle size analysis of optimized formulation

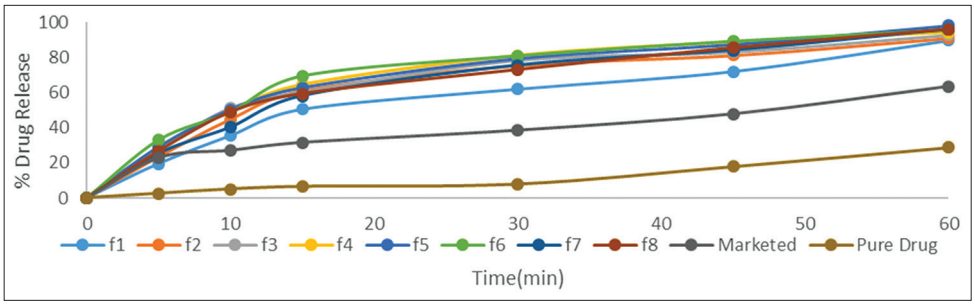

Figure 2: *In vitro* drug release profile of screened self-nanoemulsifying drug delivery systems formulations

Dissolution test

Concentration of surfactant helps in reducing the size of the particle when compared to the cosurfactant concentration; the small-sized particles obtained due to closely packed surfactant film at O/W interface, which helps in enhancing through lymphatic. When compared with a pure drug (28.8%), SNEDDS had shown increased drug release from the formulation (comparative dissolution profile illustrated in Figure 2), which was clear evidence for enhanced dissolution of RST calcium which may be attributed to the spontaneous formation of nanoemulsion *in vitro* with a decreased particle size that leads to the increased surface area leaving the drug RST as finely dispersed particles in dissolution media that can be attributed to good compositions of the three components used in the formulation from all the dissolution values the formulation F5 has shown 98.3% of drug release, respectively.

CONCLUSION

In this study, SNEDDS for oral delivery of highly lipophilic drug RST calcium was successfully designed with the significantly superior features based on different component ratios. The present approach demonstrated the substantial increase in oral bioavailability of highly lipophilic drugs through the use of SNEDDS that adopts intestinal lymphatic route along with para- and transcellular route.

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