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Research Article

Development of Microemulsion Formulation For Oral Delivery Of Rosuvastatin Calcium

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ABSTRACT

The purpose of conducting this study was to prepare an oral microemulsion formulation of rosuvastatin calcium (RC) to improve its water solubility. Oil in water microemulsion was formulated using oleic acid, Tween 80, and Polyethylene Glycol-400 (PEG-400) as oil, surfactant, and co-surfactant, respectively. The ideal proportion of surfactant: co-surfactant (Smix) was chosen by constructing pseudoternary diagrams. The microemulsion formulations, which proved to be stable after thermodynamic stability testing, were further evaluated for physical characteristics. Selected formulations were evaluated for droplet size, zeta potential, polydispersity index, viscosity, and % drug content. Results suggested that optimized microemulsion formulation (F2) was thermodynamically stable and clear, having a droplet size of 74.29 nm and zeta potential of -18.44. *In-vitro* dissolution study for optimized microemulsion was performed using a dialysis bag method and cumulative % drug release was determined. The result from the release study was indicative of improved solubility of RC, which may serve to boost up the oral bioavailability of the drug.

Introduction

Hyperlipidemia is a serious ailment, including abnormal blood levels of various lipids, specifically cholesterol, triglycerides, phospholipids, and/or plasma lipoproteins. [1] It is one of the primary factors leading to atherosclerosis and several other serious cardiovascular diseases (CVDs). [2] The incidences of elevated lipid concentrations in hyperlipidaemic individuals are of major concern among the healthcare community because fatalities from CVDs have increased recently.[3] The repercussions could be severe to such an extent that CVDs in the coming year will globally become a leading cause of death.[4]

Patients with higher lipid levels who are prone to CVDs are subjected to treatment with lipid-lowering drugs or making lifestyle changes, such as dietary modifications, increased physical activity, etc. to regulate lipid levels in the body.[5-6] Statins are effective in the reduction of low-density lipoproteins and the prevention of developing CVDs in individuals at risk.[7-8] Rosuvastatin is a highly potent statin drug, is used to reduce the levels of cholesterol, triglycerides, etc. in patients with hyperlipidemia. This can help to lower the progression of atherosclerosis, which is considered to be a major risk factor for CVDs.[9] Currently, only tablets and capsules are available in the market as oral formulations of RC.[10] It is a BCS class II drug, and the main hindrance with its oral formulations is poor aqueous solubility. Thus, a novel formulation of RC is needed to resolve its solubility and bioavailability issues.[11]

A microemulsion is a transparent dispersion of a colloidal nature containing a system of oil, water, and surfactants, which is generated spontaneously and thermodynamically stable.[12] Lipid-based microemulsions can be used to enhance the aqueous solubility of RC, which can improve its oral bioavailability.[13-14] Many reports in the

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literature suggested that the use of microemulsions can enhance oral bioavailability of loaded drugs by preventing enzymatic degradation and improving membrane permeability.[15]

The investigation was focussed on preparing and optimizing oral microemulsion of RC by using physicochemical parameters such as percent transmittance, droplet size, zeta potential, viscosity, etc. The selected formulation was then studied for *in-vitro* release of RC from the microemulsions.

Materials and Methods

Materials

RC was a generous gift from Intas Pvt. Ltd., Ahmedabad, India. Oleic acid, polyethylene glycol, and propylene glycol were procured from Chemco®, Chemdyes Corporation (India). Olive oil, castor oil, ethyl oleate, and soyabean oil were from Ozone® International Ltd. (India). Isoprpyl Myristate, Tween® 20 (polyoxyethylene 20 sorbitan monolaurate), Tween® 80 (polyoxyethylene 20 sorbitan monooleate), Span® 80 (Sorbitan monooleate), Span® 20 (Sorbitan monolaurate), ethanol, potassium dihydrogen phosphate were acquired from Central Drug House (P) Ltd. (India). The chemicals used for the investigation were of analytical reagent grade.

Excipient Selection by Using Solubility Studies

The oil, surfactant, and co-surfactant were selected on the basis of solubility of RC in various excipients. Solubility was analyzed by adding a surplus amount of drug in about 2 mL of selected excipients by thoroughly blending them in vials using a vortex mixer. The vials were further ultra-sonicated for 72 hours to equilibrate the samples and then centrifuged at 6,000 rpm for 10 minutes. The supernatant fluid obtained was filtered and analyzed for the concentration of RC in samples using an ultraviolet spectrophotometer at 243 nm.[16]

Drug-excipient Compatibility Study

The surfactants used for the preparation of microemulsion must be evaluated beforehand for their interactions with the drug. For this purpose, a known amount of drug was mixed with a combination of surfactant and co-surfactant (1:1) in a glass vial. The sample was stored for a period of one month at 25°C and observed for physical changes like precipitation, crystallization, phase separation, and color change. Chemical incompatibility was checked by performing an assay of drug in the sample using a UV-visible spectrophotometer, and a significant loss of potency (more than 10%) will serve as a sign of chemical interactions.[17]

Development of Pseudoternary Phase Diagrams

Pseudoternary diagrams were prepared to characterize the microemulsion region and to find out the optimum combination of components (oil, surfactant, and co-surfactant) used for the preparation. The water titration method was used, wherein fixed proportions of surfactant: co-surfactant (Smix), i.e., 1:2, 1:1, and 2:1 w/w was taken. Smix and oil were mixed in a ratio of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 and water are added dropwise to each oil-Smix mixture under continuous moderate stirring. The mixtures were assessed visually when it changes from clear to opaque, indicating the traversing from microemulsion to coarse emulsion zone. The plotting pseudoternary diagrams were done using SigmaPlot application Software.[18-19]

Characterization of Microemulsions

Appearance and Clarity

The appearance was determined by examining the formulation visually under light alternatively against white and black backgrounds for the presence of turbidity. The clarity of microemulsions was determined in terms of %Transmittance when checked against distilled water. A known amount of formulation was diluted 100 times with distilled water, and % transmittance is measured using a UV-visible spectrophotometer.[20]

Thermodynamic Stability Studies

Thermodynamic stability studies assessed the physical stability of microemulsion. The formulation was subjected to six heating-cooling cycles between temperatures of 4°C and 40°C stored for not less than 48 hours. The formulation was then centrifuged at 5000 rpm for 30 minutes, followed by three free thaw cycles at temperatures of -20°C, 4°C, and +25°C for not less than 48 hours.[21]

Physical Characteristics of Microemulsion

Droplet Size Distribution and Zeta-potential

For evaluating droplet size and zeta potential, the formulation is first diluted to about ten times with distilled water is agitated gently for about 10 minutes. The resultant emulsion analyzed for distribution of droplet size and zeta potential using laser diffraction, Malvern Instruments, UK.[22-23]

- Polydispersivity Index (PDI)

Polydispersivity confers the size range of particles in the system, and the results obtained from droplet size analysis were used to calculate PDI.

- Viscosity Measurement

Viscosity was measured using Brookfield viscometer DVII plus pro, Brookfield engineering laboratory, U.S.A.) at 30°C with 60 rpm using spindle LV1.[24]

Drug Content

The drug content of RC microemulsion was estimated by diluting 100 µL of the optimized formulation with methanol, and volume was made up to 100 mL. It is followed by computing absorbance of resulted solution at 243 nm using a UV spectrophotometer.[25]

In-vitro Drug Release Study

The *In-vitro* release study of microemulsion formulation was conducted using a dialysis bag method. Dialysis bag is soaked overnight into a phosphate buffer pH 6.8 as a saturation step before using it for an experimental procedure. It was then filled with about 0.5 mL of RC microemulsion, and both the ends of the bag were tied using thread. Dialysis bag containing formulation is set in a dissolution vessel filled with 900 mL of phosphate buffer pH 6.8 that was maintained 37 ± 1°C. The dissolution was carried out, and samples were taken out periodically from the medium. Samples were assayed for the concentration of RC at predetermined time intervals by using UV spectrophotometer.[26]

Results and Discussion

Excipient Selection by Using Solubility Studies

Outcomes of the solubility study of RC are shown in Table 1. Based on the results from solubility studies, oleic acid as an oil phase, Tween 80 as a surfactant, and PEG 400 as a co-surfactant were chosen.

Drug-excipient Compatibility Study

The surfactants selected from solubility studies were evaluated for compatibility with RC, as a generally very high quantity of surfactants is required in the preparation of microemulsions. The combination of Tween 80: PEG-400 did not show any indications of physical and chemical incompatible reactions, and, therefore, can be further used for the formulation purpose.

Pseudoternary Phase Diagrams

The water titration method was employed for the assessment of Smix ratios of 1:2, 1:1, and 2:1 for their capability to generate a higher microemulsion region (Fig. 1). Noticeably, formulations with 2:1 Smix ratio allow incorporation of more water but limiting the drug solubility. On the other hand, at Smix ratio of 1:2 increased drug solubility, but lesser allowance for the

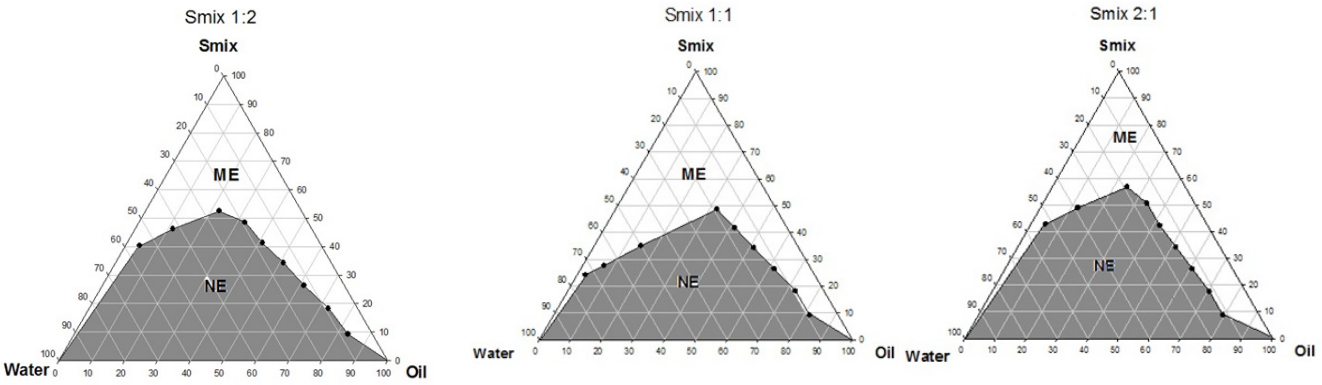

Fig.1: Pseudo-ternary phase diagrams of Oleic Acid (oil), Tween 80: PEG 400 (surfactant: co-surfactant) and water (tween 80: PEG 400 = 2:1, 1:1 and 1:2). ME: Microemulsion Region, NE: Non-microemulsion region

Table 1: Solubility of rosuvastatin calcium in various excipients at 25°C

Oils	*Drug solubility (mg/mL)*
Olive oil	7.08
Castor oil	12.13
Isopropyl myristate	19.74
Ethyl oleate	15.30
Oleic acid	46.58
Soyabean oil	8.26
Surfactants	*Drug solubility (mg/mL)*
Tween 80	59.70
Span 80	52.35
Tween 20	32.15
Span 20	26.4
Co-surfactants	*Drug solubility (mg/mL)*
Glycerine	21.306
PEG-400	42.56
Propylene glycol	28.58
Ethanol	6.36

incorporation of water. In addition to that, the existence of the microemulsion region was relatively higher for ternary plots with the Smix ratio of 1:1 when compared to the other ternary plots and thus selected for further studies. Microemulsions were obtained by mixing an appropriate quantity of RC in oil and adding surfactant and co-surfactant, which is titrated with water add dropwise to the oily phases with magnetic stirring.

Characterization of Microemulsions

Appearance and Clarity

Microemulsions selected based on phase diagram were evaluated for appearance and clarity by diluting them with distilled water. They remained clear upon dilution but appeared as transparent yellow in color due to the presence of oils and polysorbates as surfactants. The clarity of microemulsion was quantified in terms of transmittance (% T). The values of %T of all formulations were higher than 98%, and therefore microemulsions showed better clarity, which is also indicative of minimal droplet size.

Thermodynamic Stability Studies

Microemulsions containing a higher concentration of oils showed signs of physical instability and therefore failed thermodynamic stability test. RC microemulsions that conceded stability tests were further evaluated (Table 2).

Physical Characteristics of Microemulsion

Microemulsions with small droplet sizes are more stable than the larger ones because they are less prone to coalescence and creaming. It was deduced that F2 formulation produced droplets with smallest size. The outcomes of the size distribution study are shown in Table 3.

The zeta potential value asserting stable and deflocculated microemulsion system should ideally be between -10 to -30 mV. Zeta potential results were found in the range of -5 to -15 mV. (Table 3) The only formulation which lies in the ideal zeta potential range was F2.

The PDI values were less than 0.3 for F1, F2, and F3, displaying homogeneity of dispersed particles. Contrarily, PDI of F4 was found to be greater than 0.3.

The viscosities of RC-microemulsions were observed to be within the range of 0.892 to 0.916 cP. The data for viscosity determination (Table 3) point towards very low viscosities which were expected, because microemulsions have characteristically low viscosity values.

The results of % drug content in the selected formulations were higher than 98% of the total amount of drug added. These results revealed the capability of the system for entrapment of a high amount of drug.

In-vitro Drug Release Study

The analysis of release of drugs from microemulsion formulation is a useful method for predicting its *in-vivo* behavior. The *in-vitro* release of RC-Microemulsion was examined for optimized formulation (F2) was performed using a dialysis bag method. It was determined from the experiments that 35.71, 65.04, and 89.63% of RC was released during 1, 3, and 7 hour, respectively (Fig. 2). The drug release was possibly affected due to very small size of RC-containing dispersed oil globule. The oil globules might act carrier molecules facilitating the diffusion of drugs through the membrane of the dialysis bag.

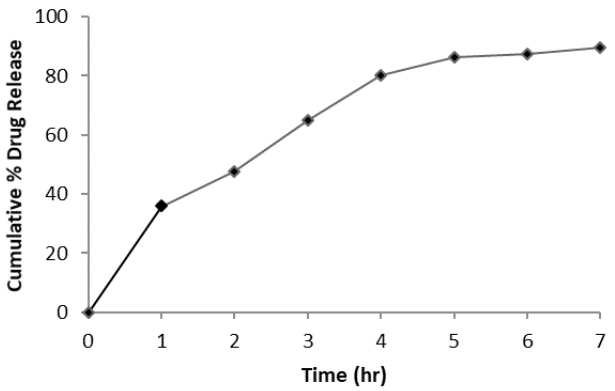

Fig. 2: *In-vitro* dissolution profile of F2 formulation

Table 2: Compositions of selected microemulsions

Component	*F1*	*F2*	*F3*	*F4*
Rosuvastatin calcium (mg)	10	10	10	10
Oleic acid (%)	5	16.5	24	32.5
Tween 80 (%)	22.5	19.25	18	16.25
PEG-400 (%)	22.5	19.25	18	16.25
Water (%)	50	45	40	35

Table 3: Physicochemical parameters of RC-microemulsion

Parameters	*F1*	*F2*	*F3*	*F4*
Droplet size (nm)	152.76 ± 2.1	74.29 ± 1.6	136.15 ± 3.3	159.03 ± 2.5
Zeta potential	-7.18	-18.44	-10.39	-5.82
PDI	0.226 ± 0.07	0.152 ± 0.04	0.129 ± 0.1	0.268 ± 0.09
Viscosity (cps)	0.892 ± 0.08	0.905 ± 0.09	0.916 ± 0.03	0.899 ± 0.02
Drug content (%)	99.17 ± 0.24	99.02 ± 0.59	98.83 ± 0.93	98.41 ± 0.73

Conclusion

This study was undertaken to formulate, develop and optimize microemulsion formulations of RC to manipulate the release characteristics of a poor water-soluble drug. It was evident from the study that microemulsions may be employed to improve the bioavailability of those drugs whose absorption is limited due to their solubility. The ratio of Tween 80: PEG 400 (Smix) and Oleic acid: Tween 80 played an important role in preparing RC loaded microemulsion. To select optimum ratio of excipients, three different combinations of Tween 80 and PEG 400 were analyzed with Oleic acid by using Pseudo ternary phase diagram. The 1:1 ratio of Tween 80: PEG 400 produced a better microemulsion region than 1:2 and 2:1 ratios. The four formulations from the Smix ratio of 1:1 were selected for further evaluation because of their better stability and ability to incorporate high amount of water.

The selected formulations F1–F4 were further evaluated for droplet size, PDI, zeta potential, viscosity, drug content and *in-vitro* drug release. The viscosities and % drug content for all the formulations were similar; however, droplet size and zeta potential results were highly in favor of F2 formulation. From the results obtained, F2 formulation found out to be optimum, which contained oleic acid, Tween 80, PEG 400 and water in 16.5, 19.25, 19.25, and 45%, respectively. The *in-vitro* release data of F2 showed that 89.63% of drug released in 7 hr, which may also pave its way to improve oral bioavailability of RC. Thus, it can be inferred that stable microemulsion formulations were prepared for oral delivery of RC.

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