Original Article

Evaluation Of SNEDDS For Poorly Water Soluble BCS Class 2 Rosuvastatin: Preparation, In-Vitro And In-Vivo Assessment

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Doi: 10.47750/pnr.2022.13.S08.424

Abstract

The objective of this study is to improve dissolution rate, and oral bioavailability of the poorly water-soluble rosuvastatin calcium, this study set out to develop a one-of-a-kind self-nanoemulsifying drug delivery system to demonstrate the effects of oil, surfactant, and cosurfactant on drug solubility, pseudoternary phase diagrams were designed. The liquid SNEDDS formulation with Capmul PG8 (oil), Tween 20 (surfactant), and Transcutol P (cosurfactant) among the liquid SNEDDS formulations tested, it produced the smallest emulsion droplet size. Solubility studies served as the foundation for the selection of the lipids, surfactants, and co-surfactants used in SNEDDS. The effect of the concentration of the oil (X1), surfactant (X2), and cosurfactant (X3) as independent variables was investigated on the droplet size (Y1), and the as dependent variables, the percentage of cumulative drug release (Y2) was used by D-Optimal mixture design. To study *in vivo* Pharmacodynamic potential of SNEDDS formulation on Wistar rats. An optimized SNEDDS formulations with droplet sizes in the nano range of less than 200 nm (58.82 nm), the D-optimal combination design yielded *in vitro* drug release >90% within 30 minutes. When compared to pure drug and commercially available tablets, the developed formulation had a greater antihyperlipidemic capability, according to an *in vivo* pharmacodynamic study performed on rats. Rosuvastatin calcium initial drug release profile from improved formulation F5 was shown 99.98 release of drug which is significantly faster than that of marketed rosuvastatin calcium, according to *in vitro* drug release experiments. The mixture design showed significant systemic and successful developments of SNEDDS with better *in vitro* and *in vivo* performance as compared to market formulation. The study showed promising approach for standard doses.

Keywords: D-optimal mixture design, Nanotechnology, SNEDDS, In vitro lipolysis, Study

INTRODUCTION

One of the most prevalent pathological illnesses affecting people is hyperlipidemia, which is caused on by a problem with the body's lipid metabolism and raises serum lipid content over normal levels. Hyperlipidemia has increased dramatically in occurrence over time and is now one of the most prevalent pathological disorders in humans as a result of inheritance, diet, nutrition, medicine, and other variables [1]. Hyperlipidemia is a condition when the blood has higher than normal levels of lipids. Preventive medicine consumes a significant amount of time and resources for modern primary care doctors. Primary care physicians frequently diagnose and treat hyperlipidemia to ward off cardiovascular disease. According to statistics from a study of 1492 physicians conducted by the Centers for Disease Control, hyperlipidemia comes in second place behind hypertension among the 10 most prevalent chronic illnesses seen. Numerous clinical studies have demonstrated that statins effectively reduce LDL cholesterol and reduce the risk of cardiovascular disease in people with dyslipidemia and metabolic disorder [2]. atherosclerosis, which eventually results in cardiovascular disease [3]. It includes a variety of genetic and acquired conditions that are characterized by high lipoprotein levels in the body are known to be a substantial risk factor for deadly disorders such as cardiovascular disease (CVD), type 2 diabetes mellitus, pancreatitis, and even some types of cancer [4,5]. Although clinical studies have indicated that reducing serum lipid levels can repair cardiac dysfunction and non-ischemic heart failure. Hyperlipidemia not only has a direct effect on systolic function and cardiac electrophysiological response, but it also has an indirect effect on heart function by promoting the development of atherosclerosis, oxidative stress, proinflammatory and mitochondrial dysfunction [6].

Hyperlipidemia makes the liver more susceptible to developing cirrhosis and cholestasis as well as oxidative damage and higher amounts the presence of radicals in body. Majority of these are in their early phases and will necessitate extra scientific research [7-9]. It is a disease characterized by high lipid levels in the human body and includes a number of inherited and acquired conditions. It occurs frequently all across the world, although it is more prevalent in the

Western Hemisphere. In contrast, a more accurate definition of hyperlipidemia includes low density lipoproteins, TC, TG or lipoprotein in $90^{t\%}$ or low level of HDL 10%. It is intriguing that there is a connection between total cholesterol and the onset of cardiovascular diseases (CVDs), and that lipid metabolism can have a negative impact on the amount of angiotensin II produced. This is accomplished by lowering the amount of adiponectin, a hormone made by adipose tissue and essential for reducing inflammation and promoting coronary heart disease. Thus, a combination therapeutic strategy that takes into account hyperlipidemia and hypertension are both effective ways to lower the frequency of cardiovascular problems in metabolic syndrome patients [11].

The most popular, effective, profitable, and feasible method of drug administration is by the oral route. It has a number of drawbacks, including low bioavailability, low stability and first pass effect but due to the high rate of patient compliance, it is the most effective way to give the drug [12]. The high percentage of drug that are not well soluble in water has resulted in a number of issues, and the nanomedicine industry has emerged as a viable and effective approach. The use of nanomedicine for pharmaceutical applications is made possible by the fact that shrinking such chemical molecules to the nanoscale can drastically alter their physical characteristics [13]. The literature frequently mentions methods to increase oral drug candidates' bioavailability, although poor pharmacokinetic profiles of those candidates are nevertheless a cause for worry. In the past, methods were limited to molecular optimization of the drug molecule, which over time led to the development of formulations in micro- and nanometer sized. Since they are so small, nano formulations are well-known for overcoming pharmacokinetics challenges [14]. The term "brick dust" refers to drugs that are poorly soluble in both water and lipids. It is particularly difficult to synthesize these drugs into a dosage form that would effectively increase their bioavailability after oral administration [15].

Lipid based carrier drug delivery system increase oral bioavailability of less soluble drugs [16, 17] and its primary goal is to decrease hepatic degradation [18]. One lipid-based medication delivery approach that is currently being researched for its benefits is the self-emulsifying drug delivery system (SEDDS). It has a large interfacial area that separates the medication from the gastrointestinal fluid and oil. The approach improves the oral bioavailability of p; oorly water soluble drugs in the form of tiny droplets [19, 20]. With gentle agitation, SNEDDS can spontaneously produce homogeneous lipid mixtures of oil in-water (o/w) nanoemulsions (NE). The advantage of nano system is that it requires lower amount of energy and high physical and kinetic stability in aqueous dispersion [21].

A promising nanoplatform developed to improve the oral bioavailability of pharmaceuticals is self-nanoemulsifying drug delivery system (SNEDDS) [22]. SNEDDS are mostly helpful for increasing the pace and extent of assimilation of hydrophobic or lipophilic drug classified as BCS classes II and IV (with low solubility), which show a dissolution rate-limited absorption [23]. Among lipid-based formulations, the SNEDDS has undergone more thorough physicochemical characterization. SNEDDS are an isotropic blend of co-surfactant, surfactant, and oil that are gently stirred together. When they come into contact with water, they combine to form an emulsion. After dilution, a SNEDDS typically produces droplet sizes ranging from 20 to 200 nm. Improved dissolving rates and bioavailability can be obtained from the generated nano-sized droplets [2].
Rosuvastatin calcium is a BCS class II drug that acts as an HMG CoA reductase inhibitor to reduce cholesterol. Cosolvents, Microemulsion, self-emulsification, drug complexation used to improve the dissolution characterstics. Liquisolid system shows promising strategy for dissolution [24].

The goal of the current investigation was to examine the rosuvastatin-loaded SNEDDS formulation *in vitro* and *in vivo*. This study used Capmul PG8 (oil), Tween 20 (surfactant), and Transcutol P (co-surfactant) to form SNEDDS. D-optimal mixture design was used to optimize the formulation. To choose the optimal formulation, many SNEDDS were assessed. Formulation was characterized for DSC, FTIR< Globule size, Zeta potential and stability studies. Food effect in vitro and invivo studies performed for developed SNEDDS [21]. The shape was estimated using TEM. The information provided by an optimization strategy permits for consistent product and process development. Experimental design was seen as a crucial technique for maximizing data while requiring the least amount of experimental activity [25]. Rosuvastatin's limited bioavailability can be attributed to its low solubility. There are many ways to improve Rosuvastatin's ability to dissolve, including complexing it with -cyclodextrin, solid dispersion, hydrotropy, micellar solubilization, microemulsion, and nanoemulsion [27, 27]. In order to increase Rosuvastatin's solubility, dissolution, and therefore bioavailability, the current effort used liquisolid compact technology.

MATERIALS AND METHODOLOGY

Materials

Sun Pharmaceutical Laboratories, Gurugram, provided a generous sample of pure rosuvastatin. IMCD, Delhi, generously gave Capmul PG8 and Capmul MCM. Croda supplied Tween 20, Gattefosse supplied Transcutol P, and Loba Chemie supplied Pancreatin. Rosuder 10 mg pills, batch no. SPT210329, were purchased from pharmacy. Analytical grade reagents were used for studies.

METHODOLOGY

Investigation of Rosuvastatin Solubility in Different Ingredients

Solubility is an important requirement in achieving the desired drug concentration in systemic circulation to elicit the desired pharmacological reaction. The solubility of oil, surfactant, and co-surfactant was all determined. By pouring an excess of medication into 1 ml of each medium, the solubility of ROS in various oils, surfactants, and co-surfactants was investigated. An excess of rosuvastatin (2 g) was mixed with each excipient in a screw-capped glass vial (2 g). A vortex blender (Genius, India) was utilised to ensure the optimum possible mixing of rosuvastatin with the components [28]. The mixture was combined for 72 hours at 100 rpm using a thermostatic control shaker (Calton, Germany). The supernatant was collected, and the solution was filtered through 0.45m membrane filters [25]. The calcium concentration of rosuvastatin was determined using a UV Spectrophotometer (Shimadzu UV-1800, Japan) at 241 nm. Following sample collection, 5000rpm centrifugation (Remi, Mumbai) was conducted. The experiment was carried out in triplicates.

Construction of ternary phase diagrams

The goal of this study is to choose the right oil and surfactant. The pseudo ternary phase diagram is a three-component system that is created by fixing the first two components and adding the water as the third component. The water titration method is another name for this technique. A phase diagram investigation reveals which ratio or co-surfactant combination best produces a nanoemulsion region. The water dilution method was used to draw the phase diagram employing chemix ternary plot software. Oil: Surfactant: Cosurfactant were used in different concentrations to develop SNEDDS. Surfactants and cosurfactants, which were chosen as excipients based on the solubility studies of ROS, were used to develop ternary phase diagrams [29]. Each model uses a different concentration of Rs (5-30%). Samples were then vortexed and sonicated to form oily liquid mixtures and then gradually mixed with distilled water. The resulting samples were then evaluated whether samples show any cracking, drug precipitation or coalescence while stirring were excluded. Chemix software version 4.5 was used to plot phase diagrams based on the aforementioned requirements [30]. Ternary mixes containing the three separate excipients were made in quantities totaling 1g. To discover which ratio provided the biggest region for the creation of nano-emulsions, Smix was blended in four ratios of 3:1, 1:2, 2:1 and 1:1. In different weights in ratio 1:9 to 9:1 was mixed in glass vials [31]. The water dilution test was performed to produce outlines of nanoemulsion region (transparent/clear region) with low consistency [33]. It was developed with Chemix version 4.50.

Design of Expert to optimize SNEDDS

SNEDDS formulation was optimized using experimental design (Design Expert 13 software) using two parameters and 14 experimental trials formulation in which independent variables were chosen as CQAs for SNEDDS at low, medium and high levels. Capmul PG8(X1), Tween 20 (X2) and Transcutol P (X3) as independent variables with least droplet size and maximum %CDR as dependent variables. Two regression equations and a desirability index was aided in selection of SNEDDS formulation [35, 25].

Evaluation of Globule Size

Formulation was diluted with distilled water (1:100w/v) and mixed for 1 minute before evaluating.
On a Malvern, U.K, Zetasizer ZS of the nano series, dynamic light scattering was used to measure the average particle size and poly dispersity index (PDI) [36-38].

Dissolution study

At 37°C±0.5°C, 900 mL of pH 6.6°C0.05 sodium citrate buffer medium of 0.05M was dissolved using a paddle type dissolution machine (Distek, USA) at 50 rpm. Before the paddle revolution commenced, the capsules containing SNEDDS (equivalent to 10 mg of rosuvastatin per capsule) were inserted in the cradle media. After 30 minutes, aliquots (5 mL) were collected and examined at 241 nm with a UV-spectrophotometer (UV 1800, Shimadzu, Japan). This analysis was conducted in triplicate [25].

Self-emulsification Time

This study was conducted on each generated formulation using the reported approach. In brief, 1g of SNEDDS formulation was mixed with filtered water (500ml) and agitated with magnetic stirrer (Remi, Mumbai) at 100rpm. Time required for dispersibility and nanoemulsion formation was recorded [39].

Evaluation Parameters

An optimal formulation was selected for characterization, transmittance test, cloud point, zeta potential and globule size.

Test for % Transmittance

There is a possibility of drug precipitation on dilution in the lumen of the gutwhile forming SNEDDS orally, and this percentage transmittance is measured. Ros SNEDDS were reconstituted with distilled water and examined for turbidity. The composition was then diluted with 1 g of pure water (100 mL). Thereafter, its % transmittance was measured at 241 nm using UV spectrophotometer (Shimadzu UV 1800) using distilled water as the blank in triplicates [29].

Robustness Dilution Test

To optimize drug loaded SNEDDS formulation, robustness for formulation on dilution was used as uniform emulsion formation from SNEDDS is critical at various dilutions because drugs might precipitate at higher dilutions *in vivo*. Nanoemulsions were diluted with water

The nanoemulsions were diluted with water and in simulated intestinal fluid (phosphate buffer pH 7.4) after 24 hours of storage [28]. After 100 times dilution, the formulation was tested in numerous media, including pH 1.2 of 0.1 N HCl [30], pH 4.5 of Acetate buffer, and pH 6.8 of phosphate buffer. These formulations were examined for phase separation/drug precipitation by eye examination after 24 hours [40]. The resulting emulsion was determined to be in the permissible nanoemulsion zone, demonstrating its dilution resistance. This outcome ensures the possibility of a consistent medication release profile in vivo [41].

Viscosity Estimation

A Hydromotion viscometer (Brookfield Engineering, USA) was used to determine the rheological parameters of mixtures. Generated nano emulsions were tested for o/w or w/o kind [42].

Cloud Point Estimation

The cloud point is a critical component of the SNEDDS made up of non-ionic surfactants, and it is crucial for the development of a stable nanoemulsion. When the temperature rises above the cloud point, irreversible phase separation occurs, and the cloudiness of the preparation has a negative impact on ROS Calcium absorption due to polyethylene oxide moiety dehydration. As a result, to avoid phase separation in the gastrointestinal system, the cloud point for SNEDDS should be above 37 ºC. A stable nanoemulsion is generated *in vivo* at physiologic temperature [29]. The improved SNEDDS formulations were diluted 1:100 with distilled water. The diluted samples were immersed in water, and the temperature was gradually increased at a rate of 10°C per minute increment. The cloud point was determined spectrophotometrically as the temperature at which cloudiness abruptly developed. The TCloud value, which indicates the stability of created SNEDDS at physiological temperature, was used to investigate the effect of temperature on the phase behaviour of enhanced SNEDDS. The optical clarity, drug precipitation, and phase separation of the samples were all visually assessed [43].

Drug content Analysis

Before being centrifuged for 30 minutes at 10,000 rpm the formulation was diluted with methanol and then supernatant was diluted with 2.5 times methanol, drug content was determined by using UV spectrophotometer at 241nm in triplicates [44, 45].

Investigation of droplet size, PDI (polydispersity index) and Zeta potential

The surface charge influences the stability of the nanoemulsion. The samples were diluted with distilled water in a 1:100 (v/v) and stirred for 1 minute then zeta potential of formulation was determined by Zetasizer ZS nanoseries (Malvern UK) which was used to assess particle size distribution, PDI and Zeta potential of droplets [46].

Drug Entrapment Efficiency

The percentage of entrapment (% EE) is commonly employed as an indication for measuring entrapment of drug [47]. In a 10ml volumetric flask the resulting Ros nanoemulsion was diluted with water. 3.0 mL of this emulsion was placed in a centrifuge [35]. The ultrafiltration procedure (3500 Da) was used to extract free rosuvastatin from SNEDDS, followed by centrifugation at 3000 rpm for five minutes and stipulation with UV spectrophotometer at 241nm. The rosuvastatin entrapment efficiency (EE) was calculated by the following equation:

$$EE = \frac{Wt - Wf}{Wt} x100$$

where Wt denotes total rosuvastatin in the nanoemulsion and Wf denotes free rosuvastatin [48].

CHARACTERIZATION

Based on the enhanced results, optimum SNEDDS were chosen to add evaluation parameters and to complete characterization.

Differential Scanning Calorimetry

DSC2-00347 (192.168.10.2) was used to characterize the thermochemical properties of pure rosuvastatin, physical mixture (rosuvastatin + Capmul PG8 + Tween 20 + Transcutol P), and rosuvastatin-loaded SNEDDS at a heating rate of 10°C/min to a temperature of 300 °C, using dry nitrogen as a carrier gas with a flow rate. The findings of the analysis were compared between 10-300°C. To calibrate the temperature and energy scales of the DSC apparatus, indium was utilized as the standard reference material [49]. TA Universal Analysis software was used to automatically to overlay thermogram, calculate the peak melting temperature for each endothermic curve. (TA Instruments Trios V4.1).

Fourier Transform Infrared Spectroscopy (FT-IR)

Figures 12, 13 show the spectra of pure medication, excipients used, and formed batches used to determine the interactions between constituents. In SNEDDS, drug peaks were constant. There were no further peaks found, indicating that solely interactions between drug molecule and other components. There was no substantial chemical incompatibility between the medicine, carrier, and other constituents, implying that excipients are compatible.

The FT-IR spectrum of rosuvastatin showed evidence of several characteristics peaks (figure 14): intense broad bands due to O–H stretching vibrations; sulfone asymmetric stretch symmetric stretch, aromatic C N stretching vibrations, aromatic C–F stretching vibration and vibration of the –CH and CH2 groups. Moreover, minor shifting of rosuvastatin characterizing peaks was observed. This could indicate a sort of physical interactions related to the hydrogen bond formation between polymers and drug [49]. To validate drug entrapment within the PVP polymer, FTIR spectra of pure ROS and optimal nanoformulation were acquired. ROS calcium distinctive FT-IR peaks were found [50].

Self-emulsification Time

Time required to preconcentrate a homogeneous mixture after dilution is self-emulsification time when SNEDDS disappear visually [28]. 1g of SNEDDS formulation was mixed in 500ml distilled water using magnetic stirrer (Remi, Mumbai) at room temperature with a rotation speed of 100 rpm. The SNEDDS were timed in order to generate a homogeneous nano-emulsion [38, 39]. The self-emulsification time was determined by diluting SNEDDS about 0.5ml in glass beaker in distilled water and mixing them with magnetic stirrer at 100 rpm in order to generaye homogeneous emulsion. UV spectrophotometer at 241nm were used to determine clarity of nanoemulsion with distilled water as blank [52].

Estimation of Cloud Point

Temperature effect on formulation phase behavior was examined by measuring at Tcloud value at 71℃ as it defines the stability of produces SNEDDS at physiological temperature and also visually tested for optical clarity, drug precipitation and phase separation [43].

Drug content analysis

10 times dilution with methanol was done before being centrifuged for 30 minutes at 10,000rpm then supernatant was diluted with methanol (2.5 times) and determined with UV spectrophotometer at 241nm in triplicate [53,54]. RST drug content was determined to be in the range of 98-99.89% in all formulations, indicating full drug solubilization in formulation. A similar dilution of blank SNEDSS revealed no noticeable peak at 241 nm, suggesting the lack of any interference. When the transmittance percentage reaches 100%, it indicates that the formed systems are clear and the prepared globules are nanometric in size.

Measurement of globule size, PDI (polydispersity index) and zeta potential

Optimized formulation was diluted at a ratio of 1:100w/v and blended and evaluated using Zetasizer ZS nanoseries (Malvern UK). This was done in triplicate. When the transmittance percentage reaches 100%, it indicates that the formed systems are clear and the prepared globules are nanometric in size [53, 54].

Multimedia dissolution testing

SNEDDS (packaged in size "00" capsules) were inserted in a paddle-type dissolution device at 37°C±0.5°C in pH 1.2, 4.5 acetate, and 6.6 citrate buffered dissolution solutions (Distek, USA). Samples (5 mL) were collected at regular intervals, with an equal amount of new fresh medium added after each sampling. 0.45m nylon Millipore membrane filters were used to filter the samples. The drug discharge rate was calculated in triplicate using UV spectrophotometer at 241nm [25].

Comparative investigation of SNEDDS with pure and marketed drug in terms of dissolution

Pure rosuvastatin, SNEDDS, and a commercial formulation (Rosuder-10 with batch no. SPT210329) were tested in a "USP Type-II dissolving assembly (Distek, USA) with 900 mL of 0.05M sodium citrate dissolution media of pH 6.60.05 at 50 rpm as suggested by the USFDA dissolution database. An equal volume of rosuvastatin-loaded optimized SNEDDS, commercial tablets, and pure medicine were placed on a cellulose membrane dialysis sac (MWCO 12,000 g/mole; Sigma-Aldrich) (rosuvastatin equivalent to 10 mg). These combinations were dumped at the bottom of the vessels using an appropriate sinker. Based on previously defined time intervals, 5 mL aliquots were collected and analyzed for drug release using a UV spectrophotometer [55].

Dynamic *in vitro* Lipolysis Study for Investigation of Food Impact

The literature database confirmed that SNEDDS uses drug release to avoid food impact on the formulation. To verify this theory, optimal SNEDDS were dissolved in modified fed and fasted state simulated intestinal fluid.

Conducting in vitro testing of the drug to mirror in vivo conditions as closely as reasonably possible is crucial for greater experimental relevance. This investigation is required for two specific justifications. Initially, the degree and rate of lipolysis are measured, which aids in the investigation of formulation solubility and dispersion properties. The

second is a post-lipolysis analysis to forecast whether the medication will be solubilized or precipitated after the reaction is completed. This model can predict the upper limit of these preparations' potential to improve oral retention of drugs with low water solubility.
The dynamic in vitro lipolysis evaluation used in this work was an interpretation of the methodology recently presented by Mohsin [56]. 510 mg of SNEDDS received 36 mL of both media, the formula for which is presented in Table 1 [57, 58]. Ca2+, bile salt (BS), phospholipids (PLs), and sodium chloride (NaCl) compositions were linked to mimic normal concentrations in Fa/FeSSIF V-2. Using NaOH or HCl, a pH of 6.5/5.8 0.05 was maintained during the early stages of dispersion. Before adding the pancreatic extract solution containing pancreatic lipase enzyme, the SNEDDS were emulsified on the magnetic stirrer with a heated plate at 37°C. 4 mL pancreatic concentrate prepared by dissolving 1 g pancreatin powder in 5 mL digestion buffer and vortexed for 15 minutes [59]. To collect the supernatant with a pH of 6.5/ 5.8 and a pancreatic lipase activity of 800 TBU/mL, ultracentrifugation was applied to digestion buffer, which activates lipolysis, and was continued for 30 minutes. The production of unsaturated fats (FAs) during lipolysis raises medium pH; a 0.2M NaOH solution was used to keep the pH constant at 6.8/5.8. The path of drug discharge was tracked using UV examination at 241 nm [60, 61]. The current technique was deemed robust, and the examined outcomes were determined to be reproducible when this procedure was followed.

Table 1: Composition of Biorelevant Media used during *in vitro* lipolysis

Chemicals	FaSSIF V-2	FeSSIF V-2
Sodium oleate (mM)	-	5
NaCl (mM)	68.62	125.5
Glyceryl monoleate(mM)	-	2
Sodium taurochlorate (mM)	3	10
NaOH(mM)	34.8	81.65
Maleic acid(mM)	19.12	55.02
Lecithin(mM)	0.2	0.8
pH	6.5	5.8

Abbreviations: Fasted state simulated intestinal fluid V-2 (FaSSIF V-2) and fed state simulated intestinal fluid V-2 (FeSSIF V-2)

Stability Study
In a 60cc HDPE bottle, 50 capsules of SNEDDS (equal to 10mg of rosuvastatin capsule) were stored [62]. Following closure, these bottles were placed in the stability chamber (Thermolab, India) in an accelerated setting (40±2°C/75±5% RH) for six months. At regular intervals, test samples were evacuated and assessed for physical appearance, drug discharge percentage, and time of breakdown [63, 64].

Pharmacodynamic Investigation
It has been noted that rosuvastatin has a dose-dependent pharmacodynamic influence, which is the reason this in vivo study with a marketed tablet was carried out.
The ethical approval for this investigation of rosuvastatin SNEDDS was granted by Institutional Animal Ethical Committee (IAEC), GD Goenka University, Gurugram (Protocol No. GDGU/IAEC/2022/09, dated 08/18/2022). Male Wistar rats having weight 150-200g were maintained as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

All rats were housed in plastic cages, with six rats in a single cage. All rats were separated into five groups (total of 30 rats; n=6 rats in each group), namely the control treatment group (CTG), the placebo treatment group (PTG), the reference treatment group (RTG), the test (TTG), and the marketed treatment group (MTG) [65]. Acacia solution (2.0%) will be used to dilute the marketed, test, reference, and placebo formulations. For four weeks, each group had a high-fat diet (dalda and coconut oil [3:2]) at a dose of 10 mL per kg per day body weight. The test, reference, marketed, and placebo formulations will be provided for 4 weeks to the test, reference, marketed, and placebo treatment groups, respectively. The test and reference formulations will be given orally at a dose of 2 mg/kg/day of rosuvastatin.

Rosuvastatin lowers total cholesterols (CH), low-density lipoproteins (LDL), and triglycerides (TGs) levels in the blood. Furthermore, it causes an increase in plasma high-density lipoproteins (HDL), which aids in the removal of CH. Rosuvastatin pharmacodynamic potential is dose-dependent, therefore it was utilized to compare the in vivo performance of optimized test, reference, placebo, and marketed formulation [67-69].

Blood samples were taken from the tail vein before and after treatment at 7, 14, 21, and 28 days to determine TC and TG. Throughout the experiment, daily food consumption and body weight changes were observed. Blood samples were obtained using a tail-vein method at preset intervals, namely before and after treatment, in polo simple clot activator glass tubes [70]. Serum was separated by ultracentrifugation for 10 minutes at 3000 rpm (Remi), and samples were evaluated for biochemical tests such as CH, HDL, and TGs levels using in vitro diagnostic kits (Erba® Mannheim) and analyzer (Erba® with software Chem-5 Plus V2) [71].

The modified Roeschlau's method was used to determine total cholesterol (CH). The Wako method and its adaptation by McGowan and Fossati were used to determine triglycerides (TG). Burstein's approach was used to calculate high-density lipoprotein (HDL) [72]. Total CH, TG VLDL, and HDL were measured for each therapy group (0 and 28 days), as well as CTGs, PTGs, TTGs, and RTGs. The differences in mean between groups were examined using one-way ANOVA with the Dunnet's test in Graph Pad version 4.01 software. A p-value of less than 0.05 is considered statistically significant.

RESULT AND DISCUSSION

Investigation of Drug Solubility (screening of oil, surfactant and co-surfactant)

SNEDDs are primarily composed of oil, surfactant, co-surfactant, and drug. When all components are placed in an aqueous phase at room temperature, they should generate a clear monophonic solution, allowing the medication to be presented in solution [30]. The purpose of drug solubility in the various excipients used in SNEDDS was to establish a robust self-emulsifying zone with a large boundary size in the ternary phase diagram and to form micro droplets to improve in vivo activity [28]. The selection of an adequate excipient blend is essential for self-emulsification. This analysis revealed that Tween 20 with the highest concentration of Capmul PG8 had been emulsified, as shown in figure 1. That is why the combination of Tween 20 and Capmul PG8 was chosen. Figure 1 shows that Transcutol P had a greater nano-emulsion area than Labrasol. As a result, co-surfactant was chosen. The surfactant produces a layer around the oil globules, reducing surface tension between the oily and aqueous phases. Furthermore, their optimal quantity improves the instant self-emulsification time. The increase in cosurfactant quantity reduces the zone for emulsion generation; nevertheless, it has no influence on the decrease in terms of interfacial tension. A higher HLB number is necessary to create an o/w type emulsion. These are generally utilized in rosuvastatin SNEDDS to limit the quantity of surfactant present. Transcutol P was added to the formulation to improve the solubilization of hydrophobic drugs [73].

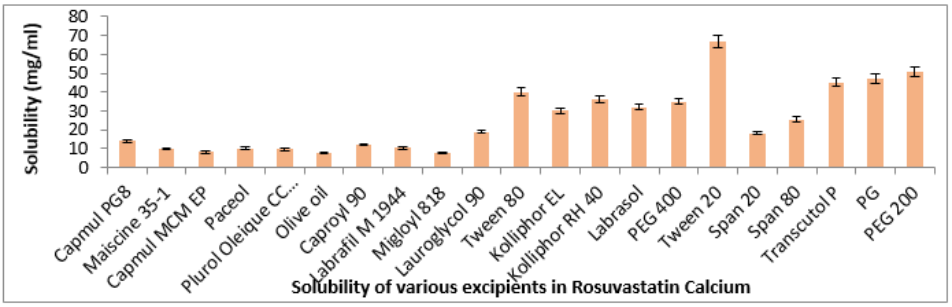

Figure 1: Solubility of rosuvastatin in oil excipients, surfactants, and cosurfactants (n = 3).

Ternary Phase Diagram

Figure 1 D-F shows region of self-emulsification in pseudo ternary phase diagram and optimize the different percentage of various excipients for formulation. SNEDDS composed of oil: surfactant: cosurfactant: drug in which all components are placed in an aqueous phase at room temperature [30]. Transcutol P and Tween 20 were utilized as cosurfactants and surfactants, respectively, to generate phase diagrams to form self-emulsification region. Surfactant concentrations of 20-50% created turbid emulsions, which was significant. The propensity to spontaneously as the amount of co-surfactant in the SNEDDS increase, the ability to generate an emulsion inside the self-emulsification zone increased. For self-emulsification, the minimum Smix ratio was 45-75%. The effectiveness of self-emulsification of the SNEDDS formulation was improved when the Smix ratio (combination of surfactant and co-surfactant) was greater than 60%. The influence of surfactants and co-surfactants on droplet size was evaluated in order to construct a phase diagram, and it was discovered that as the surfactant concentration grew from 30% to 60%, droplet size decreased from 270 to 58.82 nm. The greater mean droplet size is the result of increased surfactant concentration upto 70% [2].

Figure 2 shows transparent and reduced viscosity when the oil concentration is less than 20%. However, The nano-emulsion zone expanded as the Tween 20/Smix ratio (2:1) was increased. However, increasing the Transcutol P concentration (1:2 and 1:3) resulted in a decrease in the nano-emulsion zone. This could be because Tween 20 forms a layer around the oil globules, reducing interfacial tension up to a degree. Transcutol P, on the other hand, had little influence on interfacial tension. As a result, raising the concentration of Transcutol P will not appreciably reduce interfacial tension while retaining the thermodynamic stability of the resulting systems after dilution.

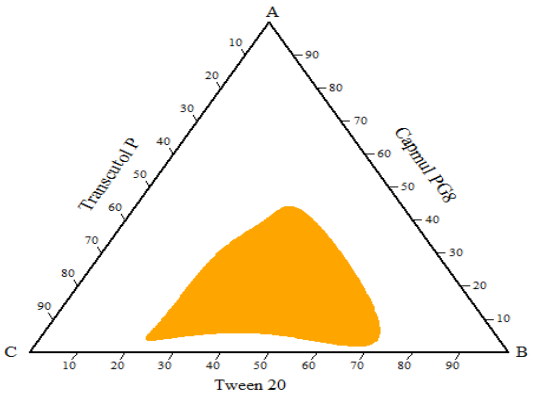

Figure 2: Capmul PG8, Tween 20, and Transcutol P ternary phase diagrams at Smix ratios of (A) 1:1, (B) 2:1, (C) 1:2, and (D) 1:3. The colored zone (orange) represents the region of nano-emulsion formation.

D-Optimal Mixture Design for Optimization

Variance associated coefficient estimates were reduced in D-optimal design as a starting point, the software chose a collection of candidate spots [75]. The influence of rosuvastatin SNEDDS factors (X1-X3) Using experimental design software, the effects of globule size, %CDR, and self-emulsification time were investigated. The best formula was adopted, with the smallest globule size, highest %CDR, and quickest self-emulsification time. With Design Expert® software, rosuvastatin SNEDDS were optimized using a mixed design. Table 2 shows 14 trial trials with two center points. Y1 ranged from 60 to 128 nm, and Y2 from 88 to 99%. Equations 1–3 explain the effect of different component proportions on the responses Y1 and Y2. Figure 4 depicts contour plots (2-Dimensional) that demonstrate the effect of X1, X2, and X3 variables on Y1 and Y2 responses. Figure 5 depicts three-dimensional response plots that demonstrate the effect of X1, X2, and X3 variables on Y1 and Y2 responses. It was discovered that as oil concentration increases, globule size (Y1) increases and drug release rate (Y2) decreases. However, an increase in surfactant concentration resulted in a reduction in globule size and an increase in drug release rate. Transcutol P concentration increased globule size due to the A substantial number of Transcutol P molecules enter the oily phase, causing the interfacial layer and particle size to expand [76].

According to the 2-D and 3-D contours, a higher proportion of oily phase greatly reduced globule size, although other excipients elevated it to a particular point in the formulation before it began to develop. Responses Y1 and Y2 yielded the same results. Table 3 displays the ANOVA results. Furthermore, the results of the target criteria, as well as the preset lower and higher limitations, and the optimum formula for D-optimal proposals was developed. The chosen formulas were derived from D-optimal mixture design theories, ensuring that the procedure was validated. The desirability index is used to optimize parameters in a multi-response system by transforming all responses from various scales into a scale free value. Its values vary from 0 to 1. Figure 6 shows that the value for SNEDDS was found to be 1. This suggests that the model is important. The optimization of the formulation was aided by three regression equations (Equations S1, S2, and S3) and the desirability index. Based on these two criteria, Formulation 10 (F-10) was determined to be the optimal batch, containing Capmul PG 8 (13%), Tween 20 (45%), and Transcutol P (42%), with a particle size of 58.82 nm and 99.5% dissolving in 15 minutes.

Formulation Code	Capmul PG8 (%)	Tween 20 (%)	Transcutol P (%)	Particle size (nm)	Drug release (%)
F-1	25	20	55	128	88.5
F-2	17	37	46	79	92.5
F-3	05	50	45	60	98.9
F-4	08	32	60	68	97.23
F-5	16	24	60	65	93
F-6	24	50	26	85	89.9
F-7	30	31	39	74	86.45
F-8	30	40	30	90	87.5
F-9	18	50	32	75	91.2
F-10	**13**	**45**	**42**	**58.82**	**99.98**
F-11	22	40	38	91	90
F-12	5	41	54	69	98.94
F-13	30	50	20	120	87.4
F-14	25	20	55	125	88.5

Table 2: Design Expert® 13 Suggestions for Various SNEDDS Formulations and Their Reactions

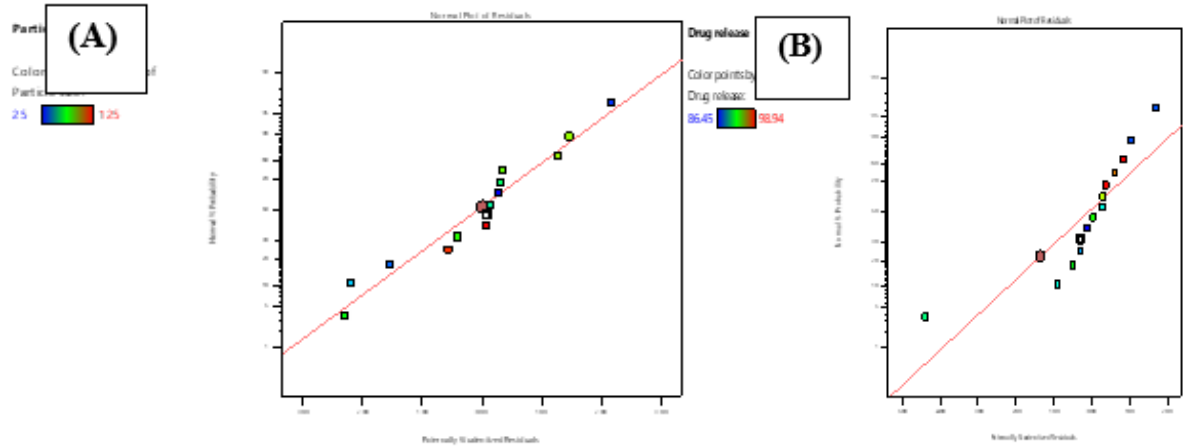

Figure 3: Actual versus predicted graph for response: (a) Particle size (b) drug release.

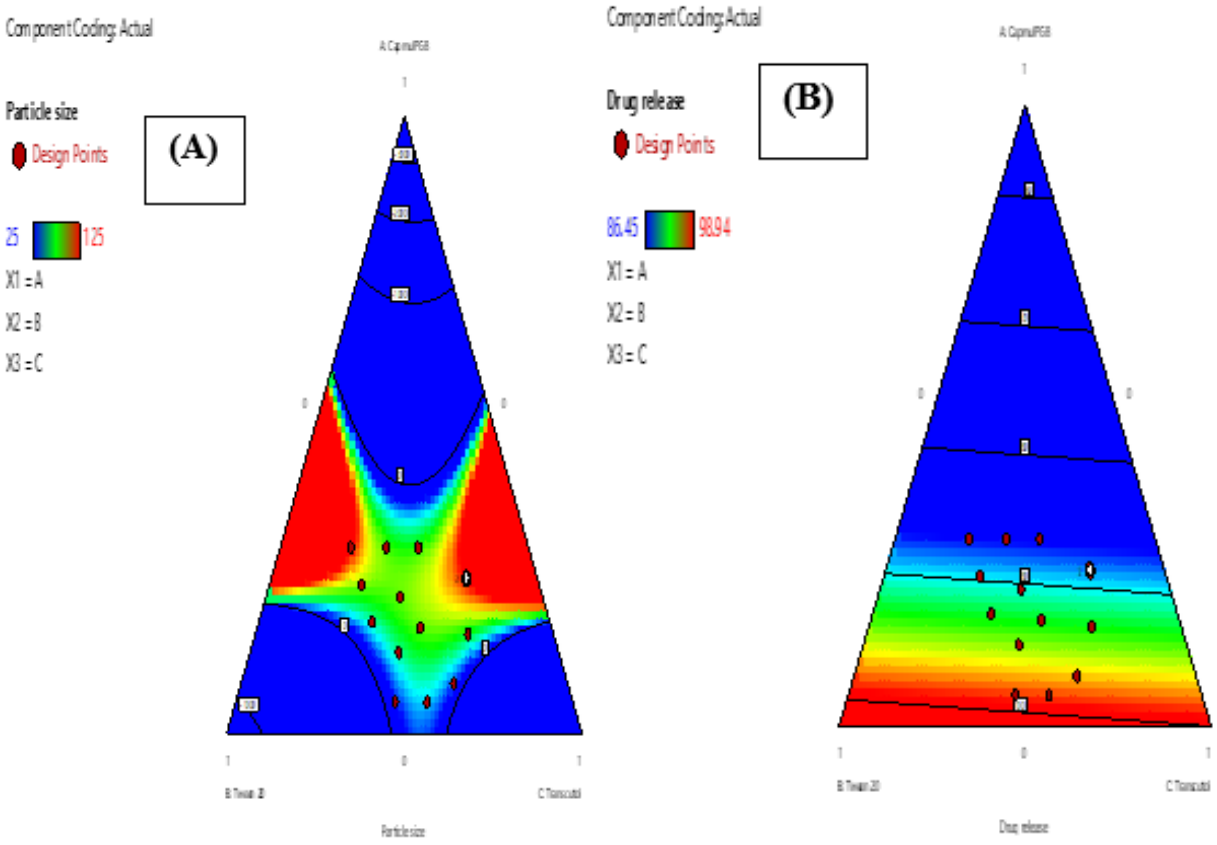

Figure 4: 2D response plot for (a) particle size (b) globule size

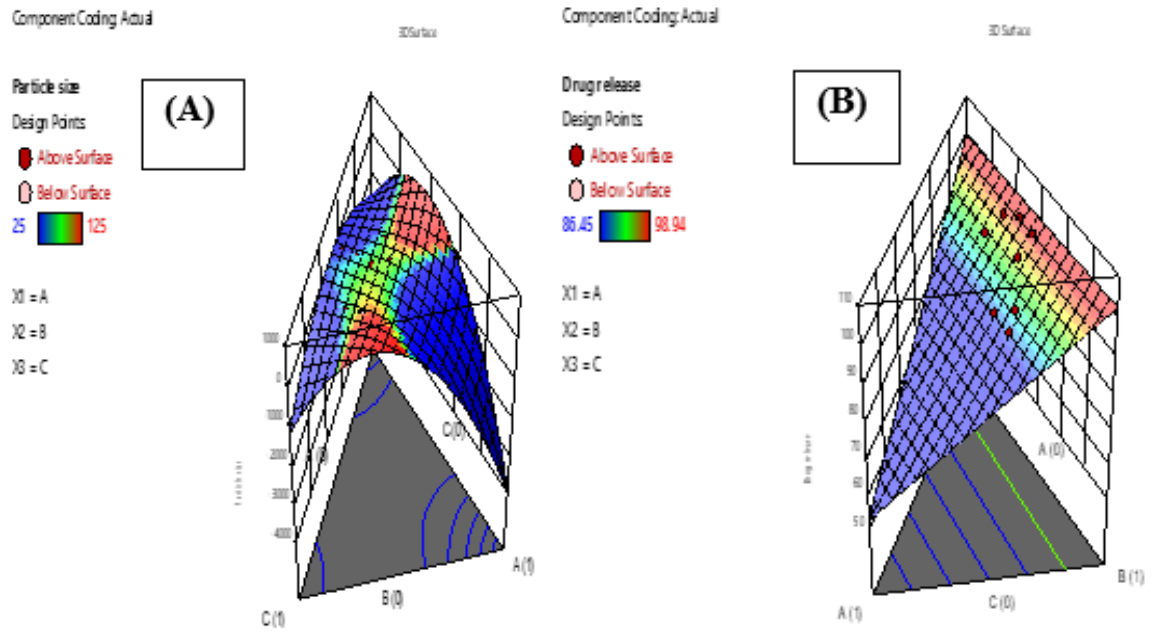

Figure 5: 3D response plot for (a) particle size (b) drug release

Table 3: Results of ANOVA

Results of ANOVA						
Response	**Sum of Squares**	**Df**	**Mean Square**	**F-value**	**p-value**	**Model**
Y1	14666.92	6	2444.49	49.96	< 0.0001	significant
Y2	235.03	2	117.51	371.83	< 0.0001	significant

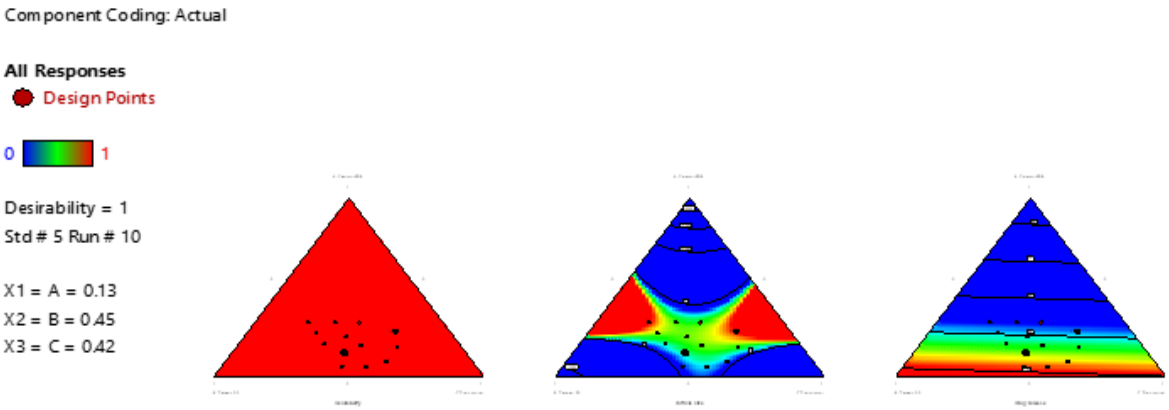

Figure 6: Desirability index for optimized rosuvastatin-loaded SNEDDS.

Mixture design tool in optimization and statistical analysis

Blend design utilizing Design Expert13 for the optimization of the rosuvastatin-loaded SNEDDS composition. Fourteen experimental runs using this dual Centre design were suggested, as seen in Table 2 above.

The following equations could explain how different component proportions affect globule size and drug release:

The fitted model's equation for-

Globular size:

-3984.09X1-1528.55X2+-1091.96X3+12308.89X1X2+10884.61X1X3+5336.79X2X3-24445.74X1X2X3
Eq(1)

%CDR

+52.83X1+102.13X2+100.02X3

X1 = Capmul PG8 Conc (Oil)

X2 = Kolliphor EL concentration (Surfactant)

X3 = Transcutol P Conc (co-surfactant)

Figure 4,5 depicts 2-D contour plots and 3-D response plots illustrating the impacts of X1, X2, and X3 on the variables Y1 and Y2. It has been discovered that increasing the oil content causes an increase in bead size while decreasing the drug release rate. Increasing the surfactant content, on the other hand, resulted in a reduction in bead size and an

increase in drug release rate. While figure 3 depicts the actual versus predicted plot for responses, it can be seen that the two are usually extremely similar. The triangle image's non-gray area denotes the region with the smallest possible bead size, the highest possible CDR, and the shortest possible self-emulsification time. High quantities of oil were seen in the 2-D and 3-D contours as well as in Equations (1, 2), this had significantly reduced the size of the beads in the mixture while surfactant and co-surfactant had increased them to their maximum size. Response Y2 exhibits a similar pattern up to a certain point before beginning to rise, as evidenced by the prediction profiler in figure 3, Desirability index in figure 6. The regression's Equations 1, and 2 were used to create the optimal formulation. The ANOVA's outcomes are displayed in Table 3.

EVALUATION PARAMETERS

% Transmittance

The Rosuvastatin calcium were reconstituted with distilled water and visually examined for turbidity. Following that, its % transmittance was determined using a UV spectrophotometer (Shimadzu UV 1800) against distilled water as the control [29]. It was found to be 99.9±0.4% at 241nm, confirms its transparency, drug precipitation and solubilization limit for dispersion into buffer medium (Table 4) [77].

Table 4: Percent Transmittance

Formulation %	Transmittance
F3	97.52
F4	99.27
F10	99.9

Robustness to dilution

The formation of homogenous nano-emulsions from SNEDDS is critical in a variety of mediums because medications can precipitate out in vivo, compromising drug digestion. After 100 times dilution, the optimized formulation was subjected to various mediums to imitate in vivo circumstances. Even after 24 hours, the optimized formulation showed no symptoms of precipitation, haziness, or phase separation, indicating that it was stable.

Viscosity

The viscosity for optimized formulation was found to be 121±5℃ cps with Brookfield hydromotion viscometer in triplicate assured that SNEDDS can be easily transferred or filled into capsule shells for their stockpiling.

Drug Entrapment Efficiency

EE is an important measure for assessing nano-pharmaceutical drug loading capacity; the higher value better the drug loading capacity. Rosuvastatin has an EE of 99.64±01 in an optimized SNEDDS formulation.

Determination of Cloud point (Tcloud)

It aids in the investigation of the effect of temperature on formulation phase behaviour, which is one of the most difficult problems with nanoemulsions, especially when nonionic surfactants are utilised. "The temperature above which the purity of the formulation becomes hazy." As a result, to avoid phase separation in the gastrointestinal system, the cloud point for SNEDDS should be above 37°C in Table 5. It results in stable nanoemulsion formation and phase behavior of SNEDDDS was assessed by measuring T cloud value at different temperatures [29]. The samples were assessed visually for optical clarity, drug precipitation, and phase separation [43].

Table 5: Cloud point (TCloud) temperature

Formulation	Temperature (℃)
F3	68
F4	70
F10	72

Drug content

The drug content in the optimized SNEDDS formulation was tested spectrophotometrically and determined to be 100.02±1.2%, confirming the formulation's dosage accuracy.

Droplet size, PDI and Zeta potential

Droplet size, PDI, and zeta potential are the most important considerations since they affect drug solubility and bioavailability. These properties have a direct impact on the emulsion's stability and also play a vital role in drug release and absorption. Smaller particles have a greater surface area, which allows for faster drug breakdown. Because of the smaller droplet dimension, an optimum mixture of oil, surfactant, and cosurfactant produces SNEDDS, which experiences impulsive emulsion production. The length of the fatty acid chain in the oil and surfactant, as well as its unsaturation, have a significant impact on the stability of the nanoemulsion [41]. Droplet size is one of the metrics used

to assess the stability properties of SNEDDS nano-emulsion. This leads in improved medication retention and oral absorption. As a result, the medicine is retained longer and has a higher oral bioavailability. Its nano dimension verifies a wider interfacial surface zone for increased drug absorption and bioavailability Its nano range may now enable more effective medicine delivery. The droplet size of SNEDDS indicates that the emulsion's globules have a nanometric range (58.82 nm) with PDI less than 0.16, demonstrating uniformity in droplet size distribution (figure 7) [78]. The zeta potential was discovered to be -17.0 mV (figure 8). The obtained formulation size appears to be too small for the kidney to rapidly remove molecules from the vascular compartment, mostly in their injected forms, and hence renal surface charge inhibits droplet buildup. When potential is high, repulsion outweighs attraction. As a result, the system will be disseminated and deflocculated, ensuring that it does not fail. The current experiment reported a negative value for the non-ionic surfactant used in the formulation. At neutral pH, they form a negatively charged contact [79,80].

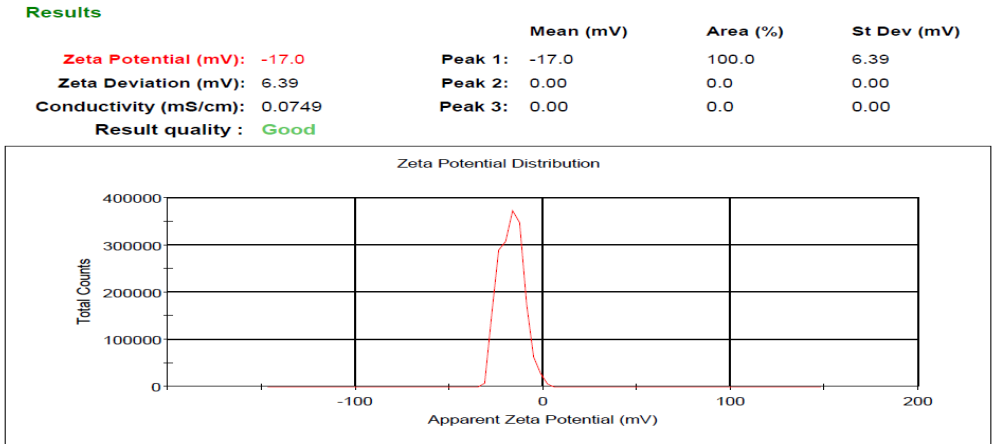

Figure 7: Zeta potential of optimized formulation.

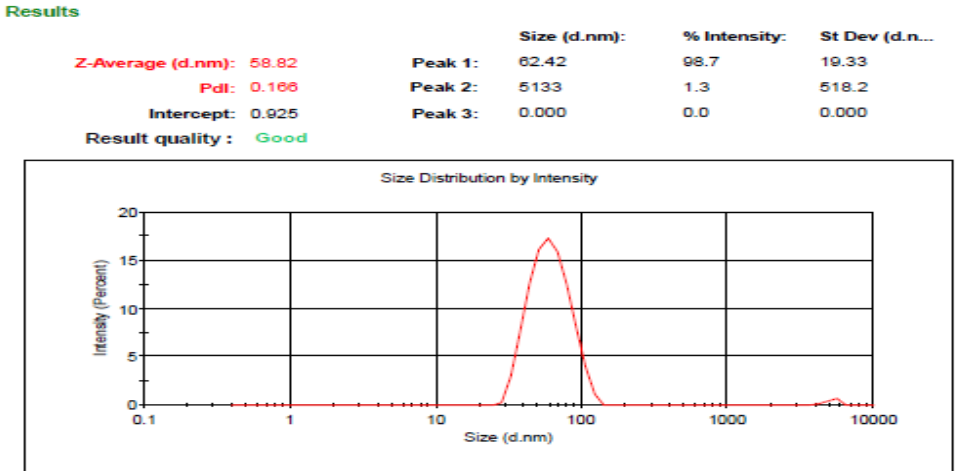

Figure 8: Globule size and PDI

DSC

Differential scanning calorimetry is a quick and dependable approach for determining drug-excipient compatibility by delivering the most information regarding potential interactions [81]. Using a differential scanning Calorimeter, thermograms of pure rosuvastatin, a physical mixture of both, and optimal L-SNEDDS were obtained [82]. DSC thermogram was shown in Figure 9 for pure rosuvastatin that shows no thermal transitions, showing that the medication is amorphous. A sharp endothermic peak at 217.58°C was seen in the thermogram of a physical mixture (Capmul PG8+ Tween 20+ Transcutol P+ Rosuvastatin), confirming the transformation of crystalline to liquid crystal form (figure 6B). This could be due to the crystalline form of rosuvastatin disintegrating [31]. The peaks of rosuvastatin and excipients vanished from the DSC curve of the optimized SNEDDS formulation due to the amorphous condition (figure 10, 11). These data indicated that rosuvastatin was completely dissolved in the excipient blend and that interactions between the medication and the excipients occurred. Interactions between rosuvastatin and excipients aided in rosuvastatin entrapment in the drug-loading mechanism and the development of rosuvastatin-loaded SNEDDS formulation [83].

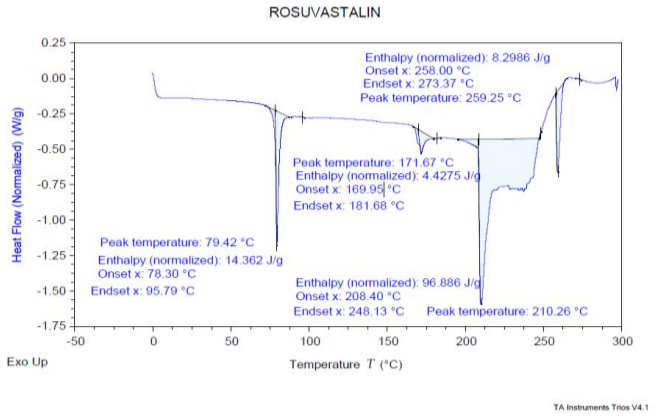

Figure 9: Pure rosuvastatin

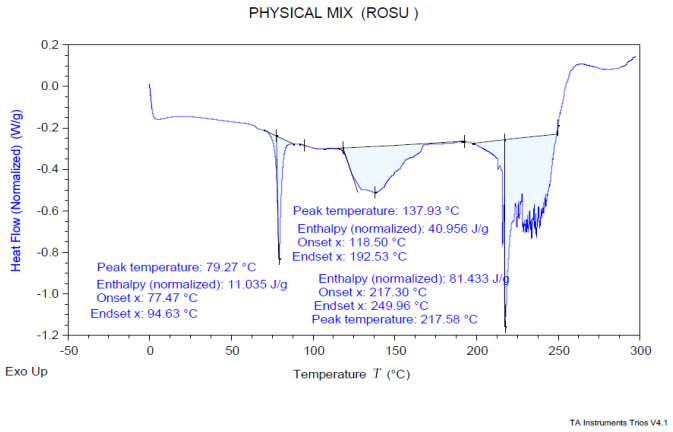

Figure 10: Physical mixture

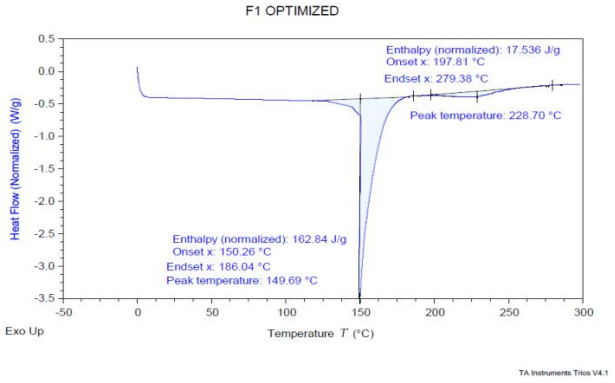

Figure 11: Optimized SNEDDS formulation

FT-IR

As indicated in Figure 12, the stretching vibration peak of aromatic N-H was 3383.78 cm^{-1}, the vibration peak of S=O was 1150.75 cm^{-1}, the peak of aromatic C-H bending was 1069 cm^{-1}, and the stretching vibration peak of C=C was 1652 cm^{-1}. The FT-IR spectrum of rosuvastatin and excipients was measured with SNEDDS (figure 13), which was nearly the superposition of rosuvastatin and ingredient FT-IR spectra. When compared to pure rosuvastatin, physical mixes, and SNEDDS, the FTIR spectral data of RC revealed a shift in the strength of the absorption bands. In SNEDDS, drug peaks were constant. There were no further peaks found, indicating that solely physical interactions between the drug molecule and the physical interactions exist. Other components and drug show no physical interaction. There was no substantial chemical incompatibility between the medicine, carrier, and other constituents, implying that excipients are compatible. The primary rosuvastatin absorption shifts were detected in both the physical combination and the improved SNEDDS formulation. Overall, the FTIR results showed there was no chemical interaction between the medication and its excipients [41]. The samples for the FTIR overlay of rosuvastatin calcium and SNEDDS formulation (F10) were scanned for IR spectra ranging from 4000 to 400 cm^{-1}.

The FT-IR spectrum of rosuvastatin showed evidence of several characteristics peaks (figure 14): intense broad bands at 3327 cm^{-1} due to O–H stretching vibrations; sulfone asymmetric stretch at 1069 cm^{-1}, aromatic C N stretching

vibrations at 1349 cm^{-1} and aromatic C–F stretching vibration at 1375 cm^{-1}. The vibration of the –CH and CH2 groups appeared at 1652 cm^{-1} and 1518 cm^{-1}.

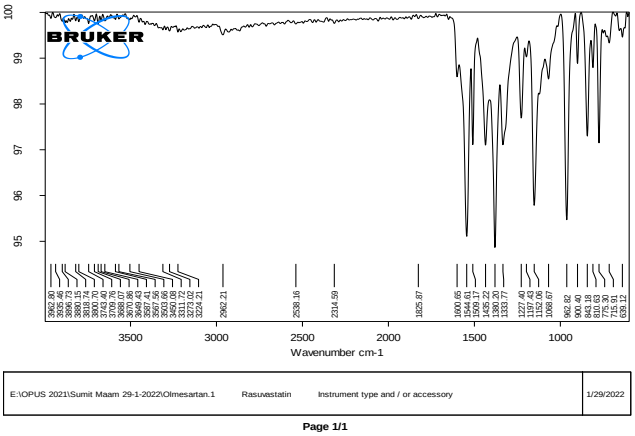

Figure 12: FT-IR spectrum of pure rosuvastatin

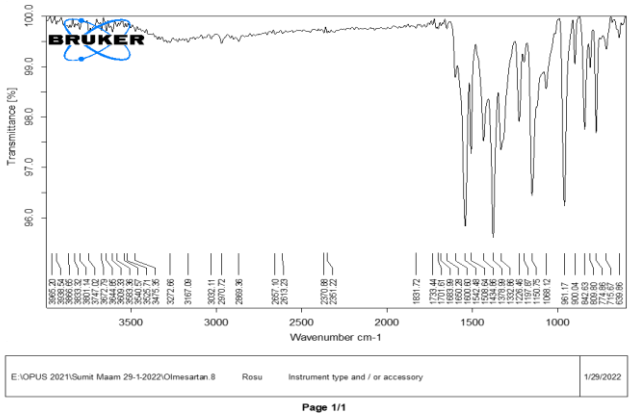

Figure 13: Physical mixture of drug & excipients

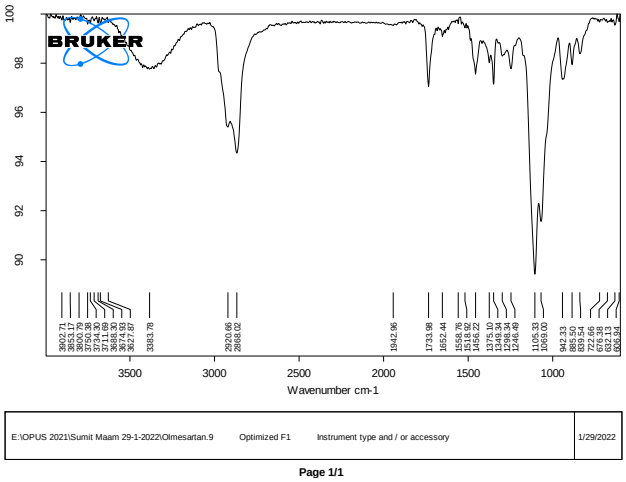

Figure 14: Optimized SNEDDS formulation.

Transmission Electron Microscopy (Surface morphology determination)

The morphology of the produced liquid ROS-SNEDDS was studied using transmission electron microscopy (TEM) [35]. According to transmission electron microscopy (TEM-Hitachi (H-7500)), the optimal SNEDDS formulation (F10) had a spherical shape structure with varied diameters of globules, as shown in figure 15. The shape of the generated nano-emulsion was determined using (1:100 dilutions). For 3 minutes at room temperature, the nano-emulsion was negatively stained with 2% phosphotungstic acid. Before being examined under an electron microscope. It had spherical droplets that did not aggregate, and its size was comparable to that established by dynamic light scattering [45].

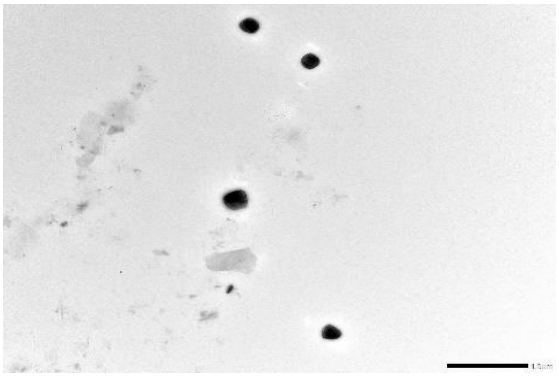

Figure 15: TEM of optimized SNEDDS formulation

Dissolution (Multi-Media) Testing

The findings of the solubility investigation revealed that rosuvastatin was well soluble in selected media. Figure 16 depicts the results of the dissolution profile of improved SNEDDS using three dissolving media. According to the findings, drug release in all medium was greater than 80% in a short period of time. However, at pH 1.2 and 4.5, there was a slight decrease or shift in drug discharge. Overall, the SNEDDS formulation resulted in significantly improved drug discharge in multimedia [84, 85].

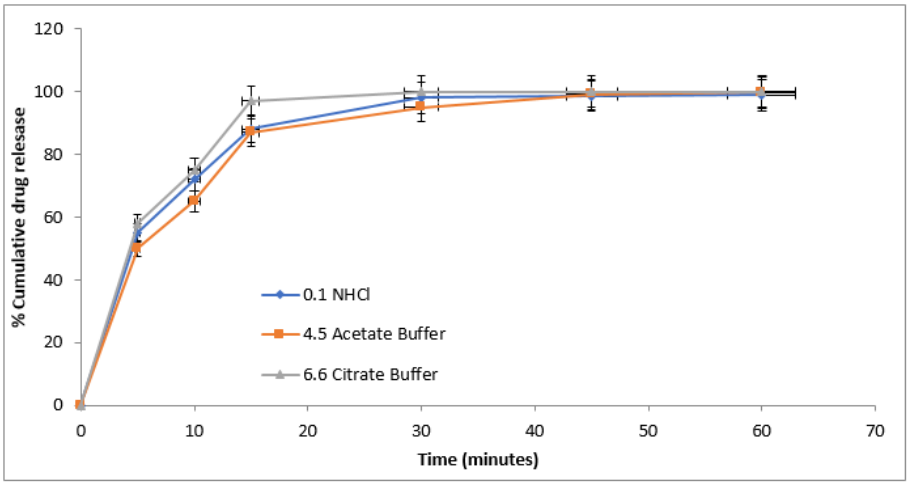

Figure 16: Dissolution (multi-media) testing of rosuvastatin SNEDDS. Each value represents the mean ± SD (n=3).

Comparative Investigation of SNEDDS with marketed and pure drug

Study was conducted using SNEDDS, marketed and pure drug as shown in Figure 17 which increased dissolution rate up to 1.11 and 1.64 times when compared to marketed and pure drug. SNEDDS converts to solid-to-liquid phase quickly changing crystalline nature into amorphous form result in enhanced dissolution. It is widely known that structural transformation accelerates breakdown and so increases bioavailability [86]. Another hypothesis is that the drug is present as a solubilized form. All of the studies showed that pure rosuvastatin in dissolving media released the medication faster.

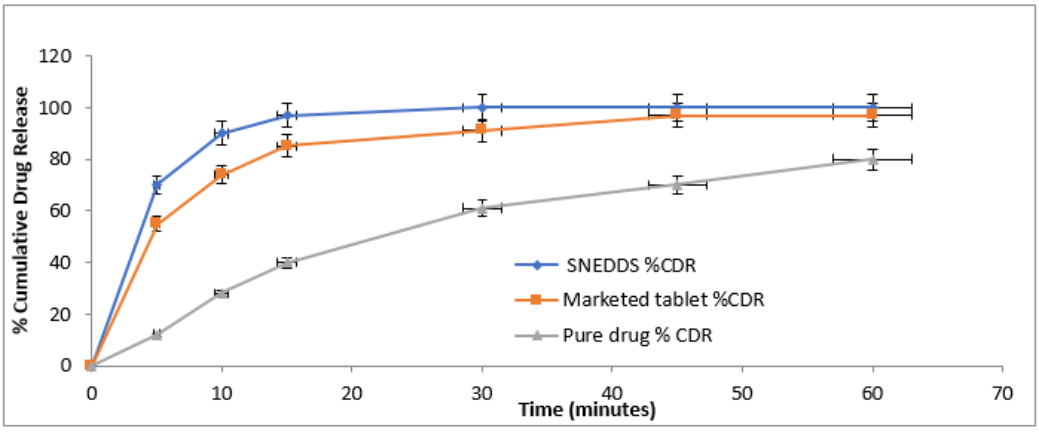

Figure 17: Dissolution of rosuvastatin-loaded SNEDDS, pure drug, and marketed tablet in a citrate buffer (pH 6.6). Each statistic indicates the mean standard deviation (n=3).

Investigation of food impact for dynamic *in vitro* lipolysis

One of the most amazing and undervalued abilities of SNEDDS is their ability to "collaborate" with the GI content that directly determines their potential. Dietary TG is generally digested quickly in the SI, and a variety of non-ionic esters act as a substrate for pancreatic lipase or other esterase [87]. This method aids in drug scattering in the presence of BS/PLs from formulation and promotes assimilation. Thus, evaluating lipid digestion can be critical because it speculates on the possibility of formulation for drug precipitation in SI. Changing the limit of solubilization that emerged throughout this approach was critical for assessing food impact using in vitro lipolysis methods [62]. It was critical to determine whether there was any likelihood of drug precipitation within 30 minutes throughout this trial. The fasting condition results showed that rosuvastatin was present in SNEDDS in the solubilized form, resulting in approximately 98.32±0.05% drug discharge. Under fed conditions, similar results (98.52±0.05%) were achieved, indicating that the SNEDDS formulation was capable of retaining a solubilized form of rosuvastatin, which is crucial for drug retention. As a consequence, SNEDDS avoided the influence of meals on drug release, as indicated in various works of literature, and was proven to be a correct theory for SNEDDS. This indicates that SNEDDS has overcome the influence of meals on medicine absorption. In this regard, SNEDDS can improve patient compliance, particularly in patients who are unable to take their drug with meals.

Stability Study

During a six-month accelerated stability trial at 40°C/75%RH, the SNEDDS formulation was found to be physically and chemically stable. Table 6 summarizes the findings of the rosuvastatin SNEDDS stability study. The study showed no significant difference in SNEDDS formulation after six months which concludes passed trials.

Table 6: Data on the Stability of an Optimized Rosuvastatin SNEDDS Formulation (n=6)

Test parameter	Initial	1 month	3 months	6 months
Description	Whitish colored capsules with clear liquid	Whitish colored capsules with clear liquid	Whitish colored capsules with clear liquid	Whitish colored capsules with clear liquid
% CDR (n=6)	98.9±1.2	95.7±2.1	96.9±0.7	99.8±1.0
Disintegration time	5±0.5 min	5±0.3min	6±1 min	6±0.2 min

Notes: %CDR-Percentage of cumulative drug release. Data are presented as the mean ± SD.

Pharmacodynamic Studies

Rosuvastatin SNEDDS (F-2), marketed pills, and the pure drug were all employed in this trial. The overall CH, TG, and HDL levels for each group are summarized in Table 7 and Figure 18. The proportion of parameters in the CTG, PTG (no drug), MTG (rosuvastatin tablet 10 mg), RTG (pure drug), and TT (SNEDDS) groups differed significantly in this investigation ($p<0.001$). Following a 28-day high-fat diet, all groups had a significant rise in blood lipid content, indicating a hyperlipidemic state in rats. Figure 18 shows that TTG treatment resulted in a substantial difference in blood lipidic contents including CH, TG, HDL, LDL, and VLDL when compared to PTG, MTG, and RTG. After 28 days of treatment, all groups showed the commencement of their pharmacodynamic effects in changing serum lipid levels ($p<0.001$). TTG reduced the CH concentration of serum more effectively than PTG, MTG, and RTG, but not as effectively as CTG. TG, LDL, and VLDL values all followed a similar pattern. When compared to CTG, TTG exhibited a significantly bigger percent expansion in HDL content level in serum than PTG ($p<0.001$), MTG ($p<0.01$), and RTG ($p<0.05$). This study found that when the medicine was designed as a SNEDDS formulation, it was extremely successful. This suggests that they were able to improve the therapeutic efficiency of rosuvastatin when compared to the suspension of marketed tablets and pure drug. Following the experiment, SNEDDS had a considerable impact on the lipid profiles of the rats. As a result of these findings, the tested formulation surpassed pure drug suspension in terms of in vivo pharmacodynamic response.

Table 7: Experimental animals lipid profile with Mean± Std. Deviation (n=6)

Parameters	0 Day	Control	Placebo	SNEDDS	Marketed Tablet	Pure drug
TG (µg/dl)	77.3±0.5	224.3±2.12	212.3±0.85	90.50±1.74 $^{***\$\wedge\wedge\wedge}$	118.0±1.2$^{**\wedge\wedge}$	167.5±1.5*
CH (µg/dl)	75±1.7	210.7±2.55	208.3±2.53	76.83±1.64$^{***\$\wedge\wedge\wedge}$	89.33±1.55$^{**\wedge\wedge}$	123.2±1.48*
HDL (µg/dl)	19.8±1.8	21.67±0.32	22.83±0.95	45.50±0.87$^{***\$\wedge\wedge\wedge}$	39.00±0.41$^{**\wedge\wedge}$	30.17±0.15*
LDL (µg/dl)	32.7±1.5	150.9±2.5	124.6±1.12	15.17±1.82$^{***\$\wedge\wedge\wedge}$	31.27±0.65$^{**\wedge\wedge}$	66.97±0.94*
VLDL(µg/dl)	15.9±1.6	42.13±1.5	38.87±0.54	14.17±0.45$^{***\$\wedge\wedge\wedge}$	16.07±1.6$^{**\wedge\wedge}$	26.03±1.2*

Data are presented as the mean standard deviation. ***$p<0.001$ (very significant); **$p<0.01$ (significant); *$p<0.05$ (significantly less significant) (*as compared to the control). $p<0.01$; ($ in comparison to the marketed tablet). $p<0.001$; $p<0.01$ (in comparison to pure medication). $p<0.001$ (very significant); $p<0.01$ (meaningful); $p<0.05$ (less significant). CH stands for cholesterol; HDL stands for high-density lipoprotein; LDL stands for low-density lipoprotein; TG stands for triglyceride; and VLDL stands for very low-density lipoprotein.

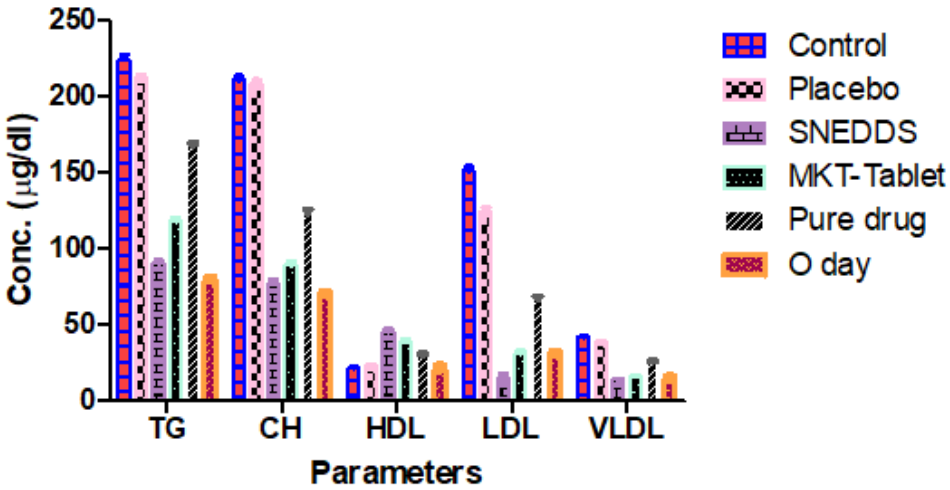

Figure 18: Results of comparative lipid profile testing. Each value represents the mean ± SD (n=6)**.**

CONCLUSION

In this research, we developed SNEDDS with improved aqueous solubility and oral bioavailability
We developed SNEDDS with increased water solubility and oral bioavailability in this study.
By using the Qbd method. Capmul PG8, Tween 20, and Transcutol P were found to be suitable as oils, surfactants, and cosurfactants in solubility and emulsification investigations, accordingly.
Ternary diagram studies indicated the nanoemulsification zone as well as a number of elements that should be used in the DoE. The DoE approach was utilized to develop SNEDDS by selecting the optimal excipient concentration. It was demonstrated that this sort of formulation had a rapid rate of dissolving in contrast to commercialized tablets and pure medication due to the nano-range of droplet size and negative charge of the zeta potential for this formulation. This increases the surface area available for medication release. Following an in vitro lipolysis study, it was established that there was no significant variation in solubilized rosuvastatin concentration between fasted and fed states. All physicochemical assessments were carried out in biorelevant media to better predict in vivo behavior. This implies that SNEDDS can avoid the impact of meals on drug release. When compared to commercial tablet suspension and pure drug, SNEDDS improved pharmacodynamics in male rats. That is why SNEDDS was established: to greatly increase bioavailability. This study investigated the use of SNEDDS to optimize drug release profile, reduce dietary effect, and increase pharmacodynamics. All of these findings suggest that SNEDSDS is a viable method for administering rosuvastatin with improved bioavailability, patient compliance, and fewer side effects.

ABBREVIATIONS

BCS stands for biopharmaceutical classification system; CH stands for cholesterol; CTG stands for control treatment group; and DoE stands for design of experiment. Fa/FeSSIF V-2 stands for fasted/fed state simulated intestinal fluid V-2; HDL stands for high-density lipoprotein; MTG stands for marketed treatment group. NaCl is for sodium chloride; o/w stands for oil in water; PDI stands for polydispersity index; PLs stand for phospholipids; and PTG stands for placebo treatment group. RTG stands for reference treatment group. SI is for small intestine; SNEDDS stands for self-nanoemulsifying drug delivery system. TTG stands for test treatment group; TG stands for triglyceride.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The investigation was approved by IAEC under Protocol no. GDGU/IAEC/2022/09, dated 18/08/2022.

ACKNOWLEDGEMENT

The authors would like to extend their sincere appreciation to Sun Pharmaceuticals Pvt. Ltd. Gurugram, GD Goenka University, Gurugram for their cooperation during the project.

CONTRIBUTIONS FROM AUTHORS

Authors contributed to analysis of data, drafting or revising the article, agreed on journal to be submitted, provided final approval to the version published and agreed to accept responsibility for all elements of work.

DISCLOSURE

There is no conflict of interest, according to the authors.

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