# Design and Development of a Lipid-Based Self-Nano Emulsifying Drug Delivery System of Rosuvastatin Calcium

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**Abstract**

The work aimed at design and development of a self-nanoemulsifying drug delivery system to increase the solubility and further to evaluate pharmacokinetic parameters of Rosuvastatin calcium (SNEDDS). Various oils were tested for their ability to self-emulsify with the help of suitable surfactants and co-surfactants. Ternary phase diagrams were drawn and used for the optimization of the system based on the solubility of Rosuvastatin calcium. The optimized formulations were tested for their duration of emulsification, dilution test, particle size, and zeta potential. The system showed stability at different pH and dilutions. The particle size was observed below 200 nanometer, which is considered acceptable for a Nano emulsion. The zeta potential of the chosen CT8P2 formulation (Capmul MCM PG 8 NF oil; Tween 80; PEG 200) was found to be with a mean globule size distribution. The CT8P2 SNEDDS showed a notable improvement in drug dissolution compared with In-vitro studies. A unique High Performance Liquid Chromatography technique was used to measure drug concentration in albino rat blood plasma, optimized Self-Nano emulsified formulation had greater absorption than the drug suspension. Lab solutions software were used to analyse the plasma drug concentration data, and the one compartment model was the best fit.

**Key words:** SNEDDS, Rosuvastatin Calcium, solubility using a pseudo-ternary phase. In-vitro dissolution and in-vivo animal studies and bioavailability, HPLC analysis, UV Spectrophotometer.

## 1. Introduction

The oral administration route is widely favoured for treating various diseases. Not only is it more convenient for patients, but the oral drug delivery system also offers several other benefits. When considering the oral route, one crucial factor to consider is aqueous solubility since gastric fluids are mostly composed of water. Unfortunately, more than forty percentage of newly developed compounds with low soluble in water, resulting in inadequate oral administration of pharmaceuticals [1, 2]. Thus, it is imperative to address this issue in order enhance the effectiveness of oral drug administration. Different methods are utilized to improve the ROS solubility that have low water solubility for their administration through the mouth. These include reducing particle size, employing hydro-trophy, utilizing solid dispersions, adjusting pH, incorporating surfactants, forming complexes, employing supercritical fluids process, utilizing co-solvency, forming salts, and employing lipid-based formulations.

Rosuvastatin calcium is a type of medication that helps lower lipids in the body. It is also known as (3R, 5S, 6E)-7-{4-(4-flurophenyl)-6-(1-methylethyl)-2- [methyl (methyl sulfonyl) amino] pyrimidin-5-yl}-3, 5-dihydroxyhept-6-enoic acid calcium salt. However, the drug has a low solubility in water, which means that only 20% of it can be absorbed when taken orally. To overcome this issue, it is important to improve the solubility of the drug so that it can have a better therapeutic effect by avoiding metabolism in the liver. When taken orally, the drug reaches its highest concentration in the blood (Mohsin Kazi, Athba Alqahtani et.al 2023).

## 1.1 classification system of BCS

The BCS, introduced by Professor Gordon Amidon and his colleagues in 1995, is a scientific framework utilized for the classification of substances according to their aqueous solubility and intestinal permeability. Its primary objective is to minimize the requirement for in-vivo bio-equivalency studies by substituting them with INVITRO dissolution tests as surrogates.
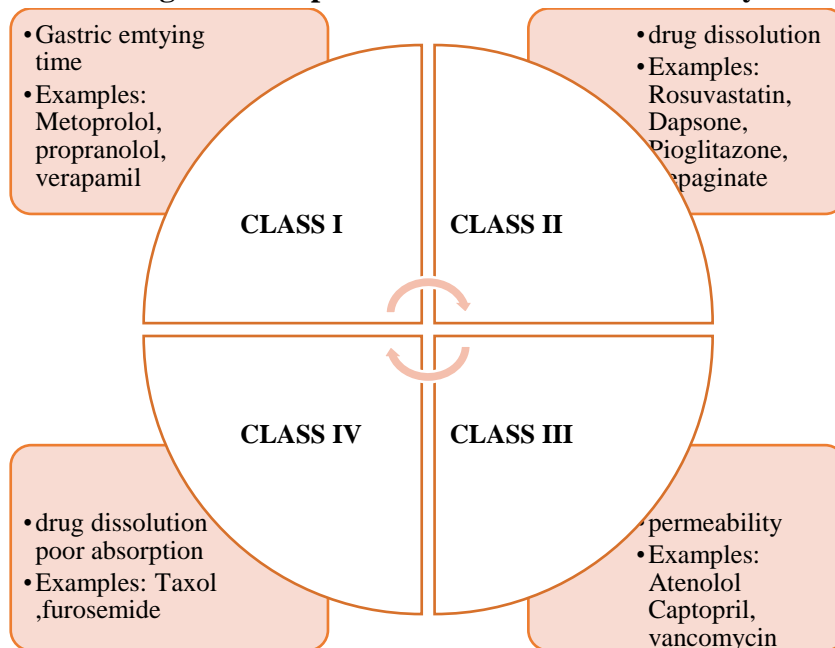
## 1.2.1 Purpose of BCS

A) The BCS expands its regulatory application and suggests ways to drugs.

B) bio-equivalence studies can be requested based on the BCS approach.

## 1.2.2 Concept and Objectives

1. It recommends a method for classifying drugs based on their dissolution in the dosage form and solubility-permeability characteristics.
2. It aims to enhance the efficiency of the drug development and review process by suggesting expendable clinical bio-equivalence tests.
3.

**Figure 1: Biopharmaceutical Classification System**



- Gastric emtying time
- Examples: Metoprolol, propranolol, verapamil

CLASS I

- drug dissolution
- Examples: Rosuvastatin, Dapsone, Pioglitazone, repaginate

CLASS II

- drug dissolution poor absorption
- Examples: Taxol ,furosemide

CLASS IV

CLASS III

- permeability
- Examples: Atenolol Captopril, vancomycin

### 1.3 Enhancement techniques

### 1.3.1 pH Adjustment

Poorly water soluble drugs can become more soluble in water by adjusting the pH. This is because the drug solubility alters parts of its molecule that can be protonated or de-protonated. By changing the pH, the drug's solubility can be changed, making it easier to dissolve in water. One advantage of pH adjustment as a solubility enhancement technique is that it requires small quantities of compounds and allows for high thorough out evaluations. Additionally, it is relatively simple to formulate, analyse, and produce.

### 1.3.2 Reduction of Particle size

The absorption of low soluble drugs is closely related to the droplet size. Various techniques, such as Micronization, Nano suspension, Sonocrystalization, and the use of a Colloid mill, can be employed to reduce particle size. Decrease the size of droplet, the area of surface improve for good interaction between the drug and the solvent, thereby enhancing solubility. However, conventional methods like spray drying, which rely on mechanical stress, are not suitable for thermo-sensitive drugs.

### Micronization

Micronization is a method that enhances the rate at which drugs dissolve by enlarging their surface area. This is accomplished by utilizing milling techniques.

### Nanonization

Nanonization, on the other hand, involves converting drug powder into nanocrystals with sizes ranging from 200 to 600nm. An example of a drug that undergoes Nanonization also known as a Nano suspension.

### Sonocrystalization

Reduces the size of particles by using ultrasound to induce crystallization. By utilizing the power of ultrasound, it increases the rate of nucleation and allows for effective control over the size distribution of active pharmaceutical ingredients.

### 1.3.3 Supercritical fluid process

An emerging technique for reducing particle size and enhancing solubility is the utilization of supercritical fluid (SCF) processes. This precise control over solubilisation can be utilized in the context of drug delivery, where a drug can be solubilized within a supercritical fluid and subsequently recrystallized into significantly smaller particle sizes.

### 1.3.4 Spray freezing liquid

This process quickly freezes the solution, creating solid particles. The SFL method is advantageous because it allows for precise control of particle size and preserves the stability of the API by using cryogenic conditions.

### 1.3.6 Solvent deposition

The solvent deposition system represents a solid preparation method wherein a drug is deposited onto the surface of a matrix from a solvent. This deposition step is typically achieved through the simple evaporation of the solvent used for distributing the drug onto the matrix. By carefully controlling the

solvent evaporation process, it becomes possible to achieve a uniform and controlled drug deposition, leading to the formation of a solid drug-matrix composite.

### 1.4 Self Nano Emulsifying Drug Delivery System

The Pharmaceutical formulations utilizing lipids for drug delivery in SNEDDS requires careful consideration in the selection of excipients. There are numerous excipients available in the market, and it is essential to have a comprehensive understanding of their properties before making a specific choice. Several criteria need to be taken into account when choosing an excipient, including its miscibility, self-dispersion ability, digestibility, and its potential to enhance the formulation. Additionally, the excipient's ability to dissolve in the solvent, as well as regulatory concerns such as irritancy, toxicity, stability, compatibility, melting point, and purity, must also be considered. SNEDDS are liquid blends made up of oil, surfactant, drug, and co-emulsifier or solubilizer. These blends are dry and uniform. When mixed with water and stirred gently, they automatically create oil-in-water Nano-emulsions that are around 200 nm or smaller in size (Mehran ashfaq et al 2022).

### Advantage of SNEDDS

1. Protection of sensitive drug substances: SNEDDS offer a protective environment for delicate drug substances, shielding them from degradation or other forms of damage.
2. Selective targeting of drugs: SNEDDS can be designed to target specific absorption windows in the gastrointestinal tract (GIT), allowing for enhanced drug delivery to the desired site of action.
3. Thermodynamic stability: SNEDDS belong to a thermodynamically stable system, which ensures their ease of storage and maintenance of their desired properties over time.
4. SNEDDS can enhance the absorption of drugs taken orally, which means that less of the drug is needed to achieve the desired effect.
5. SNEDDS offer a greater surface area for drugs to be distributed between oil and water phases, leading to more effective absorption and release compared to oil solution

### Disadvantage of SNEDDS:

SNEDDS face a significant disadvantage due to the absence of appropriate laboratory models to assess these formulations. Conventional dissolution techniques are inadequate for evaluating SNEDDS because these formulations often need to be digested before the drug is released. This creates a difficulty in accurately predicting their effectiveness and optimizing their composition.

**1.4.1 Composition of SNEDDS**

**Figure 2: Composition of Self-Nano Emulsifying drug delivery system**
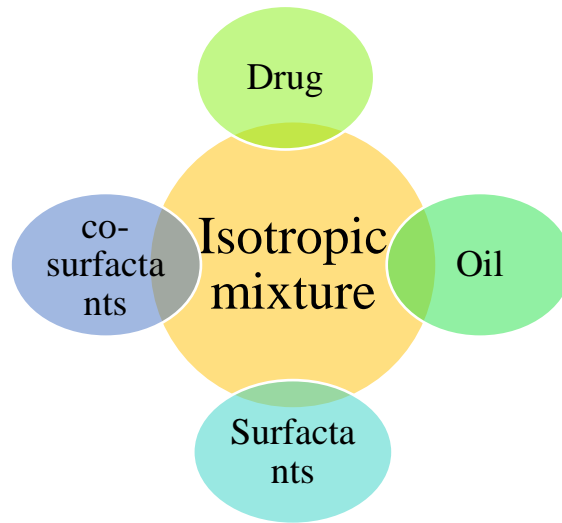


**1.4.2.1 Oils**

Oils or lipids are commonly used in the formulation to enhance the solubility of lipophilic drugs. The selected oil should have the ability to produce a Nano emulsion with small droplet size. Natural edible oils cannot solubilize a substantial amount of the hydrophobic medication. Therefore, oils that are saturated or hydrolysed oils are used. Medium chain TA is selected in the formulation because it has the highest solvent capacity and is less prone to oxidation. Examples of oils that can be used include medium chain mono and triglycerides (Campul MCM, Labrafac CC), propylene fatty acid esters (Capryol90, Lauroglycol90, and Campul PG-8 NF), coconut oil, almond oil, castor oil, among others.

**1.4.2.2 Surfactants**

Surfactants play a crucial role formulation of SNEDDS. Specifically HLB greater than 12 are utilized in the creation of SNEDDS. The concentration of surfactant in the formulation typically ranges from 30% to 60% w/w in order to achieve a stable SNEDDS. These surfactants aid in the spontaneous formation of oil-in-water globules and facilitate the formulation's quick dissemination in the aqueous phase. This is essential for maintaining the stability of the established SNEDDS formulation. Some examples of surfactants commonly employed in SNEDDS formulation include polysorbates (such as Tween40 and Tween80), castor oil (known as cremophore EL), and polyglycolyzed glycerides (such as labrafil)

**1.4.2.3 Co-Surfactants**

In addition to surfactants, co-surfactants are also utilized in SNEDDS formulation. Co-surfactants serve two main purposes: they help dissolve hydrophobic drugs in the lipid base and enhance the loading capacity of the drug. Examples of co-surfactants commonly used in this context include Propylene glycol, PEG 200, and PEG 400 are different chemicals.

**1.4.3 Rationale for formulation development**

Formulation development for Rosuvastatin calcium is based on the rationale of addressing its low water solubility, elevated partition ratio (log p=4.19), bioavailability (20%) caused by poor dissolution. Enhance the drug's ability to dissolve, absorb, and increase its bioavailability by SNEDDS is considered as one of the most effective techniques. The chosen SNEDDS formulations have successfully resolved the issues

related to the poorly soluble drug and have demonstrated the ability to produce consistent and superior dissolution profiles for Rosuvastatin calcium.

### 1.4.4 Consideration of SNEDDS

Co-surfactants play a crucial role in SNEDDS formulation, alongside surfactants. They serve two primary functions: aiding in the dissolution of hydrophobic drugs in the lipid base and increasing the drug's loading capacity in SNEDDS Commonly used co-surfactants in this context include propylene glycol, PEG 200, and PEG 400.

### 1.5 MECHANISM OF SELF–EMULSIFICATION

The addition of a binary mixture (oil/ surfactant) to water results in formation of an interface between the oil phase and aqueous continuous phase. Solubilization of water in oily phase takes place due to aqueous penetration through the interface. These will take place until the solubilization limit is reached close to the interface. Aqueous penetration results in formation of the dispersed LC phase. Once formed, rapid penetration of water into the aqueous cores aided by the gentle agitation during self-emulsification process, causes disruption of interface and then droplet formation. The high stability of self-emulsifying systems against coalescence is due to the LC interface surrounding the oil droplets.

$$\Delta G = \Sigma Ni\pi r^2 \sigma$$

Where,

**ΔG** is the free energy associated with the process (ignoring the free energy of mixing), **N** is the number of droplets of radius **r** and **σ** represents the interfacial energy

### 1.6 PSEUDO TERNARY PHASE DIAGRAMS

Pseudo ternary phase diagrams help in determining optimum concentration of different excipients necessary to obtain homogenous pre-concentrates, self-emulsification ability and drug loading. Each corner of diagrams represents 100% of particular components (oils, surfactant, co-surfactant, water). They are generally generated by water titration method in which water is added to pre concentrates in drop wise manner and visually observed for transparency. A pseudo-ternary phase diagram is shown in figure3.

**Figure 3: pseudo ternary phase construction**
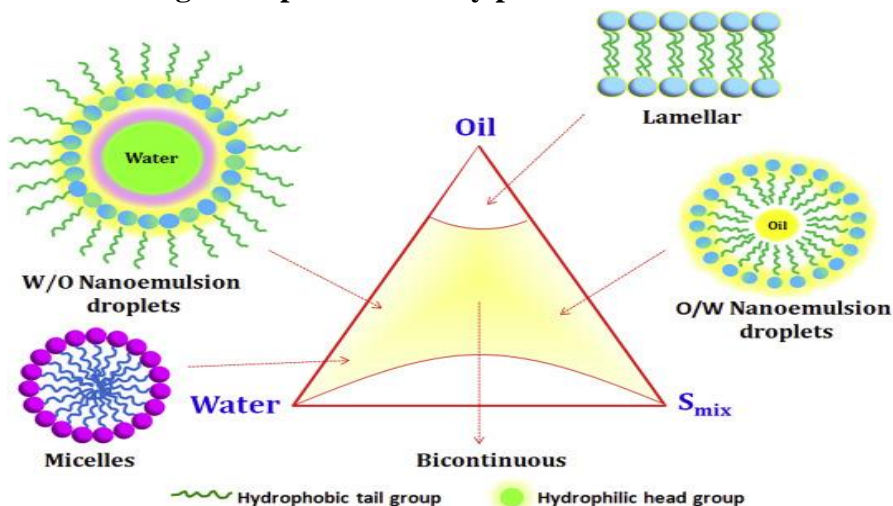
## 2. Materials & Methodology

The ingredients used in the formulation include Rosuvastatin calcium, methanol, Span 20, Span 80 (Sorbitan monooleate), PEG-200, propylene glycol (PEG-400), PEG (propylene glycol), Maisine CC, Campul PG-8NF, Tween80 (polyoxyethylenesorbitanmonooleate), Tween20 (polyoxyethlenesorbitanmonolaurate), Almond oil, Castor oil, Coconut oil, Olive oil, Campul MCM, Labrafac liophile WL 1349 (medium-chain triglycerides), labrasol (caprylocaproyl macrogol-8 glycerides), and Lauroglycol 90 (propylene glycol monocaprylate).

### 2.1 Methodology

### 2.1.1 UV-Spectrophotometric analysis of Rosuvastatin Calcium

**2.1.1.1 Preparation of standard stock solution in Methanol:** To prepare the standard stock solution of Rosuvastatin calcium (ROS) 10mg drug, a 10ml volumetric flask was utilized. The drug was dissolved in methanol and the solution was brought up to the mark with methanol. This process was carried out for duration of 2 to 3 minutes.

**Preparation of working standard solution:** The process of preparing a working standard solution involves pipetting out 0.1 ml of the stock solution into a 10ml volumetric flask and diluting it with methanol to obtain different concentrations of 1ppm, 2ppm, 3ppm, 4ppm, and 5ppm.

**Calibration Curve of Rosuvastatin calcium in Methanol:** The calibration curve for Rosuvastatin calcium was established using methanol as the solvent. A quantity of 10mg of the drug was accurately weighed and then diluted with 10ml of methanol in a 10 ml volumetric flask, resulting in a concentration of 1000 µg/ml. Subsequently, 0.1 ml of this solution was pipette out and further diluted with 10ml of methanol, yielding a concentration of 10µg/ml. From this concentration, various ranges such as 1ppm, 2ppm, 3ppm, 4ppm, and 5ppm were prepared. The absorbance of each solution was measured at 241nm using a UV-Spectrophotometer, with methanol serving as the reference solution.

**2.1.1.2 Preparation of standard stock solution in 0.1 N HCL:** To prepare the standard stock solution in 0.1 N HCL, the ROS sample of Rosuvastatin calcium was utilized. Initially, 10mg of the drug was measured and placed in a 10mL volumetric flask. The drug was then dissolved by adding 1ml of methanol. Subsequently, the volume was adjusted by adding 0.1N HCL until the flask was filled. Finally, the solution was sonicated for duration of 2 to 3 minutes.

**Preparation of working standard solution:** In order to prepare the working standard, extract 0.1 ml of the stock solution and transfer it into a 10 ml volumetric flask. Proceed to dilute this solution with 0.1N Hcl to obtain various concentrations of 1ppm, 2ppm, 3ppm, 4ppm, and 5ppm. Subsequently, all of these concentrations were subjected to UV scanning using the SHIMADZU UV-1800 Spectrophotometer within the wavelength range of 200-400nm. The resulting spectrum was observed to have a peak at 239 nm.

**Calibration curve of Rosuvastatin calcium in 0.1N Hcl:** The Rosuvastatin calcium calibration curve was established utilizing 0.1N Hcl as the solvent. Initially, 10mg of the drug was weighed and diluted with 10ml of Hcl in a 10 ml volumetric flask, resulting in a concentration of 1000 µg/ml. Subsequently, 0.1 ml of this solution was pipette out and diluted into 10ml to obtain a concentration of 10ug/ml. This concentration was then utilized to prepare various ranges such as 1ppm, 2ppm, 3ppm, 4ppm, and 5ppm. The absorbance was measured at 239nm and 241nm against methanol using a UV-Spectrophotometer.

## 2.2 Solubility studies of Rosuvastatin calcium

The saturation solubility of Rosuvastatin calcium was assessed in various mediums, including oils, surfactants, and co-surfactants. A screw capped glass vial containing 500mg of the medium was utilized, and an excess amount of the drug was added to it. To enhance the solubilization process [4].the resulting mixtures of drug and medium were cyclomixed using a cyclomixer. Subsequently, the mixtures were heated in a thermostatic water bath at 40°C for 10 minutes to further promote solubilization. Following this, the mixture was placed in a rotary shaker for 48 hours at 25°C and left to equilibrate at room temperature for 24 hours. Once equilibrium was reached, the sample was centrifuged at a speed of 5000rpm for 10 minutes the supernatant was then separated through filtration using o.45µ filters. The samples were analysed using UV Spectrophotometer at a wavelength of 241nm after dilution. The concentration of Rosuvastatin calcium in each medium was determined by utilizing a calibration curve [5, 6].

## 2.3 Phase diagram construction

To determine the concentration of components in the SNEDDS range, a pseudo ternary phase diagram was created using the aqueous titration method at room temperature. Various combinations of oils, surfactants, and co-surfactants were grouped together for phase studies. Different compositions with varying proportions of surfactant/co-surfactant mixtures (Smix) were tested to emulsify the selected oil. These Smix ratios, such as 1:1, 3:1, 4:1, were chosen to increase the concentration of surfactant relative to co-surfactant and vice versa [7,8]. To prepare each phase diagram, different volume ratios of oil and S mix were mixed in glass vials. The volume ratios used were 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, 1:2, 1:3, 1:3.5, 1:5, 1:6, 1:7, and 1:8. The mixture was vortexed to form a clear and homogeneous system, followed by titration with the aqueous phase. The clarity of the phases was evaluated visually during titration, noting the volume of water required to transition from transparency to turbidity. Pseudo ternary plots were then constructed using CHEMIX School software (Chetan Amrutkar kishor salunkhe et.al).

## 2.4 Preparation of Rosuvastatin calcium L- SNEDDS

After the identification of Self-nanoemulsifying, a formulation of SNEDDS with desired component ratios was prepared. The optimization of the surfactant to co-surfactant Smix ratio was achieved using pseudo ternary phase diagrams. By optimizing the ratios of surfactant to co-surfactant and Oil to Smix, two successful liquid SNEDDS formulas were developed. Throughout the formulation process, the content of Rosuvastatin remained constant. The oil, surfactant, and co-surfactant were accurately weighed and mixed in a stopper glass vial using a vortex mixer to ensure a thorough mixture. The Rosuvastatin was then dispersed into the mixture of oil and Smix with continuous mixing until the drug was completely dissolved. The prepared formulas were subsequently stored at room temperature for future use [11, 12].

## 2.5 Characterization and evaluation of L-SNEDDS

The physical stability of lipid-based formulations, including emulsions, is of utmost importance for their performance. However, the thermodynamic stability of these emulsions can be compromised by the precipitation of the drug within the excipients matrix. Furthermore, inadequate physical stability of the formulation can result in phase separation of the excipients, impacting not only the formulation's performance but also its visual appearance. The performance of lipid-based formulations, such as emulsions, relies heavily on their physical stability. However, the presence of drug precipitation within

the excipients matrix can negatively impact the thermodynamic stability of these formulations [13]. Moreover, if the formulation lacks proper physical stability, it can lead to phase separation of the excipients, thereby affecting both the performance and visual aspects of the formulation.

### 2.5.1 Centrifugation study

The laboratory centrifuge (REMI R-8C) was utilized to centrifuge the optimized formulations at 5000 rpm for duration of 30 minutes. Following this, the optimized formulations were assessed for any potential instability issues, including phase separation, creaming, or cracking. The stable formulation was then chosen for further investigation.

### 2.5.2 Heating and cooling cycle

The formulations underwent heating and cooling cycles ranging from 4∘C to 40∘C, with each temperature being maintained for a minimum of 24 hours. Subsequently, the formulations were carefully examined for any signs of physical instability, such as phase separation or precipitation. Following this evaluation, the selected formulation was utilized for subsequent testing purposes.

### 2.5.3 Freeze thaw cycle

The stability of Rosuvastatin Calcium loaded SNEEDS was assessed using freeze-thawing. The formulations underwent three freeze-thaw cycles, involving a freezing step at -4∘C for 24 hours followed by thawing at 40∘C for 24 hours. Subsequently, the optimized formulation was subjected to centrifugation at 5000 rpm for 5 minutes. The formulations were then examined for any occurrence of phase separation. Only the formulations that successfully passed all three tests and had the lowest Smix concentration were considered.

### 2.5.4 Self-Emulsification Time

The evaluation of self-emulsification efficiency is conducted through the utilization of a dissolution apparatus. A volume of 1ml of SNEDDS is dissolved in 250ml of water at a controlled temperature of 37±0.5∘C. To facilitate the process, a gentle agitation is applied by means of a basket rotating at a speed of 60RPM. The assessment of SEDDS is based on visual observations, taking into consideration the rate of emulsification as well as the ultimate appearance of the emulsion. Additionally, any occurrence of precipitation is visually monitored.

### 2.5.5 Dispersibility Test

The standard USP I dissolution apparatus is utilized to evaluate the effectiveness of self-emulsification in oral Nano emulsion. Each formulation, measuring one ml, is dissolved in 500 ml of water and 0.1N Hcl at a temperature of $37 \pm 1$∘C. To ensure gentle agitation, a standard stainless steel dissolution basket with a rotating speed of 50 rpm is employed. The in vitro performance of the formulations is visually evaluated using a grading system.

### 2.5.6 Percentage Transmittance

The SNEDDS of Rosuvastatin calcium were reconstituted by means of Methanol and 0.1N Hcl. The resulting Nano-emulsion was visually inspected for any signs of turbidity. Subsequently, the %

transmittance of the emulsion was determined at 239nm using a UV–VIS spectrophotometer (Shimadzu UV 1800) with distilled water serving as the blank.

### 2.5.7 Droplet size measurement
**Droplet size analysis:**

The SNEDDS formulation (10 µl) underwent dilution with 10 ml of deionised water in a beaker while being constantly stirred with a glass rod. The resulting emulsion was then analysed for particle size using the Dynamic light scattering (DLS) technique with a zeta-sizer (Nano ZS, Malvern Instruments, and UK). The droplet size was determined using a He-Ne red laser with a power of 4.0 mV and a wavelength of 633 nm, at a temperature of 25°C.

**Polydispersity Index**

The Polydispersity index (PDI) is a metric that gauges the uniformity of droplet size, with a range of 0.0 to 1.0. It is calculated by dividing the standard deviation by the mean droplet size in the formulation. A higher PDI indicates a lower level of homogeneity in droplet size, while a PDI value closer to zero signifies a more uniform distribution of droplet sizes.

### 2.5.8 Cloud point measurement

The diluted samples of the optimized SNEDDS formulations were mixed with distilled water in a ratio of 1:250. These diluted samples were then subjected to gradual temperature increase in a water bath. The cloud point, which indicates the temperature at which cloudiness suddenly appears, was determined using spectrophotometer [14].

### 2.5.9 Drug content determination

ROS Calcium SNEDDS (100 mg) was dissolved in 10 ml of methanol in 10 ml volumetric flask separately, 0.1 ml of stock solution measured accurately and transferred to 10 ml volumetric flask to which 10 ml methanol was added and filtered through 0.45µl filter paper. The above solutions were analysed by UV Spectrophotometer (Shimadzu UV 1800) at $\lambda_{max}$. 241nm. the amount of ROS Calcium present in the formulation was determined using the prepared standard calibration curves of ROS calcium in methanol.

### 2.5.10 Robustness to Dilution:
The SNEDDS formulations that were prepared underwent dilution using ratios of 1:100 and 1:1000 folds with 0.1 N HCL. The resulting diluted Nano emulsions were then stored for a duration of 24 hours. During this time, they were visually examined for any indications of phase separation or drug precipitation.

### 2.5.11 Drug loading efficiency

The drug concentration in the formulation was assessed using UV-Spectrophotometer. Precisely 10mg of each formulation was weighed and then diluted to 100ml with methanol. The resulting solutions were analysed using spectroscopic methods after appropriate dilution. The drug loading efficiency was determined using the equation:

$$\textbf{Drug loading efficiency} = \frac{\textbf{Amount of drug in a known amount of formulation}}{\textbf{Intial drug load}} \times \textbf{100}$$

## 2.6 Fourier Transformed Infrared Spectroscopy (FT- IR)

The FT-IR spectrum of the pure drug and the drug-excipients combination was acquired using a FT-IR Spectrophotometer (Bruker - Alpha). The spectrum of the drug, excipients, and drug-excipients mixture was obtained by accumulating 24 scans and setting a resolution of 4cm-1 within the range of 400-4000cm-1. Subsequently, the obtained spectrum of the drug-excipients mixture was compared to the spectrum of the pure drug to identify any potential interactions.

## 2.7 IN – VITRO drug release study

The dissolution study of S-SNEDDS, which were encapsulated in appropriately sized capsules, was conducted using the USP-Type I dissolution test apparatus (DS 1800 Lab India). The study was performed in 900ml of 0.1N Hcl at a temperature of $37\pm0.5$°c, with a rotating speed of 100 rpm. At specific time intervals (5, 10, 15, 20, 30, 40, 50, 60, and 80), samples were withdrawn and filtered through a 0.45μ filter. To maintain a constant volume, an equal amount of dissolution medium was replenished after each sampling. The samples were then analyzed using a UV-Spectrophotometer at a wavelength of 239nm. The amount of drug released was determined by calculating the concentration and absorbance of the samples [15, 16].

## 2.8 FORMULATION OF SOLID–SNEDDS

A solid SNEDDS formulation was prepared by incorporating Neusilin into a liquid SNEDDS containing Rosuvastatin Calcium. The Neusilin was added in ratios of 3:1, 1:1, and 4:1 to the liquid SNEDDS in small quantities. To ensure uniform distribution of the formulation, the contents were mixed using a glass rod after each addition. The resulting damp mass was then passed through a sieve no.120 and dried at room temperature. The dried formulation was stored for future use. This formulation exhibited good stability, self-Nano emulsification property, and demonstrated a smaller particle size and lower PDI (Polydispersity index). Additionally, it showed a favorable dissolution rate [17].

### 2.8.1 Preparation of Rosuvastatin calcium loaded Solid-S-SNEDDS:

Two optimized solid self-nanoemulsifying drug delivery systems (SNEDDS) of Rosuvastatin L-SNEDDS formulation were chosen for solidification through the spray drying technique. Neusilin US2 was utilized as the solid carrier in this process. The ratio of S-SNEDDS Neusilin US2 (1:1.5) was determined to be the optimized formulation. To prepare the solid SNEDDS, 2g of the SNEDDS formulation and 1000 mg of Neusilin US2 were suspended in 1000 mg of Oil: Smix. The suspension was continuously stirred until an isotropic mixture was formed. The mixture was then left at room temperature ($25 \pm 2$ °C) for 24 hours to equilibrate. Subsequently, the obtained mixture was subjected to spray drying using specific parameters. The inlet temperature was set at 60 °C, the outlet temperature at 35 °C, and the aspiration at 85%. The suspension was fed into the spray dryer at a rate of 5 ml/min, while the atomization air pressure was maintained at 5 pka.

### 2.8.2 Characterization and Evaluation of S-SNEDDS
**Flow properties of S-SNEDDS**
### 2.8.2.1 Angle of repose

The funnel method was used to calculate the angle of repose of S-SNEDDS. The height of the funnel was adjusted so that its tip barely touched the top of the powder pile [18]. The precisely weighed sample was

let to freely flow onto the surface through the funnel. The powder cone's diameter was measured, and the equation was used to determine the angle of repose.

$$\text{Tan}\,\theta = \frac{h}{r}$$

Where

h & r are height and radius of powder cone.

### 2.8.2.2 Bulk Density & Tapped Density

A quantity of 2grm of S- SNEDDS was introduced into 10ml measuring cylinder. Initial volume was noted and cylinder was allowed to fall under its own weight into a hard Surface from a height of 2.5cm at 2sec intervals. Tapping was kept up until there was no more audible change.. Bulk density and tapped density were calculated using the following equation.

$$\text{Bulk Density} = \frac{\text{Weight of powder}}{\text{volume of powder}}$$

$$\text{Tapped Density} = \frac{\text{Weight of powder}}{\text{tapped volume}}$$

### 2.8.2.3 Compressibility Index

Carr's compressibility index (%) = TD-BD/TD × 100

$$\text{Carr's Idex} = \frac{\text{Tapped Density} - \text{Bulk Density}}{\text{Tapped Density}} + 100$$

### 2.8.2.4 Hausner Ratio

Hausner ratio is a number that is correlated to the flow ability of powder material. Can be calculated by following equation

$$\text{Hausner Ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}}$$

### 2.9 Pharmacokinetic studies

Pharmacokinetic studies were carried out using albino rats to evaluate plasma levels of Rosuvastatin. Three groups of animals (n = 3) were created.). All animal studies were performed and certify that proposal no IAEC-III-PRIP-FEB-2023 after approval of the protocol 05 PULLA REDDY INSTITUTE OF PHARMACY-Ethics Committee. Formulations were given orally by using oral feeding cannula. Control group A: was orally administered with Saline (at 0.5ml/kg body weight to albino rats Standard Group B: Rosuvastatin calcium pure drug was orally administered with (dose equivalent to 40 mg/kg body weight of albino rats) distilled water. Test group C: L-SNEDDS (dose equivalent to 40 mg/kg body weight of albino rats) re-dispersed in about one millilitre of distilled water. The rats were anesthetized using diethyl ether and blood samples (0.2 ml) were withdrawn from the tail vein of a rat at 0 (pre-dose), 1, 2, 4, 6, 8, 12, 16, and 24 hr. Micro centrifuge tubes coated with EDTA were used to collect the samples. After collection, blood was centrifuged at 10,000 rpm for 10 min, and plasma was separated and kept at −20°C for further processing through HPLC. Frozen plasma samples were thawed at room temperature. The samples were then centrifuged again at 5000 rpm for 5 min and the supernatant (10 µL) were filtered

and directly injected into the HPLC column and peak area values were recorded. To precipitate the protein, 100 µL of plasma samples were separated, and 0.9 ml of Acetonitrile was added to each sample. Rosuvastatin concentration-time profile in plasma after oral delivery was determined by Pharmacokinetic parameters ($C_{max}$: maximum concentration of drug, AUC0-24: area under the curve between 0 and 24 h and t1/2: half-life) in plasma for Rosuvastatin Calcium pure drug and optimized L-SNEDDS were evaluated by using software (lab solutions) HPLC Shimadzu for each group. All the data were statistically analyzed 19, 20, and 21].

### 2.9.1 Preparation of Standard Solutions
The stock solution of RC was prepared by dissolving accurately weighed quantity of 10 mg of the drug in 10 ml of Acetonitrile. From this stock solution, standard solution containing 100µg/ml RC was prepared by suitably diluting the appropriate volume of stock solution with mobile phase. Different calibration standards ranging from 5, 10, 15, 20, 25 and 30 µg/ml were prepared by appropriate dilution of standard solution (100µg/ml) with mobile phase.

### 2.9.2 Preparation of sample solution
Take 0.2ml of L –SNEDDS (CT8P2) 3:1 and make up with Acetonitrile this solution was sonicated for 15 min and filtered through whatsman filter paper no. 41. Further dilution was done with mobile phase to get concentration of 10 µg/ml.



### 2.9.3 Protein precipitation method
Choose an appropriate receptacle for the precipitation process. This experimental procedure necessitates the addition of significant quantities of extra liquid to the original sample, thus the container must be adequately spacious and suitable for centrifugation. The maximum sample sizes for each container volume are as follows: 1.5 ml can accommodate up to 0.16 ml of sample, 2 ml can hold up to 0.22 ml, 10 ml can contain up to 1.1 ml, 15 ml can accommodate up to 1.6 ml, and 50 ml can hold up to 5.5 ml. Place the sample into the chosen container. Take note of the sample volume, as this information will be used to determine the appropriate amount of reagents to add in subsequent steps. Thoroughly mix the plasma sample with volumes of methanol. Utilize a vortex mixer to ensure proper mixing. Centrifuge the mixture at 5000 rotation per minute and -4°C for five minutes. After centrifugation, it is expected that two distinct phases will be observed:  The protein of interest will be located at the interface between these two phases. It may appear as a white film, or it may not be visible at all if the protein concentration is low [22, 23, 24].
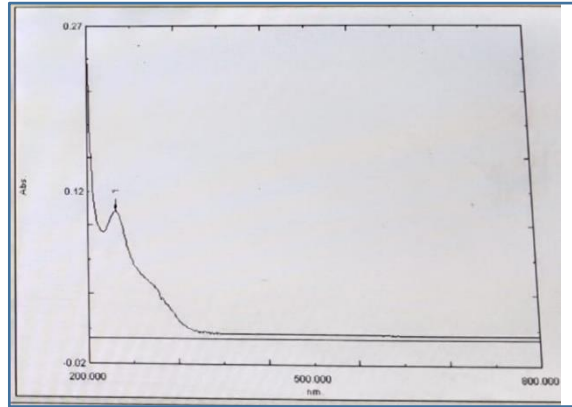
## 3. RESULTS AND DISCUSSION

**3.1 Standard graph of Rosuvastatin calcium:** Determination of $\lambda$ max of Rosuvastatin calcium in Methanol $\lambda$ max of Rosuvastatin calcium in methanol was found to be 241nm. 239nm found in 0.1N Hcl.

**Fig1: UV spectrum of ROS in methanol**
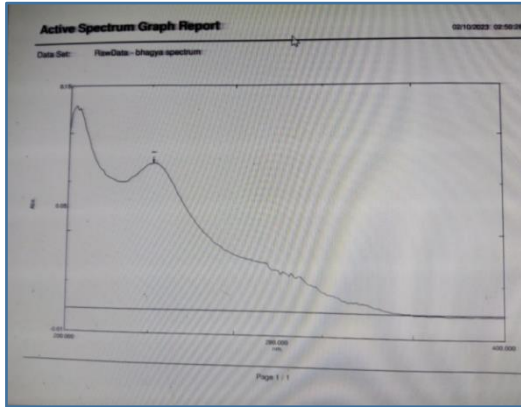


**Fig 2: UV-spectrum of ROS in 0.1N Hcl**



## 3.2 Calibration Curve of Rosuvastatin Calcium in Methanol & 0.1n Hcl

The standard graph was plotted by taking the Absorbance on Y-axis and Concentration on X-axis. The regression coefficient was found to be 0.9972, 0.9812.

**Table 1: Calibration of Rosuvastatin calcium in methanol**

| Concentration(µg/ml) | Absorbance |
|---|---|
| 1ppm | 0.106 |
| 2ppm | 0.158 |
| 3ppm | 0.232 |
| 4ppm | 0.285 |
| 5ppm | 0.347 |

**Figure 3: Standard Graph of Rosuvastatin Calcium in Methanol**
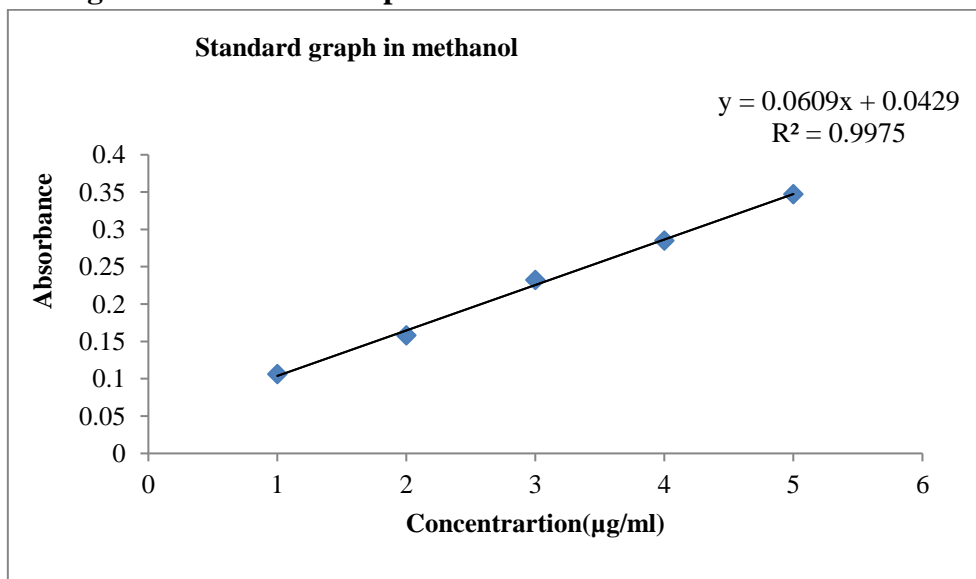
**Table 2: Calibration of Rosuvastatin calcium in 0.1N HCL**

| Concentration (µg/ml) | Absorbance |
|---|---|
| 1ppm | 0.011 |
| 2ppm | 0.016 |
| 3ppm | 0.021 |
| 4ppm | 0.028 |
| 5ppm | 0.03 |

**Figure 4: Standard graph of Rosuvastatin calcium in 0.1N Hcl**



Calibration of Rosuvastatin calcium in 0.1N Hcl

$y = 0.005x + 0.0062$
$R^2 = 0.9812$
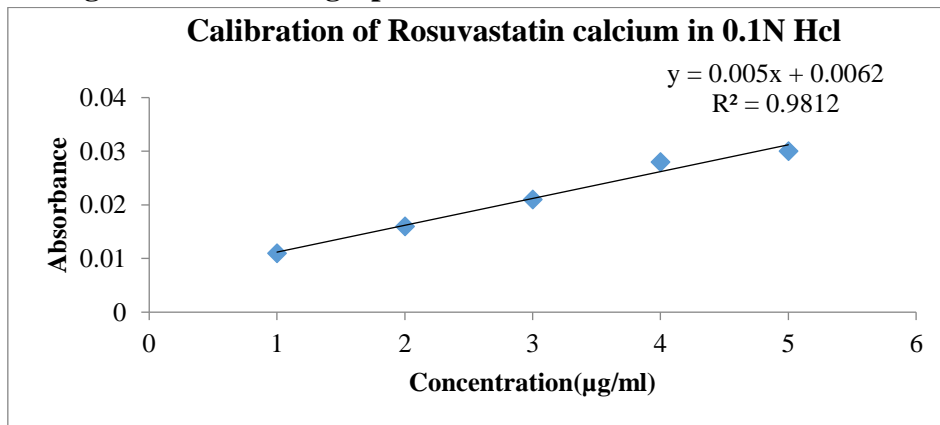
## 3.4 Solubility studies of Rosuvastatin calcium

Solubility of Rosuvastatin in various oils was determined by UV spectrophotometer. The saturation solubility of Rosuvastatin in various oils is shown in table Capmul PG 8NF oil was selected for the formulation Solubility of Rosuvastatin calcium was determined in various surfactants from these surfactants Tween 80 is selected for formulation which has highest solubility and good emulsifying ability. Solubility of Rosuvastatin calcium in various co-surfactants was determined. From this span80 selected for the formulation

(All values are expressed as Mean (n=3).

**Figure 5: Solubility of Rosuvastatin calcium in various oils**

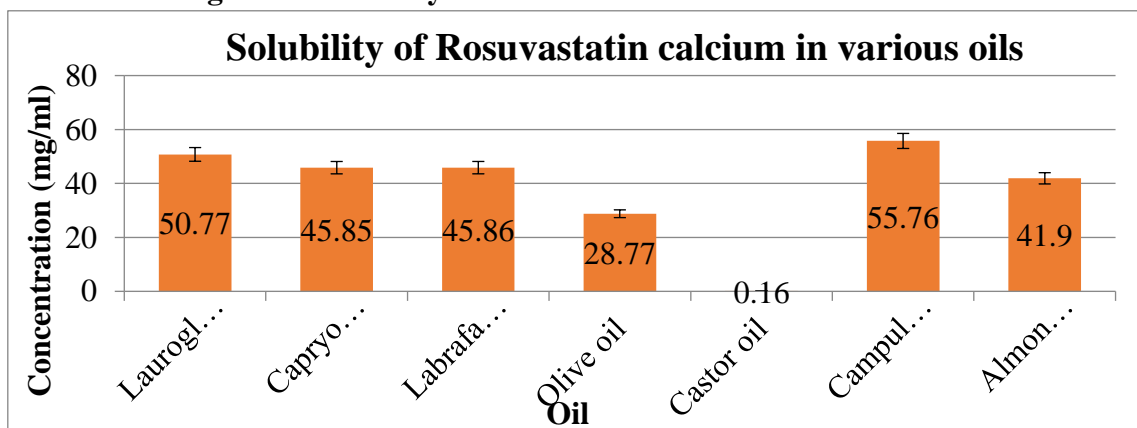

Solubility of Rosuvastatin calcium in various oils

Figure 6: Solubility of Rosuvastatin calcium in various surfactants



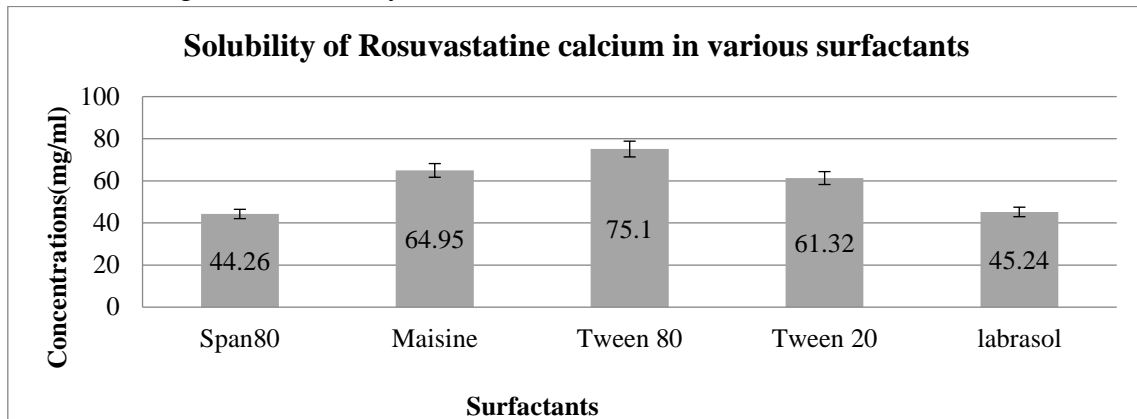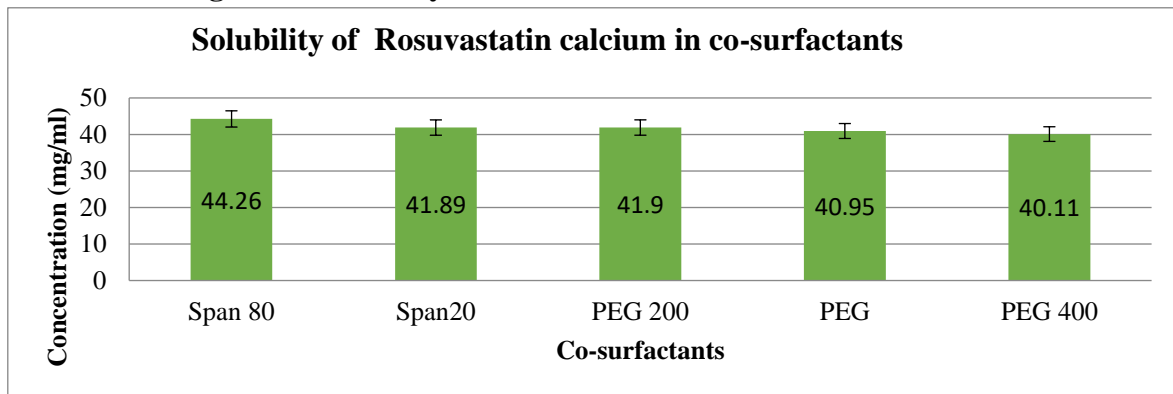Figure 7: Solubility of Rosuvastatin in various co-surfactants



## 3.5 Pseudo- ternary phase diagrams:

Pseudo – ternary phase diagrams are constructed to identify the Nano emulsion and to identify suitable composition of oils, surfactant and co-surfactant for formulation of SNEDDS. From pseudo- ternary phase diagrams, it has been found that the systems consisting of Campul PG 8 NF as oily phase tween80 as surfactants and span 80 as co-surfactant. for Smix 3:1 ratio formulation $CT_8P_2$ showed clear transparent emulsion for Oil: Smix 2:8, 4:6; formulation $CT_8P_2$ showed milky white emulsion for Oil: Smix 1:9, 5:5, 6:4,7:3, 8:2, 9:1.Based on the solubility studies done on various oils, surfactants and co-surfactants, excipients which have shown more solubility were selected for the formulation.
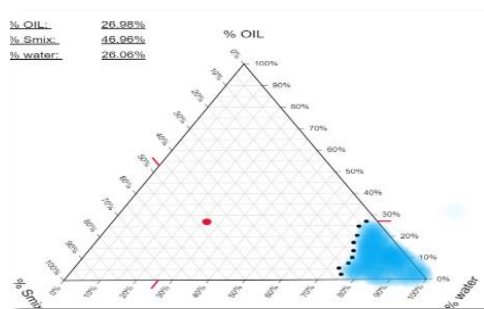
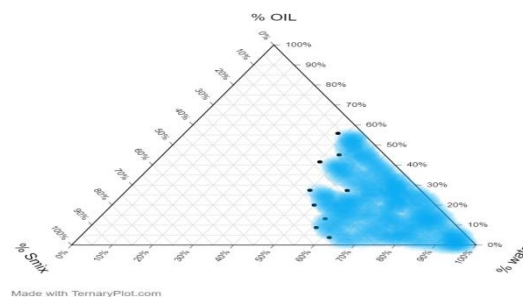**Figure 8:Smix (3:1)**          **Figure 9: Smix (4:1)**

**Figure 10: Pseudo-ternary phase diagram of optimized formulation (3:1 & 4:1)**



## 3.6 Size and Zeta potential determination of optimized formulation

Prepared formulations are analyzed in zeta size for the determination of size and potential, PDI. The droplet size was found to be in between 12.07 to 150nm and PDI of all the formulation was found to be less than 0.5, zeta potentials -12.6 to 15.2mV. Hence there is uniform distribution of particle size and zeta potential. The optimized formulations CT8P2 (3:1) results are shown in the following figure. CT8P2 has less particle size and best formulation and it should stable for a period of time compared to other formulations.

Table3: Size and Zeta potential determination (L-SNEDDS)

| Oil: Smix | Size of emulsion droplets | Region | Zeta potential | PDI |
|---|---|---|---|---|
| 3.1 CT8P2(1:9) | 12.07nm | Nano | -12.6 | 0.123 |
| 3.1 CT8P2(2:8) | 150nm | Nano | -15.2 | 0.268 |

**Table4: Size and Zeta potential determination (S-SNEDDS)**

| Oil: Smix | Size of emulsion droplets | Region | Zeta potential | PDI |
|---|---|---|---|---|
| 3:1 CT8P2 (1:9) | 12.07nm | Nano | -24.7 | 0.123 |
| 3:1 CT8P2 (2:8) | 150nm | Nano | -29.4 | 0.268 |
| 4:1 CT8P2 (1:9) | 31.20nm | Nano | -7.02 | 0.525 |

## 3.7 Evaluation of Rosuvastatin Calcium SNEDDS

**3.7.1 Self-emulsification and visual assessment:** According to visual assessment formulations are graded for self-emulsification time. Self-Emulsifying mixtures should disperse rapidly in aqueous medium with mild shaking. Self-Emulsification time that was determined for prepared SNEDDS the prepared SNEDDS of Rosuvastatin Calcium were emulsified less than 1min (24-30 sec) all optimized formulation was said to be good.

**Table 5: Self-emulsification time (seconds)**

| Formulation Name | Self-Emulsification Time(Min)With Dist. Water | Result |
|---|---|---|
| CT8P2 (3:1)(1:9) | 24± 0.09s | Good |
| CT8P2 (3:1)(2:8) | 30± 0.77s | Good |

### 3.7.2 Cloud point measurement

The optimized SNEDDS formulation were diluted with distilled water (1:250) Diluted sample are placed in water bath the temperature was increased gradually Cloud point was determined spectrophotometer as shown in the table. (n=3)

**Table 6: cloud point measurement**

| Formulation | Cloud point temperature(◦C) |
|---|---|
| 3:1 ct8p2(1:9) | 60±0.08 |
| 3:1ct8p2(2:8) | 80±1.15 |

### 3.7.3 Dispersibility test

The optimized formulation showed the following results when the test is performed in distilled water and result as shown in table (n=3). Dispersibility test which gives slightly appearance of blue colour.

**Table 7: Dispersibility test (n=3)**

| Sl. No | Formulation | Observation | Grade |
|---|---|---|---|
| 1 | 1:9 CT8P2 | Rapidly forming clear emulsion with slightly blue appearance | A |
| 2 | 2:8 CT8P2 | Appearance of blue | B |

### 3.7.4 Phase Separation and Stability Study of Emulsions

The prepared formulations are observed for precipitation and phase separation of drug at intervals 2, 4, 6, 8, 12, 24 hrs. Periods of time and it was found that all the formulations showed neither precipitation nor phase separation of drug. As in the following table.

**Table 8: Phase separation and precipitation of the drug (n=3)**

| Sl. No | Formulation | Precipitation | Phase separation |
|---|---|---|---|
| 1 | CT8P2(1:9)(3:1) | No | No |
| 2 | CT8P2(2:8)(3:1) | No | No |

### 3.7.5 Robustness to Dilution

No precipitation (or) phase separation is found which indicates all formulation are robust to dilution No - indicates no phase separation and no precipitation. As shown in table.

**Table 9: robustness to dilution (n=3)**

| Sl. No | Formulation | Distilled water | 0.1N HCL |
|--------|-------------|-----------------|----------|
| 1 | CT8P2(1:9)(3:1) | No | No |
| 2 | CT8P2(2:8)(3:1) | No | No |

### 3.7.6 Percentage Transmittance

Each diluted samples was observed for % transmittance at 241nm. All the formulations showed % transmittance more than 95% indicating emulsions. All values are expressed as Mean± SD (n=3)

**Table 10: Percentage Transmittance**

| Sl. No | Formulation | Distilled water | 0.1N HCL |
|--------|-------------|-----------------|----------|
| 1 | CT8P2(1:9)(3:1) | 98.82± 0.78% | 98.72± 0.7% |
| 2 | CT8P2(2:8)(3:1) | 95.76±0.68% | 97±0.096% |

### 3.7.7 Drug loading efficiency

40mg of each SNEDDS formulation was diluted with 100ml Methanol and 0.1N Hcl. Resultant solutions are analyzed UV Spectrophotometricaly following suitable dilution. Absorbance of each solution is measured at 241nm. The formulations have drug loading efficiency more than 90%.                All values are expressed as Mean± SD (n=3)

**Table 11: Drug loading efficiency of formulations**

| Sl. No | Formulation | Drug loading efficiency |
|--------|-------------|------------------------|
| 1 | CT8P2(1:9)(3:1) | 95.23±0.34% |
| 2 | CT8P2(2:8)(3:1) | 85.05±0.01% |

### 3.7.8 FT-IR Studies of Rosuvastatin Calcium Pure Drug

The spectrum of drug- excipient mixtures and the formulation so obtained were compared with spectrum of pure drug for any interactions. FT-IR spectrum of pure drug and the formulation were almost similar because of same functional groups. It indicates there was no interaction between Rosuvastatin and excipients used in formulation. As shown in figure 14
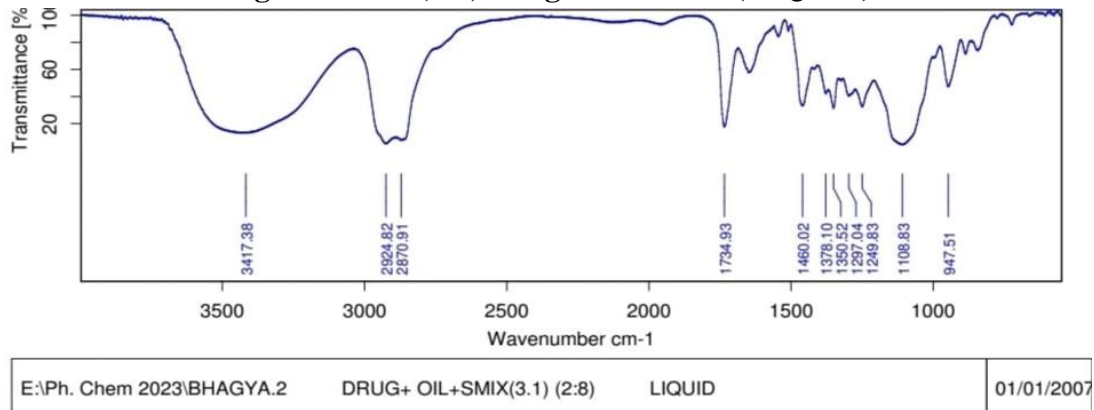
**Figure 14: FT-IR SPECTRUM OF PURE DRUG**
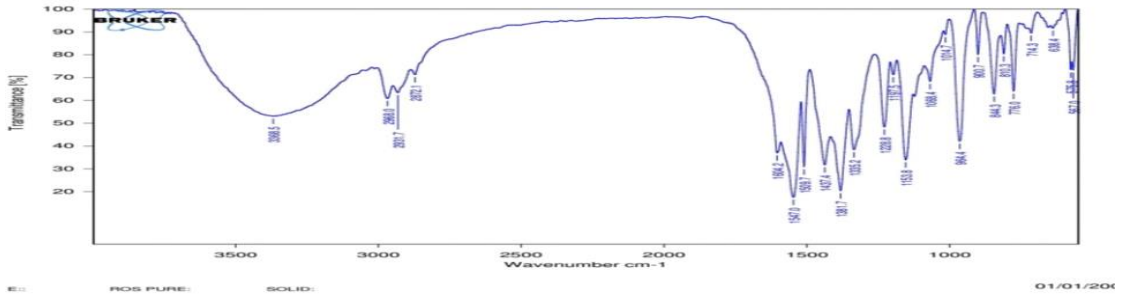


**Figure 15: 3:1(1:9) Drug +Oil + Smix (LIQUID)**
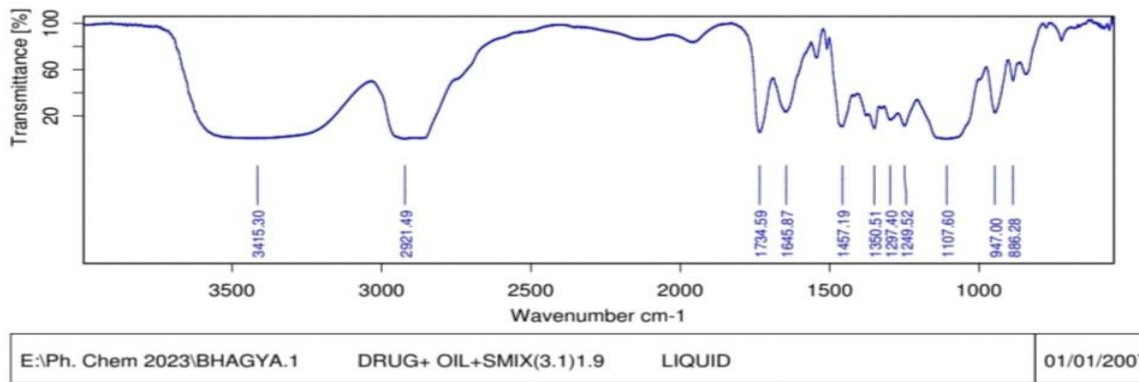


**Figure 16: 3:1(2:8) Drug +Oil +Smix (LIQUID)**
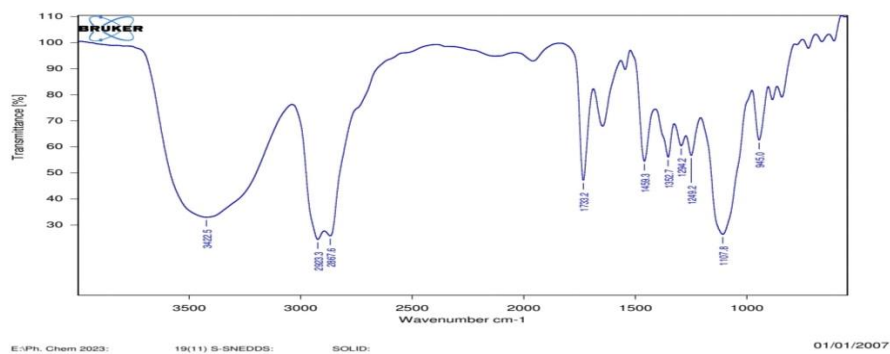


**Figure 17: 3:1(1:9) (SOLID - SNEDDS)**
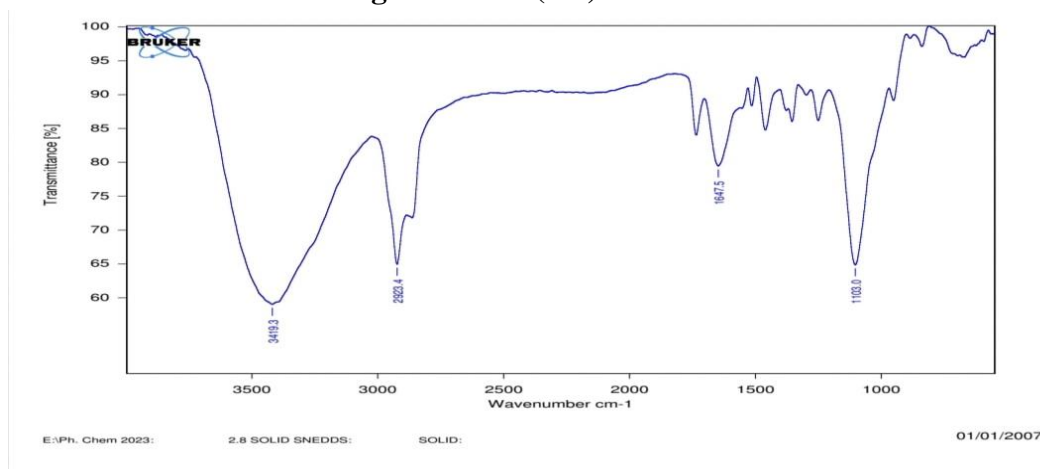
**Figure 18: 3:1(2:8) S-SNEDD**



**Table 12: INTERPRETATION OF PURE DRUG & SNEDDS FORMULATION**

| Functional Group | Pure drug | CT8P2 L-SNEDDS 3:1(1:9) | CT8P2 L-SNEDDS 3:1(2:8) | CT8P2 S-SNEDDS 3:1(1:9) | CT8P2 S-SNEDDS 3:1(2:8) |
|---|---|---|---|---|---|
| N-H | 3423.16 | 3415.30 | 3417.38 | 3415.30 | 3416.70 |
| C-H | 2924.14 | 2921.49 | 2924.82 | 29221.49 | 2924.31 |
| C=O | 1734.02 | 1734.02 | 1735.02 | 1734.59 | 1734.93 |
| C=C | 1646.93 | 1650.87 | 1650.44 | 1650.87 | 1645.27 |
| Alkanes | 1460.48 | 1457.19 | 1460.02 | 1457.19 | 1460.01 |
| C-O | 1352.75 | 1350.51 | 1378.10 | 1350.51 | 1378.09 |
| C-O/C-H | 1294.82 | 1297.40 | 1297.04 | 1297.40 | 1297.04 |
| C-N | 1249.61 | 1249.52 | 1249.83 | 1297.40 | 1259.02 |
| C-O-C | 1108.41 | 1107.60 | 1108.83 | 1105.57 | 1108.65 |
| Polysaccharides | 945.53 | 947.0 | 947.51 | 944.00 | 947.0 |
| Aromatic group | 886.07 | 886.26 | 886.26 | 886.08 | 886.05 |

**3.7.9 Thermodynamic stability studies**

Thermodynamic stability study is designed to identify Meta stable formulation. The SNEDDS are subjected to centrifugation study Jond Freeze thaw cycle. The emulsions are stable during centrifugation at 3500 rpm and alternative temperature cycle of -20∘c and +25∘c.There is no precipitation and phase separation of formulations as shown in table. (n=3)

**Table 13: thermodynamic stability studies**

| Sl. No | Formulation | Centrifugation(3,500 rpm for 30 minutes) | Freeze thaw cycle(-20 |
|--------|-------------|------------------------------------------|-----------------------|
| 1 | CT8P2(1:9)(3:1) | Stable | Stable |
| 2 | CT8P2(2:8)(3:1) | Stable | Stable |

## 3.7.10 IN VITRO DISSOLUTION STUDIES IN 0.1N HCL

Figure 19: INVITRO % cumulative drug release of optimized SNEDDS formulation. Each value represents the mean ± SD (n=3).
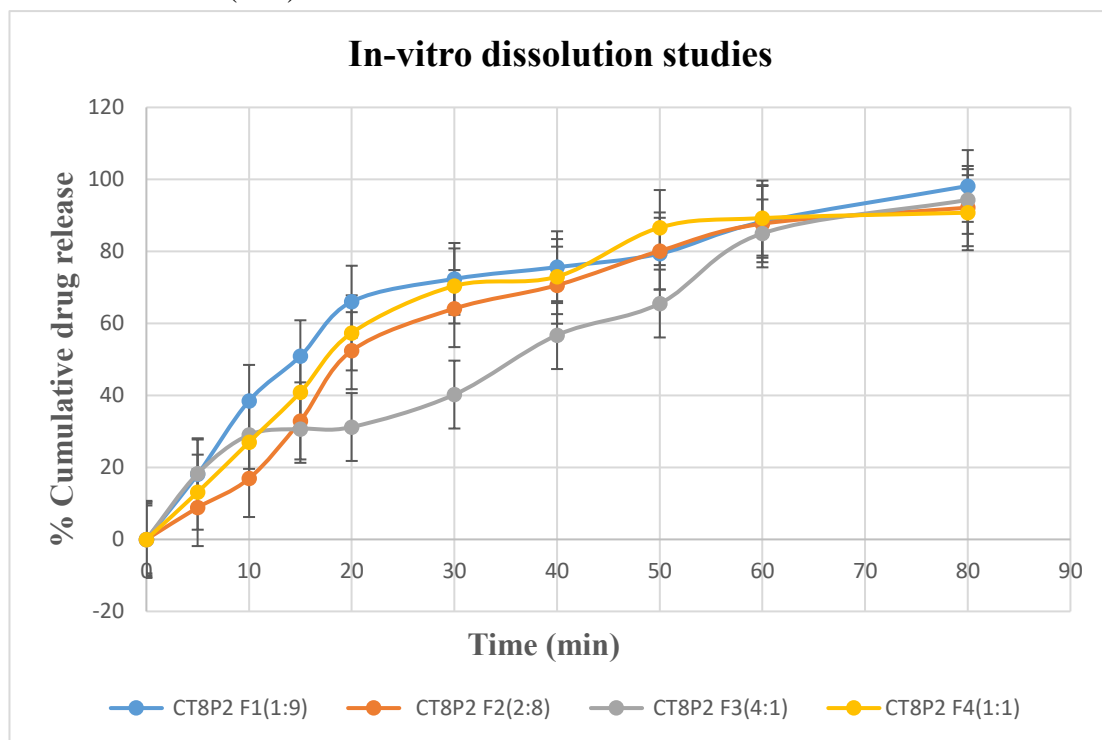


## 3.7.11 EVALUATION OF SOLID SNEDDS

All values are expressed as average standard deviation (n=3)

**Table 14: Evaluation of optimized S- SNEDDS**

| Formulation | Angle of repose | Bulk Density(g/ml) | Tapped Density(g/ml) | Carr's Index (%) | Hausner ratio |
|-------------|-----------------|--------------------|----------------------|------------------|---------------|
| CT8P2(1:9)(3:1) | 24.38± 0.05 | 0.46±0.04 | 0.54±0.07 | 18.2±0.4 | 1.08±0.05 |
| CT8P2(2:8)(3:1) | 19.95± 0.03 | 0.48±0.03 | 0.68±0.04 | 20.11±0.6 | 1.25±0.02 |
| CT8P2(1:9)(4:1) | 23.74± 0.02 | 0.44±0.04 | 0.61±0.77 | 15.3±0.2 | 1.39±0.04 |
| CT8P2(1:9)(1:1) | 20.65± 0.01 | 0.45±0.01 | 0.58±0.02 | 13.33±0.3 | 1.28±0.03 |

### 3.7.12 Linearity of Rosuvastatin Calcium in HPLC
### 3.7.13

**Figure 20: Linearity of Rosuvastatin calcium**



**Standard graph of Rosuvastatin calcium by HPLC**

$y = 0.060x + 0.042$
$R^2 = 0.997$
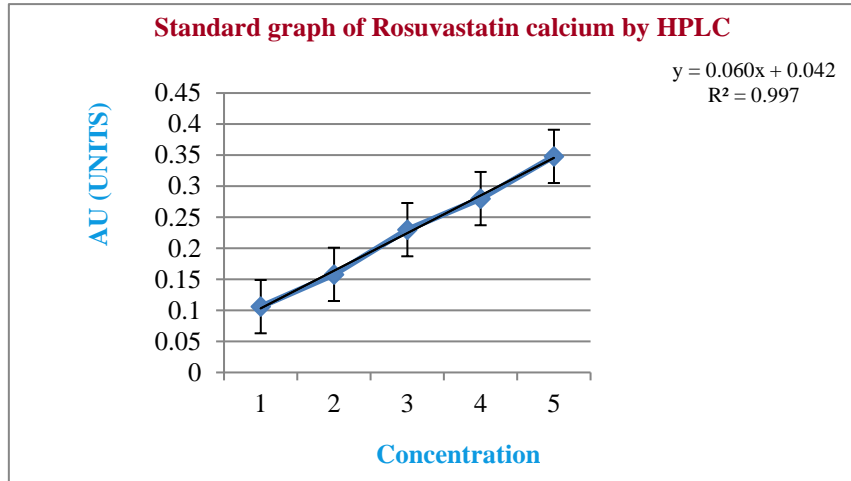
## 3.7.13 Pharmacokinetic Study of Plasma Drug Concentration

Bioavailability of Rosuvastatin Optimized SNEDDS Formulation(3:1)(1:9)



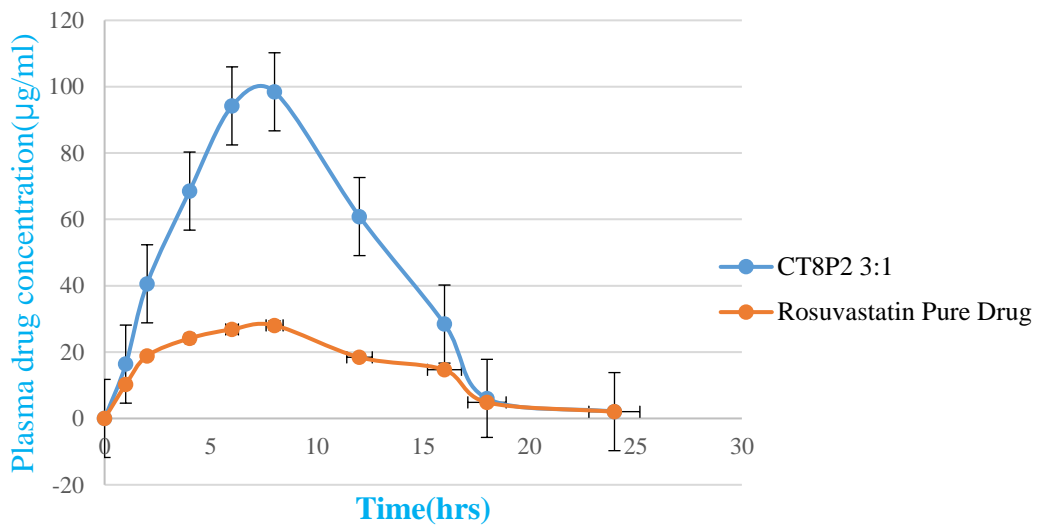**Figure 21: plasma drug concentration time**

**Table 15: Pharmacokinetic parameter**

| PK parameter | CT8P1 (3:1) | Rosuvastatin calcium |
|---|---|---|
| Cmax(g/L) | 98.45 | 28 |
| T max(hrs) | 8hr | 8hrs |
| AUC(mg/ml) | 117.4 | 40.17 |
| t(1/2) | 14.53 | 14.62 |

### 3.7.14 Stability Studies for Optimized Formulation CT8P2 (3:1) 1:9

From the dissolution study it was concluded that the formulation CT8P2 (1:9) 3:1 showed more % drug release than other formulation. So it is selected as the best formulation.

**Table 16: stability study of optimized formulation CT8P2**

| Time (months) | Particle size(d.nm) | PDI | Zeta potentials | Drug content (%) |
|---|---|---|---|---|
| 1 | 14.87 | 0.425 | -13.0 | 98.17±1.2% |
| 2 | 15.49 | 0.324 | -12.8 | 95.24±0.25% |
| 3 | 20.18 | 0.512 | -16.4 | 94.34±0.45% |

### 4. Conclusion

Drug compounds with low aqueous solubility and BCS classes II SNEDDS is a promising method. For lipophilic drugs, this is the best approach because the emulsification that results provides faster absorption and dissolution rates. Different self-emulsifying formulations of ROS based on its solubility data. Capmul MCM PG 8 NF Oil, Tween 80, Polyethylene glycol 200 compositions has shown best self-emulsifying property for ROS. The droplet size of the CT8P2 SNEDDS formulation was less than 200 nm, which resulted in better solubility of the compound. The optimized formulation complied with the requirement of zeta potential for stability. In-vivo Pharmacodynamics potential of SNEDDS formulation on albino rats. An optimized SNEDDS formulations with droplet sizes in the Nano range of less than 200 nm (12.07 nm) the in vitro drug release 98.17% within 80 minutes. When compared to pure drug suspension with optimized formulation had a greater anti-hyperlipidemic capability, according to an in vivo pharmacodynamics study performed on rats. Rosuvastatin calcium initial drug release profile from improved formulation F1 CT8P2(1:9) 98.45±1.25% release of drug which is significantly faster than that of Rosuvastatin calcium suspension 28.05±0.30%, according to in vitro drug release experiments.

### Conflict of Interest

All authors declare no conflict of interest

### Acknowledgement

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