JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Solubility Enhancement of selected statins using different approaches.

Miss. Mufashira Salim Bargir[1], Mr. Negesh C.[2]
Student[1] Professor[2]
Maratha Mandal College of Pharmacy Belgaum 590016 Karnataka

ABSTRACT

The main aim of the present research work was to improve bioavailability of Rosuvastatin calcium by preparing solid dispersion, solid lipid nano particles, and nono particles. These were prepared using different concentration surfactants, carrier and stabilizer by solvent evaporation method. The prepared formulation of solid dispersion, nano particles and solid lipid nano particles were evaluated for material balance, compatability studies, solubility, surface morphology, entrapment efficiency, drug content, zeta potential, *in-vitro* release profile, kenitic data analysis and stability studies

For solid dispersion nine total formulation were prepared DF1-DF9 among these DF7 was found to be better because it has solubility of 0.41±0.02, entrapment efficiency 68.95% and drug release was found to be 89.98±0.28%.

For solid lipid nano particles six total formulations were prepared (SF1-SF6) among these SF6 were found to be better because it has solubility of 0.56±0.01, entrapment efficiency 49.24% and drug release was found to be 93.54±0.92%.

For nano particles eight total formulations were prepared (NF1-NF8) among these NF4 was found to be better because it has solubility of 0.37±0.25, entrapment efficiency 57.12% and drug release was found to be 91.23±0.05%.

Selected formulation DF7, SF6, and NF4 were used for stability studies. Stability data reveals that the preparation are found to be stable with negligible in content, solubility, dissolution etc. Among the different techniques of solubility enhancement NF4 was found to be best formulation.

Key words:- Rosuvastatin calcium, solid dispersion, solid lipid nanoparticles and nanoparticles. Solvent evaporation , solubility, drug entrapment and *in-vitro* studies.

INTRODUCTION

The therapeutic effectiveness of drug depends upon the ability of the dosage form to deliver the medicament to its site of action at a rate and amount sufficient to elicit the desired pharmacological response. This attribute of the dosage form is reffered to as physiologic availability, biologic availability or simply bioavailability. For most drugs, the pharmacologic response can be related directly to plasma levels. Thus the term bioavailabilty is defined as the rate and extent to which the ingredients active moiety is absorbed form the drug product and becomes available at the site of action. A drug with poor bioavailability is due to poor aqueous solubility, slow dissolution rate in biological fluids, poor stability of dissolved drug at physiological p^H, extensive presystemic metabolism. Bioavailability of poorly water soluble drug is major problem[1].

For most medicines that are taken orally, the active ingredients are released in the gastro intestinal (GI) tract and arrive at their site of action via the systemic circulation. Blood concentration of the active ingredients and or their activemetabolites there by provide a marker for the concentration at the site of action and valid measure of bioavailability.

A blood concentration ; time curve (achieved by serial measurement over time) reflects not just the release of the active ingredients from the dosage form and its absorption from the GI tract, but also other factor including pre systemic metabolism, distribution and elimination.
A hypothetical drug given orally has a bioavailability of 50%, this is due to.

- Incomplete absorption of drug in GI tract so that only 70% of the intial dose isabsorbed.
- Subsequent metabolism of a further 20% before it reaches the systemiccirculation (first pass through the liver).

Therefore only 50% of the original oral dose reaches the systemic circulation[2].

Various physiological factors reduce the availability of drugs prior to their entry into the systemic circulation, These may be, Physicochemical properties of the drug (hydrophobicity, P^{Ka}, solubility.), the drug formulation (Immediate release, excipients used, manufacturing methods, modified release delayed release, extended release etc), whether the drug is administered in a fed or fasted state, gastric emptying rate, enzyme induction/ inhibition by other drug /food.

Poor solubility of a drug is in most cases associated with poor bioavailability. The contents of gastrointestinal tract are aqueous and hence a drug having poor aqueous solubility has a low saturation solubility which is typically correlated with a low dissolution velocity, resulting in poor oral bioavailability. About 10% of the present drugs are poorly soluble, about 40% of the drugs in the pipeline possess a poor solubility, and even 60% of drugs coming directly from synthesis have a solubility below 0.1 mg/ml[3].

Poor aqueous solubility of active pharmaceutical ingredients is due to the following reasons. These include,

- Inappropriate partition coefficient
- First-pass metabolism
- Degradation in the gastrointestinal tract

To improve oral bioavailability of drugs enhancement of solubility and dissolution rate are very important. Solubility is defiend as maximum quantity of solute that can enter in a certain quantity of solvent or quantity of solution at a specified temperature. As the solubility increase bioavailability increases. On the basis of solubility, solute/drugs are classified as per **table no 1:**

Table no 1: Definition of solubility

Definition	**Parts of solvent required for one part of solute**
Very soluble	<1
Freely soluble	1-10
Soluble	10-30
Sparingly soluble	30-100

Slightly soluble	100-1000
Very slightly soluble	1000-10,000
Insoluble	>10,000

BCS (Biopharmaceutics classification system) classify the drug in to four classes according to their solubility and permeability[4]. As for BCS class II & IV drugs rate limiting step is drug release from the dosage form and solubility in gastric fluid and not the absorption, so increasing the solubility in turn increase the bioavailability for BCS class II & IV drugs. BCS classification systems with examples are as follow[4].

- BCS class I (High permeability and high solubility)
- BCS class II (High permeability and low solubility)
- BCS class III (Low permeability and high solubility)
- BCS class IV (Low permeability and low solubility)here are various techniques available to improve the solubility of poorly solubledrugs. These are.

- The traditional method include,

a. Solid Dispersion,

b. Complexation and,

c. pH adjustments

- Newer methods includes,

a. Liquisolid

b. Hydrotropy

c. Sonocrystallization

d. Self emulsifying system[5].

- Novel formulation approaches,

a. liposomes,

b. microemulsion,

c. Nanoparticles,

d. solid dispersion,

e. solid lipid nanoparticle

f. nanocrystal.

In the present study attempts have been made to increase solubility of poorlysoluble drugs by solid dispersion, SLN, nanoparticles, nanocrystals.

Solid Dispersions:

The term solid dispersion refers to a group of solid products consisting of at least two different components, generally a hydrophilic matrix and a hydrophobic drug. The matrix can be either crystalline or amorphous. The drug can be dispersed molecularly, in amorphous particles (clusters) or in crystalline particles[6].

Solid lipid nanoparticles:

The solid lipid nanoparticles are spherical particulate system with average diameter between about 50 to 500nanometers in range. Solid lipid nanoparticles contain a solid lipid core matrix that can be solubilize lipophilic molecules. The lipid core is stabilized by surfactants like natural, synthetic and semi synthetic surfactants. SLN are more stable than liposomes due to their relatively rigid core consisting of hydrophobic lipid that are solid at room and body temperature, bounded by a monolayer of phospholipids[7].

Nanoparticles:

Nanoparticles are sub nanosized colloidal structures composed of synthetic or semisynthetic polymer. Nanosphers are solid core spherical particulate which are nanometric in size. They contain drug embedded within the matrixs or adsorbed on to the surface. Nanocapsules are vesicular system in which drug is essentially encapsulated with in the central volume surrounded by an embryonic polymeric sheath. In nanocystals drug is mainly encapsulated in the solution system[8]. For the present study rosuvastatin calcium was selected as a model drug. Which is suitable statin class will be selected, as majority of the statins are belongs to BCS II drug category. Statin (or HMG – COA reductase inhibitors) is a class of cholesterol lowering drugs that Inhibit the enzyme HMG- COA reductase which play a central role in the production of cholesterol. High cholesterol levels have been associated with cardio vascular disease. Statin have been found to reduce cardio vascular disease mortality in those who are at high risk[9].RESEARCH OBJECTIVES

The objective of the present investigation is to enhance the solubility of rosuvastatin calcium using different approaches.

Work is aimed to,

- Perform preformulation studies.
- Prepare solid dispersion, nanoparticle and solid lipid nanoparticle's usingdifferent excipients.
- Characterize the prepared solid dispersion, nanoparticles, solid lipidnanoparticles.
- Perform stability studies as per ICH guiddlines.

PLAN OF RESEARCH WORK

- Preformulation study ,
    a) Determination of melting point.
    b) Identification of drug.
    c) Compatibility study.
- Preparation of standard calibration curve for rosuvastatin calcium.
- Development of formulations.

Evaluation parameters as,

- Practical yield by mass balance ratio.
- Vesicle size by suitable method.
- Surface morphology of vesicle by scanning electron microscopy (SEM).

- Zeta potential measurement by zeta meter.
- Drug content by UV spectrophotometer.
- Drug entrapment efficiency by using an appropriate method.
- *In vitro* drug release profile.
- Stability studies.

METHODOLOGY

MATERIALS AND METHODS:

Materials used: The materials used were either AR/LR grade or the best possible grade available as supplied by the manufacturer without further purification or investigation.

Table No. 2: Materials Used for the Formulation

Sr.No.	Materials	Manufacture
1.	Rosuvastatin calcium	Micro Labs, Bangalore.
2.	Span 60	Vasa scientific co, Bangalore.
3.	Cholesterol	Vasa scientific co, Bangalore.
4.	SLS	Kemphasol,Mumbai
5.	Ethanol	Changshu yangyuan chemical,china.
6.	Polaxamer	Vasa scientific co, Bangalore.
7.	Eudragit	Yarrow chemical products, Bangalore.
8.	Acetone	Milton chemicals, Mumbai.
9.	Urea	Ozone international, Mumbai.
10.	Pvpk25	Loba chemie laboratory, Mumbai.
11.	PEG6000	Otto chemika biochemika reagents, Mumbai.
12.	Methanol	Himedia laboratory Pvt. Ltd Mumbai.

Table No. 3: Equipments and instruments used

Sr.No.	Name of Instrument	Manufacturer
1.	UV-Visible Spectrophotometer (Model UV-1800)	Shimadzu corporation, Japan.

2.	Dissolution Apparatus (TDT-08L)	Electolab Mumbai, India.
3.	Centrifugation	Remi motors.
4.	Sonicator	Equitron Instruments, Bangalore.
5.	Scanning electron microscope	JEOL-JSM-6360, Japan
6.	Contech electronic balance	Mumbai, India
7.	Zeta potential apparatus	Malvern Instrument Ltd
8.	Distillation apparatus	Bhanu scientific instruments Company, Bangalore.
9.	Test sieves	Jayant scientific Ind, Mumbai,
10.	Stability chamber	Remi instrument, Mumbai, India.

Preformulation studies:
Preformulation testing is an investigation of physical and chemical properties of drug substance alone and when combined with excipients. It is the first step in the rational development of dosage forms.
The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bio-available dosage forms. The scope of preformulation parameters maximizes the changes in formulating an acceptable, safe, efficacious and stable product.

Melting point determination:
Melting point of Rosuvastatin calcium was determined by open capillary method in Thiel's tube[43].

Identification of drugs:
Since every type of bond has a different natural frequency of vibration and since two of the same type of bond in two different compounds are in two slightly different environments, no two molecules of different structures have exactly the same infrared absorption pattern (infrared spectrum) and hence it is easy to identify the drug and other excipients by IR spectroscopy. The obtained sample was examined by infrared absorption spectral analysis and was compared with the reference standard IR spectrum of Rosuvastatin calcium[44].

Compatibility studies:
The infrared (IR) spectroscopic studies were used to find the interaction between pure drug, lipids, surfactants and its physical mixture by KBr pellet technique using FTIR spectrophotometer (Shimadzu, RX 1, Japan). The IR spectrum of the physical mixture was compared with the spectrum of pure drug (rosuvastatin calcium) to assess the compatibility of the excipients and drug.

Method- The pellet (pressed-disk) technique depends on the fact that dry, powdered potassium bromide (KBr) can be compacted under pressure in vacuo to form transparent disk. The sample (0.5-1.0 mg) is mixed with 100 mg of KBr in a mortar. Then the pressure of 10000-15000 psi is applied on mixture to form transparent disk known as pellet. This pellet is inserted in sample holder of IR spectrophotometer. IR radiations are passed through it to give absorption spectrum[36].

Preparation of 0.1N HCl:
Exactly measured 8.5ml of conc. HCl was taken in 1000ml volumetric flask and final volume was made up to 1000ml with distilled water to get 0.1N HCl.

Preparation of buffer and reagents:

Preparation of phosphate buffer (pH 7.4): Dissolve 2.38g of disodium hydrogen phosphate, 0.1g of potassium dihydrogen phosphate and 8.0g of sodium chloride in sufficient water to produce 1000ml.Adjust the pH, if necessary[45].

Determination of λ_{max}

The λ_{max} of Rosuvastatin calcium were determined in 0.1N HCl and pH 7.4 buffer solutions, which was scanned between 200-400 nm in the UV spectrophotometer.

Standard calibration curve of rosuvastatin calcium:

Accurately weighed 100mg rosuvastatin calcium was dissolved in 30ml of methanol and add 70ml of 0.1N HCl to get the first stock solution of 100µg/ml. From the 1st stock solution 1ml aliquot withdrawn and further diluted to 100ml with 0.1N HCl to get second stock of 100µg/ml. From the second stock, aliquot of 2ml,4ml,6ml,8ml and 10ml were withdrawn and make volume up to 10ml with by 0.1N HCl solution to get concentration of 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, & 10µg/ml respectively. The absorbance of the solutions was measured at 241nm by using UV-visible spectrophotometer.

Same procedure is followed to obtain standard calibration curve of Rosuvastatin calcium in phosphate buffer (pH 7.4), only difference is that we had used phosphate buffer (pH 7.4) instead of 0.1 N HCl as a dilution medium.

Preparation of solid dispersion of rosuvastatin calcium:

Solid dispersion were prepared by solvent evaporation method by dissolving required amount of drug and carriers in weight ratio of 1:3,1:6 and 1:9 into methanol:acetone 1:1 solvent system. The solvent was evaporated at 60^0c on water bath with continuous stirring to obtain dry mass. The dry mass was pulverized by passing through sieve no.44 followed by sieve no.60. The dried mass was stored in dessicator until further use[46].

Table no 4: Composition of solid dispersion of rosuvastatin calcium

Sl.no.	Ingredients (mg)	Formulation code								
		DF1	DF2	DF3	DF4	DF5	DF6	DF7	DF8	DF9
1.	Rosuvastatin calcium	100	100	100	100	100	100	100	100	100
2.	Urea	300	600	900	-	-	-	-	-	-
3.	PVP k25	-	-	-	300	600	900	-	-	-
4.	PEG6000	-	-	-	-	-	-	300	600	900
5.	Methanol	1ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
6.	Acetone	1 ml	1 ml	1ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
7.	Total	402	702	1002	402	702	1002	402	702	1002

Preparation of solid lipid nanoparticles of rosuvastatin calcium:

Solid lipid nanoparticles were prepared by solvent evaporation method. Rosuvastatin calcium, cholesterol and span 60are dissolved in ethanol and kept for some time in bath Sonicator. The aqueous medium is prepared by dissolving tween80distilled water and kept for stirring in magnetic stirrer for 15mins. The lipid phase is slowly added into the aqueous phase under continuous stirring. The nanoparticles dispersion is formed in the aqueous medium. The solution was kept in Homogenization at different RPM speed[7].

Table no 5: Composition of solid lipid nanoparticles of rosuvastatin calcium

SI.no	Ingredients (mg)	Formulation code					
		SF1	SF2	SF3	SF4	SF5	SF6
1.	Rosuvastatin Calcium	20	20	20	20	20	20
2.	Cholesterol	100	100	100	200	200	200
3.	Span 60	50	50	50	100	100	100
4.	SLN	0.5	1.0	1.5	0.5	1.0	1.5
5.	Ethanol (ml)	10	10	10	10	10	10
6.	Water	50	50	50	50	50	50
7.	Total	670	1170	1670	820	1320	1820

Preparation of Nanoparticles of rosuvastatin calcium:

Nanoparticles were prepared by Nanoprecipitation technique. Polymer was dissolved in acetone. The drugs were soluble in polymer/ acetone solution. This organic phase was added to an aqueous solution containing polaxamer. This final nanosuspension was centrifuged to separate the drug polymeric aggregates. Then it filtered through 0.22 µm membrane filter[8].

Table no 6: Composition of Nanoparticles of rosuvastatin calcium.

Sl.no.	Ingredients (mg)	Formulation code							
		NF 1	NF2	NF3	NF4	NF5	NF6	NF7	NF8
1.	Rosuvastatin Calcium	100	100	100	100	100	100	100	100
2.	Pvp k25	250	500	750	1000	-	-	-	-
3.	Eudragit	-	-	-	-	250	500	750	1000
4.	Polaxamer	10	10	10	10	10	10	10	10
5.	Acetone	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml
6.	Water	15ml	15ml	15ml	15ml	15ml	15ml	15ml	15ml

7.	Total	380	630	880	1130	380	630	880	1130

EVALUATION PARAMETERS:

I. Percentage yield:

Total amount of preparation of solid dispersion, nanoparticles and solid lipid nanoparticles obtained were weighed and the percentage yield was calculated; by considering the weight of drug and other excipients.

$$\%\ \text{yield} = \frac{\text{Actual weight of product}}{\text{Total weight of drug and excipients}} \times 100$$

II. Solubility:

Solubility of prepared formulation of nanoparticles solid lipid nanoparticles was tested in different solvents such as distilled water, acid buffer (pH 1.2) and phosphate buffer (pH 7.4). The excess amount of pure drug and preparation were added to the 150ml of different solvents in separate flask. Then this mixture was magnetically stirred for 24 hrs at 25^0 C temperatures. The supernatant liquid were collected and filtered through 0.2µ membrane filter and analyzed by UV visible atwave length 241nm and the solubility of pure rosuvastatin calcium were calculated[47].

III. Surface morphology study:

Shape and surface characteristics and size of solid lipid nanoparticles / nanoparticles were studied by using scanning electron microscope (SEM). The sample was placed in the scanning electron microscope (JEOL- JSM-6360, Japan) equipped with a digital camera, at acceleration voltage of 20kV and then photographs were taken[48].

IV. Zeta potential analysis:

Zeta potential analysis is done for determining the colloidal properties of the prepared formulations (SLNs, nanoparticles). The suitably diluted preparations are determined using zeta potential analyzer based on electrophorectic light scattering and laser Doppler velocimetery method the temperature is set at 25^0C. Charge on vesicles and their mean zeta potential values with deviation of 5 measurements are obtained directly from the measurements.

V. Entrapment Efficiency:

a) For solid dispersion.

Entrapment efficiency was determined by dialysis method. Weighed quantity of solid dispersion was placed inside the dialysis membrane and it was suspended suitably in a beaker containing 100ml diffusion medium (pH 7.4 phosphate buffers) which was constantly stirred at 37°±1°C on a magnetic stirrer for 10min and then transferred to next beaker containing same medium maintained in similar conditions. Each time samples were diluted suitably and assayed spectrophotometrically at 241nm using Shimadzu UV Spectrophotometer 1800. The same procedure is followed to separate unentrapped drug until negligible reading was obtained. The sum of each release gives the total unentrapped drug. The time required to release the unentrapped drug was noted. Entrapment efficiency was calculated by subtracting the amount of the drug dialyzed from the total drug used in the formulation.

$$\%\ \text{Entrapment efficicency} = \frac{\text{Total drug} - \text{Unentraped drug}}{\text{Total drug}} \times 100$$

Entrapped drug is cross checked by triturating the retained solid dispersion and then assayed spectrophotometrically at 241nm after suitable dilutions[49-51]

a) For solid lipid nanoparticles and nanoparticles.

The entrapment efficiencies of prepared solid lipid nanoparticles and nanoparticles were determined by measuring the concentration of free drug in the dispersion medium. The unentrapped drug was determined by adding 0.1ml of nanosuspension to 9.9ml methanol in order to dissolve the unentrapped drug the obtained suspension was centrifused for 45min at 6000 rpm. The supernatant was separated and then filtered through filter paper. The filtrate was diluted using methanol and measured spectrophotometrically[52].

The entrapment efficiency was calculated using the following equation**.**

$$\%\ \text{Entrapment efficicency} = \frac{\text{Total drug} - \text{Unentraped drug}}{\text{Total drug}} \times 100$$

II. Drug content:

Take equivalent quantity of weight of solid dispersion / nanoparticles /solid lipid nanoparticles. Containing Rosuvastatin calcium. Triturate the preparations in small quantity of 0.1N HCl in a mortar and transfer into 100ml volumetric flask. Rinse the mortar and pestle with 0.1N HCl and transfer into 100ml volumetric flask. Then make up the volume to 100ml of 0.1N HCl and sonicating for few min. Filter the solution by using whatman filter paper and from this first stock solution pipette out 1 ml solution and diluted to 100ml in another volumetric flask with 0.1N HCl. Absorbance of this second stock solution is seen in UV-visible spectrophotometer at 241nm to determine the drug content.

Same procedure is followed to determine drug content in phosphate buffer (pH 7.4) but only difference is that; here we have used pH 7.4 except 0.1N HCl solution.

II. Release studies:

a) For solid dispersion and nanoparticles:

Drug release studies of solid dispersion / nanoparticles formulations were carried out using USP type XXIV apparatus, using paddle by dialysis method. Initially solid dispersion / nanoparticles equal to one dose were taken in dialysis bag pre-soaked for 24hr and sealed it properly. Then these bags were placed in 900 ml of

0.1 N HCl for 2 hours then followed by pH 7.4 phosphate buffers for next 10hrs which was maintained at 37°C and paddle was rotating with the speed of 50rpm. The 5ml of sample were withdrawn manually at definite time intervals replacing with fresh buffer to maintain sink condition and analyzed for *in-vitro* drug release of rosuvastatin calcium using a UV-visible spectrophotometer at 241 nm[53].

Table 7: conditions of Release studies

Conditions for	Solid dispersion	Nanoparticles
Temperature	37^0C	37^0C
Volume of jar	900ml	200ml
Stirring	50	60

a) For solid lipid nanoparticles:

Drug release studies of SLN preparation were carried out by diffusion cell. Initially SLN equal to one dose were taken in celluffin membrane pre-soaked for 24hrs and sealed it properly. Then these membranes were placed in 20mainedl of 0.1N HCL for 2 hrs then followed by pH 7.4 phosphate buffers for next 10hrs which was maintained at 37^0C and stirre was rotating with speed of 50rpm. The 1ml of sample were withdrawn manually at definite time intervals replacing with fresh buffer to maintain sink condition and analyzed for in vitro drug release of rosuvastatin calcium using a UV visible spectrophotometer at 241nm.

II. Drug Release Kinetics and Data Analysis:

To analyze the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted into, zero order, first order, higuchi matrix and krosmeyer and peppas model. In this by comparing the r – values obtained, the best fit model was selected.

1. Zero order kinetics

Zero order describes the system where the release rate of drug is independent of its concentration. The equation is

$$C = C_o - K_o t$$

Where, C = Amount of drug release or dissolved

C_o = Intial amount of drug in solution

K_o = Zero order rate constant

t = time.

For study of release kinetics, the graph plotted between cumulative amount of drug released verses time. This relationship can be applied to describe the drug dissolution of drug from several types of modified release Pharmaceutical dosage form as in the case of some transdermal system as well as matrix tablet with low soluble drugs in coated forms, osmotic system. Such models are important in certain classes of medicines intended,

example for antibiotic delivery, heart and blood pressure maintenance, pain controland antidepressant.

1. First order kinetics

The drug release which follows the first order kinetic can be expressed by theequation,

$$\text{Log } C = \text{Log } C_o - Kt/2.303$$

Where, C =Amount of drug released.

C_o=Initial concentration of drug.

K =First order constant.

t = time.

The data obtained are plotted as log cumulative percentage drug remainingverses time, which yield a straight line with slop=K/2.303.

This relationship can be use to describe the drug dissolved in Pharmaceutical dosage forms like those contained water soluble drugs in porous material.[59]

II. Stability Studies:

The stability of the Solid lipid nanoparticles and nanoparticles formulation was ascertained by monitoring the physical appearance, particle size and percentage retention of drug, since instability of the formulation would reflect in drug leakage and a decrease in the percentage drug retained. To determine the stability of preparations, the optimized batch was stored in airtight sealed glass vials. At definite time intervals (0, 1, 2, 3 months), the solid dispersion was analyzed by UV-visible spectrophotometer at 241 nm for drug content, *in vitro* dissolution study. ICH guidelines suggests stability studies for the dry preparation meant for reconstitution should be studied for accelerated stability at 40ºC/75% relative humidity as per international climatic zones and climatic conditions[36-54]

RESULTS AND DISCUSSION

The present investigation was planned to conduct preformulation studies, formulation design, characterisation of prepared formulation and stability studies as per ICH guidelines.

PREFORMULATION STUDIES

Identification tests:

1. Determination of melting point

Melting point of rosurvastatin calcium was found to be 182ºCwhich is similar to the melting point which is reported i.e. 182ºC - 186ºC which confirms the purity of sample.

2. IR spectroscopy:

The IR spectrum of pure rosurvastatin calcium drug sample was recorded by FTIR spectrometer, which was compared with standard functional group frequencies. The spectrum of rosurvastatin calcium is shown in following **table 9.**

Table No. 8: The IR frequencies of Rosurvastatin calcium

Groups	Peaks (cm^{-1})
OH	3600-3500
CH-aromatic	3100-3010
CH-aliphatic	2990-2910
C = O	1700-1600
S = O	1600-1550
C = C	1550-1500
C ─ F	900-800
CNC	700-650

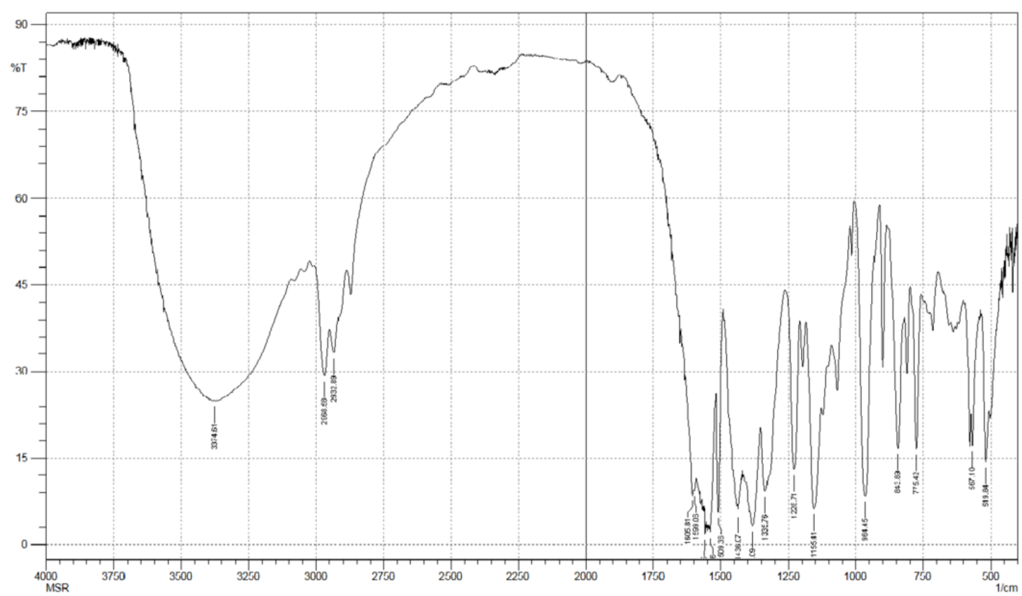

Figure No. 1: IR spectrum of Rosuvastatin calcium Drug excipients compatibility studies:

The proper design and formulation of a dosage forms require consideration of the physical, chemical and biological characteristics of all drug substances and excipients used in formulating the final products. The stable and effective solid dosage form depends on the careful selection of the excipients that are added in the formulations. The drug and excipients must be compatible with one another to produce a product that is stable, efficacious and safe. The characteristic peaks of rosuvastatin calcium are present in spectra at respective wavelengths which indicate the compatibility between drug and excipients.

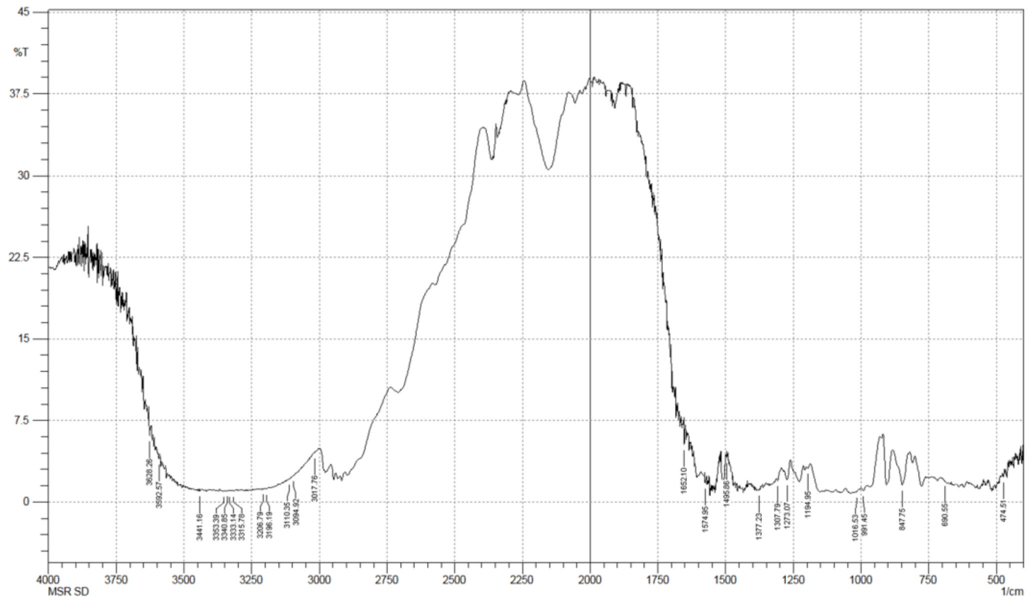

Figure No. 2: The IR spectrum of solid dispersion formulation

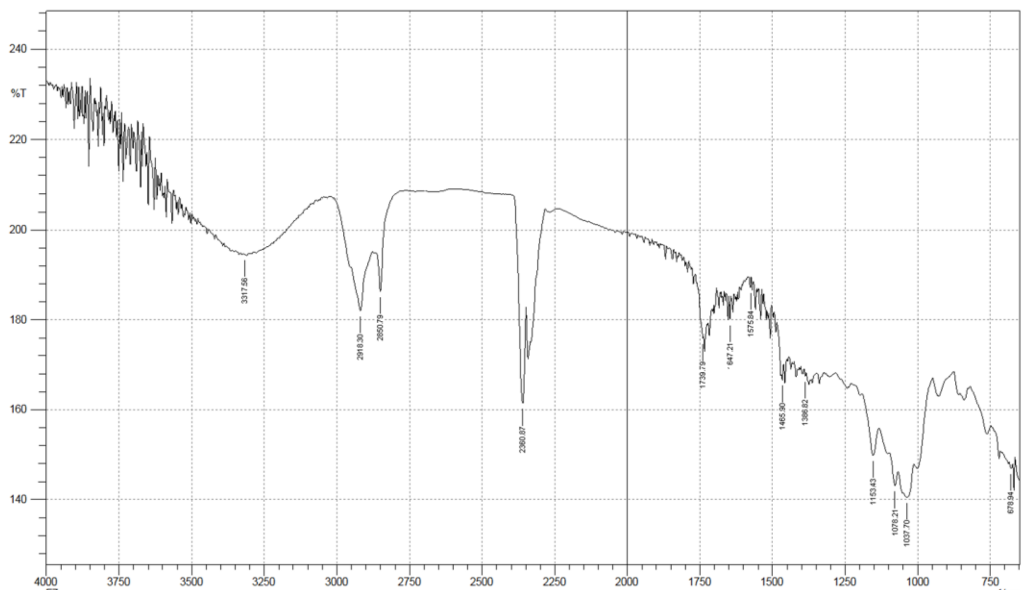

Figure No 3: The IR spectrum of solid lipid nanoparticles

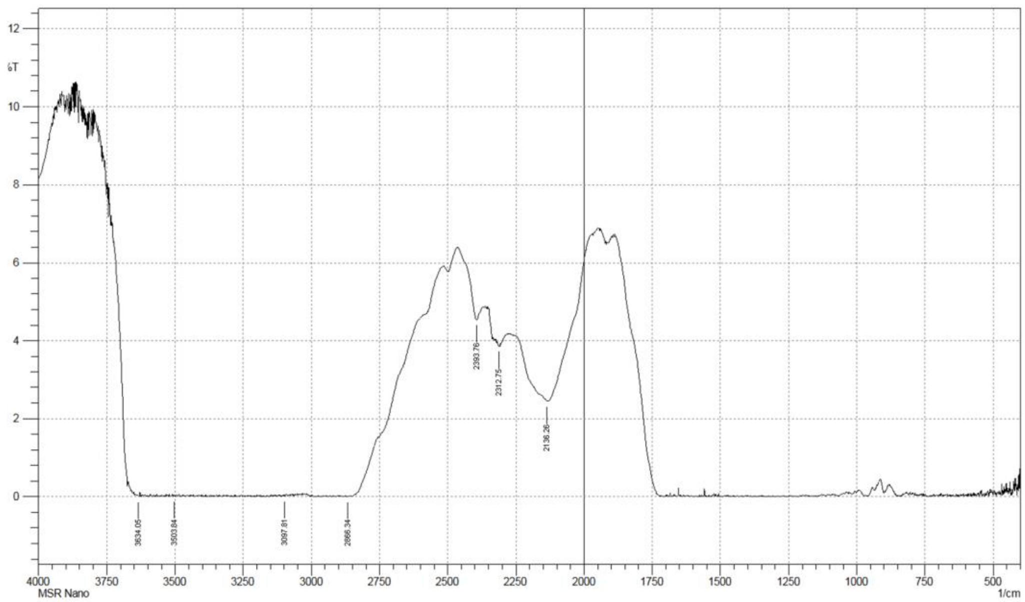

Figure No 4: The IR spectrum of nanoparticles

Determination of λmax of rosuvastatin calcium in water, 0.1N HCl and phosphate buffer of 7.4pH.

The separate solution of 100µg/ml concentration of rosuvastatin calcium was prepared in water, 0.1N hydrochloric acid and phosphate buffer of 7.4 pH and scanned over a range 200-400nm to determine its λ_{max}. The peak was observed at 241nm in water, 0.1N hydrochloric acid and phosphate buffer of 7.4 pH as shown in **figure no .9,10 and 11**. The observed λmax of rosuvastatin calcium is similar to that of standard λmax (241nm), so this result confirms the purity of drug.

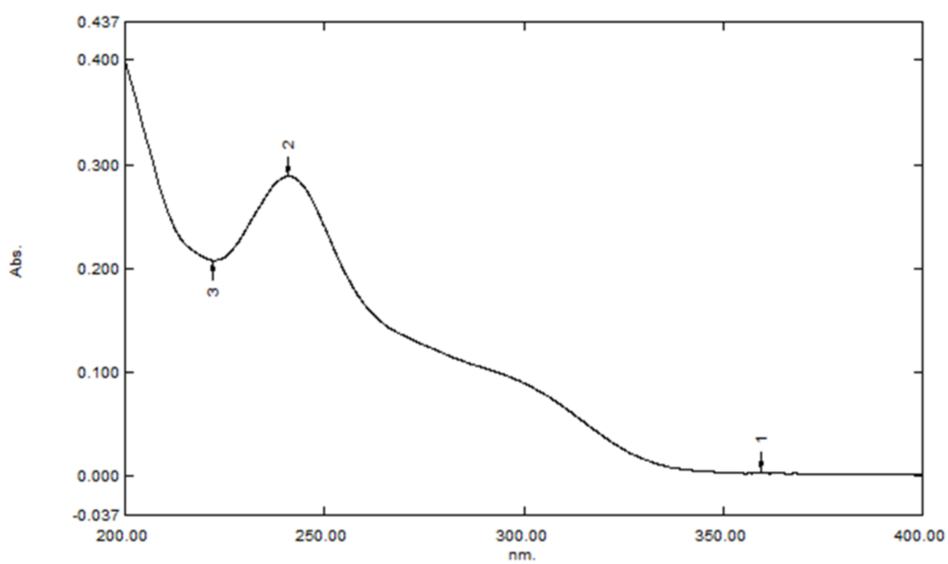

Figure No.5: UV – Visible spectra of rosuvastatin calcium in water

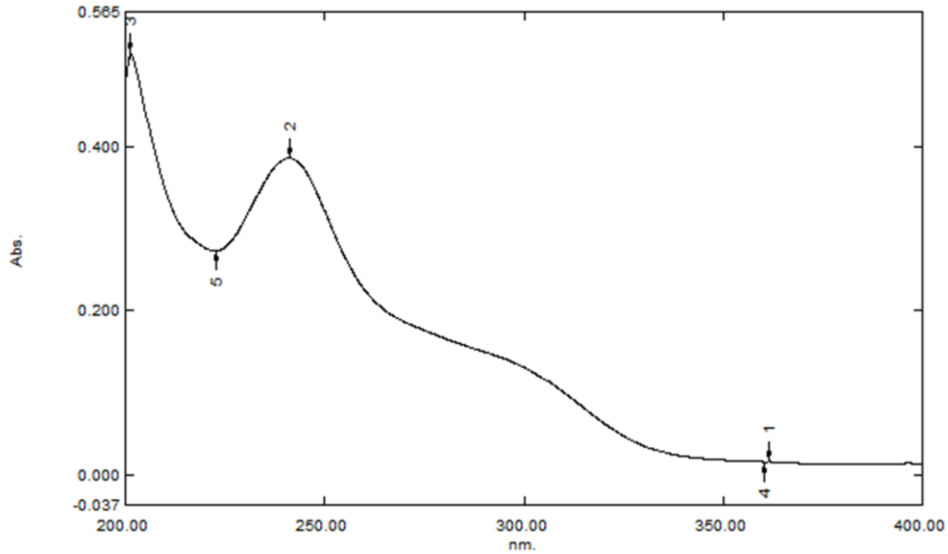

Figure No.6: UV – Visible spectra of rosuvastatin calcium in 0.1N HCl

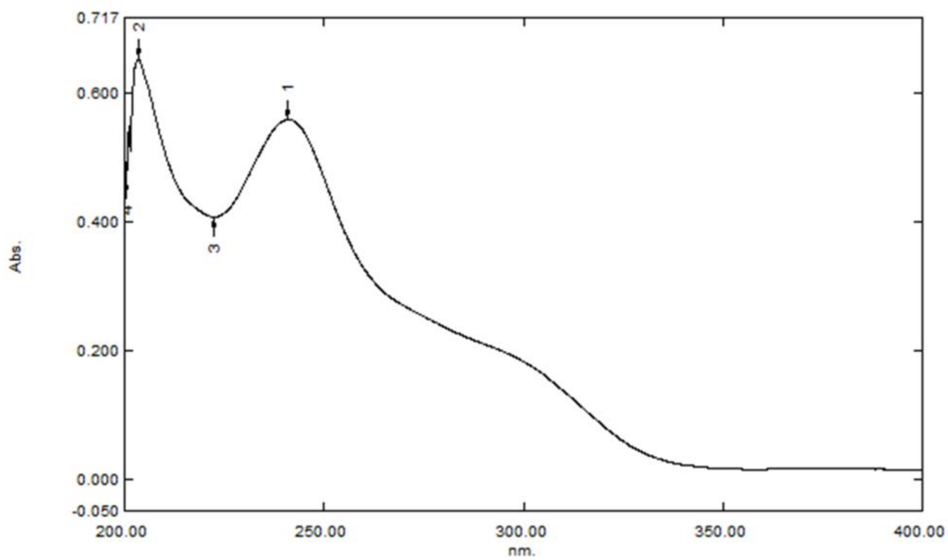

Figure No. 7: UV – Visible spectra of rosuvastatin calcium in phosphate buffer of 7.4 pH

Standard curve of rosuvastatin calcium in water, 0.1NHCland phosphate buffer 7.4pH.

The absorbance of rosuvastatin calcium standard solutions containing 2 to 10µg/ml (Beer's range) in water, 0.1N hydrochloric acid and phosphate buffer of 7.4 pH was shown in **figure no.12,13 and 14** respectively with slope and regression coefficient values. The curve was found to be linear in the range of 2-10µg/ml at λ_{max} 241nm in water, 0.1N hydrochloric acid and phosphate buffer of pH 7.4.Table No.9: Absorbance values of rosuvastatin calcium in water, 0.1 N HCl andphosphate buffer of 7.4 pH

Sr .no	Concentration (µg/ml)	Absorbance		
		Water	0.1 N HCL	7.4 Ph
1	0	0	0	0
2	2	0.08 ±0.001	0.093 ±0.002	0.085 ±0.003

3	4	0.162±0.001	0.184±0.004	0.161±0.0011
4	6	0.243±0.0011	0.273±0.0040	0.243±0.005
5	8	0.321± 0.001	0.366± 0.004	0.324± 0.013
6	10	0.406±0.001	0.450±0.0015	0.401±0.030

Note: The values presented are ± SD's of three determination

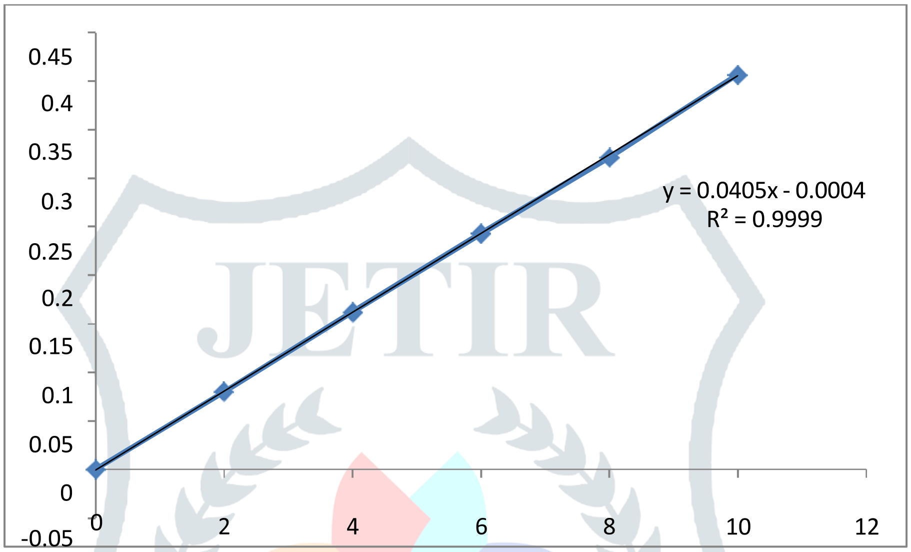

Figure No. 8: Standard curve of rosuvastatin calcium in water

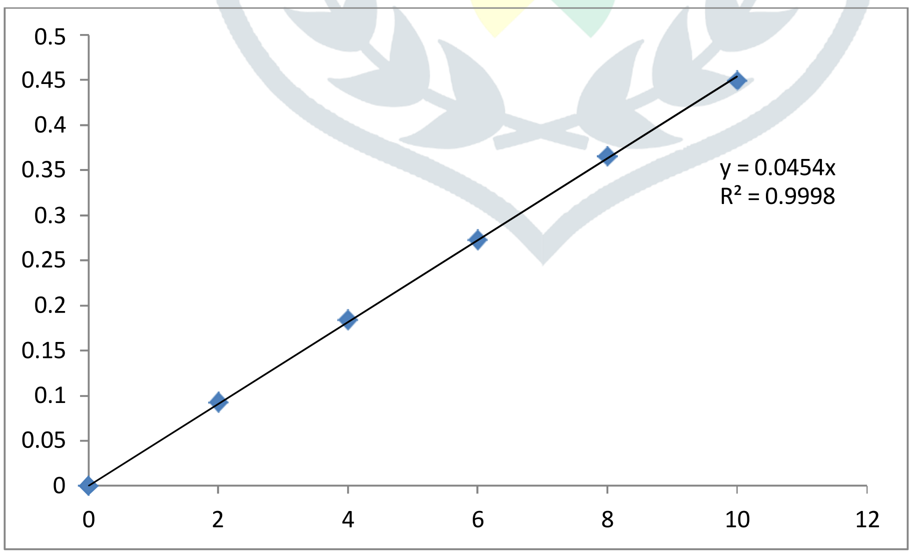

Figure No. 9: Standard curve of rosuvastatin calcium in 0.1N HCL

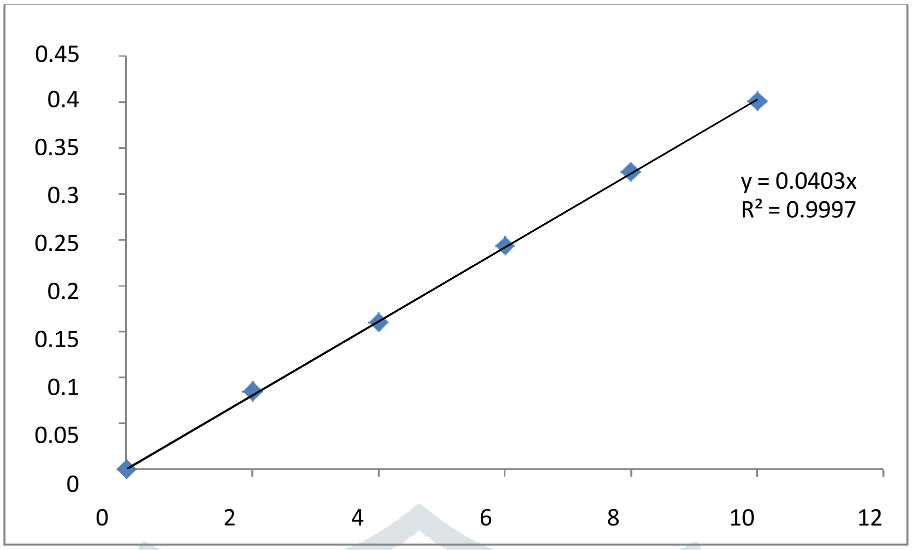

Figure No 10: Standard curve of rosuvastatin calcium in phosphate buffer ofpH 7.4

FORMULATION DESIGN:

The present study was carried out to develop solid dispersion, solid lipid nanoparticles and nanoparticles drug delivery system of rosuvastatin calcium, to enhance the solubility. We have prepared nine formulations of solid dispersion, six formulations solid lipid nanoparticles and eight nanoparticles formulations. The formulations were prepared as per **table no.4,5 and 6** by using solvent evaporation method.

EVALUATION PARAMETERS

1. Percentage yield:

The percentage yield of all the formulations were given **table no 10, 11 and**

12. The results indicated that the percentage yield of solid dispersion was good and found to be in the range of 96.50±0.5% to 94.59±0.16 due to less wastage of material during the formulation. Percentage yield of nanoparticles found in the range of 85.83±0.2 to 91.26±0.09 and solid lipid nanoparticles found in the range of 87.81±0.22 to 96.88±0.30.

The difference in the percentage yield values may be due to change in the concentrations of excipients present in the formulations.

Table No 10: percentage yield of solid dispersion

Sr no.	Formulation code	Percentage yield(%)
1	DF1	96.00±0.50
2	DF2	97.85±0.14
3	DF3	92.14±0.169
4	DF4	96.58±0.69
5	DF5	91.00 ±3.32
6	DF6	91.62±0.570
7	DF7	98.33±0.38

8	DF8	86.14±0.428
9	DF9	94.59±0.169

Table No 11: percentage yield of solid lipid nanoparticles

Sr no.	Formulation code	Percentage yield(%)
1	SF1	87.81±0.22
2	SF2	78.74±0.63
3	SF3	90.55±1.21
4	SF4	87.70±2.22
5	SF5	87.12±1.19
6	SF6	96.88±0.30

Table No 12: percentage yield of nanoparticle

Sr no.	Formulation code	Percentage yield (%)
1	NF1	85.83±0.20
2	NF2	97.37±0.59
3	NF3	97.74±0.17
4	NF4	99.51±0.20
5	NF5	97.50±0.55
6	NF6	94.86±0.62
7	NF7	99.22±0.29
8	NF8	91.26±0.09

2. Solubility:

Solubility data of rosuvastatin calcium solid lipid nanoparticles, nanoparticles and solid lipid nanoparticles formulation is shown in **table 13,14 and 15**. Amongall the formulations of solid dispersion, solid lipid nanoparticles and nanoparticles DF7 (0.42 mg/ml), SF6(0.36mg/ml and NF4(0.37mg/ml) are showing highest solubility in water as compared with pure rosuvastatin calcium (0.22mg/ml).

Table No 13: Comparison solubility of rosuvastatin calcium solid dispersion with pure rosuvastatin calcium in different solvent.

Formulation code (mg/ml)	Solvents		
	Water	Acid buffer(pH1.2)	Phosphate buffer(pH7.4)
Pure drug	0.10±0.02	0.18±0.01	0.22±0.02
DF1	0.08±0.02	0.10±0.02	0.12±0.02

DF2	0.07±0.05	0.08±0.02	0.15±0.01
DF3	0.09±0.03	0.10±0.05	0.13±0.05
DF4	0.08±0.02	0.10±0.05	0.19±0.02
DF5	0.013±0.02	0.16±0.09	0.28±0.02
DF6	0.027±0.03	0.30±0.21	0.39±0.05
DF7	0.028±0.01	0.19±0.05	0.41±0.02
DF8	0.08±0.02	0.10±0.02	0.12±0.03
DF9	0.080±0.05	0.087±0.02	0.15±0.07

Table No 14: Comparison solubility of rosuvastatin solid lipid nanoparticles withpure rosuvastatin in different solvent.

Formulation code (mg/ml)	Solvents		
	Water	Acid buffer(pH1.2)	Phosphate buffer(pH7.4)
Pure drug	0.1±0.02	0.18±0.01	0.22±0.02
SF1	0.06±0.02	0.10±0.02	0.13±0.02
SF2	0.07±0.05	0.08±0.02	0.13±0.07
SF3	0.05±0.03	0.10±0.05	0.132±0.02
SF4	0.08±0.025	0.11±0.05	0.13±0.02
SF5	0.12±0.02	0.17±0.09	0.24±0.02
SF6	0.27±0.03	0.30±0.02	0.56±0.01

Table No 15: Comparison solubility of Rosuvastatin calcium nanoparticles with pure rosuvastatin calcium in different solvent.

Formulation code (mg/ml)	Solvents		
	Water	Acid buffer(pH1.2)	Phosphate buffer(pH7.4)
Pure drug	0.10±0.05	0.18±0.01	0.22±0.02
NF1	0.03±0.03	0.05±0.01	0.06±0.03
NF2	0.04±0.03	0.05±0.04	0.08±0.03
NF3	0.04±0.02	0.06±0.05	0.09±0.07
NF4	0.25±0.03	0.28±0.01	0.37±0.02
NF5	0.21±0.02	0.21±0.01	0.25±0.03
NF6	0.18±0.01	0.19±0.02	0.23±0.03
NF7	0.17±0.03	0.2±0.01	0.24±0.03
F8	0.20±0.03	0.21±0.01	0.24±002

2. Surface morphology study:

Surface morphology of best formulation was studied in scanning electron microscope in 20kv with magnification of 20000X. The image was obtained. It shown that the particle is grossly spherical in shape and size of is solid lipid nanoparticles is about 100µm, and the size of nanoparticles is about 105 µm. The surface of the solid lipid nanoparticles and nanoparticles shows layer of surfactant on carrier particle.

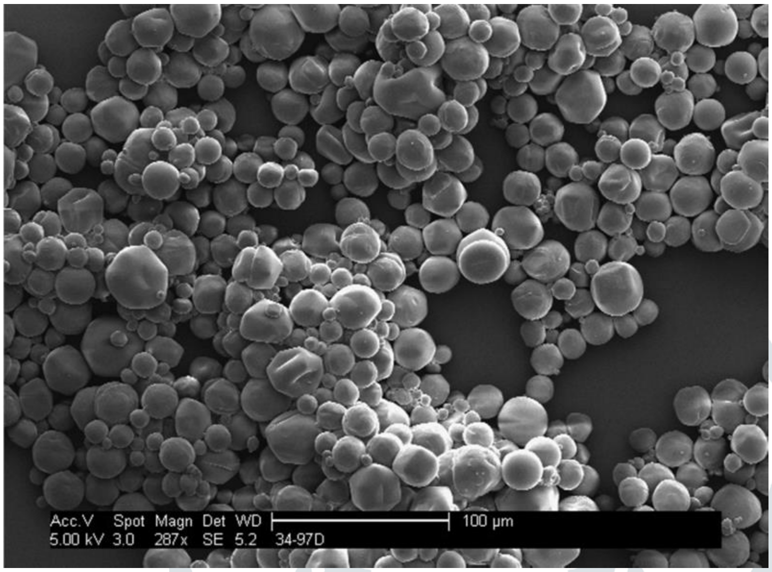

Figure No 11: Image of scanning electron microscopy of solid lipid nanoparticlesof SF6 formulation

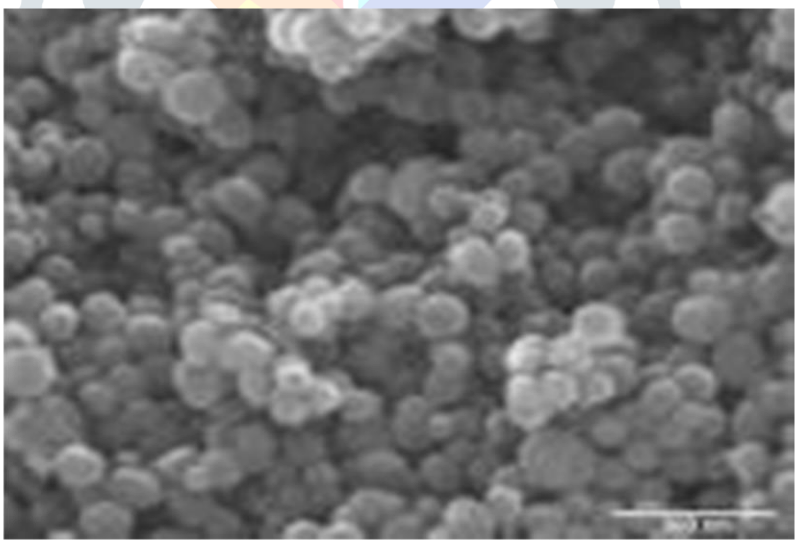

Figure No 12: Image of scanning electron microscopy of nanoparticles of NF4 formulation

2. Zeta potential

The surface charge of solid lipid nanoparticle and nanoparticles of best formulation (SF6, NF4) was determined in Malvern zeta analyser. The surface charge was found to be 26.5 mV and 10.2mV it is in the acceptable range.

3. Entrapment efficiency:

a) For solid dispersion

The entrapment efficiency was determined after separating unentrapped drug from entrapped drug. The formulation DF7 had shown maximum entrapment of rosuvastatin calcium 68.95%and formulation DF1 had shown least entrapment 35.28%So the result shows that as PEG 6000 concentration increases, the entrapment efficiency also increases. The result is shown in **table no 16.**

Table No.16: Entrapment efficiency of solid dispersion formulation

Formulation code	Total drug	Unentrapped drug	Entrapped drug	% Entrapment
DF1	96.08±0.04	33.90±0.04	62.18±0.01	35.28%
DF2	97.16±0.02	44.92±0.03	52.24±0.02	46.23%
DF3	95.08±0.04	33.90±0.04	62.18±0.01	37.28%
DF4	91.78±0.06	32.49±0.5	59.29±0.05	35.39%
DF5	93.96±0.04	54.17±0.02	42.79±0.01	55.86%
DF6	94.85±0.01	60.94±0.03	53.89±0.03	64.26%
DF7	96.936±0.03	40.05±0.04	53.18±0.02	68.95%
DF8	92.40±0.02	50.40±0.02	42.16±0.03	54.54%
DF9	99.02±0.01	52.92±0.01	46.16±0.02	53.44%

b) For solid lipid nanoparticles and nanoparticles

The entrapment efficiency was determined after separating unentrapped drug from entrapped drug. The formulation of SLN's SF6 had shown maximum entrapment of rosuvastatin calcium 32.24% and formulation SF1 had shown least entrapment 25.57%. So the result shown that as cholesterol concentration increases, the entrapment efficiency also increases. and nanoparticles NF4 had shown maximum entrapment of rosuvastatin calcium57.21% and NF1 had shown least entrapment 39.67% result shown that as PVP concentration increase, the entrapment efficiency also increases. The result shown in **table no 17and 18.**

Table No.17: Entrapment efficiency of solid lipid nanoparticles formulation.

Formulation Code	Total drug (mg)	Unentrapped drug	Entrapped drug	% Entrapment
SF1	19.12±0.02	4.89±0.02	14.23±0.01	25.57%

SF2	17.83±0.01	5.20±0.03	12.23±0.03	29.16%
SF3	18.33±0.03	5.23±0.01	13.10±0.03	28.53%
SF4	19.48±0.04	7.98±0.03	11.50±0.02	40.96%
SF5	19.03±0.01	5.93±0.01	13.01±0.04	43.16%
SF6	19.41±0.02	6.23±0.02	13.12±0.03	49.24%

Table No.18: Entrapment efficiency of nanoparticles formulation

Formulation Code	Total drug	Unentrapped drug	Entrapped drug	% Entrapment
NF1	79.95±0.03	31.92±0.04	48.20±0.03	39.67%
NF2	93.01±0.01	41.02±0.03	52.10±0.03	44.05%
NF3	90.17±0.03	40.94±0.01	49.23±0.01	45.40%
NF4	93.78±0.01	53.66±0.03	40.12±0.01	57.21%
NF5	79.95±0.03	31.92±0.04	48.20±0.03	39.67%
NF6	96.78±0.01	48.66±0.03	41.12±0.01	48.21%
NF7	96.23±0.03	48.11±0.01	48.12±0.02	49.99%
NF8	99.84±0.01	47.74±0.02	52.10±0.01	47.81%

Note: The values presented are ± SD's of three determination

2. Drug content:

The percentage drug content of Rosuvastatin calcium in solid dispersion was found to be within63.40±0.96 to 96.88±3.63 and 72.08±0.28to97.91±0.14in 0.1N HCl and in phosphate buffer (pH 7.4) respectively, nanoparticles was found to be within 69.55±2.32 to 92.59±3.39 and 52.66±0.38 to 96.66±1.44 in 0.1N HCl and in phosphate buffer (pH 7.4) respectively, solid lipid nanoparticles was found to be within the range of 70.07±0.67 to 96.0±0.22 and 70.75±0.66 to 94.58±0.52.The results have shown that the drug content of all the batches was found to be uniform this confirms the mixing of content was proper. The results are shown in the **table no.19,20 and 21.**

Table No 19: Percentage drug content of solid dispersion in 0.1NHCl andphosphate buffer (pH7.4)

Formulation code	Drug content	
	1.2pH	7.4 pH
DF1	63.40±0.84	96.25±0.25
DF2	68.20±0.96	95.50±0.90

DF3	74.40±0.22	72.08±0.28
DF4	84.96±0.64	92.91±0.14
DF5	84.14±1.09	91.02±1.04
DF6	80.59±0.89	79.16±0.52
DF7	96.88±3.63	97.91±0.14
DF8	94.29±0.92	89.75±0.51
DF9	80.81±3.77	79.33±1.81

Note: The values presented are ± SD's of three determination.

Table No 20: percentage drug content of nanoparticles in 0.1NHCl andphosphate buffer (7.4pH)

Formulation code	Drug content	
	1.2pH	7.4Ph
NF1	89.62±1.28	52.66±0.38
NF2	91.85±1.28	55.16±2.64
NF3	92.59±3.39	90.10±2.50
NF4	85.92±1.28	96.66±1.44
NF5	82.22±4.25	81.25±0.90
NF6	91.48±0.12	92.58±1.66
NF7	69.55±2.32	79.91±0.38
NF8	92.44±0.38	82.33±0.38

Note: The values presented are ± SD's of three determination.

Table No 21: percentage drug content of solid lipid nanoparticles in 0.1NHCl and phosphate buffer (7.4pH)

Formulation code	Drug content	
	1.2pH	7.4 pH
SF1	84.81±0.46	76.40±0.80
SF2	70.07±0.67	77.50±0.25
SF3	82.22±4.25	79.08±0.62
SF4	72.14±0.71	70.75±0.66
SF5	86.88±0.22	74.50±4.41
SF6	96.01±0.22	94.58±0.52

Note: The values presented are ± SD's of three determination.

2. *In-vitro* Dissolution study

a) For solid dispersion

In-vitro drug release study was performed using USP type XXIV dissolutiontest apparatus. The condition were maintained are 50rpm using 900ml of 0.1N hydrochloric acid for first 45 min and phosphate buffer of pH 7.4 for next 90min at temperature 37±0.5°C. *In- vitro* drug release profiles for (DF1-F4) formulations andfor (DF5-DF9) formulations were tabulated in the **table no. 22 and 23.**From the results of *in-vitro* dissolution studies the following observation are made,

- DF1 formulation has shown the drug release of about 76.74±0.36% ofrosuvastatin calcium at the end of 90 min.
- DF2 formulation has shown the drug release of about 76.74±0.36% ofrosuvastatin calcium at the end of90 min.
- DF3 formulation has shown the drug release of about 84.58±1.48% ofrosuvastatin calcium at the end of 90min.
- DF4 formulation has shown the drug release of about 92.05±0.77% ofrosuvastatin calcium at the end of 90min.
- DF5 formulation has shown the drug release of about 87.54±0.91% ofrosuvastatin calcium at the end of 90min.
- DF6 formulation has shown the drug release of about 72.92±0.91% ofrosuvastatin calcium at the end of90min.
- DF7 formulation has shown the drug release of about 89.98±0.28% ofrosuvastatin calcium at the end of 90min.
- DF8 formulation has shown the drug release of about 88.90±0.41% ofrosuvastatin calcium at the end of 90min.
- DF9 formulation has shown the drug release of about 82.46±0.89% ofrosuvastatin calcium at the end of 90min.

Table No. 22: *In-vitro* drug release of rosuvastatin calcium solid dispersions forformulations DF1-DF4

Sr.no	Time (min)	Cumulative % drug release of rosuvastatin calcium (DF1-DF4)			
		DF1	DF2	DF3	DF4
1	15	47.57±0.18	39.37±0.28	30.58±1.48	40.30±0.18
2	30	54.53±0.18	46.47±0.28	46.13±0.59	57.32±0.18
3	45	56.35±0.18	52.21±0.28	55.60±3.25	75.22±0.18
4	60	74.55±0.18	60.47±0.30	67.60±2.71	78.34±0.37
5	75	74.72±0.15	71.88±0.53	83.33±1.48	85.03±0.49
6	90	76.74±0.36	76.74±0.36	84.58±1.48	92.05±0.77

Note: The values presented are mean ± SD's of three determination.

Table No. 23:*In vitro* drug release of rosuvastatin calcium solid dispersions forformulations DF5-DF9

Sr.no	Time (min)	Cumulative % drug release of rosuvastatin calcium (DF5-DF9)				
		DF5	DF6	DF7	DF8	DF9
1	15	34.93±4.90	43.72±0.49	42.59±0.18	36.56±0.30	38.89±0.51
2	30	37.23±3.65	46.46±0.60	54.31±0.18	40.79±0.30	45.28±0.51
3	45	42.72±4.77	48.96±0.60	63.05±0.18	44.74±0.30	50.43±0.51
4	60	84.57±1.32	48.96±0.57	82.93±0.36	86.12±0.71	75.96±0.55
5	75	86.58±1.74	68.24±0.76	89.53±0.31	87.10±0.35	75.96±0.55
6	90	87.54±0.91	72.92±0.91	89.98±0.28	88.90±0.41	82.46±0.89

Note: The values presented are mean ± SD's of three determination.

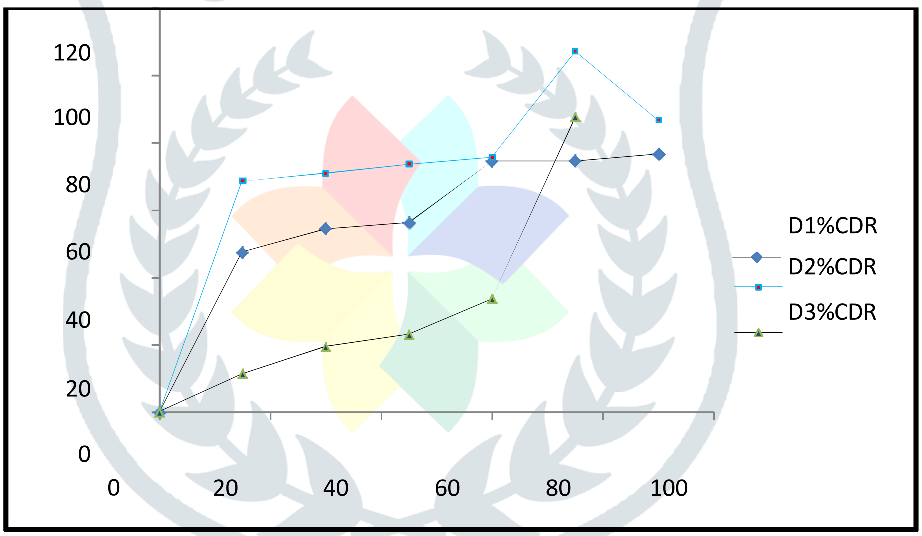

Figure No.13: Zero order release profile for formulation DF1 –DF3

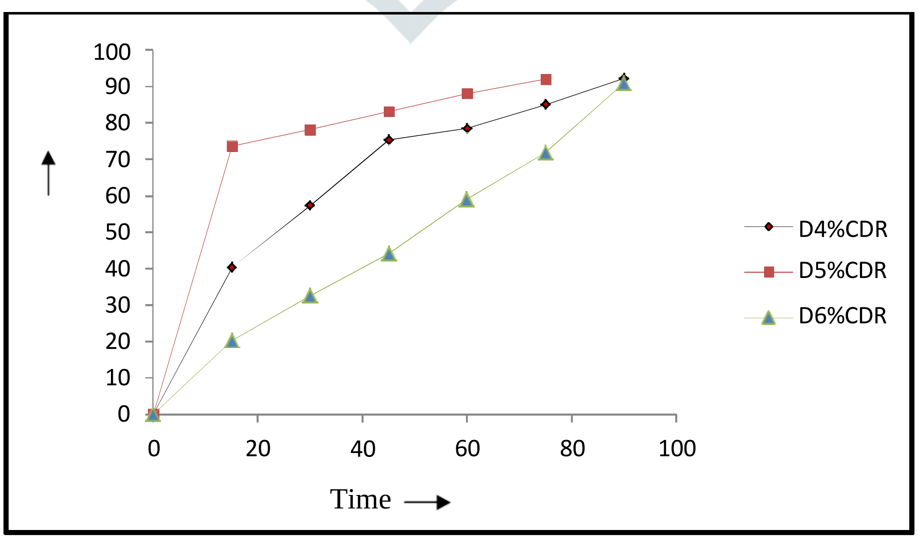

Figure No.14 : Zero order release profile for formulation DF4 –DF6

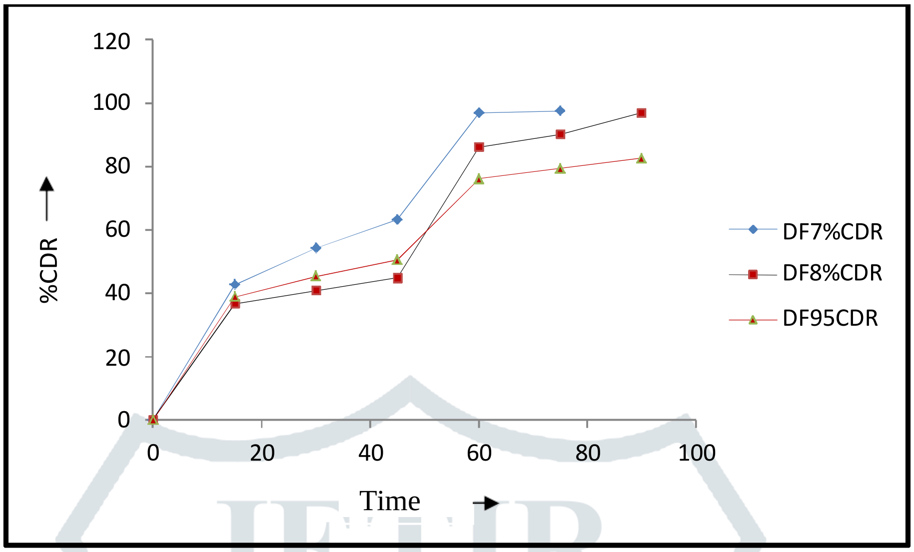

Figure No. 15 : Zero order release profile for formulation DF7 –DF9

a) For nanoparticle

In-vitro drug release study was performed using USP type XIIV dissolution test apparatus. The condition were maintained are 50rpm using 900ml of 0.1N hydrochloric acid for first 45 mins and phosphate buffer of pH 7.4 for next 90 mins at temperature 37±0.5°C. *In -vitro* drug release profiles for (NF1-NF4)

formulations and for (NF6-NF8) formulations were in the table no. 24 and 25. From the results of *in-vitro* dissolution studies the following observation are made,

- NF1 formulation has shown the drug release of about 88.20±0.21% of rosuvastatin calcium at the end of 90mins.
- NF2 formulation has shown the drug release of about 95.13±0.20% of rosuvastatin calcium at the end of 90mins.
- NF3 formulation has shown the drug release of about 87.47±0.12% of rosuvastatin calcium at the end of 90mins.
- NF4 formulation has shown the drug release of about 91.23±0.05% of rosuvastatin calcium at the end of 75mins.
- NF5 formulation has shown the drug release of about 85.75±0.38% of rosuvastatin calcium at the end of 90mins.
- NF6 formulation has shown the drug release of about 85.84±0.11% ofrosuvastatin calcium at the end of 90mins.
- NF7 formulation has shown the drug release of about 92.04±0.15% of rosuvastatin calcium at the end of 90mins.
- NF8 formulation has shown the drug release of about96.42±0.17% of rosuvastatin calcium at the end of 90mins.

Table No. 24:*In vitro*-drug release of rosuvastatin calcium nanoparticles forformulations NF1-NF4.

Sr.no	Time (min)	Cumulative % drug release of rosuvastatin calcium (NF1-NF4)			
		NF1	NF2	NF3	NF4
1	15	21.96±0.10	17.83±0.09	24.01±0.05	44.88±0.55
2	30	22.93±0.10	18.71±0.14	97.03±0.05	47.64±0.05
3	45	26.33±0.10	23.96±0.09	30.88±0.05	50.52±0.05
4	60	46.06±0.11	43.29±0.09	41.01±0.11	83.75±0.05
5	75	67.01±0.12	63.69±0.096	64.41±0.20	91.23±0.05
6	90	88.20±0.21	95.13±0.20	87.47±0.12	-

Note: The values presented are mean ± SD's of three determination.

Table No. 25: *In-vitro* drug release of rosuvastatin calcium nanoparticlesfor formulations NF5-NF8

Sr.no	Time (min)	Cumulative % drug release of rosuvastatin calcium (NF5-NF8)			
		NF5	NF6	NF7	NF8
1	15	32.19±0.1	30.91±0.06	37.57±0.06	56.67±0.09
2	30	35.15±0.10	32.0±0.06	40.99±0.06	59.98±0.09
3	45	43.25±0.10	35.96±0.06	42.97±0.06	61.40±0.09
4	60	56.60±0.21	55.84±0.10	62.03±0.10	74.84±0.19
5	75	70.71±0.29	61.34±0.10	67.44±0.08	85.99±0.20
6	90	85.75±0.38	85.84±0.11	82.04±0.15	87.42±0.17

Note: The values presented are mean ± SD's of three determination4

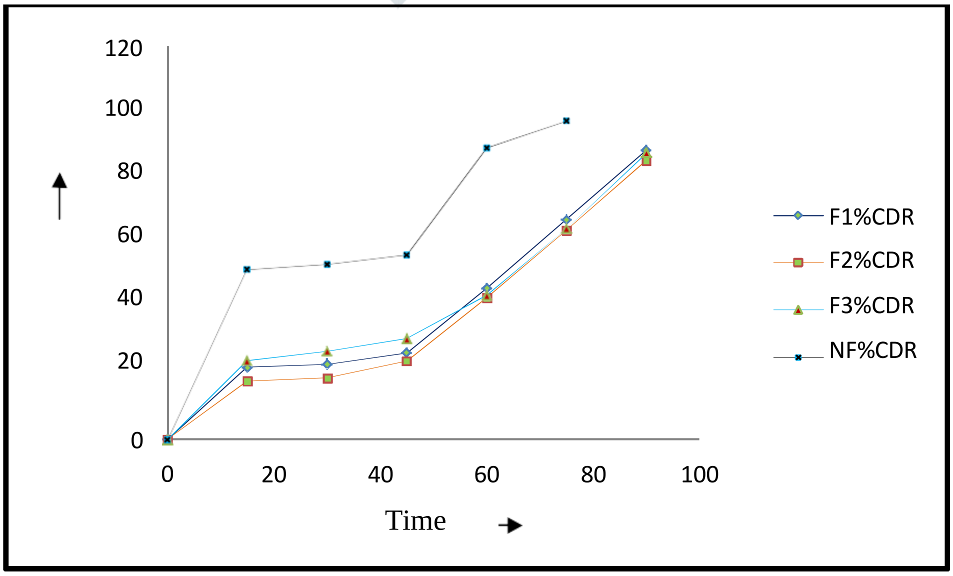

Figure No.16 : Zero order release profile for formulation NF1-NF4

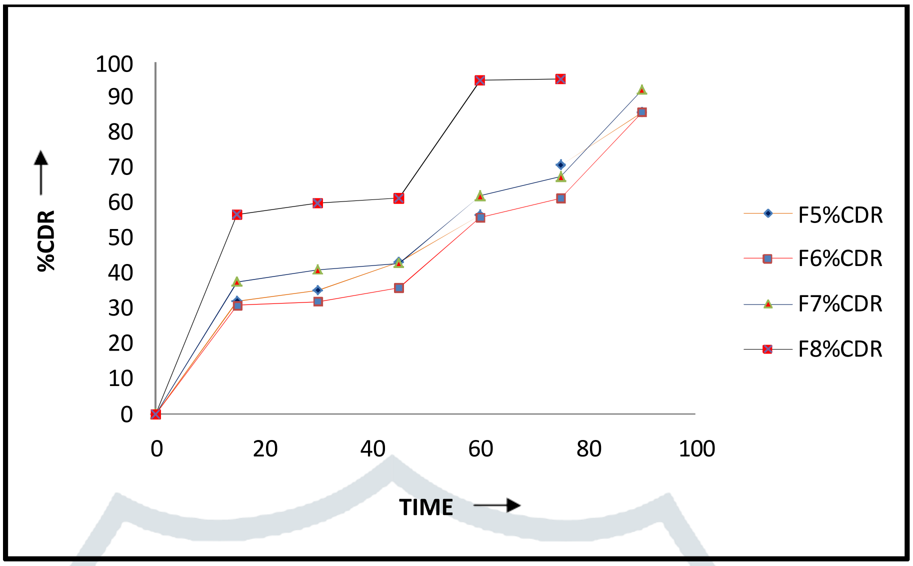

Figure No. 17: Zero order release profile for formulation NF5-NF8

b) For Solid Lipid Nanoparticle

In-vitro drug release study was performed using franz diffusion apparatus. The condition was maintained are rpm 50 using 20ml of 0.1N hydrochloric acid for first 45 mins and phosphate buffer of pH 7.4 for next 90 mins at temperature 37±0.5°C.*In - vitro* drug release profiles for (NF1-NF4) formulations and for (NF6-NF8)

formulations were in the table no. 24 and 25. From the results of *in-vitro* diffusion studies the following observation are made,

- SF1 formulation has shown the drug release of about 75.27±0.74% ofrosuvastatin calcium at the end of 90mins
- SF2 formulation has shown the drug release of about 83.11±0.87% ofrosuvastatin calcium at the end of 135mins.
- SF3 formulation has shown the drug release of about 79.69±0.85% ofrosuvastatin calcium at the end of 120mins
- SF4 formulation has shown the drug release of about 76.50±1.11% ofrosuvastatin calcium at the end of 105mins
- SF5 formulation has shown the drug release of about 91.03±0.76% ofrosuvastatin calcium at the end of 120mins
- SF6 formulation has shown the drug release of about 93.54±0.92% of rosuvastatin calcium at the end of 75mins

Table No. 26: *In vitro* drug release of rosuvastatin calcium solid lipidnanoparticles for formulations SF1-SF3

Sr.no	Time (min)	Cumulative % drug release of rosuvastatincalcium (SF1-SF3)		
		SF1	SF2	SF3
1	15	21.79±1.28	14.12±0.68	19.60±1.94
2	30	35.09±0.77	28.21±1.80	33.26±2.04
3	45	61.70±0.66	45.46±1.50	49.29±1.69
4	60	68.77±0.97	55.46±2.46	55.28±1.79
5	75	74.72±0.97	66.28±1.98	73.60±2.25

6	90	75.27±0.74	79.75±0.92	77.55±1.28
7	105	-	81.37±0.72	79.46±1.01
8	120	-	83.00±0.72	79.69±0.85
9	135	-	83.11±0.87	-

Note: The values presented are mean ± SD's of three determination

Table No. 27: *In vitro* drug release of rosuvastatin calcium solid lipidnanoparticles for formulations SF4-SF6.

Sr.no	Time (min)	Cumulative % drug release of rosuvastatin calcium (SF4-SF6)		
		SF4	SF5	SF6
1	15	22.04±2.25	14.81±1.55	25.55±0.83
2	30	32.91±1.80	31.99±1.05	39.79±0.52
3	45	50.16±2.32	45.68±1.35	50.17±1.33
4	60	56.07±0.95	62.12±1.66	63.97±1.54
5	75	70.92±0.87	76.24±2.26	93.54±0.92
6	90	75.22±1.93	83.13±1.59	-
7	105	76.50±1.11	90.69±0.53	-
8	120	-	91.03±0.76	-

Note: The values presented are mean ± SD's of three determination

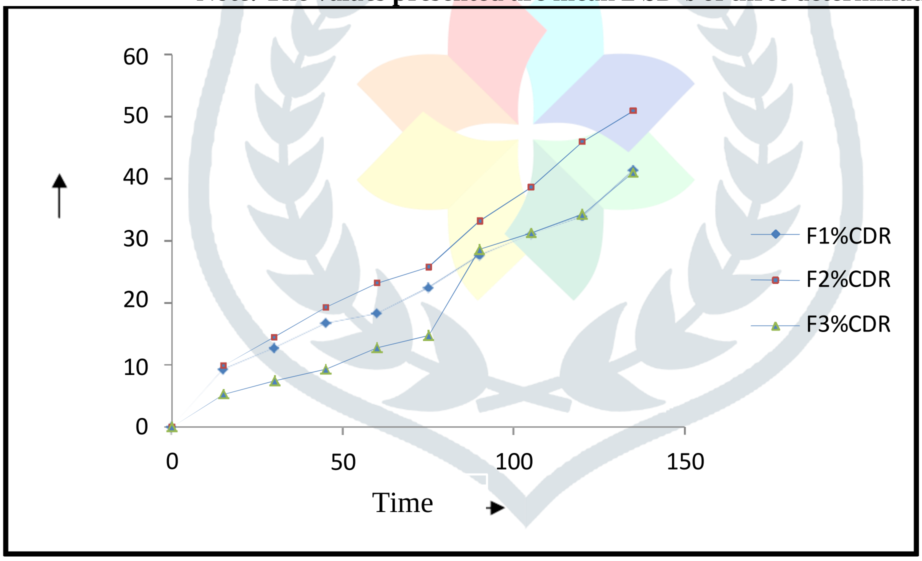

Figure No.18 : Zero order release profile for formulation SF1-SF3

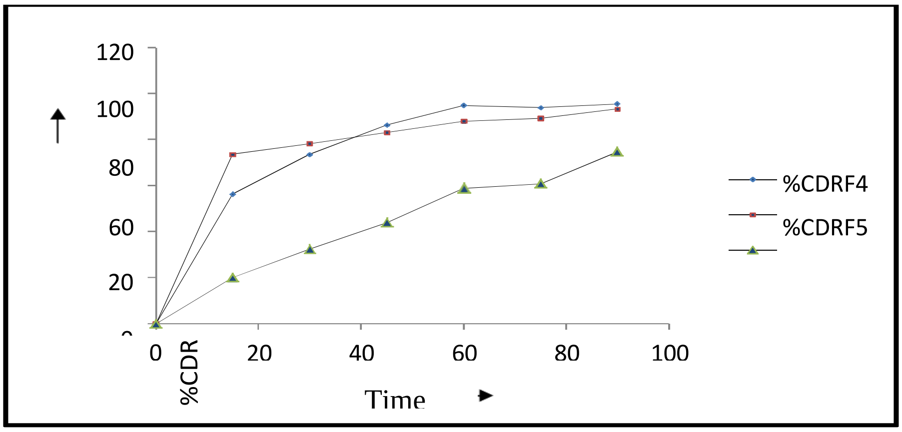

Figure No. 19: Zero order release profile for formulation SF4-SF6

b. Release kinetic data:

The results obtained in the *in-vitro* drug release studies were plotted in following models of data analysis,

- Cumulative percent drug release v/s time (zero order kinetics).
- Log cumulative percent drug retained v/s time (first order kinetics).

Release kinetic data of solid dispersion, solid lipid nanoparticle and nanoparticle formulations was tabulated in **table no. 28,29 and 30**. From the data, it was observed that release of rosuvastatin calcium in DF1 to DF9, SF1 to SF6 AND NF1toNF8 Formulation follows first order kinetics.

Table No.28: Release kinetic data of DF1-DF9 formulation

Formulation code	Mathematical model (kinetics)	
	Zero order (R^2)	First order (R^2)
DF1	0.765	0.971
DF2	0.632	0.951
DF3	0.780	0.912
DF4	0.860	0.987
DF5	0.618	0.823
DF6	0.884	0.916
DF7	0.825	0.954
DF8	0.720	0.892
DF9	0.891	0.896

Table No.28: Release kinetic data of SF1-SF6 formulation

Formulation code	Mathematical model (kinetics)	
	Zero order (R^2)	First order (R^2)
SF1	0.881	0.873
SF2	0.928	0.961
SF3	0.953	0.972
SF4	0.728	0.960
SF5	0.579	0.964
SF6	0.972	0.972

Table No.30: Release kinetic data of NF1-NF8 formulation

Formulation code	Mathematical model (kinetics)	
	Zero order (R^2)	First order (R^2)
NF1	0.829	0.960
NF2	0.830	0.959
NF3	0.832	0.961
NF4	0.868	0.880

NF5	0.754	0.949
NF6	0.829	0.958
NF7	0.910	0.994
NF8	0.826	0.853

Stability study:

The accelerated stability studies were carried out according to ICH guidelines. Best formulation DF7, SF6 and NF4 were packed in airtight sealed glass vials and was stored in ICH certified stability chambers maintained at 40ºC and 75%RH for 3 month. The formulation DF7, SF6 and NF4 was evaluated at definite time intervals(0, 1, 2, 3 months) for change in appearance, the drug content and *in-vitro* drug release profile.

It was noted that the DF7, SF6 and NF4 formulation was dry, free flowing without any microbial or fungal growth or bad odour and shows slight decrease in drug release and drug content. The results of drug content and drug release data are shown in **table no. 31, 32 and 33.**

Table No.31: Stability study for DF7

Time (days)	Physical appearance	Drug content %		% CDR at the end of 2hrs
		1.2pH	7.4pH	
30	No change	95.40±1.05	96.41±1.42	88.93%
60	No change	93.77±1.05	95.41±0.52	87.31%
90	No change	92.88±1.05	94.83±0.63	86.98%

Table No.32: Stability study for SF6

Time (days)	Physical appearance	Drug content %		%CDR at the end of 2hrs
		1.2pH	7.4pH	
30	No change	95.40±0.33	90.83±0.38	93.39%
60	No change	94.07±0.33	89.33±0.38	92.41%
90	No change	93.40±0.33	88.75±0.38	92.18%

Table No.33: Stability study for NF4

Time (days)	Physical appearance	Drug content %		%CDR at the end of 2hrs
		1.2pH	7.4pH	
30	No change	85.18±1.28	96.5±1.44	91.09%
60	No change	84.22±1.28	95.16±1.44	90.98%
90	No change	83.03±1.28	94.58±1.44	89.88%

CONCLUSION

A satisfactory attempt has been made to increase the solubility of rosuvastatin by formulating solid dispersion, solid lipid nanoparticles and nanoparticles of rosuvastatin calcium. Rosuvastatin is a hypolipidimic agent, it act HMG - CoA reductase enzyme

The aim of the present investigation is to enhance the bioavailability poorly soluble drug i.e. rosuvastatin calcium. The following conclusions are drawn from the results of experimental studies,

- Biocompatible, PVP K25, urea, PEG6000, surfactant span 60 along with cholesterol and sodium lauryl sulphate, Eudragit S-100, Polaxamer along with solvent were used to formulate solid dispersion, solid lipid nanoparticles and nanoparticles.
- The size of the nanoparticles, solid lipid nanoparticle were found to be below 100µm and 105 µm therefore it may improve the bioavailability.
- From *in-vitro* drug release study, it was found that the solid dispersion prepared using the PEG6000 (DF7) shows the drug release upto75 mins. the solid lipid nanoparticles prepared by using the cholesterol , span60 (SF6) and nanoparticles prepared using PVPk25 ,Polaxamer (NF4) have shown the drug release upto 75 mins.
- By considering the results of practical yield, drug entrapment and drug release, the formulations DF7, SF6 and NF4 were selected as best formulations from each method. These formulations were taken for further studies.
- The surface topography study of solid lipid nanoparticles (SF6) and nanoparticles (NF4) by scanning electron microscopy shows that the particles are grossly spherical in shape.
- Zeta potential study of solid lipid nanoparticles and nanoparticles revealed that, there is no aggregation of particles so that the formulation may remain stable for long duration.
- All the formulation followed first order.
- There was no significant change observed in the DF7, SF6, NF4 formulation after drug stability studies for the period of 3 months, as per the ICH guidelines.

BIBLIOGRAPHY

1. Himani B, Seema B,. Bioavailability enhancement. *International Journal of Pharma and Bio Sciences*. 2011April; 2(2):202-216.
2. Bioavailiability and Bioequivalence. *Best practice journal* Generic 2009; 4-8.
3. Thakkar H, Patel B. A review on techniques for oral bioavailability enhancement of drugs. *International Journal of Pharmaceutical Sciences Review and Research*. 2010September; 4(3):203-223.
4. S.Kadam, D.Shinkar,. Review on solubility enhancement techniques. *International Journal of Pharmacy and Biological Sciences*. 2013JUL-SEP; 3*(3)* 462-475.
5. Sandeep K, Pritam S,. Various techniques for solubility enhancement. *Pharma Innovation Journal* 2016; 5(1): 23-28.
6. Mogal S., Gurjar P. Solid dispersion technique for improving solubility of some poorly soluble drugs. *Der Pharmacia Lettre*, 2012, 4 (5):1574-1586.
7. K.Shahul Hameed M, Thirumoorthy N. Design and charecterization of solid lipid nanoparticle by solvent evaporation method followed by homoginization. *International Journal Of Biopharmaceutics.*2014, 5(3) 190-96.
8. M Suganeshwari, Anto S, K.Azhagesh. Preparation and charecterization and evaluation of nanoparticles containing hypolipidemic drug and antihypertensivedrug*: International Journal Of Pharmaceutical And Biological Archives*; 2011, 2(3); 949-53.
9. https://en.wikipedia.org/wiki/statin(accesed at 12:10pm on 16th Dec 2016.
10. Onwe PE., Folawiyo MA., Anyigor -Ogah CS., Umahi G., Okorocha AE and Afoke AO,. Hyperlipidemia: Etiology and Possible Control .*IOSR Journal of Dental and Medical Sciences*; 14(2):93-100.
11. K.Harikumar, S. Abdul , Kishore k, M. Ramunaik, CH. Suvarna *International Journal Of Novel*

Trends In Pharmaceutical Sciences;.2013Oct, 3(3):59-71.
12. www.actavis.ie/en/products/Rosuvastatin(accesed at 4:43pm on 24Jan 2016.
13. N.K. Jain, Hari G, Pharmaceutical technology. *Pharmaceutical Nanotechnology*;1-19.
14. Vyas & Khar. Targeted controlled drug delivery system; 13-15.
15. Vyas & Khar. Targeted controlled drug delivery system; 346-48.
16. Mogal S, Gurjar P. N1,Yamgar D. S2 And Kamod A.C.2 Solid dispersion technique for improving solubility of some poorly solubledrugs *Der Pharmacia Lettre*, 2012, 4 (5):1574-1586.
17. Ladan N, Gurinder S, Gaurav S. Solid Dispersion: Methods and Polymers to increase the solubility of poorly soluble drugs. *Journal of Applied Pharmaceutical Science* Vol. 2 (10), pp. 170-175, October, 2012.
18. https://en.wikipedia.org/wiki/sorbitanmonostereate (accessed at 3:42pm on Nov2016.
19. https://en.wikipedia.org/wiki/cholesterol (accessed at 3:42pm on Nov2016.
20. Peltonen L. Cholestrol. In: Raymond CR, Paul JS, Marion EQ (eds); *Handbook Of Pharmaceutical Excipients*. 6th edition USA: pharmaceutical pross; 2009, 178-80.
21. https://en.wikipedia.org/wiki/polaxamer (accessed at 4:20pm on Jan 2017.
22. https://en.wikipedia.org/wiki/polyvinylpyrolidine (accessed at 11:20pm on Jan 2017.
23. https://en.wikipedia.org/wiki/EudragitS100 (accessed at 3:20pm on Jan 2017.
24. https://en.wikipedia.org/wiki/Sodium lauryl sulphate (accessed at 10:30pm on Jan 2017
25. https://en.wikipedia.org/wiki/polyethyleneglycol6000 (accessed at 11:00pm on Jan 2017
26. Swapna V, Vinyas M, Rama M. Formulation, Development And Evaluation Of Rosuvastatin Calcium Immediate Release Tablets; *European Journal Of Pharmaceutical And Medical Research*.2016 Feb;3(3)., 351-358.
27. Netish K, Sumon D, Abinash S,. Formulation and Evaluation of Rosuvastatin Immediate Release Tablets 10 Mg; *Journal of Pharmacy and Biological Sciences*. 2016Sep- Oct.11(5),.1-5.
28. Kurti D, Pranav S. Formulation optimization of rosuvastatin calcium loaded solid lipid nanoparticle by 3 factorial design: *Nano World Journal*; 2015; 112-21.
29. Ramkumar P, Janakiraman K. Formulation and characterization of rosuvastatin calcium nanoparticles: *Indo American Journal Of Pharmaceutical Research*. 2015; 5(2), 767-79.
30. B Arunprasath1, S A Sreenivas. Formulation and evaluation of nanoparticles containing antihypertensive agent and hypolipidaemic Drug; *International Journal Of Pharma Research And Health Sciences*;2015, 3-(4);.824-831.
31. Ashok J, Ramkanth S. Enhancement of saturation solubility and *in vitro* dissolution of carvedilol nanoparticles by high pressure homogenization technique;. *International Journal of Current Pharmaceutical Review and Research* ;. November 2015; 6(6); 269-273.
32. D Karthikeyan, M Shrinivas. Formulation and evaluation of stavudine nanoparticles: *International Journal Of Novel Trends In Pharmaceutical Science;* Feb2013, 3(1), 24-32.
33. Amulya Ratna B, Sunit K. Preparation and evaluation of glibenclamide-loaded biodegradable nanoparticles. *Tropical Journal Of Pharmaceutical Research*. June2012; 11(3), 345-50.
34. S Tamizhrasi, A Shukla. Formulation and evaluation of lamivudine loaded polymethacrylic acid nanoparticles: *Internatioinal Journal Of Pharm Tech Research*. July-Sep2009; 1(3), 411-15.
35. Abather S, Alaa A, Abdul R. Formulation and evaluation of silibinin loaded solid lipid nanoparticles for peroral use targeting lower part of gastrointestinal tract. *International Journal Of Pharmacy And Pharmaceutical Science*. 2014; 6(1), 56- 67.
36. Abdul H, Sathali.A, Nisha.N. Development of solid lipid nanoparticles of rosuvastatin calcium: *BioMedRx*; 2013; 1(5),536-48.
37. Upadhyay N, Mohan G. Formulation development and evaluation of atorvastatin calcium loaded solid lipid nanoparticles: *Journal Of Fundamental Pharmaceutical Research*; 2013. 1(1); 46-58.
38. Viveksarathi K, Kannan K. Formulation and evaluation of nanoscale solid lipid particles containing a hydrophilic drug-rasagiline mesylate: *Journal Of Pharmaceutical Scienc;* May 2016, 6(5), 44-50.
39. Yifan L, Dawei C. Solid lipid nanoparticle for enhancing vinpocetine's oral bioavaliability: *Journal Of Controlled Release*. May2006; 114, 53-59.
40. Francois H, Lyes M. Solid dispersion for oral administration and overview of the methods for their preparation: *Current Pharmaceutical Design*; 2016, 22(0), 1-17.
41. Arunprasad K, Narayana N. Preparation and evaluation of solid dispersion of turbinafine hydrochloride: *International Journal Of Pharmaceutical Science Review And Research*; July-

Aug2010, 3(1), 130-34.

42. Apparao B, Shivalingam M. Formulation and evaluation of aceclofenac solid dispersion for dissolution rate enhancement: *International Journal Of Pharmaceutical Science And Drug Research.* 2010; 2(2), 146-50.
43. United state pharmacopea 31st National Formulary 26,2008.Vol 1,Asian Ed, P 297.
44. Pavia D, Lampman G, Kriz G,. Infrared Spectroscopy. 4th edition USA: Books cole cengage learning; 2010.
45. Indian Pharmacopoeia-2007, Volume 1. Buffer Solution 477-78.
46. Vyas Jigar, Vyas Puja, Patel Jayavardan. Formulation and evaluation of solid dispersions of rofecoxib for improvement of dissolution profile: *African Journal of Pharmacy and Pharmacology*; May 2011, 5(5), 571-81.
47. Arun k, Rajkumar M. Design and development and evaluation of nanocrystals of valsartan for solubility enhancement: *World Journal Of Pharmacy And Pharmaceutical Science;* 3(3); 1414-27.
48. Alsara A, Bosela A. Proniosomes as a drug carrier for transdermal delivery of ketorolac. *European Journal of Pharmacy and Pharmaceutical Sciences;* 2005, 59: 485-90.
49. Sathali A, Rajalakshmi G. Evaluation of transdermal targeted niosomal drug delivery of terbinafine hydrochloride. *International Journal of PharmTech Research* 2010; 2(3):2081-89.
50. Sathali A, Lingan MA, Kumar MR, Gokila A. Formulation and evaluation of topical drug delivery system containing clobetasol propionate niosomes. *Scientific Reviews and Chemical Communications* 2011; 1(1):7-17.
51. Akhilesh D, Hazel G, Kamath JV. Proniosomes – a propitious provesicular drug carrier. *International Journal of Pharmacy and Pharmaceutical Science Research* 2011; 1(3):98-103.
52. Upadhyay N, Mohan G, Sharma S. Formulation development and evaluation of atorvastatin calcium loaded solid lipid nanoparticles. *Journal of Fundamental Pharmaceutical Research* 2013; 1(1) :46-58
53. United state pharmacopoeia 31, National formulary 26. Dissolution 2008. Vol- 1,Asian edition ,p268-69.
54. Kumar S, Gupta R, Gupta N, Kumar V, Prajapati SK, Kumar A. The proniosomes development and optimization as a surrogated drug carrier for oral delivery of gliclazide. *World Journal of Pharmacy and Pharmaceutical Sciences* 2014; 3(9):275-94.