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Formulation and Evaluation of Nanosuspension of Rosuvastatin using Nanoprecipitation Technique

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Abstract **Original Research Article**

In this work a new attempt was made to enhance the solubility, poor water solubility and slow dissolution rate are issues for majority of upcoming and existing biologically active compounds. The aim of present work was to increase the dissolution rate of Rosuvastatin Calcium, a poorly water soluble drug and hence improve its oral bioavailability by Nanosuspension technology. In the present work Nanosuspension is made by nanoprecipitation technique in the presence of Poloxamer 407 as a surfactant, Tween 80 as a wetting agent and HPMC as a stabilizer. The formulated nanosuspensions were characterised by Scanning Electron Microscope (SEM) and FTIR. The formulations were evaluated for drug content, entrapment efficacy, Zeta potential and In-Vitro dissolution. SEM results showed the particle size of the formulated nanosuspensions in nanosize. FTIR spectrum revealed that there are no interactions between drug and carriers. Finally it was concluded that formulating poorly soluble drugs in the form of Nanosuspension would be a promising approach in delivery of poor water soluble drugs by oral route in a simple and effective way.

Keywords: Rosuvastatin, Poloxamer 407, Tween 80, HPMC, Solubility, Formulation and Evaluation.

INTRODUCTION

The design and formulation of a dosage form require consideration of the physical, chemical, and biological characteristics of all the drug substances and pharmaceutical ingredients to be used in its preparation. An important property of a drug substance is solubility, especially aqueous system solubility [1]. One of the critical problems associated with poorly soluble drugs is too low bioavailability and erratic absorption because of their low dissolution rates [2]. The solubility–dissolution behavior of a drug is a key factor to its oral bioavailability. Nanotechnology can be used to solve the problems associated with these conventional approaches for solubility dissolution and bioavailability enhancement. In Nanosuspension technology, the drug is maintained in the required crystalline state with reduced particle size (i.e. increase in the surface area) leading to an increased dissolution rate and therefore improved bioavailability [3]. Reduction of drug particles to nanometer range leads to an enhanced dissolution rate not only because of increased surface area but also because of saturation solubility. Rosuvastatin Calcium is a synthetic, enantiomerically pure antilipemic agent that competitively inhibits hydroxyl-methyl-glutaryl-co-enzyme A (HMG-CoA) reductase. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonic acid, the rate-limiting step in cholesterol biosynthesis. Rosuvastatin belongs to a class of medications called statins and is used to reduce plasma cholesterol levels and prevent cardiovascular disease. RVS is a white, crystalline, poorly soluble in water. It is generally considered that compounds with very low aqueous solubility will show dissolution rate-limited absorption. Improvement of aqueous solubility in such case is a valuable goal to improve therapeutic efficacy. The dissolution rate is a function of the solubility and the surface area of the drug, thus, dissolution rate will increase if the solubility of the drug is increased, and it will also increase with an increase in the surface area of the drug [4, 5]. In this present study, nanoprecipitation technique is used where a drug solution in a water miscible organic solvent is mixed with an aqueous solution containing a surfactant(s). Upon mixing, the supersaturated solution leads to nucleation and growth of drug particles, which may be stabilized by surfactants [6]. The aim of this work is to formulate the Nanosuspension by nanoprecipitation method and enhance the dissolution rate. The optimize formulation was further characterize by Scanning Electron Microscopy (SEM). Dissolution

study of Nanosuspension formulations was performed in distilled water [7].

MATERIALS AND METHODS

Materials: Rosuvastatin calcium was obtained as a gift sample from Mumbai, Acetone Lactic acid chitosan, HPMC E5, Tween 80 (0.2%) and Poloxamer 407 was purchased from New Delhi, All other reagents and chemicals used were of analytical reagent grade.

Methods

Preformulation Studies

Identification of Drug: Infrared spectroscopy (IR), ultra violet (UV) and melting point are used for identification and purity of drug sample. Rosuvastatin was identified by various techniques which are following:

Organoleptic Property of the Drug

Drug (Rosuvastatin) was Physically Characterized on the basis of colour, odour and taste. All these parameter ware recorded and compared with standard.

Identification of Drug by U.V Spectroscopy

10 mg of Rosuvastatin was taken in volumetric flask and volume make up to 100 ml with methanol, 10 ml of above solution is diluted with methanol up to 100 ml and then it was scanned between 200 nm to 400 nm. The solution showed absorbance maximum at 238 nm in Fig. 1.

Identification of Drug by I.R. spectroscopy

The FTIR spectral analysis was carried out by pressed pellet technique. IR spectrum of any substance gives information about the group present in a specific substance. An IR spectrum of drug was taken using (KBr potassium bromide) pellets. Small quantities of drug sample were mixed with oil, and a drop was placed between KBr pellets and spread uniformly. The pellets were placed in the holder, and an infrared spectrum was taken. The range of scanning was 400-4000 cm^{-1}, Different peaks in the infrared spectrum were interpreted for presence of various group in the structure of the drug. The observed IR spectra of the drug are shown in Fig. 2 and Fig.3.

Differential Scanning Calorimetric Study

A DSC-60 Differential Scanning Calorimeter (Make - Shimdzu) equipped with an intracooler and a refrigerated cooling system was used to analyze the thermal behavior of drugs and mixture of drug and excipients in hermetically sealed flat aluminium crucibles, with temperature range from 30 to 300ºC according to predetermined melting point of drug. Blank crucible was used to calibrate the DSC temperature. Nitrogen was purged at 30 ml/min through cooling unit. The obtained peaks were analyzed for drug excipient compatibility study.

Melting Point Determination

The temperature at which the solid and liquid phases are in equilibrium is called the melting point of substance. The melting point of a drug can be measured using three techniques:

- Hot stage microscopy
- Capillary melting method
- Differential scanning calorimeters thermal analysis

A melting point determination is a good first indication of purity since the presence of relatively small amount of impurities can be detected by lowering as well as widening in the melting point range. Melting point of Rosuvastatin was determined by capillary method using melting point apparatus.

10 mg of the drug sample was weighed accurately and placed into a capillary tube. Tube was placed in the melting point apparatus and was heated to a temperature below 5-10°C of the temperature at which powder started to melt, and temperature at which the sample started to melt was observed.

Solubility Determination

The solubility study of drug was performed in different solvents (e.g. methanol, ethanol, acetone, ethyl acetate, 0.1N HCl). A known quantity of drug was transferred in series of different solvents having volume 5ml in test tubes. Excess amount of drug was added to different solvents till the solution became saturated and these test tubes were shaken by mechanical shaker for 1 hr under constant vibration at constant temperature. After this period the solution were centrifuged. The supernatant was then analyzed by U.V. spectrophotometer (Shimadzu-1700, Japan) at λ_{max} 273 nm with appropriate dilution. Three determinations were carried out before each sample to calculate the solubility of Rosuvastatin in different solvents.

Determination of Partition Coefficient of Drug

Partition coefficient of a drug is a measure of its hydrophilic-lipophilic balance (HLB). It can be defined as the ratio of unionized drug distributed between the organic and aqueous phase in equilibrium. Partition coefficient (solid water quotient of drug distribution) has a number of applications which are relevant to preformulation.

- Solubility both in aqueous and in mixed solvents
- Drug absorption *In-vivo*: applied to a homologous drug series for structure activity relationships
- Partition chromatography: choice of column (HPLC) and choice of mobile phase (eluent)

Partition coefficient of drug sample was determined by shake flask method. Equal volume of water (or phosphate buffer pH 6.8) and *n*-octanol were taken in glass stoppered flask and added accurately weight amount (10 mg) of Rosuvastatin. The mixture was shaken for 24 h at room temperature with the help of wrist action shaker. The two phases are separated by

separating funnel and the aqueous phase was analyzed spectrophotometrically at 238 nm for drug content after appropriate dilution. The drug concentration in n-octanol phase was determined by subtracting the amount in aqueous phase from the total quantity of drug. The partition coefficient P is expressed as by the equation:

$$\text{Log P} = \frac{\text{Concentration in n-octanol}}{\text{Concentration in water}}$$

N-octanol is used because the properties of n-octanol are thought to resemble those of lipid bilayer membranes. It has therefore been suggested that distribution that distribution of chemicals into n-octanol simulates, to a certain extent, their ability to passively diffuse across biological membranes.

RESULTS

Pre-formulation Studies

Rosuvastatin was procuring from Yarrow Chem, Mumbai. It was identified and characterized as per the identification test given in the Indian Pharmacopoeia (2010) and United State Pharmacopoeia.

Identification of the Drug

Organoleptic Property

- Colour: - White or almost white
- Odor: - odourless
- Appearance: - Crystalline powder

Identification of Drug by U.V Spectroscopy

Rosuvastatin was scanned between 230 nm to 360 nm. The solution showed absorbance maximum at 244 nm. (Spectra of Rosuvastatin in Methonal show below in Fig.1.

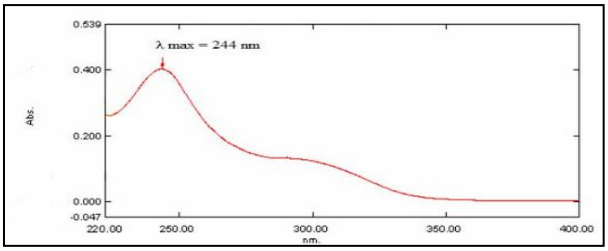

Fig-1: UV Scan of Rosuvastatin in Methanol (244 nm)

FTIR Spectroscopy

The IR spectrum of the obtained sample was done acc. to the procedure mention in section 5.2.1.4 and complied with the IR spectrum of reference standard of Rosuvastatin. IR spectra of sample drug show similar characteristic peaks. Fig.2 shows IR spectra analysis of standard drug Rosuvastatin and Fig. 3 shows the IR spectra of sample drug and the interpretation is shown in Table 1.

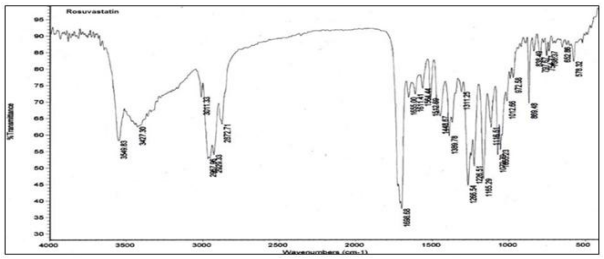

Fig-2: IR Spectra Analysis of Standard Rosuvastatin

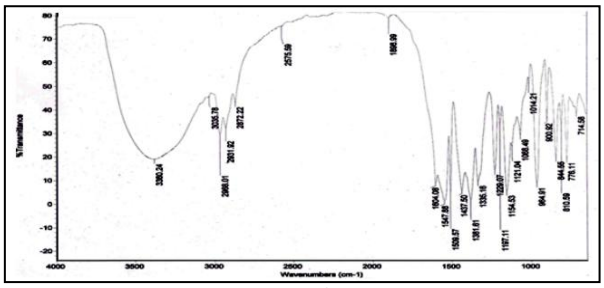

Fig-3: IR Spectra Analysis of Rosuvastatin (Sample)

Table-1: Interpretation of Rosuvastatin

S. No.	Reported peaks (cm^{-1}) in standard drug	Observed peak (cm^{-1}) of sample drug	Inference
1	1290 (1300)	1301	O-NO_2 stretching
2	1060 (1075)	1068	C-O stretching
3	1653 (1650)	1604	CO-NH
4	1371 (1310)	1335	Aromatic pyridine tertiary amine
5	3244 (3320)	2968	N-H stretching

Major functional groups like Aliphatic Ethers, Aliphatic Hydrocarbons, and Primary Aliphatic Alcohols, present in Rosuvastatin showed characteristic peaks in FTIR spectrum. The major peaks were identical to functional group of Rosuvastatin. Hence, the sample was confirmed as Rosuvastatin.

DSC: Has been used to measure the amount of heat energy absorbed (endothermic) or released (exothermic) by the drug when it is heated or cooled. The thermal curve of Rosuvastatin showed an initial flat profile followed by a sharp endothermic peak representing the melting of the substance in the range of 152.9 ºC. The thermal curves of both mixtures obtained by simple blending gave a superimposition as that of single component indicating the absence of solid-state interaction as shown in Fig. 4.

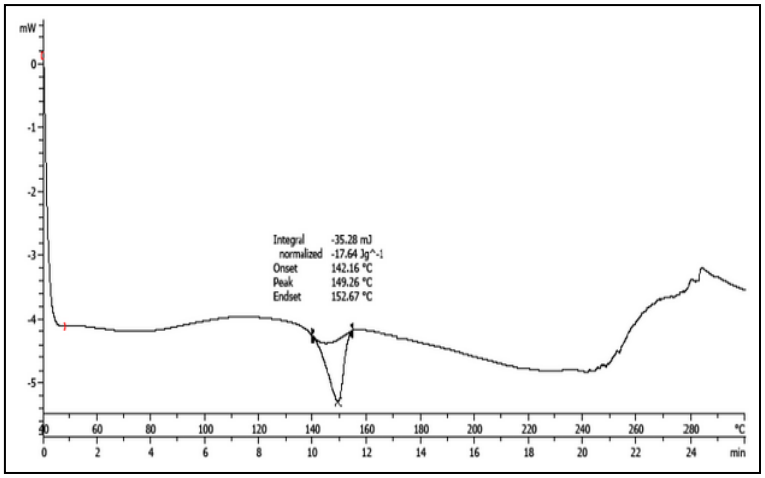

Fig-4: DSC of pure Rosuvastatin

Determination of Melting Point

Melting point range of the drug having from 152.9 ºC and Melting point of the drug was found to be 154-155 ºC. So the drug was found to be suitable for the formulation.

Solubility Study of the Drug

Qualitative: It was found that Rosuvastatin was soluble in most of the organic solvent and insoluble in water as shown in Table 2.

Table-2: Solubility Study of Drug

S. No.	Solvent	Interference
1	Water	Very slightly soluble
2	Ethanol	Very slightly soluble
3	Methanol	Soluble
4	DMSO	Sparingly soluble
5	0.1M HCl	Soluble
6	0.1 M NaOH	Slightly soluble

Quantitative: The results of Quantitative solubility of the drug are given below in the following Table 3.

Table-3: Solubility Study of Drug

S. No.	Solvent	Interference
1	Water	8.87 g/L mg of drug was present in distilled water
2	DMSO	15.5 g of drug was present in 1 m DMSO
3	Methanol	0.96 mg of drug was present in I L Methanol
4	Ethanol	0.87 mg of drug was present in 1L Ethanol
5	0.1M HCl	29.5 g of drug was present in 1 m HCl
6	0.1 M NaOH	1.5 g of drug was present in 1 m NaOH

Partition Coefficient of Rosuvastatin

Partition coefficient of the drug was determined by the procedure mention under the section 5.2.1.7 and shown in Table 4. The value of log P was found out to be 2.36. The standard value of log P for the drug is 2.40.

Table-4: Partition coefficient of Rosuvastatin

Water: n- octanol (ml)	Conc. of drug in water (µg/ml)	Conc. of drug in n-octanol (µg/ml)	Log P
1:1	6.72	14.8	2.36

Preparation of Calibration Curve of Rosuvastatin

Calibration curve of Rosuvastatin were prepared as per the procedure mentioned in section 5.2.2.

Table-5: Standard Curve of Rosuvastatin in Methanol

S. No.	Concentration (µg/ml)	Absorbance
1.	0	0.000
2.	1	0.0141
3.	2	0.0223
4.	3	0.0423
5.	4	0.0531
6.	5	0.0672
7.	6	0.0782
8.	7	0.0901
9.	8	0.1190
10.	9	0.1212
11.	10	0.1314

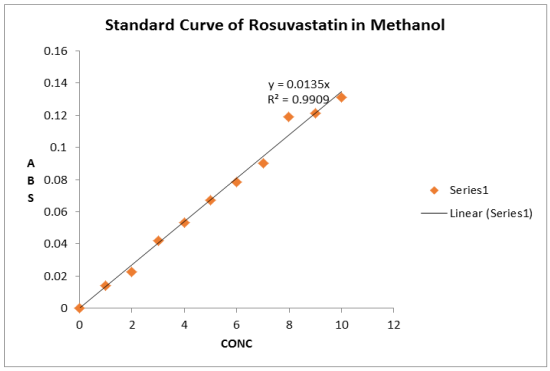

Fig-5: Calibration Curve of Rosuvastatin in methanol at λ_{max} 244 nm

Table-6: Standard Curve Data of Rosuvastatin in 0.1N HCl

S. No.	Concentration (µg/ml)	Absorbance
1	0	0.000
2	1	0.0172
3	2	0.0269
4	3	0.0413
5	4	0.0545
6	5	0.0669
7	6	0.0811
8	7	0.0934
9	8	0.1081
10	9	0.1191
11	10	0.1312

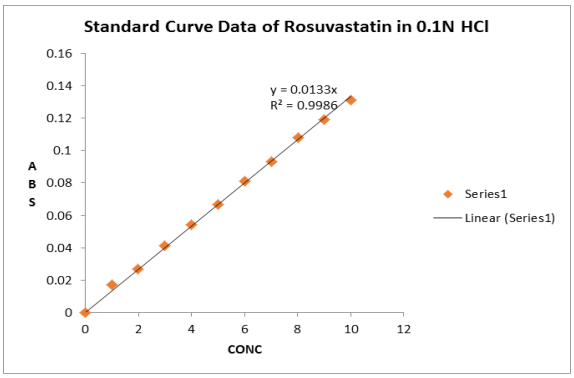

Fig-6: Calibration curve of Rosuvastatin in 0.1N HCl at λ_{max} 244 nm

Table-7: Standard Curve Data of Rosuvastatin in PBS pH 6.8

S. No.	Concentration (µg/ml)	Absorbance
1	0	0.000
2	1	0.0181
3	2	0.0344
4	3	0.0551
5	4	0.0731
6	5	0.0908
7	6	0.1101
8	7	0.1291
9	8	0.1491
10	9	0.1662
11	10	0.1893

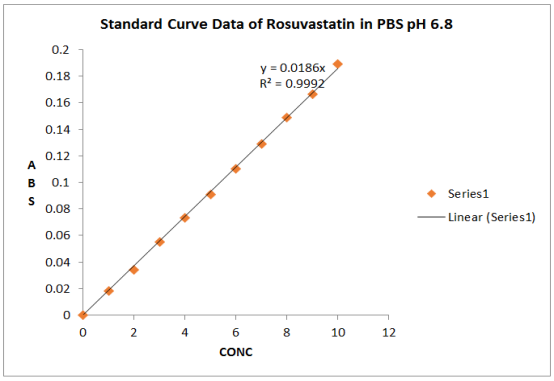

Fig-7: Calibration curve of Rosuvastatin in PBS pH 6.8 at λmax 244 nm

Table-8: Standard Curve Data of Rosuvastatin in PBS pH 7.4

S. No.	Concentration (µg/ml)	Absorbance
1	0	0.000
2	1	0.0162
3	2	0.0322
4	3	0.0591
5	4	0.0731
6	5	0.0949
7	6	0.1129
8	7	0.1321
9	8	0.1521
10	9	0.1672
11	10	0.1911

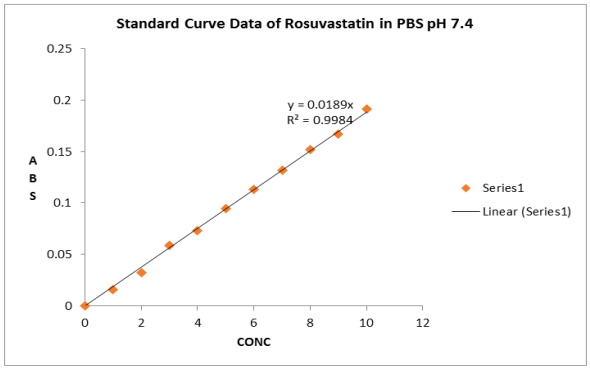

Fig-8: Calibration curve of Rosuvastatin in PBS pH 6.8 at λ_{max} 244 nm

Formulation Development and Characterisation
Preliminary Screening (Optimization):

From the compatibility study polymers, water soluble grade chitosan and HPMC E5 and surfactants, Poloxamer 407, Tween 80 were selected for further study. The safe range of HPMC in ophthalmic formulation is 0.45 -1% w/w. So the optimization trials were taken in the concentration of 0.25-1% w/w. Water soluble chitosan is a mucoadhesive biodegradable polymer. It's safe concentration for suspension use is not mentioned but in previous studies it was used in the range of 0.1 to 1% w/w. So the optimization trials were taken in 0.1-0.5% w/w. Surfactants, Poloxamer and Tween 80 is safe for preparations in the range of 0.1 to 5%w/w. So the optimization trials were taken 0.1-0.5% w/w.

Formulation of Rosuvastatin Nanosuspension

The preliminary study which was carried out to select the range of polymer and surfactant has revealed that the formulations were showing desired particle size and entrapment efficiency when the chitosan concentration was 0.3% w/v, HPMC E5 concentration was 0.5% w/v and surfactant concentration 0.2- 0.5% w/v. The appearance of the formulation was found slightly clear to clear. The Chitosan and HPMC getting aggregated when used more than 0.3% and 0.5% w/v. So this indicates that the selected concentration is more enough to entrap the drug.

Effect of Surfactant and Polymers on Particle Size

In optimized formulations the formulation with Poloxamer 407 given less particle size compared to Tween 80. But when compared with combination of Poloxamer 407 and Tween 80 has showed very less particle size. This claims that there is a synergistic effect of surfactants on particle size. There is no effect of combination of polymers on particle size. The role of polymers on drug release may be effective.

Process Optimization

Table-9: Process Optimization for Rosuvastatin Nanosuspension

S. no.	Parameter	Optimized range
1	Homogenization Speed	6000 rpm
2	Homogenization Time	15 min
3	Sonication Amplitude kHz	30 kHz
4	Sonication Time	20 min

From trial and error method many batches were taken to optimize the process parameters. The optimized process parameters are given in table for Rosuvastatin.

Characterization of Rosuvastatin Nanosuspension
pH: The pH of the formulations is shown in the table 18.

Particle Size and Charge

All the formulations prepared were having particle size below 600 nanometers with polydispersity index 0.4 to 0.9 indicating that nanoparticles are homogeneously dispersed in the dispersion. The zeta potential observed was in between -12 to -42 mV indicating that dispersions are stable.

Entrapment Efficiency

The entrapment efficiency of the formulated Rosuvastatin nanosuspension was determined by centrifugation method. All formulations RF1-RF9 have entrapment efficiency above 50%. Thus more than 80 % of the added drug was found to be entrapped in the vesicles.

Table-10: Evaluation of Rosuvastatin Nanosuspension

Formulation Code	Mean Particle Size	PDI	Zeta Potential
RF1	459±20	0.9±0.1	-38±4
RF2	498±16	0.8±0.1	-40±6
RF3	400±10	0.6±0.1	-39±4
RF4	137±12	0.8±0.2	-34±6
RF5	147±18	0.5±0.2	-20±4
RF6	85±20	0.9±0.2	-31±4
RF7	267±17	0.7±0.2	-42±4
RF8	285±22	0.5±0.2	-12±6
RF9	209±14	0.4±0.1	-34±6

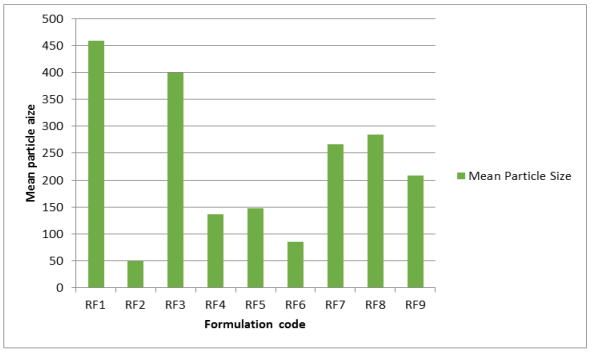

Fig-9: Mean Particle Size of Formulation

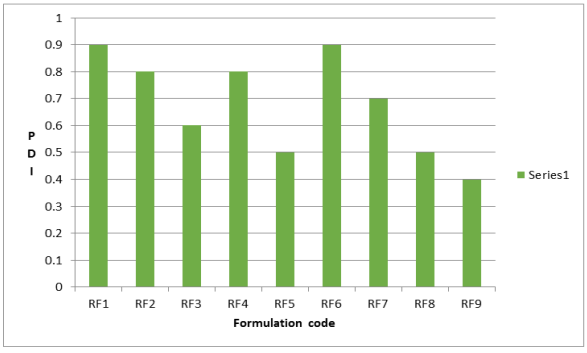

Fig-10: Poly Dispersity Index of Formulation

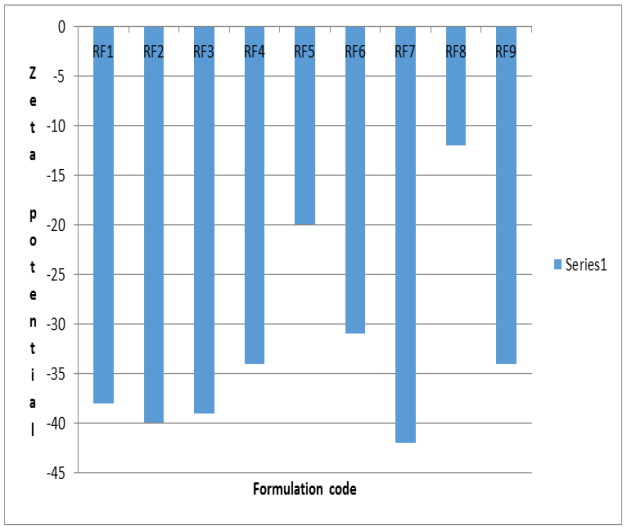

Fig-11: Zeta Potential of Formulation

Table-11: Evaluation of Rosuvastatin Nanosuspension

Formulation Code	% Entrapment Efficiency	pH	Appearance
RF1	80±2	7.2	Slightly Clear
RF2	91.71±2	7.3	Clear
RF3	94±2	7.4	Slightly Clear
RF4	89.95±1	7.3	Clear
RF5	91.71±2	7.4	Clear
RF6	90.54±1	7.2	Clear
RF7	88.20±2	7.4	Clear
RF8	93.46±2	7.3	Slightly Clear
RF9	92.29±1	7.4	Clear

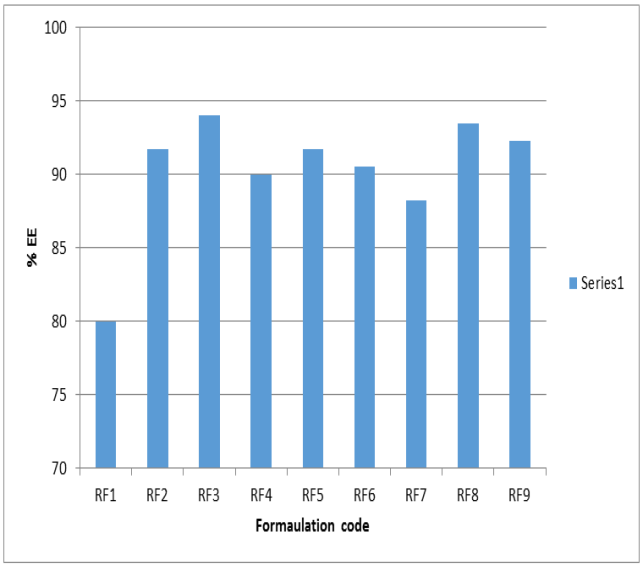

Fig-12: % Entrapment Efficiency of Formulation

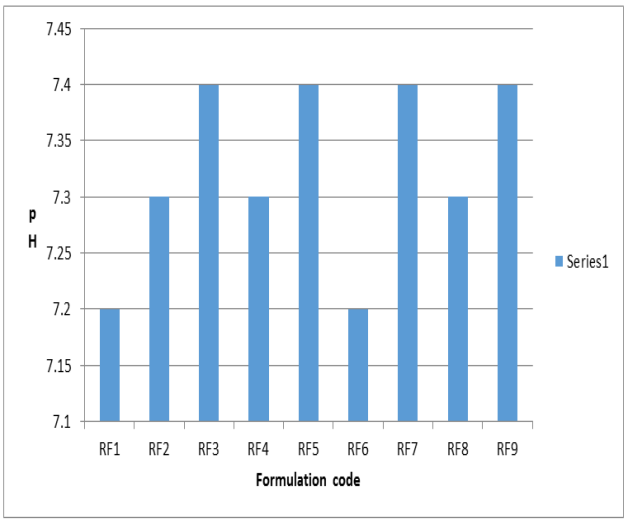

Fig-13: pH of Formulation

Zeta Potential Measurement

The sample of 1ml was taken into disposable folded capillary cell and zeta potential was determined using zeta potential measuring instrument (ZS90, Malvern Instruments, and Worcestershire, UK). In case of zeta potential, electric field of -120 to 120V applies. Zeta potential of optimized formulation was -35.2 ± 0.67 mV.

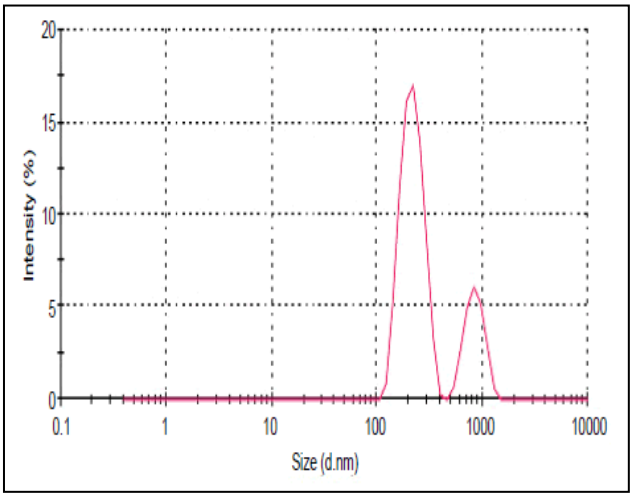

Fig-14: Zeta potential of optimized nanosuspension formulation

Transmission Electron Microscopy

TEM study of optimized NE was done to find out more information about the morphology and mean diameter of the globules of the nanosuspension system. TEM has indicated that most of the oil globules were of uniform shape (spherical) and in the nanometer range (size range 33–40 nm) (Fig. 15). The sizes of globules were in the further agreement with the results obtained using dynamic light scattering (DLS).

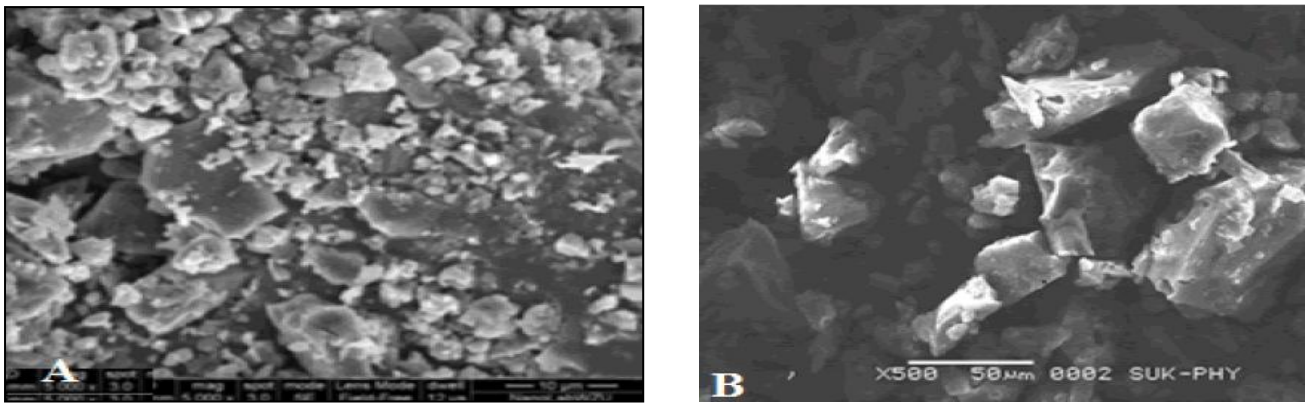

Fig-15: SEM Analysis of Rosuvastatin (A) and Optimized Batch of Nanoparticles (B)

In vitro Drug Release Study

The in vitro drug release profiles revealed that all batches extended drug release up to 6 to 9 h as shown in Fig. 16. The optimized formulation RF3 comprising of 0.3% water soluble chitosan, 0.2% Tween 80 and Poloxamer 407 in 1: 1 ratio, prolonged the drug release up to 9 h. From the above data water soluble chitosan based formulation extended drug release up to 9 h compared to HPMC based formulations which were limited up to 6 hrs. This effect might be due to the mucoadhesive effect of the water soluble grade chitosan.

Table-12: Comparative cumulative % drug release between drug solution and NS

Time (h)	Cumulative % drug release from Rosuvastatin solution (± SD)	Cumulative % drug release from Rosuvastatin - NS (± SD)
0.5	35.64± 5.1	22.22 ± 4.1
1	44.89± 5.7	28.43 ± 4.8
2	64.36± 4.3	34.01 ± 5.1
3	71.18 ± 4.6	41.10 ± 4.2
4	99.18± 4.8	46.32 ± 4.3
6	-	55.76± 4.9
8	-	69.62 ± 3.5
10	-	74.21± 5.8
24	-	89.90 ± 4.2

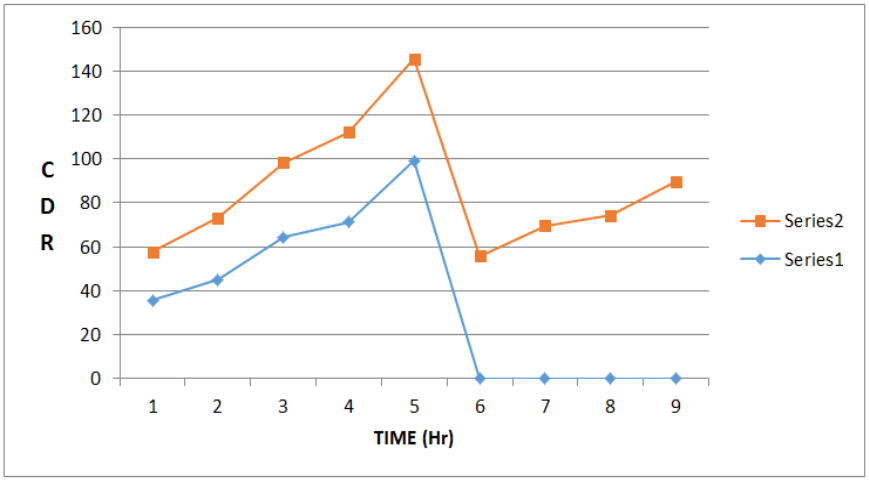

Fig-16: Comparative cumulative % Drug Release between Drug Solution & NS

Mechanism of Drug Release from Rosuvastatin loaded NS

The analysis of kinetics of release of the optimized nanosuspension was undertaken to find out the release mechanism of developed formulation.

Table-13: Kinetic analysis of Rosuvastatin loaded NS (n=3)

Time (min)	Square root of time	Log time	% Cumulative release	Fraction drug release	Log % drug released	% Drug remaining	Log % drug Remaining
30	4.77	1.577	22.2	0.203	1.426	76.8	1.796
60	6.45	1.878	28.4	0.265	1.337	72.6	1.816
120	09.54	2.179	34.54	0.305	1.631	66.4	1.719
180	12.16	2.155	41.1	0.401	1.703	58.9	1.677
240	14.91	2.138	46.3	0.453	1.656	53.7	1.937
360	17.73	2.456	55.76	0.507	1.838	44.24	1.555
480	20.08	2.581	67.6	0.646	1.923	33.4	1.423
600	23.94	2.678	74.21	0.732	1.814	25.79	1.427
1440	36.47	3.258	89.9	0.889	1.948	13.1	1.145

Table-14: Co-efficient of Correlation for Optimized NS

Zero Order		First Order		Higuchi Model		Korsmeyer-peppas	
R^2	K_0	R^2	K_1	R^2	K_H	R^2	K
0.758	0.000	0.961	-0.000	0.963	0.024	0.945	0.612

DISCUSSION

The preliminary study was carried out in order to identify and characterize the drug. The identification of the drug was carried out by UV spectroscopy, FTIR and Differential Scanning Colorimeter (DSC). The λmax of the Rosuvastatin was found to be 244 nm which was confirming to the already available literature. The FTIR spectrum was showing the characteristics peaks of the drug at different wave numbers and was confirming to the various groups available in structure of Rosuvastatin. The DSC thermogram was showing sharp endothermic peak at 152.9 ºC which was due to melting of the crystals of drug at this temperature. The melting point of the drug mentioned in official books is 152.9 ºC. Thus the sample of the drug was identified and confirmed by determining its various characteristics.

During formulation development of nanosuspension it is necessary to determine the factors which will have the potential effects on the responses like particle size, entrapment efficiency, in vitro release profile etc. which affects overall performance of the dosage form. Based on the literature survey the concentration of the polymer and surfactants which were considered as potential factors affecting performance of formulation were subjected to preliminary screening. The particle size was found to be increasing with increase in concentration of the polymer which is in confirmation of the earlier results by other groups. The more surfactant may be forming agglomeration of particles which results in increase in size. The particle size was found to be more than 950 nm when the concentration crossed above 0.3% w/v with water soluble chitosan and with 0.5% w/v with HPMC E5, so the concentration of the polymer was used below this level. The effect of surfactant concentration on particle size was studied at levels from 0.1 to 0.5 % v/v. The particle size was found to be directly proportional to the concentration of the surfactant. These results were contradictory to the earlier results. This may be attributed to high surfactant concentration, which causes particle agglomeration, suffices for changing particle morphology. Another reason may be that though intermediate surfactant concentration increases inter-particle repulsion by promoting fluctuation force; at high concentration. Based on these studies the three different concentrations of polymer (water soluble chitosan 0.2%, 0.3%, 0.4% w/v) and ethanol (HPMC E5 0.4%, 0.5%, 0.6% w/v) and the surfactants Poloxamer 407 and Tween 80 in (0.1%, 0.2% and 0.3%) were taken for trial and error batches. These are tried as individually and in combination as mentioned in experimental were selected and 9 different formulations were prepared on the basis of trial and error approach.

Formulation and Characterization of Rosuvastatin Nanosuspension

The 9 different formulations with three different levels of polymers and surfactants were prepared by nanoprecipitation method. The prepared nanosuspension were characterized for particle size, PDI, Zeta potential and entrapment efficiency. The particle size was increased with increased concentration of polymer as discussed earlier. The PDI was found to be increased with increase in the concentration of polymer and for the concentrations above 0.3 % w/v of water soluble chitosan and 0.5% HPMC E5 the PDI was more than 0.9. This indicates that the more concentration of polymer makes the suspension heterogeneous. Poloxamer 407 and Tween 80 are non-ionic surfactants and were incorporated in the formulation to prevent the growth or slugging of nanoparticles by giving steric stabilization. The concentration of Poloxamer and Tween 80 resulting in ideal particle size were designated to avoid its excess concentration, as higher amount of surfactant results in high initial burst release, and moreover, sufficient concentration of surfactant is necessary to reduce the particle size and to prevent aggregation. The combination of surfactants was found more effective as compared to individual effect. This is might be due to the synergistic effect of two surfactants. The particle

size and PDI of the optimized formulation (RF3) were found to be 400±10 nm and 0.6±0.1, respectively. Small values of PDI indicated a homogeneous population.

The entrapment efficiency of the particles was found to be increased as the concentration of surfactants was increased and the combination of surfactant showed good impact on entrapment efficiency. The entrapment efficiency was found more than 80 % for all the formulation and lower the particle size, and higher entrapment of the drug was observed.

In-vitro Release of Drug Rosuvastatin Nanosuspension

In vitro release study from Rosuvastatin loaded nanosuspension was carried out in artificial tear fluid of pH 7.4 using dialysis membrane for 12 h. In vitro release profiles of all the formulations RF1 to RF9 are shown in Fig. 16. The biphasic release pattern was observed for all the formulations. More than 25% of the drug was found to be released within initial 2 h which may be due to release of UN entrapped drug which was adhered to the polymeric surface. When these results compared with their respective particle size it was observed that the particle diffusion is the rate limiting step in the drug release. The optimized formulation RF3 comprised of 0.3% water soluble chitosan, 0.2% Tween 80 and Poloxamer 407 in 1:1 ratio prolonged the drug release up to 9 h.

From the observed data, water soluble chitosan-based formulation extended drug release up to 9 h compared to HPMC-based formulations which were limited up to 6 h. This effect might be due to the mucoadhesive effect of the water soluble grade chitosan 61.

Curve fitting of in-vitro release data of the optimized formulation was compared with a different release model to select the best fit kinetic model using PCP Disso software (version 3.0). The best kinetic model was found to be the Peppas model (R=0.9796, t test=13.79 (passes) with a critical value of n=0.7), and it follows the non-fickian transport type of drug release.

Stability Study

Stability study indicated that the formulation was physically and chemically stable when stored at the 40 ± 2°C and 75 ± 5% RH for a period of one month. It was observed that there was a slight change in all optimization parameters which have less than ±5% bias which was insignificance. Negligible difference was observed in results obtained from optimized batch before and after stability study

CONCLUSION

The Rosuvastatin nanosuspension was successfully formulated and optimized for polymer and surfactant content. The particle size of Rosuvastatin nanosuspension was found to be below 450 nm when concentration of the polymer and surfactant used was below 0.3% w/v for water soluble chitosan and 0.5% w/v for HPMC E5 and both combined surfactants concentration below 0.2% w/v. The optimized formulation RF3 was having less particle size and higher entrapment efficiency as compared to other formulations. The particle size and entrapment efficiency of RF3 were found to be 300 ± 10 nm and 94 ± 2 % respectively.

Nanosuspension formulations were found to be safe. Consequently, after the data analysis, the nanosuspension was able to sustain the release of drugs for prolonged time. The nanosuspension found stable at 5± 20^0C for six months. These results may reveal a potential application of this new formulation in bacterial conjunctivitis and glaucoma management, in order to improve patient compliance by lowering the frequency of administration and to enhance therapeutic effectiveness of conjunctivitis and glaucoma treatment. Thus the ophthalmic nanosuspension could be an effective and safe ocular drug delivery system for the drugs which are having low ocular bioavailability.

Though in this study the attempt has been done to get optimum particle size with desired characteristics by taking into consideration the percentage of polymer and surfactant as independent factors and the process parameters homogenization time, sonication time in to consideration the others can also use the other excipients and formulation method. In present study the optimization is done on the laboratory trial and error experiment. The other can use the different quality by design (QbD) approaches for preliminary screening. This study will be helpful for pilot scale production of nanosuspension and technology transfer for production level.

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