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Experimental Design Development and Characterization of Rosuvastatin Loaded Nanosuspension for Solubility Enhancement

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Abstract

The purpose of this study was to develop rosuvastatin nanosuspension with enhanced solubility and bioavailability; it was prepared by precipitation ultrasonication method. To ensure the quality of the rosuvastatin nanosuspensions, the selected formulation (F10) with particle size 200nm, entrapment efficiency 89.6% and *in vitro* drug release 83.5% at 60 min was subjected to 2^2 factorial design. The optimum composition obtained using a 2-factor, 2-level Factorial design was as follows: polyvinyl alcohol (90 mg), sonication time of 40 min. The constant regression values for particle size was 180nm, zeta potential -24.5mV, *in vitro* drug release 88.3%, entrapment efficiency 91.5%. From the data it was observed that **R2** formulation was the best formulation. Scanning Electron Microscopy revealed that the particles were prismatic in shape. Stability studies performed for a period of 3 months indicated that there were no significant changes in the *in vitro* drug release pattern and entrapment efficiency.

Keywords: Rosuvastatin, Nanosuspension, Solubility, Bioavailability

Introduction

Low aqueous solubility is the major problem in formulation development of several new chemical entities. Often, these poorly water soluble drugs need to be administered at high doses to achieve desired therapeutic plasma concentrations after oral administration. A great number of new and possibly beneficial chemical entities do not have suitable pharmaceutical dosage forms because of their poor solubility and poor dissolution rates. Nanosuspensions are colloidal dispersions of nanosized drug particles stabilized by surfactants. They can also be defined as a biphasic system consisting of pure drug particles dispersed in an aqueous vehicle in which the diameter of the suspended particle is less than 1µm in size.[1] 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. The increase in the saturation solubility and solution velocity of nanoparticle is due to increase of vapour pressure of the particles. [2]

Innovations for the planning of nanosuspension are generally divided into two different ways, top-down and bottom-up techniques. The top-down technique decrease the drug particle size without organic solvent utilizing procedures like milling (jet mill and ball mill) and high-pressure homogenization. Nonetheless, in some cases these top down techniques are hard to apply to thermolabile materials since they are high-energy process and consequently, they produce heat. Besides, a lot of energy can produce amorphous particles and deform

crystals. The bottom up technique utilizes the particle precipitation from a saturated or unsaturated drug solution. The bottom up methods incorporate different procedures,such as solvent evaporation, supercritical fluid, antisolvent precipitation, and chemical precipitation. These techniques require moderately low energy contrasted with top down technique. While applying the most generally utilized the antisolvent method, it is vital to control the residual solvent and particle growth. Improper control of particle growth is a consequence of an inadequate comprehension of the formulation and manufacturing processes. Thus, there has been a requirement for development of robust processes that do not involve additional harsh processes for organic solvent removal to prepare a nanosuspension.[2]

Rosuvastatin calcium (RC), a HMG-CoA reductase inhibitor, is widely used in the treatment of hyperlipidemia. RC promotes conversion of HMG-CoA to mevalonic acid by reducing the synthesis of cholesterol. However, RC has poor solubility and bioavailability (20 %). Of late, various kinds of formulations of RC such as self-nanoemulsifying drug delivery systems, lipid nanoparticles[9], nanolipospheres, solid lipid nanoparticle and nanostructured lipid carriers have been developed to enhance the oral bioavailability of RC. However, solid dispersion and nanosuspension of RC (NRC) have not been evaluated. The present research work deals with the development of tablets using nanosuspension and solid dispersions of RC to enhance its solubility and dissolution. [3].

Materials and Methods

Rosuvastatin was obtained from Sun Ridges Health Care Pondicherry, Poloxamer 407, PVA, and HPMC K100M was obtained from Yarrow Chem Products, Mumbai.

Preparation of nanosuspension : precititation ultrasonication method

Rosuvastatin was completely dissolved in methanol to form organic phase and deionised water containing different polymers as anti-solvent. 1ml of organic solution was quickly injected by a syringe into an anti-solvent phase under sonication for 30 min in an ice cold condition. The samples were placed in a magnetic stirrer for 3h and subsequently placed in refrigerated ultracentrifuge for 1h at 20000rpm. The supernatant was discarded and replaced by same quantity of fresh antisolvent. The solid residue was redispersed by sonication (Table 1). (3).

Table 1: Formulation of Rosuvastatin loaded nanosuspension

Formulation code	Rosuvastatin (mg)	Poloxamer 407 (mg)	HPMC 100M (mg)	PVA (mg)
F1	50	50	-	-
F2	50	100	-	-
F3	50	150	-	-
F4	50	200	-	-
F5	50	-	50	-
F6	50		100	-
F7	50	--	150	-
F8	50	-	200	-
F9	50	-	-	50
F10	50	-	-	100
F11	50	-	-	150
F12	50	-	-	200

Table 3: Particle size of the prepared drug loaded nanosuspension

Formulation Code	Particle Size (nm)	Entrapment Efficiency (%)	Drug content(%)
F1	278.3	78.6 ± 0.3	96.71
F2	268.5	80.3 ± 1.2	96.42
F3	245	84.7 ± 0.2	98.89
F4	284.5	83.9 ± 0.9	97.45
F5	310.2	68.2 ± 0.9	92.32
F6	298.3	72.2 ± 1.4	97.84
F7	328.9	74.2 ± 0.5	94.39
F8	345.2	75.5 ± 0.2	95.27
F9	211.9	85.2 ± 0.7	96.34
F10	200	89.09 ± 0.6	99.04
F11	257.4	87.1 ± 0.3	95.54
F12	283.5	87.2 ± 0.5	97.65

Evaluation of nanosuspension

Mean particle size and zeta potential

Particle size and zeta potential of rosuvastatin nanosuspensions were measured by photon correlation spectroscopy (PCS) using Malvern Zetasizer. The particle size analysis was performed at a scattering angle of 90 C at room temperature.[4]

Drug content

Drug content in the rosuvastatin nanosuspension was determined by dissolving the equivalent of 10 mg of drug in methanol (50 µg/mL). The samples were subsequently sonicated and aliquots were filtered using 0.11 µm Sartorius filter (Sartorius, AG, Germany). The study was performed in triplicate. The drug content was determined using the following equation:

Drug content (%) = (Observed drug content/ Theoretical drug content) × 100.

Entrapment efficiency

The prepared nanosuspension was ultracentrifuged, the amount of free Rosuvastatin present in the clear supernatant was measured using a UV spectrophotometer.[5]

FTIR measurements

FTIR spectra of rosuvastatin, API, and nanosuspensions were recorded using Shimadzu FTIR (Japan). 2–3 mg of a sample was mixed with crystalline KBr and pellet was prepared. Each sample was scanned through the wavenumber region of 4000–400 cm−1.

In vitro drug release by using dialysis sac

The *invitro* release of various nanosuspension formulations were performed by dialysis bag diffusion technique. The sac was hermetically sealed and filled with pH 7.4 phosphate buffer and emptied for leaks. The receptor compartment contained 100 mL of pH 7.4 phosphate buffer maintained at 37±0.5^{0}C under agitation at 500rpm using a magnetic stirrer. At specific time intervals, aliquots of 1 mL were withdrawn and immediately restored with the same volume of fresh pH 7.4 phosphate buffers. The amount of drug released was assessed by measuring the absorbance at 300 nm using a single beam UV spectrophotometer.[5]

Kinetic analysis of release data

The obtained dissolution data were fitted to zero order, first order, Higuchi- Crowell and Korsmeyer-Peppas equations to understand the rate of drug release from the prepared formulations. The correlation coefficient values were calculated and used to find the fitness of the data.[6][7]

Statistical optimization

A 2^2 factorial design was used to optimize the variables in the present study. In this design, 2 factors: concentration of PVA and sonication time evaluated, each at 2 levels: low and high and experimental trials were performed at all 4 possible combinations. Various concentrations of PVA (X1) and sonication timewere selected as independent variables. Mean particle size (Y1), Zeta potential (Y2) Entrapment efficiency and *in vitro* drug release were selected as dependent variables. Data obtained from all formulations were analyzed using Mintab 18-V 12.20.

Scanning electron microscopic study of optimized formulation

The optimized formulation was centrifuged, filtered and dried to convert to powder form. The dried powder was evaluated for SEM analysis. The morphology of rosuvastatin loaded nanosuspension was studied using SEM (S-4800, Hitachi technologies corporation, Japan). Prior to the examination, the sample is mounted onto metal stubs using a double sided adhesive tape and sputtered with a thin layer of gold under vacuum. The scanning electron microscope is operated at an acceleration voltage of 1.5KV.[8][9][10]

HPLC-analysis

The Rosuvastatin concentrations were determined by using a high performance liquid chromatography (HPLC) method. The mobile phase was based on the method of the European Pharmacopoeia: 1.4 g of di-sodium hydrogen phosphate was added to 1000 ml MilliQ-water and the pH was adjusted with phosphoric acid to 7.6. Seventy-three parts of this buffer were mixed with 27 parts of HPLC-grade acetonitrile. Flow rate was 1 ml/min; the UV detector was operated at a wavelength of 280 nm. A Eurosphere 100, C18, 5 mm column was used in the HPLC hardware from Kontron Instruments (Germany). The nanosuspension samples were prepared by diluting 10 ml of nanosuspension to 10 ml with mobile phase. A freshly prepared standard was checked with every run. To compare the stability of the nanosuspension with the rosuvastatin solution a reference specimen was prepared by dissolving an exact amount (5 mg) of Rosuvastatin in 8.4% sodium bicarbonate solution. The concentration of this reference was assayed directly without dilution.[11]

Stability study

A short term (3 months) stability studies were performed for the final optimized nanosuspension. The temperature was maintained at 40^0C / 75% RH, to monitor the extend of entrapment efficiency and *invitro* drug release.[12]

Results and Discussion

Compatibility study of drug and excipients - ft-ir

Through FTIR analysis, The IR spectra of Rosuvastatin calcium is characterized by the absorption frequency of two stretching band at 3394.72 cm-1 and that of carbonyl group at 1604.77 cm-1.[13] The results indicate that has no interactions or bondings between drug and polymers/excipients, so there was no chemical incompatibility between drug and excipients used in the formulation.

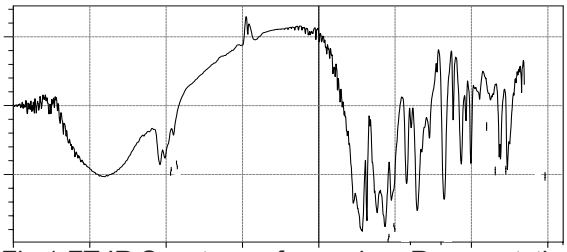


Fig 1:FT-IR Spectrum of pure drug Rosuvaststin

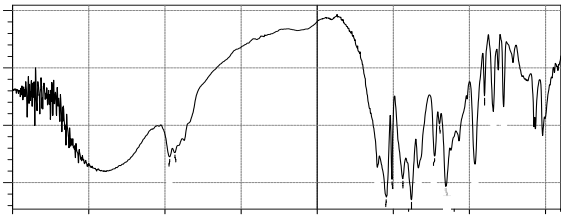


Fig 2: FT-IR Spectrum of drug and HPMC K100M

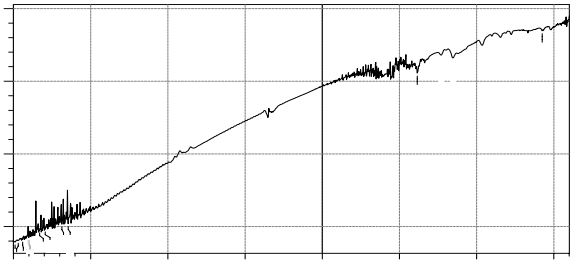


Fig 3: FT-IR study of drug and PVA

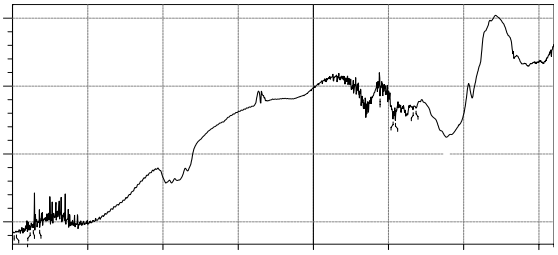


Fig 4:FT-IR study of drug and Poloxamer 407

Evaluation of nanosuspension

The nanosuspension batches containing different stabilizers may influence the droplet size upon aqueous dispersion. The average particle size distribution data obtained was in the range of 198 – 310 nm. Among the set of nanosuspensions, prepared using three different polymers lower particle sizes obtained were 245 ± 1.50 nm for Poloxamer 407 (F3),

298.3 ± 1.02 nm for HPMC K100M (F6), 200 ± 1.12 nm for PVA (F10). On comparison, F10 formulation prepared using PVA tend to show lowest particle size (Table 3). The entrapment efficiency of the formulations was in the range of 68.2 – 78.8%. When concentration of polymer is increased, the platform for binding the drug to the core is increasing.[14] Among the set of nanosuspensions, prepared using three different polymers lower entrapment efficiency obtained were 84.7 ± 0.2% for Poloxamer 407 (F3), 298.3 ± 1.02 nm 72.2 ± 1.4% for HPMC K100M (F6), 89.09 ± 0.6% for PVA (F10). On comparison, F10 was found to have highest entrapment efficiency (100mg PVA) and drug content (Table 3).

***In vitro* drug release using dialysis sac**

Drug release data for all batches of Rosuvastatin loaded nanosuspensions were in the range 52-82%. From the data, it was clear that amount of polymer directly compromises the drug release of prepared formulations. It was observed that the *in vitro* drug release of the F10 formulation was faster and higher than other formulations. The sharp increase and highest rate (83.5% at 60min), in the *in vitro* drug release was due to the increased surface area of the nanosized drug particles. According to Noyes–Whitney equation, an increase in solubility and decrease in particle size lead to an increased in rate of drug release and it has been reported that the solubility increases with decreasing particle size (nanometers in range). [15]The overall release profiles in the present investigation suggests that nanosized drug particles (≈200 nm) found to have profound impact on rate of drug release and drug solubility. The bioavailability of nanosuspension thus can be affected by the rate of drug release, where particle size reduction can significantly improve the performance of the drug.

From the above evaluation parameters F10 has shown lower particle size, higher entrapment efficiency and highest *invitro* drug release rates. So, F10 formulation was considered as the best from the trial datas.

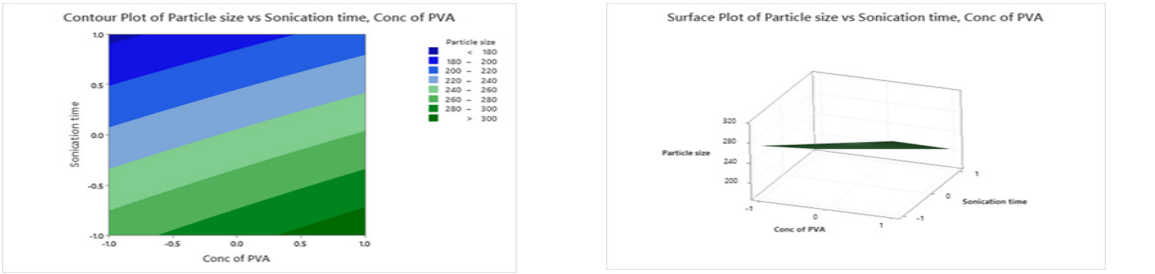
Statistical optimization

A 2^2 factorial design was used to optimize the variables.. In this design, 2 factors: concentration of PVA and sonication time evaluated, each at 2 levels: low and high and experimental trials were performed at all 4 possible combinations. Various concentrations of PVA (X1) such 90mg, 110 mg and sonication time were 20min, 40min were selected as independent variables (Table 4). Mean particle size (Y1), Zeta potential (Y2), Entrapment efficiency (Y3) and *in vitro* drug release (Y4) were selected as dependent variables. Data obtained from all formulations were analyzed using Mintab 18-V 12.20. All batches that showed particle size in the range of 175-315 nm, zeta potential -7.32 to -27.1 mV, entrapment efficiency 68.7-91.52%, *in vitro* drug release 55.9-88.3%.The various models fitted for each responses were linear and two- factor interaction models. Using the ANOVA available in the software, the polynomial equations involving the main effects and interaction factors were determined based on the estimation of various statistical parameters. [16] Obtained data were subjected to multiple regression analysis.

Table 4: Optimization batches of Nanosuspension using 2^2 Factorial Design

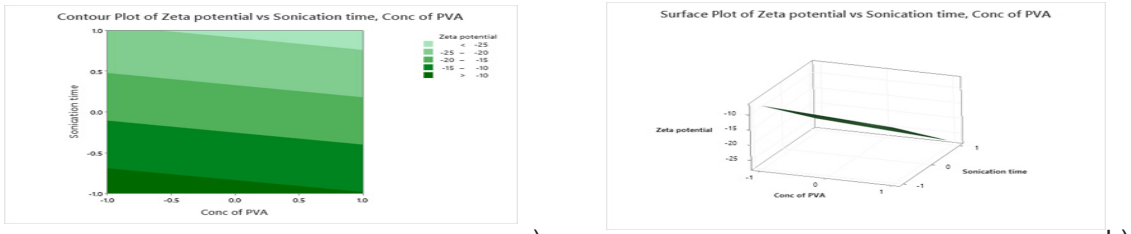
Run Order	X1	X2	Y1	Y2	Y3	Y4
R1	-1	-1	272	-7.32	68.72	62.48
R2	-1	+1	180	-24.5	91.52	88.32
R3	+1	-1	315	-9.81	73.86	55.9
R4	+1	+1	209	-27..1	88.7	78.3

X1=Concentration of PVA , X2=Sonication time, Y1=Particle size, Y2=Zeta potential,Y3=Entrapment efficiency, Y4= *In vitro* drug release



a) b)

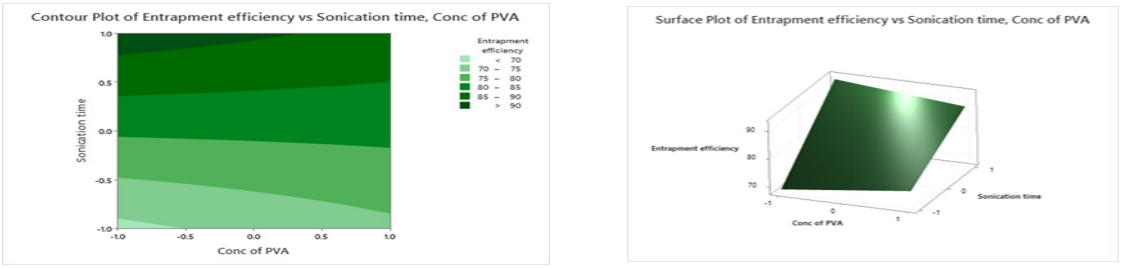
Fig. 5: a) Surface Plot of Y1 (Particle size) vs X2(Sonication time), X1 (Conc. Of PVA).b) Contour plot of Y1 (Particle size) vs X2 (Sonication time), X1 (Conc. Of PVA)



a) b)

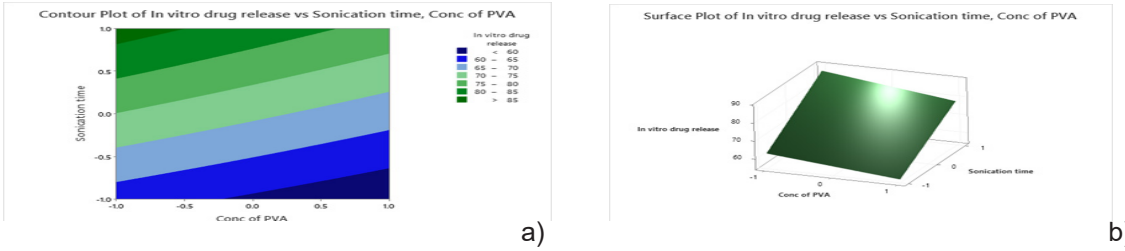
Fig. 6: a) Surface Plot of Y2 (Zeta potential) vs X2(Sonication time), X1 (Conc. of PVA)

a) Contour Plot of Y2 (Zeta potential) vs X2(Sonication time), X1 (Conc. of PVA



a) b)

Fig.7: a)Surface Plot of Y3 (Entrapment efficiency) vs X2(Conc. Of Sonication time),X1(Conc.of PVA) b)Contour Plot of (Entrapment efficiency) vs X2 (Sonication time), X1 (Conc. Of PVA)

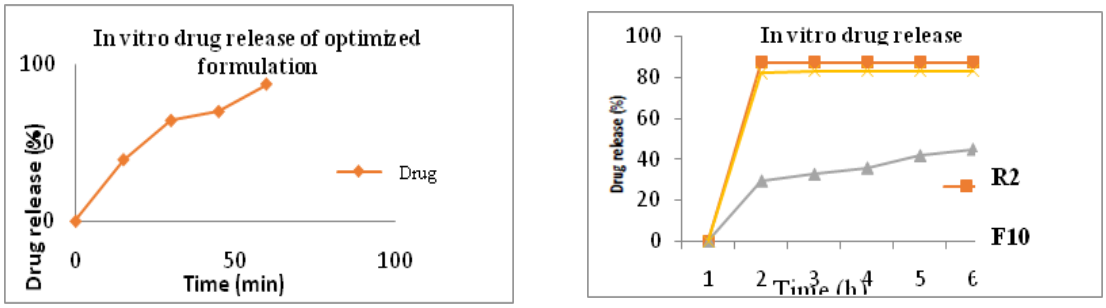


a) b)

Fig. 8: a) Surface Plot of Y4(In vitro Drug release) vs X2(Sonication time), X1 (Conc. Of PVA) b)Contour Plot of Y4(In vitro Drug release) vs X2(Sonication time), X1 (Conc. Of PVA)

In Vitro dissolution study of optimized formulation (r2)

It was observed that the *in vitro* drug release of the optimized formulation R2 was faster and higher than pure drug. The sharp increase and highest rate (87.32% at 60 min), (87.35% at 5h) was obtained in comparison with pure drug (44.68% at t_{5h}) and F10 (82.2% at t_{5h}) in the *in vitro* drug release was due to the increased surface area of the nanosized drug particles. The overall release profiles in the present investigation suggests that nanosized drug particles (≈180nm) found to have profound impact on rate of drug release and drug solubility.



a) b)

Fig.9: Percentage drug release profile of a) Optimized formulation for 60 min b) Comparison on R2, F10 and pure drug rosuvastatin

Drug release kinetics

The results of In vitro release profile obtained from the formulation code could be plotted in models of data treatment as follows (Table 5):

The Korsemeyer peppa's plot n value was found to be 0.9. Hence the optimized formulation was found to be controlled release and obeys Zero order kinetics. The mechanism is Case II transport.[17]

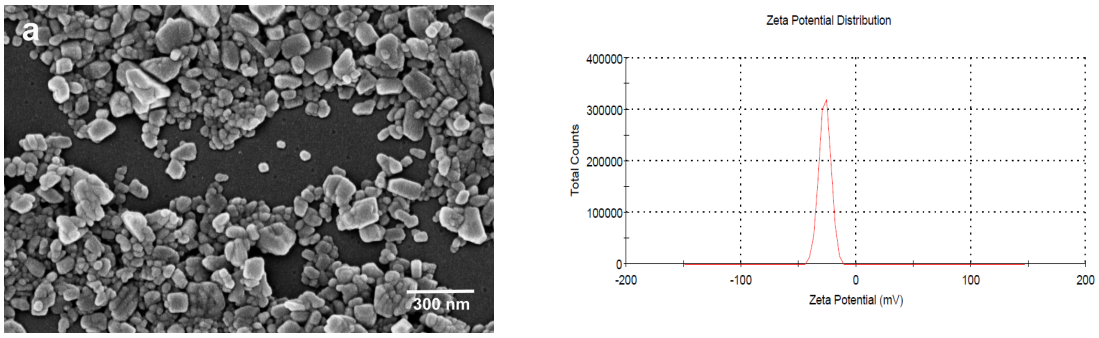
Table 5: Kinetic study of optimized formulation

Code	Coefficient Of determination($R^{2)}$				Korsmeyer plot (n)	Release Mechanism
	Zero Order R^2	First Order R^2	Higuchi R^2	Korsmeyer Peppas R^2		
R2	0.99	0.88	0.89	0.89	0.9	case II transport

- Zero order kinetics model-Cumulative percentage drug release vs time
- First order kinetics model-log cumulative percent drug remaining vs time
- Higuchi's model-cumulative % drug release vs square root of time
- Korsemeyer equation or Peppa's model-Cumulative % drug released Vs log time.

Scanning electron microscopy and zeta potential of optimized formulation

SEM was performed to analyze the morphology of the optimized formulation. The prepared nanosuspension formulation was found to be prismatic in nature and Zeta potential of the optimized formulation was found to be -24.5mV (Fig 10).



a) b)

Fig. 10: a) SEM image of optimized formulation (R2) b) Zeta potential image of optimized formulation (R2) HPLC

A reversed phase HPLC method has been developed and validated as per USP and FDA guidelines for determination of Rosuvastatin in pharmaceutical formulations by using a gradient mobile phase comprising 90% aqueous acetonitrile to 100% acetonitrile for 10 minutes at ambient temperature at flow rate of 0.7 mL/min with UV detection at 302 nm. The injection volume was kept at 20 µL for standard and all samples. The retention time of Rosuvastatin was obtained at 3.0 ± 0.2 min.

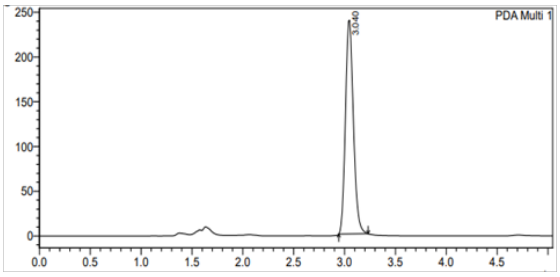


Fig.11. HPLC chromatogram of optimized Rosuvastatin nanosuspension

Short term stability studies

Optimized formulation R2 was kept for Stability studies at 40^0C/75%RH for 3 months. The entrapment efficiency and *in vitro* drug release profile of Optimized formulation of the tablets after 3 months has no change. The R^2 value of finished formulation before 3 months was 0.958 and after 3 months was0.959.

Conclusion

In conclusion, research work focused to develop Rosuvastatin loaded nanosuspension and optimization were carried out in order to enhance solubility, bioavailability and to reduce side effects. The study results indicated that nanosuspension prepared with PVA (F10 formulation) with drug to polymer ratio 1:2 has shown lower particle size, higher entrapment efficiency and higher *in vitro* drug release. On the basis of evaluation parameters, the optimized formulation (R2) can be used once in a day application which is based on the severity of the disease, and age. In conclusion the optimized formulation is suitable for large scale manufacturing. In the near future the optimized nanosuspension prepared with PVA can be extended to produce intravenously injectable nanosuspension, because the polymers are selected in such a way that they are suitable for parenteral administration.

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