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Development And Evaluation Of Nanocrystals Of Rosuvastatin For Enhancement Of Dissolution Rate.

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ABSTRACT

Nanosuspensions are fine dispersion of uniform-sized solid particles in an aqueous vehicle. The present work was aimed at the formulation and evaluation of nanocrystals of rosuvastatin calcium (ROC), a poorly water soluble anti-hyperlipidemic drug. ROC nanosuspension was prepared using probe sonicator using variable concentration of Tween 80, PVP K 25, propylene glycol and SLS as surfactants and transformed into dry powder using freeze drying with mannitol and characterized by particle size, polydispersity index (PDI), zeta potential, SEM, solubility, *in vitro* dissolution and stability studies. Optimized ROC nanosuspension was prepared by probe sonication using stabilizer, propylene glycol. The optimized formulation showed particle size of 313.4 nm and zeta potential of – 22.80 mV. SEM studies revealed that nanosuspension were nearly spherical in shape. The nanosuspension saturation solubility was significantly increased. Optimized formulation NSROC9 and pure drug exhibited the *in vitro* dissolution about 71.54% and 8.51% in 0.5% sodium lauryl sulfate (SLS) media, respectively within 5 mints. The stability results indicate that nanoformulations stored at 5 ◦C ± 3 ◦C for three months showed maximum stability. The results indicate the suitability of probe sonication method for preparation of nanocrystals of poorly soluble drugs with improved *in vitro* dissolution rate, thus potentially capable of enhancing fast onset of therapeutic activity, and bioavailability.

Keywords: Rosuvastatin calcium; Dissolution; Lyophilization; Nanocrystal; Nanosuspension

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INTRODUCTION

Rosuvastatin is a potent lipid lowering agent which acts on HMG-CoA-reductase enzyme for inhibiting the synthesis of cholesterol. It is commonly prescribed for the treatment and management of diverse conditions like dyslipidemia, familial hyperlipidemia, hypertriglyceridemia and atherosclerosis [1, 2]. It is administered through oral route in clinically recommended doses, ranging between 10 and 40 mg, and is well tolerated in all age groups. Rosuvastatin belongs to the BCS class II having low solubility and high permeability. It exhibits poor aqueous solubility, high hepatic first-pass metabolism and oral bioavailability of less than 20% [3, 4]. This reduces the efficacy of rosuvastatin in lowering the elevated serum lipid levels in hyperlipidemia and atherosclerosis.

Solubility is an essential factor for drug therapy in any route of administration. It possesses a major challenge for pharmaceutical technologists to develop new pharmaceutical products, since nearly half of the active pharmaceutical ingredients (API) being identified through the new paradigm in high-throughput screening are either insoluble or poorly soluble in water [5]. Most of the new chemical entities developed have a poor solubility in water and thus low oral bioavailability. Approximately, 60% of newly developed and investigated API's are poorly water-soluble which often leads to low bioavailability. Nanosuspension has emerged as an important tool in drug delivery to rectify these solubility conflicts [6–8]. Nanosuspensions are submicron colloidal dispersions of nanosized drug particles stabilized by surfactants [9]. Nanosuspensions consist of the poorly water-soluble drug without any matrix material suspended in dispersion [10]. These can be used to enhance the solubility of drugs that are poorly soluble in water as well as lipid media. As a result of increased solubility, the rate of flooding of the active compound increases and the maximum plasma level is reached faster.

This approach is useful for molecules with poor solubility, poor permeability, or both, which poses a significant challenge for the formulators. Preparation of nanosuspension is simple and applicable to all drugs which are water insoluble. A nanosuspension not only solves the problems of poor solubility and bioavailability, but also alters the pharmacokinetics of drug and thus improves drug safety and efficacy. They have received increasing attention due to their unique advantages, i.e., ease of manufacture and scale-up, and little batch-to-batch variation compared to other approaches [11].

Rosuvastatin is a drug with low aqueous solubility and 20% oral bioavailability. Poor aqueous solubility limits the oral absorption of rosuvastatin. This reduces the efficacy of rosuvastatin in lowering the elevated serum lipid levels in hyperlipidemia and atherosclerosis. Enhancing the solubility of rosuvastatin is a desirable approach to improve its therapeutic performance. Nanosuspension approach can be very advantageous for rosuvastatin drug. Drug particle size reduction leads to an increase in surface area and consequently in the rate of dissolution. Nanosuspension has been reported to enhance absorption and bioavailability; it may help to reduce the dose of the conventional oral dosage forms. The present study was aim to develop and evaluate rosuvastatin nanocrystals using probe sonicatoion method with the objective of increasing its dissolution rate.

MATERIALS AND METHODS

Materials

Rosuvastatin calcium (ROC) was procured from Sigma Aldrich, (St. Louis, MO, USA). Tween® 80, sodium lauryl sulphate and propylene glycol were purchased from S.D. Fine-Chem. Ltd. (Mumbai, India). Rest of the chemicals were of analytical grade and were purchased from Merck specialities private limited (Mumbai, India).

Preparation of nanosuspension

To establish the process of nanosuspension different trial batches were prepared. Aqueous solutions of surfactants i.e. Tween 80, PVP K 25, propylene glycol and SLS were prepared in various concentrations as shown in the Table 1 using purified water. Accurately weigh ROC (0.5 g) added to 100 ml of aqueous solution of surfactants. The suspensions mixed by mechanical stirrer to get homogeneous suspensions at 1000 rpm for 5 min. To the resultant homogenous suspension, probe sonication was employed at amplitude of 30%, pulse

30 sec for 10 min to produce nanocrystals (SONICS Vibra cell VC750, United States). Those surfactant which showed good stabilizing properties and able to the kept the nanocrystals without any steric barrier were selected. Only propylene glycol gave stabilized nanocrystals. After the establishment of process, the three different batches of nanosuspension were prepared (Table 2).

Table 1: Preparation of nanosuspensions of ROC

Formulation code	Formulation Composition			
	Tween 80 (%w/v)	PVP (%w/v)	PG (%w/v)	SLS (%w/v)
NSROC1	1	-	-	-
NSROC2	2	-	-	-
NSROC3	-	1	-	-
NSROC4	-	2	-	-
NSROC5	-	-	1	-
NSROC6	-	-	2	-
NSROC7	-	-	-	1
NSROC8	-	-	-	2

Table 2: Particle size (PS), polydispersity index (PDI), zeta potential (ZP), drug content of different batches of lyophilized nanosuspension

Formulation code	Rosuvastatin % (w/v)	Conc. of PG % (w/v)	Particle size (nm)	PDI	Zeta potential (mV)	% drug content (w/w)
NSROC6	0.5	2.0	449.3	0.627	-42.87	91.82
NSROC9	0.5	2.5	313.4	0.527	-22.80	93.88
NSROC10	0.5	3.0	299.8	0.512	-23.42	92.61

Lyophilization of nanosuspension

Accurately, 5% w/v mannitol as cryoprotectant was added into the nanosuspensions before deep freezing. 50 mL of nanosuspension filled in vials and frozen using deep freezer at −20 ◦C for 24 h. These frozen solids were freeze-dried using lyophilizer (Zirbus technology, Germany) at a vacuum degree of 200 Pasto produce free flowing dry powder.

Physico-chemical characterization of nanosuspension

Measurement of particle size, polydispersity index and zeta potential

The particle size (PS), PDI and zeta potential (ZP) of nanocrystals were measured using dynamic light scattering zetasizer (PSS NICOMP Z3000, **Port Richey, FL).** Prior to the measurement, the samples were diluted with double distilled water to a suitable scattering intensity and redispersed by hand shaking. Dynamic light scattering measures Brownian motion and relates to the size of the particles. It does this by illuminating the particles with a laser and analyzing the intensity fluctuations in the scattered light. The ZP is considered as one of the highest measured parameters which denotes the overall charges acquired by the particles in a particular medium and it is considered as one of the important factors for the stability of the nanoparticles. The nanosuspension of ROC was selected based on PS and ZP.

Morphology study by scanning electron microscopy (SEM)

The morphology of ROS nanosuspensions was studied by Scanning Electron Microscopy (EVO LS 10 Zeiss, Carl Zeiss Inc., Germany). Initially, the freeze dried optimized nanosuspension formulation was fixed on to the carbon coated brass stub. This was sputter coated with Platinum coating machine (JEOL, JFC-1600 Auto fine coater) and mounted in SEM for surface analysis by applying 20 kV under high vacuum and images were collected in secondary electron mode.

Determination of Drug content

Drug content in the prepare freeze dried nanosuspension were determined by dissolving about 10 mg of powder in 10 mL of methanol followed by sonication and filtering through 0.22 µm filter and analyzed using UV-Vis spectrophotometer (λ_{max} 244 nm; Shimadzu Model 1601, Tokyo, Japan).

Determination of saturation solubility

The solubility of ROC was determined by preparing saturated solutions in distilled water, maintained at 37 ◦C ± 0.5 ◦C in a water bath and continuously shaken into mechanical orbital shaker up to 24 h. Samples were taken out and filtered through 0.45 µm pore size filter paper and analyzed using UV-VIS spectrophotometer at 244 nm. In the similar way saturation solubility was determined for lyophilized nanosuspension. Each determination was carried out in triplicate.

***In vitro* dissolution studies**

In vitro dissolution studies were carried out on pure drug and freeze dried nanosuspension using USP dissolution assembly Type-II (Erweka Dissolution tester, Germany). The rotation speed of the paddles is set to 100 rpm. About 900 mL of 0.5% SLS at 37±0.5 ◦C was used as the dissolution medium. At predetermine time intervals, 5 mL samples were withdrawn, filtered through 0.45 µm membranes immediately and 5 mL blank dissolution media added for replenishing of the medium, respectively. The amount of dissolved drug is determined using UV-spectrophotometer at 244 nm. The mean results and the standard deviation will be reported.

Stability studies

The stability studies were performed according to International Conference on Harmonization (ICH) Q1A (R2) guidelines with the aim to assess the stability of lyophilized nanosuspension at 5 ◦C ± 3 ◦C with respect to PS and ZP. Optimize formulation batch in dry powder form fill in six different glass vials with rubber stoppers. Three vials were kept in deep freezer maintained at 5 ◦C ± 3 ◦C for period of 90 days. The remaining three vials were stored at room temperature for same period. After 90 days the samples were redispersed in demineralised water and checked for stability with respect to PS and ZP.

RESULTS AND DISCUSSIONS

To establish the process optimization of nanosuspension the eight different trial batches were prepared and characterized by PS, ZP and PDI (Table 1). Different surfactants like Tween 80, PVP K 25, propylene glycol and SLS were used as candidate to select the appropriate stabilizers for preparing ROS nanosuspension. PG showed the good stabilizing properties and it was able to the keep the nanocrystals without any steric barrier. So in this current study, PG was employed as stabilizers. Based on desired PS, PDI and ZP the trial batch NSROS6 was selected for the further development and process optimization. After process optimization of nanosuspension, three different formulation batches NSROS6, NSROS9 and NSROS10 were prepared and characterized by the PS, PDI, ZP and drug content.

Measurement of PS, PDI and ZP

The effect of the concentration of stabilizer on the particle size is shown in Table 2. The result exhibited that the PS reduced with the increasing of PG concentration and finally a plateau region was reached at the concentration of 3% w/v (NSROS10) from where the particle size no remarkably changed. Although nanosuspension formulation NSROS9 (prepared with 2.5% w/v PG) is considered as optimized formulation as three times less concentration of surfactant was used and it demonstrated the smallest particle size. PS, PDI and ZP for the optimized formulation batch showed in Fig.1. Polydispersity of optimized nanosuspensions was found 0.527 indicating the narrow particle size distributions of particles. PS and ZP of the optimized formulation batch NSROS9 have 313.4nm and -22.80 mV respectively. The surface charge is an important factor, influencing the stability of colloidal dispersion. It is known that ZP of - 20 to - 30 mV is required for electrostatic stabilization [12].

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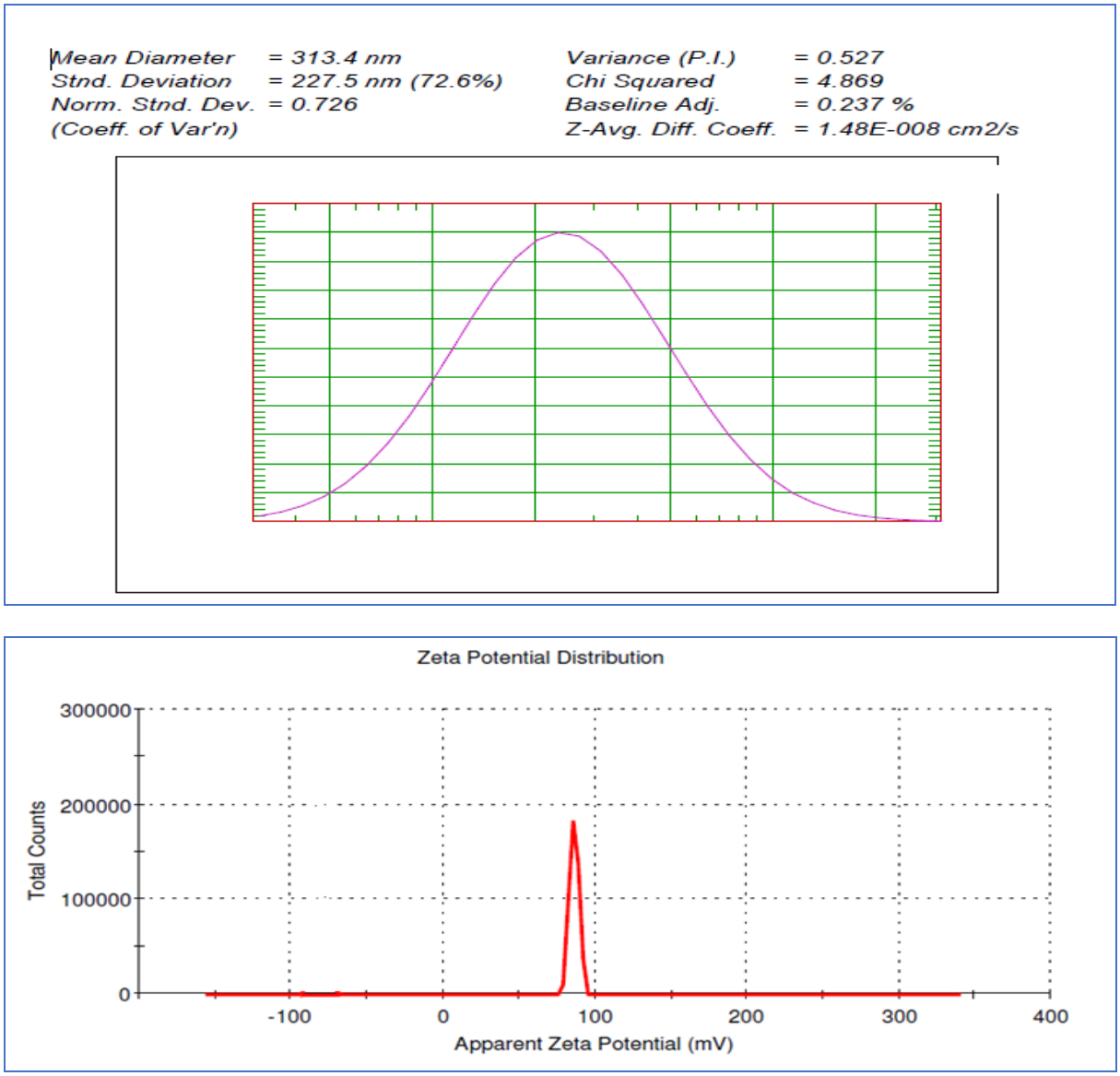

Fig 1: Partcle size and zeta potential of the optimized nanosuspension formulation batch NSROS9.

Scanning electron micrography

The particles of ROC nanosuspension were spherical to oval in shape with a smooth surface (Fig. 2). The nanoparticle size as observed by SEM was also correlated with size measured by Zetasizer.

Solubility studies

The observed solubility for pure drug and nanosuspension (NSROS9) was found to be 56.74 ± 0.46 µg/ml and 507.51± 1.12 µg/ml. Result demonstrated the enhanced solubility of ROC by 8.94 folds in nanosuspension form. Solubility enhancement of ROC nanosuspension was due to decrease in particle size which leads to providing larger surface area than the pure ROC and its amorphous nature is the second reason behind it which was only crystalline in its pure form.

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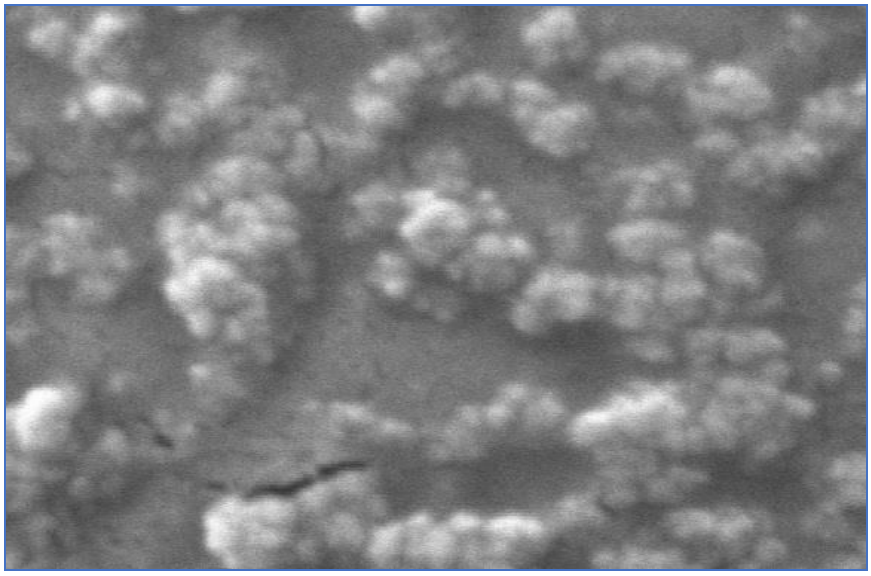

Fig 2: SEM topography of optimized ROC nanosuspension (NSROC9)

***In vitro* dissolution**

Dissolution of optimized batches of freeze dried nanosuspension (NSROC9) and pure drug was carried out in 0.5% SLS as dissolution medium. Comparison of the dissolution profiles revealed, significant increase in the dissolution rates of nanosuspension (Fig. 3). The dissolution rate had enhanced due to reduction in particle size and increase in surface area. In addition, the increase in surface wetting by the surfactants in the nanosuspension formulations most likely resulted in further enhanced dissolution rates as compared to pure drug [13].

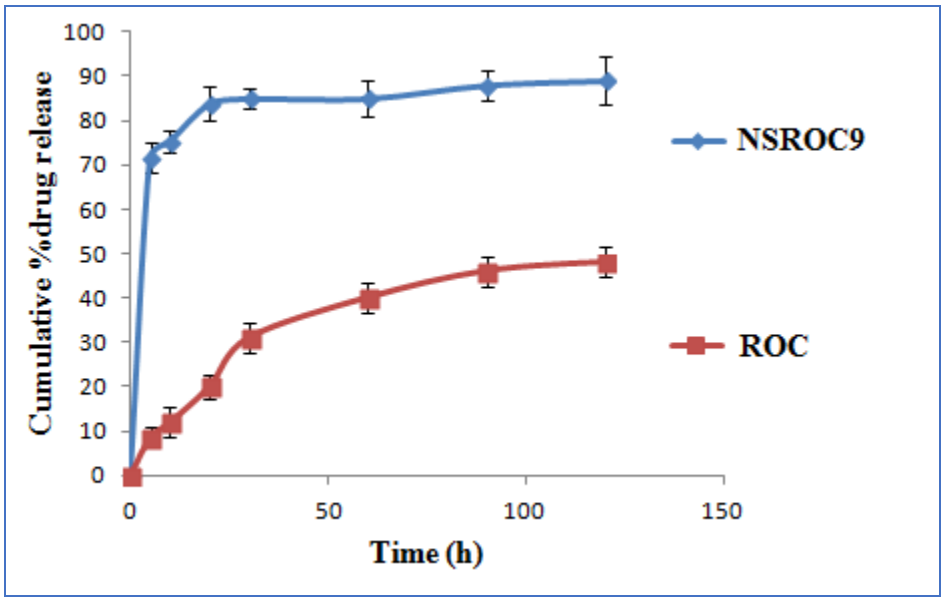

Fig 3: *In vitro* drug release profile of freeze dried nanosuspension (NSROC9) and pure drug (ROC)

Stability studies

Stability studies were conducted for finally optimized formulation (NSROC9) at room temperature and 5 ◦C ± 3 ◦C temperature for 90 days. There was no significant change in size and surface charge of lyophilized nanosuspension at 5 ◦C ± 3 ◦C but some changes were noticed in size and surface charge values at room temperature and was statistically significant, which indicated the susceptibility for stability problems during

storage at room temperature. At room temperature Ostwald ripening was more facilitated due to quicker redispersion on the particles and resulted into the larger particles. Hence, storage of prepared nanosuspension is mandatory at 5 ◦C ± 3 ◦C temperature to stabilize throughout its shelf life.

CONCLUSION

Rosuvastatin calcium is a drug with low aqueous solubility and 20% oral bioavailability. In this study, probe sonication method was successfully employed to produce stable ROC nanocrystals. This method was easy to apply, simple, cheap and promising for preparing drug nanocrystals. Formulation NSROC9 and pure drug exhibited the *in vitro* dissolution about 71.54% and 8.51% in 0.5% sodium lauryl sulphate medium, respectively witin five mints. These results indicate that dissolution rate of ROC nanosuspension was enhanced. Therefore, use of propylene glycol could be effective carrier in development of nanosuspension for low water soluble ROC to enhance the solubility. A significant decrease in particle size, enhanced aqueous solubility, improved drug dissolution collectively led to improvement in oral bioavailability of ROC. Taken together, the results of this study could be helpful in the development of biopharmaceutical formulations of ROC.

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