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Fabrication and Characterization of Multiparticulate System Containing Antihyperlipidemic for Solubility Enhancement

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ABSTRACT

Rosuvastatin calcium, an antilipidemic agent exhibits poor water solubility, dissolution and flow properties. Thus, the aim of the present study was to improve the solubility and dissolution rate of Rosuvastatin calcium by preparing microspheres by spray drying technique using Pluronic-F68 and F-127. Rosuvastatin calcium Microspheres containing different ratios of polymer were produced by spray-drying using methanol and water (1:2) as solvent system to enhance solubility and dissolution rate. The prepared formulations containing different ratios of drug and polymer were evaluated for solubility and in-vitro dissolution. The prepared formulations were characterized by DSC, FTIR, XRD and SEM. Dissolution profile of the prepared spray dried microspheres was compared with its physical mixture and pure sample.

The Rosuvastatin calcium microspheres containing 1:3 w/w (Rosuvastatin calcium: Polymer) showed highest % of drug release and solubility compare to other ratio, physical mixture and pure sample of Rosuvastatin calcium. Stability results showed that prepared microspheres stable for 6 month as per ICH guidelines. Hence, from the above result it can be concluded that spray dried microspheres of Rosuvastatin calcium is a useful technique to improve the solubility and dissolution of poorly water soluble drug like Rosuvastatin calcium.

1. INTRODUCTION

Solubility of a drug is an important property that mainly influences the extent of oral bioavailability. Enhancement of oral bioavailability of poorly water soluble drugs is the most challenging aspects of drug development. Many approaches, such as salt formation, solubilisation and particle size reduction have commonly been used to increase dissolution rate and thereby oral absorption and bioavailability of such drugs. Also there are some novel techniques such as nanoparticles, spray drying technique, microwave induced method, Self-emulsifying drug delivery systems, nanosuspensions. But they have the limitations of laboratory level scaling and cost because the materials used in the formulations are of synthetic origin and are very costly. Thus

particle size reduction is emerging as a very cost effective method that can be performed at laboratory level using simple apparatus. It is very important to find appropriate formulation approaches to improve the aqueous solubility of poorly aqueous soluble drugs.[1, 2]

Rosuvastatin calcium (RVS Ca) is a hydroxymethylglutaryl- CoA (HMG-CoA) reductase inhibitor (statin) is an antilipidemic agent. It is used orally for treatment of high LDL cholesterol (dyslipidemia), total cholesterol (hypercholesterolemia) & triglycerides (hypertriglyceridemia). The drug exhibits low bioavailability related to its poor water solubility. RVS Ca is a Biopharmaceutical Classification System (BCS) class II compound, i.e. water-insoluble, lipophilic, and highly permeable according to Biopharmaceutical Classification System. Therefore, bioavailability of RVS Ca may be improved by increasing its solubility.[8, 9]

Amorphous system exhibit significant solubility benefits, due to excess thermodynamic properties and lower energetic barrier than its crystalline form. The major reason for limited solubility benefit from amorphous system is their devitrification, on exposure to primary aqueous dissolution medium. This limited solubility can be overcome by further increases in solubility by preparing Spray Dried microspheres with polymer having high Tg value (like Pluronic). Spray drying is the transformation of an emulsion, suspension or dispersion to a dry state by atomizing the product and dispersing it through a hot gas. Microspheres increase the solubility by slowing devitrification, and increase wet ability due to hydrophilic nature .[4]

The aim of present study is to prepare the microspheres of RVS Ca by spray drying technique with low viscosity grade of Pluronic having the high glass transition (Tg) value. The physical properties of the prepared spray dried microspheres of RVS Ca were characterized by differential scanning calorimetry (DSC), scanning electron microscopy (SEM), X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR) and solubility studies.

2. Materials and Methods

2.1 Materials

Rosuvastatin calcium was gifted from Hubei Hongyuan Pharmaceutical Co., Ltd. China. Pluronic F-68 and F-127 was purchased from Sigma chemicals, Mumbai, India. The HPLC grade's Ethanol and Methanol (HPLC grade) were considered from Merck chemicals India Pvt. Ltd., Mumbai. In this study, other chemical used were in analytical reagent grade and was used as received. The materials used were listed as "generally recognized as safe" (GRAS), recommended safe for use in oral drug delivery by world health organization, and were within their acceptable limits.

2.2 Methodology

2.2.1 Compatibility Study

Drug was triturated with each excipient in 1:1 ratio and stored in petridish and covered with parafilm. The petridish containing samples were subjected to accelerated storage condition 40 ± 2˚C/ 75 ± 5% RH were observed for any significant physical change.[3]

2.2.2 Preparation of spray dried microsphere

RVS Ca microspheres were prepared by spray drying technique. Methanol and distilled water in ratio (1:2) was used as a solvent to prepare different drug/polymer ratio microspheres shown in **Table 1**. Feed solution was prepared by dissolving the drug and polymer in the solvent by using magnetic stirrer. Drug loaded microspheres were obtained by spraying the feed solution with a spray dryer (Lu, 222, Advanced, Lab ultima, Mumbai) using a standard 0.7 mm nozzle. The solution was fed to the nozzle with a peristaltic pump, atomized by the force of compressed air and blown together with heated air to the chamber where the solvent in the droplets were evaporated. The dried microspheres were harvested from the apparatus collector and kept under vacuum for 48 hours [6, 7].The spray drying parameters are described in **Table 2.**

Table 1: Formulations of rosuvastatin calcium SEDDS.

Batch no.	ROS-Ca (mg)	Polymer (mg)	Composition ratio (Drug:Polymer)	Methanol (ml)	Distilled Water
F1	400	P-F68 (1200)	2:2	30	20
F2	1200	P-F68 (400)	3:1	40	60
F3	800	P-F68 (800)	1:3	50	100
F4	800	P-F68 (1600)	2:4	70	140
F5	1600	P-F68 (800)	4:2	80	180
F6	400	P-F127 (1200)	1:3	30	20
F7	1200	P-F127 (400)	3:1	40	60
F8	800	P-F127 (800)	2:2	50	100
F9	800	P-F127 (1600)	2:4	70	140
F10	1600	P-F127 (800)	4:2	80	180

Table 2: Spray Drying Parameter.

Inlet temperature (°C)	Outlet temperature (°C)	Aspirator Speed	Feed Pump Speed
100-120	80-90	40-50%	9-10ml/min

3. Characterization Parameters

3.1 Drug loading and incorporation efficiency

The weighed amount of microspheres were dissolved in distilled water and kept overnight. The drug content was measured spectrophotometrically (UV 1800, Shimadzu, Japan) at 244 nm for pure

drug. The drug loading and incorporation efficiency (%) were calculated by using following equations [5, 6].

$$\text{Drug loading}(\%) = \frac{\text{M(actual)}}{\text{weighed quantity of powder of microspheres}} \times 100$$

$$\text{Incorporation efficiency}\ (\%) = \frac{\text{M actual}}{\text{M theoretical}} \text{X}100$$

Where, M actual is the actual drug content in weighed quantity of powder of microspheres and M theoretical is the theoretical amount of drug in microspheres calculated from the quantity added in the spray-drying process.

3.2. ATR studies

An accurately weighed amount of sample was mixed thoroughly with 100 mg potassium bromide IR powder and compacted under vaccum at a pressure of about 12 psi. The resultant disc was mounted in a suitable holder in IR spectrophotometer and IR spectrum was recorded from 4000cm^{-1} to 400cm^{-1} [10].

3.3. Differential scanning calorimetry (DSC)

An accurately weighed amount of sample was placed in open flat bottom, aluminum sample pans. Thermo grams were obtained by heating the sample at a constant rate 10ºC/min. A dry purge of nitrogen gas (60ml/min) was used for all runs [11].

3.4. X-ray Diffraction Study

X-ray diffractogram of the plane drug, blank microsphere and drug loaded microsphere were recorded by diffractogram using Philips X' Pert MPD diffractometer with Cu-Kα line as a source of radiation which was operated at the voltage 35 kV and the current 25 mA. All samples were measured in the 2θ angle range between 30 and 800 C and 0.010 step size [12, 13].

3.5. Globule size and zeta potential

An accurately weighed amount of 5mg of formulation was added to 100 ml of distilled water and was employed to assess the globule size and zeta potential using particle size analyzer (Sizer Malvern Zeta Nano Series ZS90) **[14].**

3.6. Scanning electron microscopy (SEM)

The surface morphology was observed by scanning electron microscopy (JSM-5310LV, JEOL,Tokyo, Japan) at voltage of 20 KV. Samples were mounted on a double-faced adhesive tape and sputtered with platinum for 250 sec before scanning electron microscopy [15].

3.7. Solubility study

The quantitative solubility study was done by shake flask method. Briefly, an excess amount of drug was dissolved in 5ml of different solvent like water, mixture of methanol and buffers (HCl buffers pH 1.2, phosphate buffer pH 2.5, 3.5, 4.4, 5.5, 6.0, 6.8, 7.2, 7.4). Samples were placed on shaken for 24 hrs at room temperature with 75 shakes/min. After 24 hrs, samples were centrifuge at 15,000 for 15 min. clean supernatant was taken out, filtered using whatman filter paper 1 and subsequently diluted with same media. The corresponding absorbance values were noted at 244 nm[16].

3.8. In-vitro drug release study

Drug release studies were carried out for optimized formulation using cellulose acetate membrane sac to hold the sample. Sac was immersed in 200ml buffer media (phosphate buffer pH 7.2) stirred at 100 rpm at a temperature of 37±0.5 ˚C. At pre-determined time intervals till 24 hr., an aliquot (3 ml) of the sample was collected, filtered, and analyzed for the content of ROS by the UV-visible spectroscopy. An equivalent volume (3 ml) of fresh medium was added to compensate for the loss due to sampling. It was also conducted on pure drug in an analogous manner [16, 17].

3.10. Release kinetics

The kinetics and mechanism of release were assessed by plotting the release data for zero order, first order, Higuchi model and Korsermeyer-peppas model. The regression coefficient was calculated in order to determine the kinetics and mechanism of *in vitro* release.

3.11 Confocal scanning laser microscopy

Confocal scanning laser microscopy (CSLM) (Leica TCS SP2, Leica Microsystems, Germany) was used to observe formulation globule shape from the freshly prepared formulation.

3.12. Thermodynamic Stability studies

The optimized formulation was subjected to stability studies carried out at 25±2˚C/60%±5% RH, as per the ICH guidelines for the climatic zone IV. The formulation was kept in air-tight glass vials and assayed periodically, at the time points of 0, 15, 30 and 90 days.

4. Result & Discussion

4.1 Drug Loading and Incorporation Efficiency

Incorporation efficiency was found to be high since as prepared by spray drying method. An increasing the ratio of drug to polymer, the drug loading of microspheres was increased shown in Figure 1.

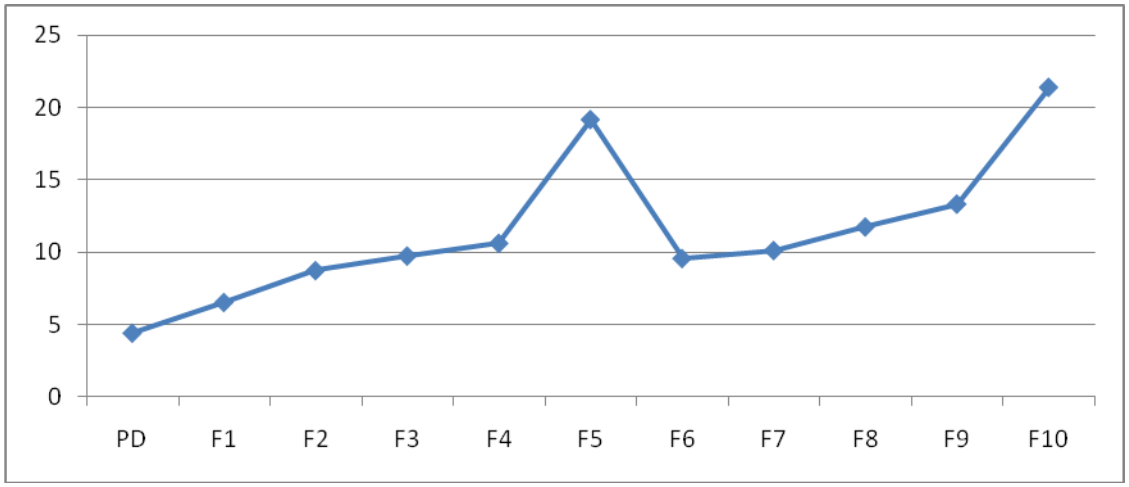


Figure 1: Drug Loading of Pure Drug and Different Formulations

4.2. ATR Studies

The ATR of optimized formulation of F10 and F5 showed the presence of drug in the final formulation in **Figure 2** and **figure 3**. The IR spectra indicates that the characteristic absorption peaks of RVS Ca was found at 3356.25 cm^{-1} and 2968.55 cm^{-1}(O-H stretch), shows strong absorption peak at 1546.96 cm^{-1}(N-O) and 1155.40 cm^{-1} (C-H). These characteristic peaks also found in the drug-polymer mixture, which indicates principle peak values of drug remain unchanged in the spray drying. Hence, it's confirmed that both drug and polymer were comparable with each other.

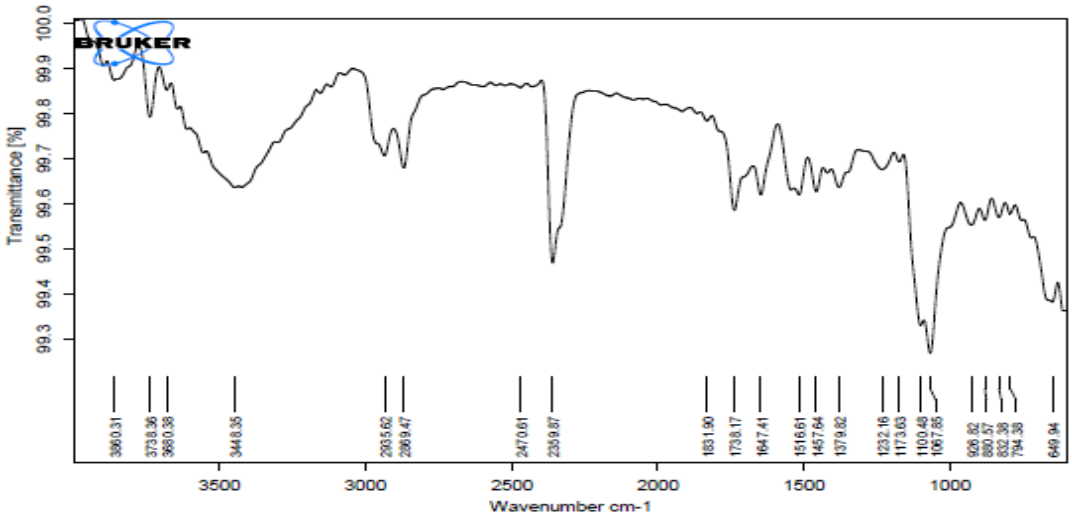


Figure 2: ATR of Pure Drug.

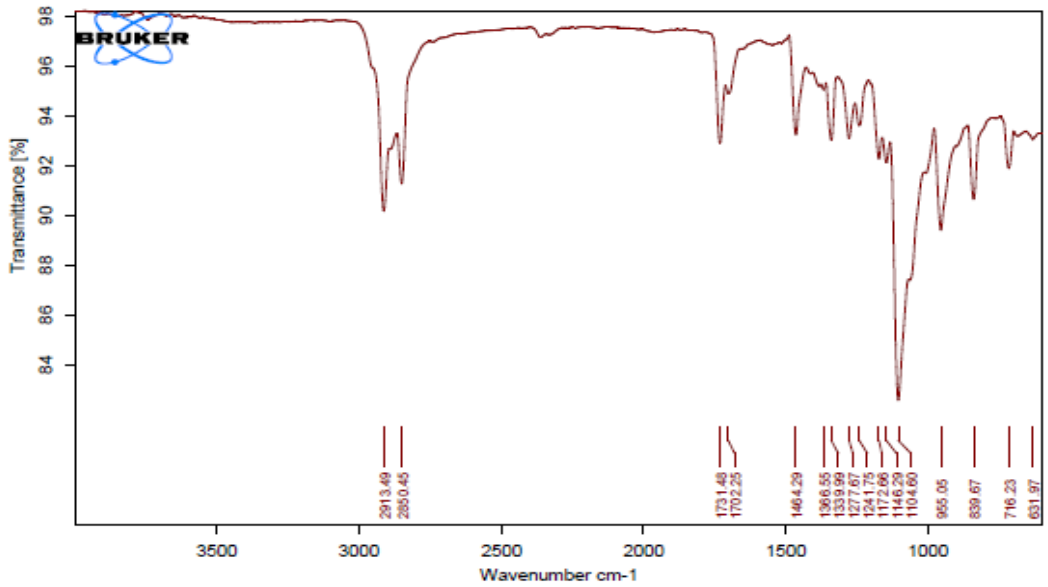


Figure 3: ATR of Physical Mixture.

4.3. Differential Scanning Calorimetry (DSC)

The DSC thermogram of Rosuvastatin Calcium was confirmed by scanning at rate of 10^0C/min it exhibits sharp melting endothermic peak at temperature of 166.07 ^{0}C as shown in **Figure 4.** The microsphere showed peak at 224.96°C for RSV Ca. However, the melting endotherm was absent on the DSC thermogram for the Microspheres suggesting absence of crystallinity and presence of an amorphous state of the drug. This could be because RSV Ca was molecularly or amorphously dispersed in the Microspheres.

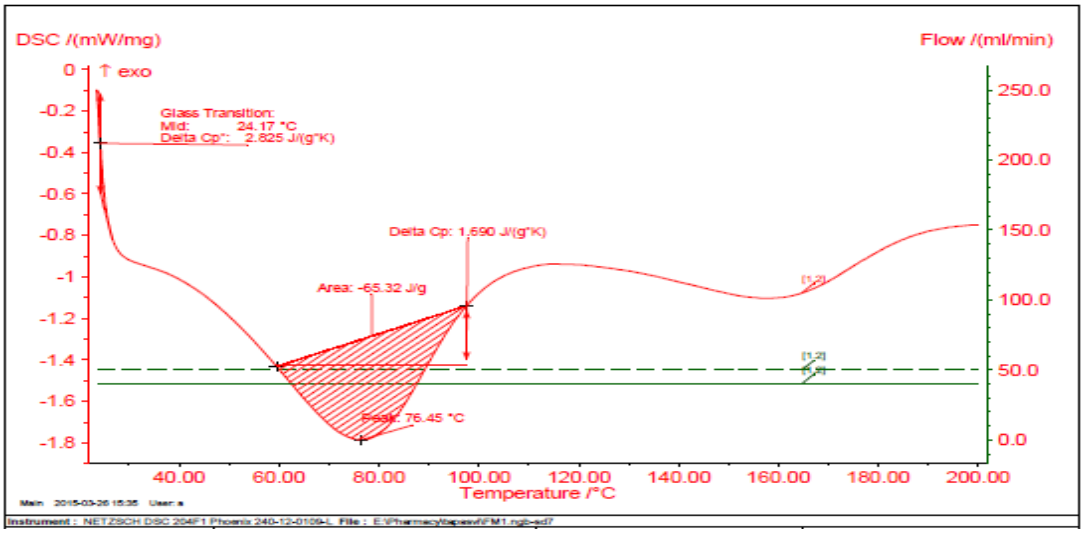


Figure 8: DSC OF PURE DRUG

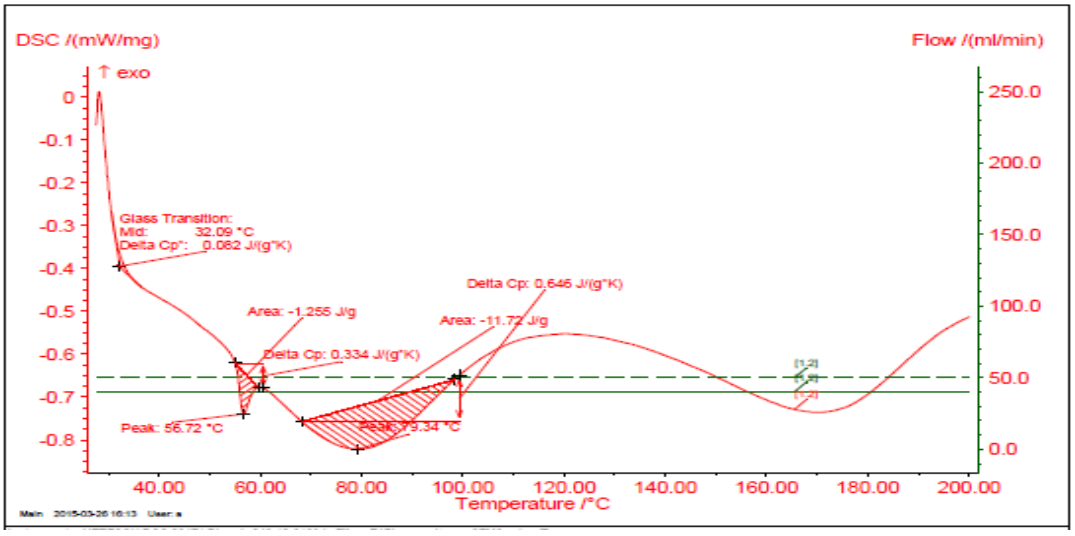


Figure 9: DSC OF PHYSICAL MIXTURE

4.4. X-ray Diffraction Study (XRD)

X- Ray diffraction was used to analyze potential changes in the inner structure of RVS Ca nanocrystals during the formulation of the Microspheres. The extent of such changes depends on the chemical nature and physical hardness of the active ingredient. The X-ray diffraction spectras were recorded for pure Rosuvastatin Calcium and drug loaded microsphere for investigating the crystallanity of the drug in the polymeric microspheres in **Figure 10** and **11**. The X-ray diffractogram of Rosuvastatin Calcium has sharp peaks and which shows a typical crystalline pattern. However Rosuvastatin Calcium, drug loaded microspheres shown peaks indicating that some amount of drug converts to amorphous form.

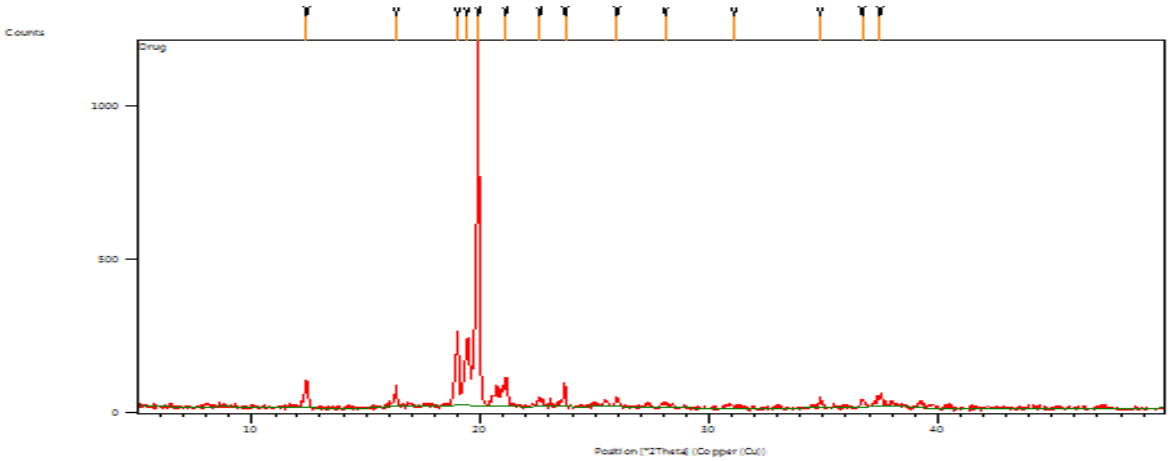


Figure 10: DSC of Pure Drug

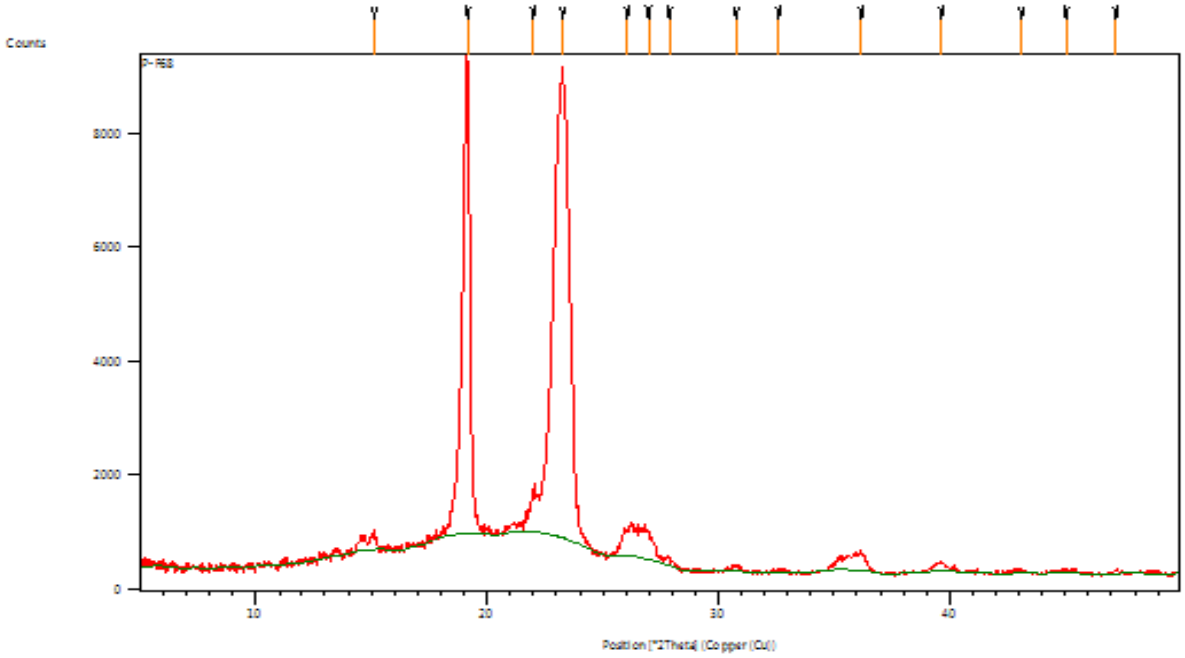


Figure 11: DSC of Physical Mixture

4.5. Determination Of Globule Size And Zeta Potential

Average particle size of microspheres ranged from 1 to 100µm, such particles are considered to be suitable for oral administration is shown in **Figure 12**.It was also noted that increasing drug to polymer ratio, slightly increased the size of microspheres in **Table 3**

TABLE 3: Particle size, zeta potential of optimized formulation.

FORMULATION	SIZE(nm)	ZETA(mV)	PDI
F4	112.9	-33.4	0.549
F5	21.20	-13.8	0.145
F9	123.5	-13.1	0.590
F10	19.75	-19.7	0.466

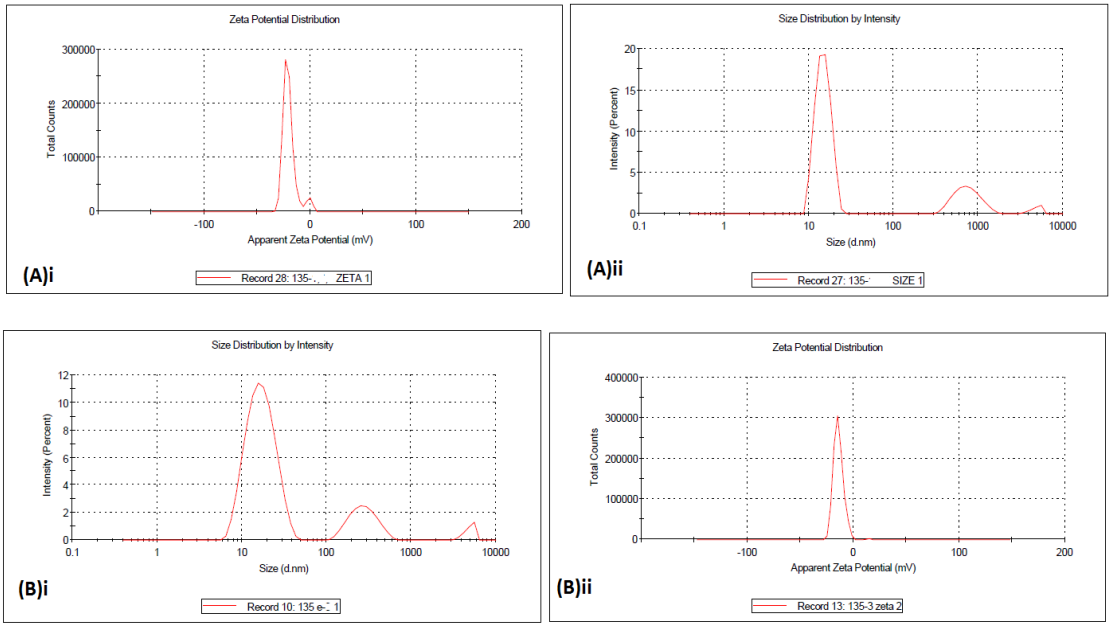
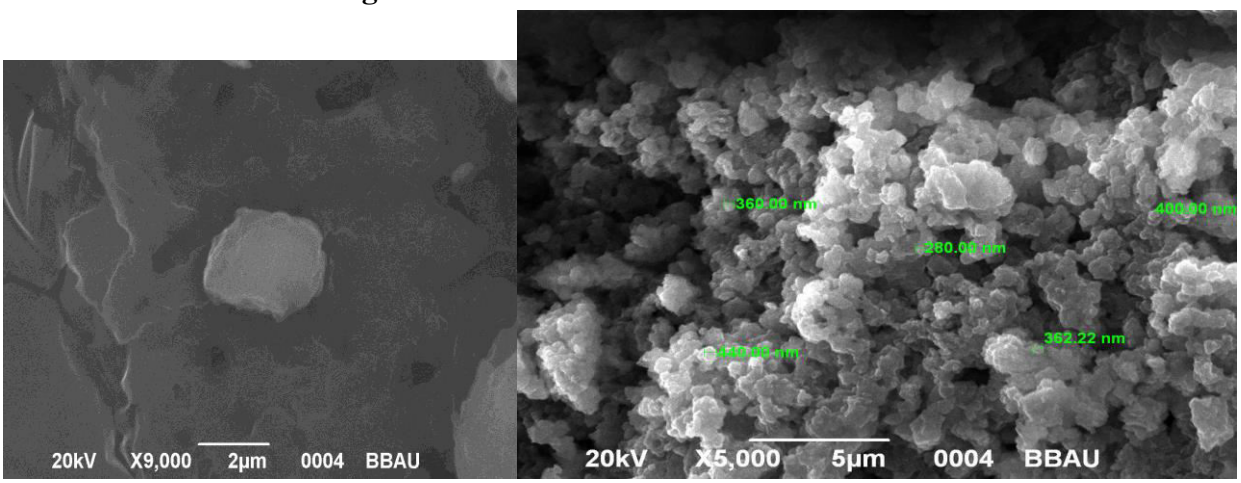


Figure 12: Images of (A)i is the zeta potential, (A)ii is the particle size of formulation F10 and (B)i is the zeta potential, (B)ii is the particle size of formulation F5.

4.6. Scanning Electron Microscopy (SEM)

For converting a liquid SNEDDS into a solid state, there is a need of highly porous powder with a good retaining capacity. The spherical shape of microspheres does not lead to cake formation during storage because of less point of contact thereby increasing the stability ofthe microsphere formulation, which is an advantage over other shapes. This could be therefore, indicate that RVS Ca particle size has been reduced, which also accelerates solubility and dissolution was shown in **Figure 13**.



(A) (B)

Figure 13: SEM image of (A) Pure Drug (B) Microsphere

4.7. Solubility Study

Increase in the solubility of RVS Ca from microspheres (0.90 mg/ml and 0.88mg/ml) was found to be nearly three times higher than the solubility of the pure drug (0.31 mg/mL) in Phosphate buffer 6.8, suggesting the presence of a high amount of an amorphous form of RVS Ca in the microspheres, indicating super-saturation. Increase in the solubility of RVS Ca from the physical mixture (PM) was nearly two times higher than pure drug. This could be due to the solubilising effect of highly water-soluble pullulan used in the formulation. The solubility results for the different formulations are shown in **Figure14**.The higher solubility of RVS Ca from Microspheres may be due to the increased surface area, wet ability and solubilising effect of highly water-soluble pullulan used in the formulations.

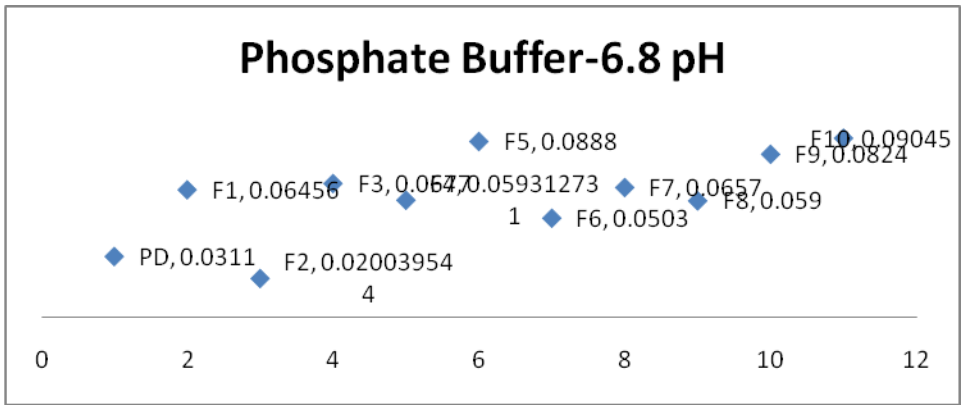


Figure 14: Solubility analysis of pure drug and different formulations in phosphate buffer 6.8 pH.

4.8. *In-Vitro* Drug Release Study

In-vitro drug release of the optimized formulations of F10, F5, F9 was compared with Pure Drug shown in **Figure 15**. Formulation after 7 hrs showed higher dissolution in comparison to marketed formulation. It was found to be 81.4% in F1 and 45.48% in marketed formulation shown in **table 4.** This indicated the sustained drug delivery of the rosuvastatin calcium formulation can be obtained for 7 hrs.

Table 4: Comparison of in-vitro drug release of various S-SNEDDS formulation.

TIME (hrs)	F10(%)	F3(%)	F9(%)	MARKETTED FORMULATION(%)
0.5	8.81	6.65	1.19	4.41
1	11.08	10.71	1.80	6.53
2	24.85	21.89	19.26	9.74
3	35.35	30.92	24.26	11.17
4	42.38	40.59	32.11	12.12
5	51.65	50.12	38.90	13.33
6	66.24	58.26	47.11	30.1
7	81.40	66.12	51.92	45.48

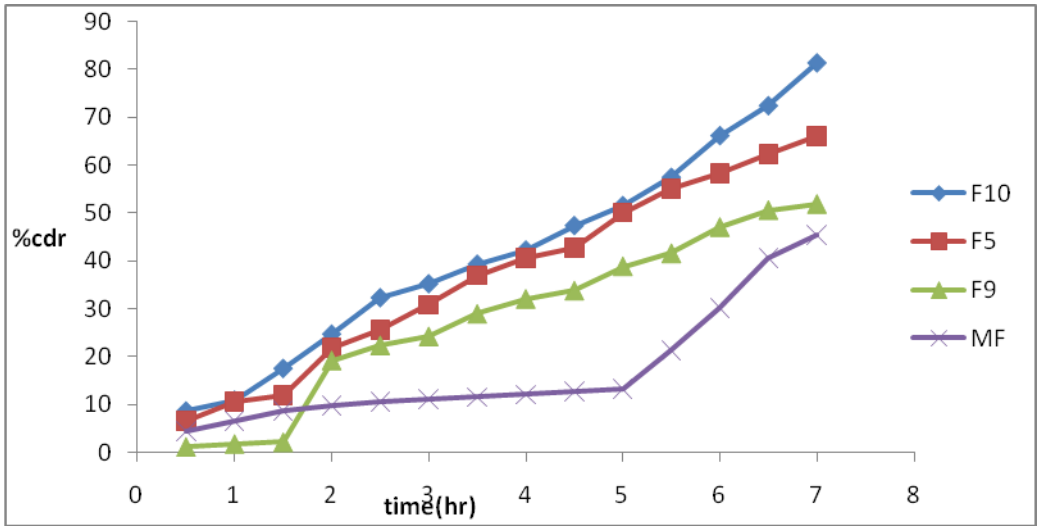


FIGURE 15: In-vitro drug release of optimized formulations and marketed formulation.

4.9. Release Kinetics

The data of *in vitro* release behavior plotted according to different kinetic models showed in table 14 that optimized formulation (F1) follows zero order kinetic with regression coefficient R^2 = 0.9873.

Table 5: Kinetics with related regression coefficient of optimized formulation (F1).

KINETICS	R^2 VALUE
KORSEMEYER PEPPER'S EQUATION	0.9186
ZERO ORDER	0.9873
FIRST ORDER	0.9047
HIXON CORNELL EQUATION	0.9448
HIGUCHI EQUATION	0.9525

4.10. Confocal Scanning Laser Microscopy

The optimized formulations (F10<F5<F9) were shows uniform distribution in F10 and F5 of fine oil droplets in spherical shape as shown in **Figure 16**.

A) F10 B) F5 C)F9

FIGURE 16: Microscopy image of optimized formulations F10, F5 and F9.

4.11. Thermodyamic Stability Studies

The stability of optimized formulation was assessed in term of particle size and zeta potential for 3 months. (Table 6). From the table it was observed that optimized formulation (F10) was stable.

Table 6: Stability study of optimized formulations.

CONDITION (75% RH, 40˚C TEMP)	FORMULATION	PARTICLE SIZE	ZETA
15 DAYS	F10	25.40	-12.2
15DAYS	F5	173.5	-13.1
15DAYS	F9	243.9	-8.14

CONDITION 75% RH, 40˚C TEMP	FORMULATION	PARTICLE SIZE	ZETA
1 MONTH	F10	115.8	-18.2
1 MONTH	F5	445.1	-15.1
1 MONTH	F9	368.4	-20.7

CONDITION 75% RH, 40˚C TEMP	FORMULATION	PARTICLE SIZE	ZETA
3 MONTH	F10	181.5	-17.5
3 MONTH	F5	544.1	-17.1
3 MONTH	F9	436.1	-19.3

5. Conclusion

In this present study, an increased solubility and dissolution rate of Rosuvastatin calcium were achieved by preparing microspheres by spray drying technique using different ratio of Pluronic having different grades. DSC, ATR and XRD studies showed that there is no change in the crystal structure during the spray drying process and showed that spray dried microspheres exhibited decreased crystallinity. The solubility and dissolution of the spray dried microspheres was improved significantly compared with its physical mixture and pure sample of Rosuvastatin calcium. The Rosuvastatin calcium microspheres containing (Rosuvastatin calcium:Pluronic) showed highest % of drug release and solubility compare to other ratio, physical mixture and pure sample of Rosuvastatin calcium. Stability results showed that prepared microspheres stable for 3 month as per ICH guidelines. Hence, from the above result it can be concluded that spray dried microspheres of Rosuvastatin calcium is a useful technique to improve the solubility and dissolution of poorly water soluble drug like Rosuvastatin calcium.

6. Acknowledgement

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