



Research Article

FORMULATION AND EVALUATION OF SPRAY DRIED MICROPARTICLES CONTAINING ANTILIPIDEMIC FOR THE ENHANCEMENT OF SOLUBILITY AND DISSOLUTION RATE

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ABSTRACT

Rosuvastatin calcium (RVS Ca), 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 RVS Ca by preparing microspheres by spray drying technique using Pullulan having low viscosity and high Tg (Glass transition temperature) value. RVS Ca Microspheres containing different ratios of pullulan 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 pullulan 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 RVS Ca microspheres containing 1:3 w/w (RVS Ca: Pullulan) showed highest 99 % of drug release and solubility compare to other ratio, physical mixture and pure sample of RVS Ca. Accelerated 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 RVS Ca is a useful technique to improve the solubility and dissolution of poorly water soluble drug like RVS Ca.

Keywords: Rosuvastatin calcium, pullulan, Spray drying, Microsphere. Solubility.

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 (SEDDS), 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 Pullulan). 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 wettability due to hydrophilic nature⁴.

The aim of present study is to prepare the microspheres of RVS Ca by spray drying technique with low viscosity grade of Pullulan 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.

MATERIAL AND METHOD**Material**

Rosuvastatin calcium- Zydus Cadila Healthcare, Mumbai.
Pullulan- Ganwal chemicals, Pvt. Ltd, Mumbai
Pluronic F68- Sigma Aldrich
Other chemical used were of analytical grade of Loba chemie PVT LTD. India

Physical mixture

Sample for ratio optimization were prepared by mixing the drug and polymer in different ratio such as 1:1 to 1:4 w/w in the mortar for 5 min and then sieving⁷.

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 (1:1 to 1:4) microspheres. 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.

Evaluation of microspheres

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^{5, 6}.

FTIR spectroscopy

The interaction between the drug and polymers was determined by using the FTIR (8400 - Shimadzu, Japan) spectroscopy wherein infrared spectra of pure drug, physical mixture and pure drug loaded microspheres were carried out using the KBr disk method^{10, 11} (2 mg sample in 200 mg KBr). The scanning range was 450 to 4000 cm^{-1} and the resolution was 1 cm^{-1} .

Differential Scanning Calorimetry (DSC)

A DSC study was carried out to detect possible polymorphic transition during the crystallization process. The thermal behaviour of plain drug, drug loaded microspheres and blank microspheres were determined using differential scanning calorimeter (Mettler, Toledo) at heating rate of 10 $^{\circ}\text{C}/\text{min}$. The measurements were performed at a heating range of 30 – 400 $^{\circ}\text{C}$ under nitrogen atmospheres^{12, 13}.

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-K α 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 2 θ angle range between 3 $^{\circ}$ and 80 $^{\circ}$ C and 0.010 step size^{12, 13}.

Particle size analysis

The microspheres were evaluated for the particle size. An optical microscope (Motic, B1, Series, and Systemic Microscope.) was used for this purpose. The microscope was equipped with the software, image manager through a camera. Analysis was carried out on the spray-dried microspheres dispersed in immersion oil. This slide was observed under the

microscope. An image was clicked and used for the particle size analysis. The average particle size of the microspheres was expressed as the volume surface diameter (μm) and standard deviation (σ) was calculated for each batch of microspheres¹⁵.

Scanning Electron Microscopy (SEM)

Scanning electron microscopic (Joel- LV-5600, USA, with magnification of 250x) photographs were obtained to identify and confirm spherical nature and surface topography of the crystals^{14, 15}.

Solubility studies

Drug solubility was determined by adding excess amounts of pure RVS Ca, their physical mixture and microspheres in distilled water and phosphate buffers 6.8 at $37 \pm 0.5^{\circ}\text{C}$ respectively at a rotation speed of 100 rpm. The solution formed were equilibrated under continuous agitation for 24 h and passed through Whatman filter paper (No. 41) to obtain a clear solution. The absorbance of the samples was measured using UV spectrophotometer (UV 1800, Shimadzu, Japan) method at 244 nm and the concentrations in $\mu\text{g}/\text{ml}$ were determined. Each sample was determined in triplicate¹⁴.

In vitro dissolution studies

The dissolution of pure RVS Ca, their physical mixture and microspheres was determined by using USP dissolution apparatus XXIV-Type II (Electro Lab, Mumbai), Shimadzu, Japan. Dissolution medium was 900 ml of pH 6.8 phosphate buffer. The amount of dissolved drug was determined using UV spectrophotometer (UV 1800 Shimadzu, Japan) at 244 nm. Each sample was determined in triplicate¹⁵.

Determination of the physical stability

To determine the physical stability of optimized Microspheres, a stability study of prepared Microspheres was carried out at 25 $^{\circ}\text{C}$ and 60% relative humidity for 6 months according to the ICH guidelines. The microsphere was packed in high density polyethylene (HDPE) container and placed in stability chamber (CHM-10S Remi, India). The samples were withdrawn at the interval of 0, 1, 3 and 6 months and evaluated for appearance, characterization by FT-IR and dissolution release and compared with initial results¹⁶.

Statistical analysis

All analyses of data were performed with a statistical software package (SPSS 13, USA). The results are expressed as means and standard deviations. Comparative statistical studies on the inclusion complex and dissolution rate were performed by ANOVA.

RESULT AND DISCUSSION

The spray drying method describe here appeared be a suitable & simple technique to prepare Pullulan microspheres loaded with RSV Ca. It is one step process, easy & rapid, as it combines drying of the feed and embedding of the drug into a one-step operation.

Table 1: Formulation composition of microspheres

Formulation code	Ratio (Drug- polymer)	Formulation composition			
		RVS Ca (mg)	Pullulan (mg)	Methanol (ml)	Distilled water (ml)
F1	1:1	400	400	30	60
F2	1:2	400	800	50	100
F3	1:3	400	1200	70	140
F4	1:4	400	1600	90	180

Table 2: Spray-Drying Parameters

Inlet Temperature (°C)	Outlet temperature (°C)	Aspirator speed	Feed pump speed
100 – 120 °C	80 – 90 °C	40 - 50 %	9-10 ml/min.

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 Table 3.

Table 3: Drug loading and incorporation efficiency of microspheres

Formulation code	Drug loading (%)	Incorporation efficiency (%)
F1	59.18 ± 0.067	35.16 ± 0.15
F2	80.18 ± 0.096	77.89 ± 0.086
F3	70.36 ± 0.12	89.42 ± 0.073
F4	52.81 ± 0.072	89.13 ± 0.069

[mean ±SD, n= 3]

FTIR spectroscopy

Drug-excipient interaction was studied by FTIR technique. The IR spectra of RVS Ca and Pullulan (physical mixture), microspheres are given in Figure 1, 2, 3 respectively. 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.

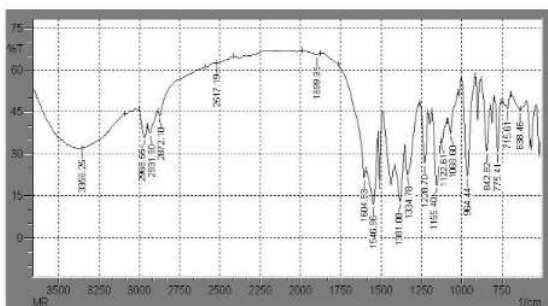


Figure 1: FTIR spectra of Rosuvastatin Calcium

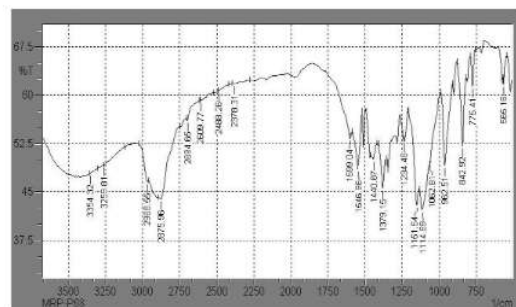


Figure 2: FTIR spectra of physical mixture

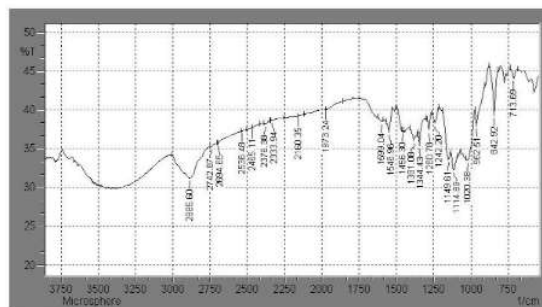


Figure 3: FTIR spectra of Rosuvastatin Calcium loaded pullulan microsphere

Differential Scanning Calorimetry

Rosuvastatin Calcium was confirmed by DSC at scanning rate of 10 °C/min it exhibits sharp melting endothermic peak at temperature of 166.07 °C as shown in Figure 4. In DSC spectra of RSV Ca with pullulan microsphere showed peak at

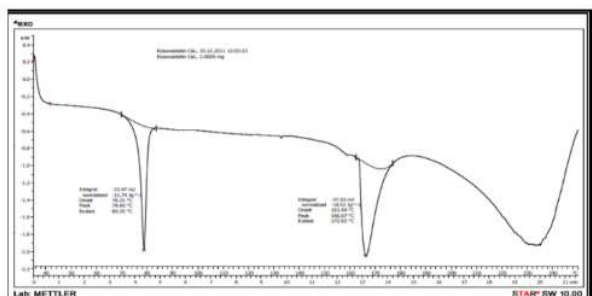


Figure 4: DSC Thermogram of pure Rosuvastatin Calcium

X-ray diffraction study (XRD)

X- Ray diffraction spectroscopy has been used to assess the degree of crystallinity of the Microspheres formulation. XRD patterns are shown in (Figure 6, 7). The X-ray diffractogram of

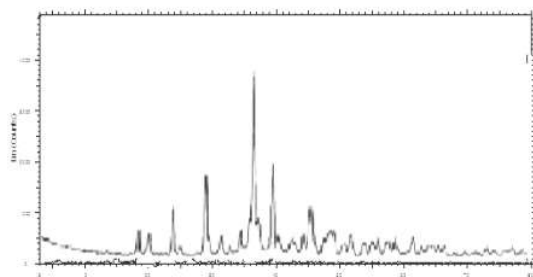


Figure 6: X-ray diffraction patterns of Rosuvastatin Calcium

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.

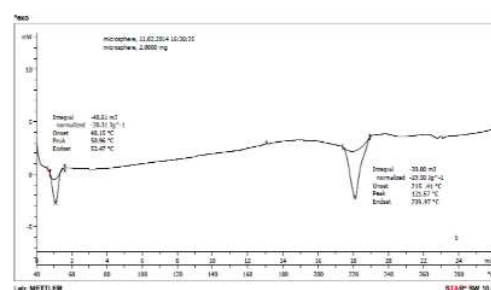


Figure 5: DSC thermogram of Rosuvastatin Calcium loaded pullulan microspheres

pure Rosuvastatin Calcium has sharp peaks and which shows a typical crystalline pattern. However Rosuvastatin Calcium drug loaded microspheres shown diffused peaks indicating that some amount of drug converts to amorphous form.

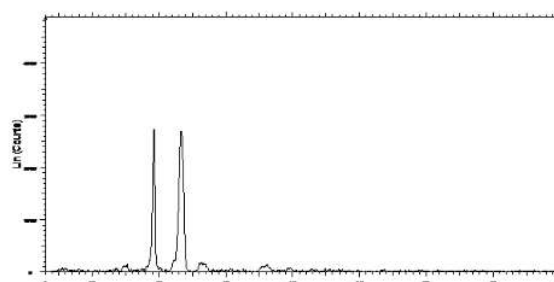
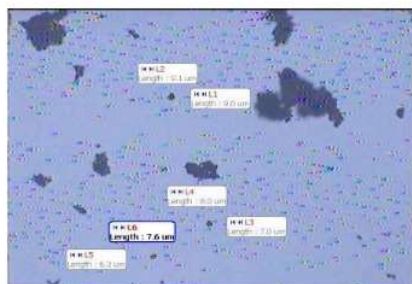


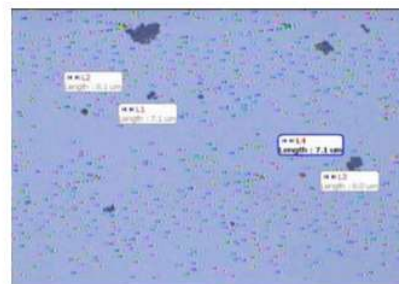
Figure 7: X-ray Diffraction patterns of microspheres by spray drying method

Particle size analysis

Average particle size of microspheres ranged from 1 to 100 μm, such particles are considered to be suitable for oral administration (Figure 8-11). It was also noted that increasing drug to polymer ratio, slightly increased the size of microspheres (Table 4).



F1



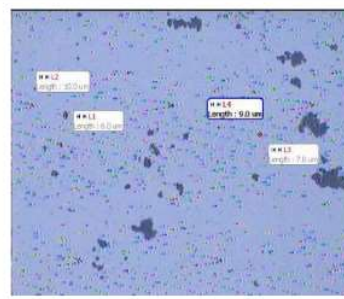
F2

Table 4: Particle size of microspheres

Formulation Code	Average Particle size (μm)
F1	7.1
F2	7.8
F3	8.0
F4	8.6



F3



F4

Figure 8: Optical microscopic images of formulation

The spray dried microspheres was analyzed by SEM for studying particle shape and surface structure (Figure 9, 10). the shape of prepared microspheres are uniform and spherical in shape with small in size 7-9 μm (Table 4). The spherical shape of microspheres does not lead to cake formation during storage

because of less point of contact thereby increasing the stability of the 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

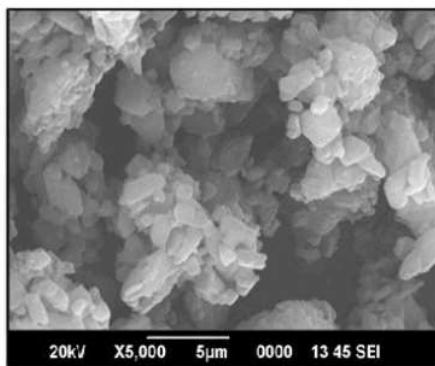


Figure 9: SEM image of Rosuvastatin Calcium

From the Figure 9, it is concluded that Rosuvastatin Calcium particles were needle, plate shaped with smooth surface, while in case of spray dried microspheres it was observed that they were of irregular shape and size. Figure 10 clearly shows that crystal shape of Rosuvastatin Calcium was completely changed in microspheres. SEM images show that the crystalline Rosuvastatin Calcium is converted to its amorphous form which was confirmed by DSC and XRD study.

Solubility study

Increase in the solubility of RVS Ca from microspheres (0.94 mg/mL) was found to be nearly three times higher than the

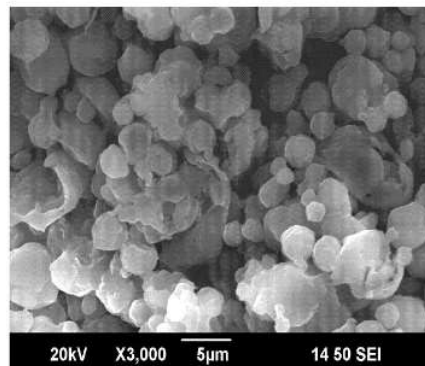


Figure 10: SEM image of Microspheres

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 supersaturation. 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 Table 5. The higher solubility of RVS Ca from Microspheres may be due to the increased surface area, wettability and solubilising effect of highly water-soluble pullulan used in the formulations

Table 5: Solubility of RVS Ca and Different formulation in distilled water and pH 6.8

Different formulation	Solubility in distilled water(mg/ml)	Solubility in phosphate buffer pH 6.8 (mg/ml)
Pure drug	0.27 $\pm$ 0.021	0.31 $\pm$ 0.015
F1	0.52 $\pm$ 0.017	0.58 $\pm$ 0.046
F2	0.70 $\pm$ 0.031	0.75 $\pm$ 0.025
F3	0.78 $\pm$ 0.042	0.82 $\pm$ 0.035
F4	0.87 $\pm$ 0.013	0.94 $\pm$ 0.024
PM1	0.30 $\pm$ 0.027	0.36 $\pm$ 0.014
PM2	0.36 $\pm$ 0.047	0.44 $\pm$ 0.019
PM3	0.54 $\pm$ 0.034	0.57 $\pm$ 0.024
PM4	0.61 $\pm$ 0.028	0.64 $\pm$ 0.025

[mean $\pm$ SD, n= 3]

Dissolution study

The dissolution profile of pure RVS Ca, physical mixture and prepared microspheres in pH 6.8 phosphate buffer shown in Figure 11. The dissolution profiles were plotted as the % release from the different microspheres versus time in minute. It was observed that the release rate from pure RVS Ca and physical mixture was very slow and incomplete compared with spray dried microspheres. The % release from ratio of (1:3 w/w) drug and polymer showed more release compared to other ratios. In

case of microspheres containing (1:3 w/w) showed 99% release in 90 min and at the same ratio of physical mixture showed 68% release in 90 min. The increase in dissolution from the microspheres and physical mixtures was probably due to the wetting, solubilizing effect and amorphizing power of pullulan of the pullulan, which could reduce the interfacial tension between the RVS Ca and the dissolution medium, thus leading to a higher dissolution rate than pure RVS Ca. The large surface area of the resulting microspheres should result in an enhanced dissolution rate and thereby improve the bioavailability.

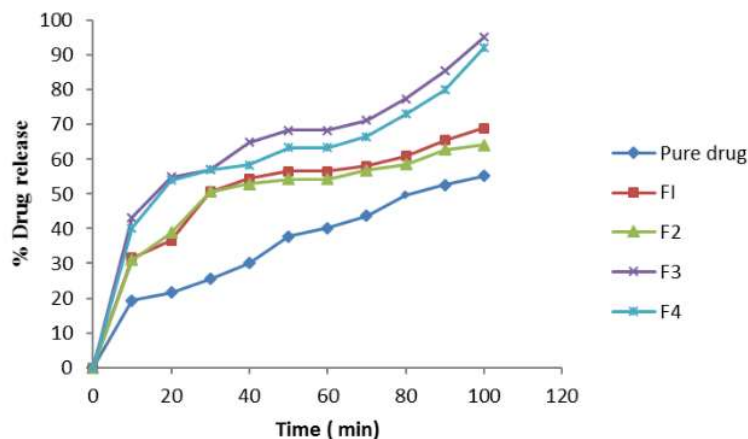


Figure 11: *In Vitro* Release of Rosuvastatin Calcium microspheres by spray drying

Determination of the physical stability

The best way to guarantee stability is by maintaining their physical state and molecular structure. The results of the stability study of prepared microspheres (1:3 w/w) of RVS Ca

Stored at 25 °C and 60% relative humidity for 6 month is presented in table 6. Prepared microspheres of RVS Ca were stable and within acceptable limit with all the properties when compared to initial results of prepared microspheres of RVS Ca.

Table 6: Stability data of spray dried microspheres

Testing interval	Description of Drug	FT-IR Study	XRD Study	%Drug loading	In vitro drug release
Initial	White to off white	As standard	As standard	70.36±0.012	99.23±0.023
1 month	Complies	Complies	Complies	69.28 ± 0.02	98.39±0.034
3 month	Complies	Complies	Complies	69.02 ±0.045	98.12 ±0.013
6 month	Complies	Complies	Complies	68.89±0.056	98.09±0.019

[mean ±SD, n= 3]

CONCLUSION

In this present investigation revealed that Rosuvastatin calcium can form spray dried microsphere with pullulan having low viscosity and high Tg value. From these result, it can be assumed that the formation of the spray dried microsphere with pullulan can increase the aqueous solubility of Rosuvastatin calcium. The improved dissolution rate may be due to increase in solubility, brought about by wetting and solubilizing effect of the pullulan, which could reduce the interfacial tension between the RVS Ca and the dissolution medium and amorphizing power of pullulan. DSC, FT-IR and XRD studies showed that there is no change in the structure of Rosuvastatin calcium during the spray drying process. 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 1:3 w/w (Rosuvastatin calcium: Pullulan) showed highest % of drug release and solubility compare to other ratio, physical mixture and pure sample of Rosuvastatin calcium. Stability results data showed that prepared microspheres stable for 6 month as per

ICH guidelines and no significant change compared to initial result. 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.

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