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FORMULATION AND *INVITRO* EVALUATION OF ROSUVASTATIN CALCIUM SOLID DISPERSION

T. Sravani[1], T. Balakrishna[2], V. Akash[3], S. Srinivasa Rao[4], A. Tejaswi[5], T. Navya Sri[6], M. Raga Mallika[7], A. Indira[8].

[1, 3, 4, 5, 6,7] Department of Pharmaceutics, V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, 521356, India, A.P.

[2] Department of Pharmaceutics, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Guntur, 522019, India, A.P.

[8] Department of Chemistry, AG & SGS Degree College, Vuyyuru, 521165, India, A.P.

Abstract:

Rosuvastatin calcium (RST) is a selective and competitive inhibitor of HMG-CoA reductase, mainly used in the treatment of hypercholesterolemia, hypertriglyceridemia and atherosclerosis. In this work a new attempt was made to enhance the solubility, dissolution rate and oral bioavailability of poorly soluble rosuvastatin by formulating it as solid dispersions using various techniques with polyethylene glycol (PEG) 6000 as a carrier. Solid dispersions were prepared by physical mixing, kneading and solvent evaporation methods using PVP K-30 as a superdisintegrant at different ratios were found to be stable and suitable for increasing the dissolution rate of Rosuvastatin. 1:2 ratio of drug and superdisintegrant respectively in every method showed highest rates of dissolution than the other ratios i.e., as superdisintegrant concentration increases the drug solubility increases. Formulations were evaluated for physical parameters and drug release by in vitro dissolution studies. drug-excipient interactions FTIR studies.

Key Words: *Rosuvastatin calcium, PEG 6000, Sodium starch glycolate, Poly vinyl Pyrrolidone, Pregelatinized starch, Mannitol.*

Corresponding author:

Ms. T. Sravani,

Assistant Professor,
Department of Pharmaceutics,
V. V. Institute of Pharmaceutical Sciences,
Gudlavalleru, 521356, India, A.P.
Sravaniatmuri123@gmail.com
9705434807

QR code

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INTRODUCTION:

The oral route of drug administration is the most common and preferred method of delivery due to convenience and ease of ingestion. From a patient's perspective, swallowing a dosage form is a comfortable and a familiar means of taking medication 1, 2. Although the oral route of administration is preferred, for many drugs it can be a problematic and inefficient mode of delivery for number of reasons. Limited drug absorption resulting in poor bioavailability is paramount amongst the potential problems that can be encountered when delivering an active agent via the oral route. [3-5] Drug absorption from the gastrointestinal (GI) tract can be limited by a variety of factors with the most significant contributors being poor aqueous solubility and/or poor membrane permeability of the drug molecule. When delivering an active agent orally, it must first dissolve in gastric and/or intestinal fluids before it can then permeate the membranes of the gastrointestinal tract to reach systemic circulation [6-8]. Therefore, a drug with poor aqueous solubility will typically exhibit dissolution rate limited absorption and a drug with poor membrane permeability will typically exhibit permeation rate limited absorption [9,10]. Hence, two areas of pharmaceutical research that focus on improving the oral bioavailability of active agents include: (i) enhancing solubility and dissolution rate of poorly water-soluble drugs and (ii) enhancing permeability of poorly permeable drugs. So, a solid dispersion.

Drug absorption from the gastrointestinal (GI) tract can be limited by a variety of factors with the most significant contributors being poor aqueous solubility and/or poor membrane permeability of the drug molecule. When delivering an active agent orally, it must first dissolve in gastric and/or intestinal fluids before it can then permeate the membranes of the gastrointestinal tract to reach systemic circulation. [6-8] Therefore, a drug with poor aqueous solubility will typically exhibit dissolution rate limited absorption and a drug with poor membrane permeability will typically exhibit permeation rate limited absorption. [9,10] Hence, two areas of pharmaceutical research that focus on improving the oral bioavailability of active agents include: (i) enhancing solubility and dissolution rate of poorly water-soluble drugs and (ii) enhancing permeability of poorly permeable drugs. So, a solid dispersion technology is used to improve the dissolution characteristics of poorly water-soluble drugs and in turn their oral bioavailability. [11-12]

In the present investigation the drug such as rosuvastatin was selected for the enhancement of solubility and bioavailability by improving its dissolution rate by preparing it in solid dispersion form. Rosuvastatin is a selective and competitive inhibitor of HMG-CoA reductase, the rate limiting enzyme that converts 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonate, a precursor of cholesterol. rosuvastatin calcium is a white amorphous powder that is sparingly soluble in water and methanol. rosuvastatin calcium after oral administration is well absorbed from gastrointestinal tract. Peak plasma concentration was reached 3-5 h following oral dosing. It has got elimination half-life of 19 h and 88 % of Rosuvastatin calcium has the tendency to protein binding.

Based on the above physicochemical and biopharmaceutical properties, rosuvastatin was selected for developing solid dispersions formulations for improving its solubility and dissolution rate.

MATERIAL AND METHODS:

Materials

Rosuvastatin was commercially procured, Poly Vinyl Pyrrolidone k-30 and Polyethylene glycol
Commercially procured from Yarrow chem. Products, Mumbai.

Saturated Solubility Studies

Saturated solubility studies of Rosuvastatin were performed in different dissolution media. 500 mg of Rosuvastatin was weighed and transferred into different conical flask. 50 ml of different dissolution media were transferred into individual conical flask and were closed appropriately. All the conical flasks were placed in the REMI incubator shaker. The shaker was allowed to operate at 50 rpm at 37º C ± 1º C for 24 hrs.[1] Then the conical flasks were removed from the incubator shaker and the samples were filtered by using whatmann filter paper. The clear solution obtained by filtration was suitably diluted with appropriate dissolution media and the absorbance values were noted at 241 nm by using corresponding dissolution media as blank solutions. The absorbance values and their corresponding solubilities were given in table 2.

Preparation of Rosuvastatin Solid Dispersions

Preparation of Solid Dispersions:

Solid dispersions were prepared by using croscarmellose sodium as a superdisintegrant by employing different techniques. The superdisintegrant concentration was maintained constant in the

investigation. They methods employed for the preparation of solid dispersions are:

1. Physical mixing.
2. Kneading method
3. Solvent evaporation

1. Physical Mixing:

Known quantity of drug (Rosuvastatin) and Superdisintegrant (PVP) were weighed separately and passed through sieve no. 80. The materials passed through sieve no.80 were collected and transferred into a clean and dry glass mortar Rosuvastatin and polyvinyl pyrrolidone were triturated together for 5 min and again screened through sieve no. 80. The mixture passed through sieve no. 80 is collected and packed in a wide mouthed amber coloured glass container and was hermetically sealed. [(2)]

Kneading Method:

In this method, superdisintegrants was triturated in a mortar with water to get slurry like consistency. Later drug was incorporated into it by continuous trituration and it was carried out for about 1hr. Slurry was then air dried at 25^0C for 48 hrs. The product was pulverized and passed through 80# sieve and stored in desicator for further studies.[(2)]

3. Solvent Evaporation:

Specified quantity of Drug (Rosuvastatin) and Superdisintegrant (CCS) were taken in a china dish and to that few ml of methanol was added and slightly heated until both drug and polymer dissolves. Then it is subsequently allowed to evaporate. The obtained mixture was dried, passed through the sieve no.80 packed in a wide mouthed amber coloured glass container, and was hermetically sealed and stored. [(3)] The composition of various solid dispersions were given in table 1.

EVALUATION OF PHYSICAL PARAMETERS OF SOLID DISPERSIONS

Physical Parameters of Solid Dispersions:

Physical parameters such as Angle of Repose, Carr's Index, Average particle size and Drug content were evaluated for solid dispersions as per the standards of official compendium.

Drug Content Uniformity for Solid dispersions:

Solid dispersions of Rosuvastatin from a batch were taken at random and were transferred into a 100 ml volumetric flask and 70 ml of methanol was added to it. It was shaken occasionally for about 30 minutes and the volume was made up to 100 ml by adding methanol. About 10 ml of the solution from the volumetric flask was taken and centrifuged. The supernatant solution from the centrifuge tube was collected and again filtered by using Whatmann filter paper. Then the filtrate was subsequently diluted with 6.8 p^H phosphate buffer and the absorbance was measured at 241nm. This test was repeated six times (N=6) for each batch of tablets. The amounts of Rosuvastatin estimated from different batches were given in table 3.

***In-Vitro* Dissolution Studies**

The dissolution test for the solid dispersions was carried out in USP Apparatus Type II (paddle) [USPNF, 2007] with 900 ml of 6.8 p^H phosphate buffer as the dissolution medium. The samples were drawn at 5, 10, 15, 20, 30, 45 minutes. Fresh volume of the medium was replaced with the withdrawn volume to maintain the sink conditions and constant volume throughout the experiment. Samples withdrawn were suitably diluted with same dissolution medium and the amount of drug dissolved was estimated by Shimadzu U.V.1800 spectrophotometer at 241nm and subsequently analysed for the cumulative percentage of drug released.

The dissolution studies on each formulation were conducted in triplicate. The drug release profiles for all the solid dispersions were shown in figures 9 to 11.

Evaluation of Dissolution Parameters:

Pharmacokinetic parameters such as zero order; first order and Hixon Crowell were calculated from the dissolution data obtained from various formulations.

Characterization

Based on the dissolution studies performed on all the formulations, some of the optimized solid dispersions were selected and further characterised by FT-IR, DSC and PXRD studies.

FT-Infra Red Spectroscopy:

IR spectrum of Pure drug and its solid dispersions were recorded using BRUKER 8400S, infrared spectrophotometer in scanning range 450 to 4000 cm^{-1}, by KBr disc method. [(5)]

RESULTS AND DISCUSSION:

Solid dispersions were prepared by physical mixing, kneading and solvent evaporation methods using CCS as a superdisintegrant at different ratios were found to be stable and suitable for increasing the dissolution rate of Rosuvastatin.

1. Physical Mixing:
Known quantity of drug (Rosuvastatin) and Superdisintegrant (PVP) were weighed separately and passed through sieve no. 80. The materials passed through sieve no.80 were collected and transferred into a clean and dry glass mortar Rosuvastatin and croscarmellose sodium were triturated together for 5 min and again screened through sieve no. 80. The mixture passed through sieve no. 80 is collected and packed in a wide mouthed amber coloured glass container and was hermetically sealed.(3)

2. Kneading Method:
In this method, superdisintegrant was triturated in a mortar with water to get slurry like consistency. Later drug was incorporated into it by continuous trituration and it was carried out for about 1hr. Slurry was then air dried at 25^0C for 48 hrs. The product was pulverized and passed through 80# sieve and stored in desicator for further studies.(4)

3. Solvent Evaporation:
Specified quantity of Drug (rosuvastatin) and Superdisintegrant (PVP) were taken in a china dish and to that few ml of methanol was added and slightly heated until both drug and polymer dissolves. Then it is subsequently allowed to evaporate. The obtained mixture was dried, passed through the sieve no.80, packed in a wide mouthed amber coloured glass container, and was hermetically sealed and stored. (5) The composition of various solid dispersions were given in table 1.

Table 1: Composition of Various Solid Dispersions of Rosuvastatin

S. No	Composition	Ratio (Drug: Superdisintegrant)
Physical Mixtures		
1	ROPH1	1:0.5
2	ROPH2	1:1
3	ROPH3	1:1.5
4	ROPH4	1:2
Kneading Method		
5	ROKM1	1:0.5
6	ROKM2	1:1
7	ROKM3	1:1.5
8	ROKM4	1:2
Solvent Evaporation Method		
9	ROSE1	1:0.5
10	ROSE2	1:1
11	ROSE3	1:1.5
12	ROSE4	1:2

*One part is equal to 20mg

Estimation of Rosuvastatin:
Several methods have been reported for estimation of Rosuvastatin by spectrophotometric and chromatographic techniques. (6,7) In the present investigation a simple, sensitive more accurate spectrophotometric method was used for the estimation of Rosuvastatin. (8, 9) The absorbance values of Rosuvastatin measured at a λ_{max} of 241 nm. Calibration curve was shown in Figure1.

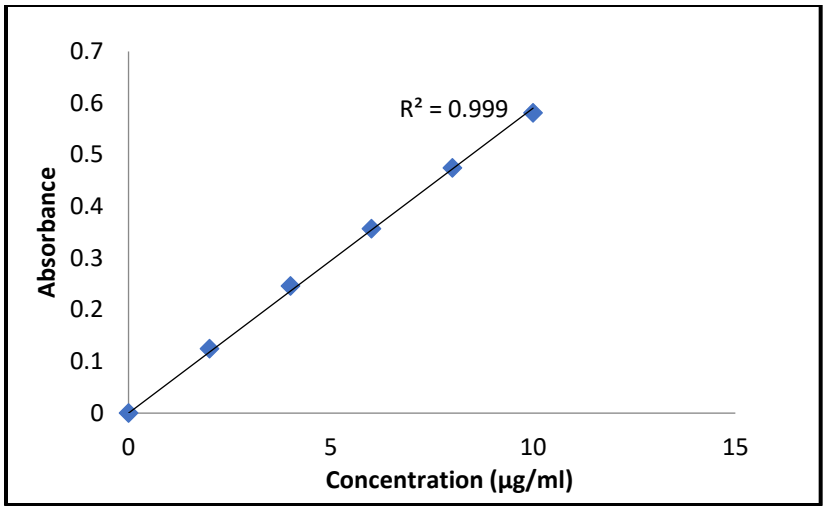


Figure 1: Calibration Curve for the Estimation of Rosuvastatin

Saturated Solubility Studies

Saturated solubility studies of Rosuvastatin were performed in different dissolution media. 500 mg of Rosuvastatin was weighed and transferred into different conical flask. 50 ml of different dissolution media were transferred into individual conical flask and were closed appropriately. All the conical flasks were placed in the REMI incubator shaker. The shaker was allowed to operate at 50 rpm at 37º C ± 1º C for 24 hrs.[1] Then the conical flasks were removed from the incubator shaker and the samples were filtered by using whatmann filter paper. The clear solution obtained by filtration was suitably diluted with appropriate dissolution media and the absorbance values were noted at 241 nm by using corresponding dissolution media as blank solutions. The absorbance values and their corresponding solubilities were given in table 2.

Table 2: Saturated Solubility Studies of Rosuvastatin

S. No	Solvent	Amount Soluble (Rosuvastatin) in mcg/ml
1	0.1N HCl	149.23
2	6.8 pH Phosphate Buffer	598.31
3	4.6 pH Acetate Buffer	431.30
4	Distilled Water	236.86

Evaluation of Physical Parameters for Rosuvastatin Solid Dispersion

The Rosuvastatin solid dispersion formulations are evaluated for physical parameters such as drug, entrapment efficiency and particle shape. [10, 11, 12] The flow properties such as Angle of repose and Carr's index evaluated for various solid dispersions were found to exhibit good flow characteristics. The angle of repose values obtained for various solid dispersions were in the range of 13-25 and Carr's index were in the range of 9 - 23%. Particle sizes of prepared solid dispersions were found to be in the range of 172-179 µ and the drug content in the range of 97.35 – 99.95%. The results were given in Table 3.

Table 3: Physical Parameters of Rosuvastatin Solid Dispersions

S. No	Solid Dispersion	Angle of Repose (^{O})	Carr's Index (%)	Particle Size (microns)	Drug Content (%)
1	ROPD	23.21	17.23	176 ± 2	------
2	ROPH1	21.45	14.56	175 ± 4	99.95±0.3
3	ROPH2	19.85	13.77	173 ± 3	97.35±0.9
4	ROPH3	19.22	13.25	177±2	98.20±1.1
5	ROPH4	18.65	11.14	178±2	99.15±0.5
6	ROKM1	16.52	13.64	176±5	97.35±2.1
7	ROKM2	15.18	12.85	176±2	97.60±0.9
8	ROKM3	15.58	12.95	175±4	98.15±1.5
9	ROKM4	13.32	09.47	174±4	97.65±0.6
10	ROSE1	21.76	15.45	172±3	98.20±0.9
11	ROSE2	19.88	14.65	173±2	97.65±0.6
12	ROSE3	18.64	13.74	173±2	97.10±0.9
13	ROSE4	16.71	12.41	179±2	99.35±0.7

***In Vitro* Dissolution Studies:**

The dissolution test was carried out in USP Apparatus Type II (paddle) with 900ml of 6.8 p^{H} phosphate buffer as the dissolution medium. The samples were drawn at 5, 10, 15, 20, 30, 45 minutes. Fresh volume of the medium was replaced with the withdrawn volume to maintain the sink conditions and constant volume throughout the experiment. Samples withdrawn were suitably diluted with same dissolution medium and the amount of drug dissolved was estimated by Schimadzu U.V.1800 double beam spectrophotometer at 241 nm and subsequently analyzed for the cumulative percentage of drug released.

The *in vitro* dissolution studies were performed for various solid dispersions in 6.8p^{H} phosphate buffer. It was found that the solid dispersions ROSE-4, ROKM-4 in the drug: superdisintegrant ratio 1:2 prepared by solvent evaporation and kneading methods respectively release the drug rapidly than the pure drug and other dispersions.

Dissolution studies were performed on all the niosomal formulations by using USP paddle -II dissolution apparatus. By using 6.8 pH phosphate buffer. The drug release from the Formulations ROPH-1 - ROPH-4 formulated by physical mixing the drug release was found to be 66.51 ± 0.48 to 89.33 ± 0.75. The drug release from the Formulations ROKM-1 - ROKM-4 formulated by kneading method the drug release was found to be 74.59 ± 0.16 to 98.83 ± 0.46. The drug release from the Formulations ROSE-1 -ROSE-4 formulated by solvent evaporation method the drug release was found to be 76.15 ± 0.29 ± 0.16 to 98.05± 0.87. The results were shown in Figures 2-4.

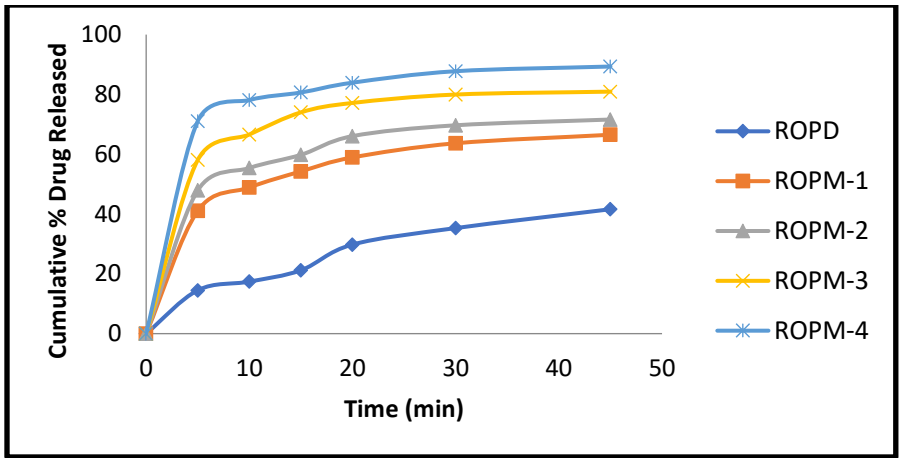


Figure 2: Drug Release Profiles of Rosuvastatin Solid Dispersions Prepared by Physical Mixing Method

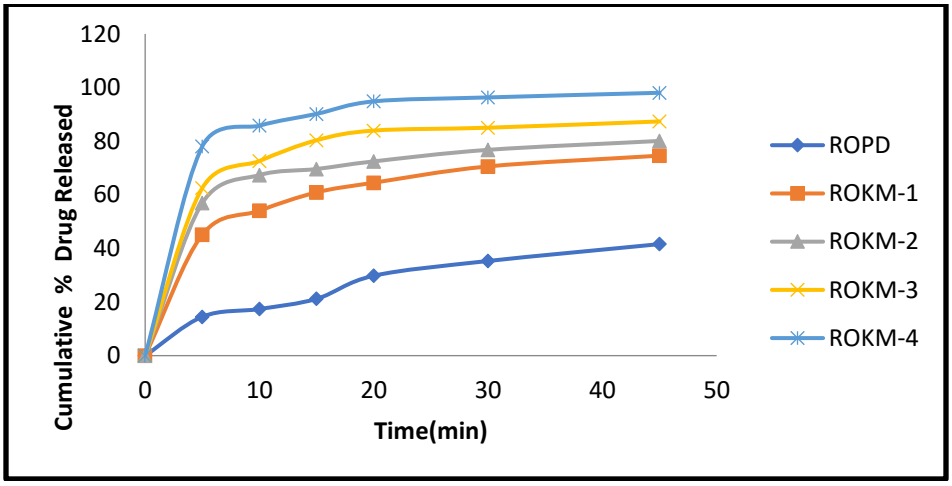


Figure 3: Drug Release Profiles of Rosuvastatin Solid Dispersions Prepared by Kneading Method

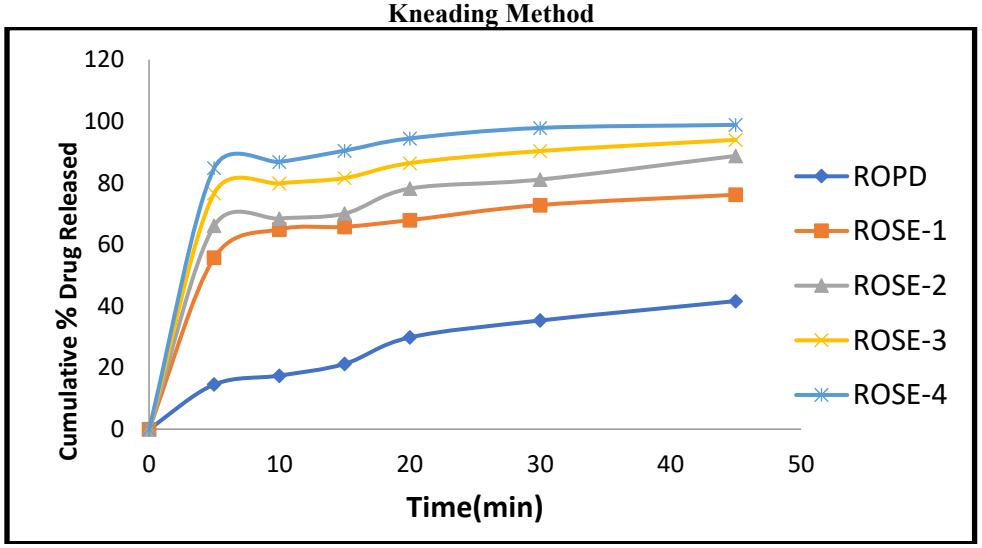


Figure 4: Drug Release Profiles of Rosuvastatin Solid Dispersions Prepared by Solvent Evaporation Method

Evaluation of Various Dissolution Parameters:

The zero order plots for all the Rosuvastatin formulations were not linear. All the Rosuvastatin solid dispersions formulations were found to be linear with first order release rate constant. Among ROKM-4, ROSE-4 solid dispersions ROKM-4 released the drug rapidly than the ROSE-4, pure drug, and other solid dispersions. The first order R^2 values of solid dispersions were in the range of 0.90 – 0.99. Thus, all the SDs were found to be linear with first order rate constant. The zero order R^2 values of solid dispersions were in the range of 0.56 – 0.70. Thus, all these SDs were found to be nonlinear with zero order rate constant. The results were given in Table 4 and shown in Figures 5-12.

Table 4: *In-vitro* Dissolution Parameters of Rosuvastatin Calcium Solid Dispersions

Solid Dispersion	Drug release parameters of Rosuvastatin Calcium Solid dispersions									
	% Drug Released at 45 mins	T_{50} (min)	T_{90} (min)	DE 30%	Zero order		First order		Hixson Crowel	
					R^2	K (mg/min)	R^2	K (min^{-1})	R^2	K ($mg^{1/3}$/min)
ROPD	41.6	>45	>45	21.66	0.512	0.603	0.885	0.013	0.925	0.016
ROPM-1	66.51	11	>45	48.33	0.599	0.191	0.971	0.012	0.841	0.015
ROPM-2	71.66	6.30	>45	53.33	0.551	0.884	0.987	0.021	0.819	0.011
ROPM-3	80.93	4	>45	63.33	0.481	0.628	0.967	0.019	0.688	0.007
ROPM-4	89.33	3	>45	73.33	0.425	0.656	0.993	0.035	0.832	0.006
ROKM-1	74.59	6.30	>45	53.33	0.611	1.368	0.952	0.021	0.838	0.010
ROKM-2	80.03	4.30	>45	61.66	0.489	0.514	0.971	0.019	0.802	0.005
ROKM-3	87.35	4	>45	70	0.472	0.312	0.988	0.030	0.692	0.006
ROKM-4	98.05	2	15	81.66	0.417	0.115	0.985	0.047	0.667	0.005
ROSE-1	76.15	4	>45	58.33	0.476	0.298	0.993	0.012	0.906	0.004
ROSE-2	88.77	4.3	>45	73.33	0.514	0.738	0.987	0.027	0.975	0.003
ROSE-3	93.98	3.30	28	75	0.429	0.661	0.939	0.017	0.940	0.015
ROSE-4	98.93	2.30	15	83.33	0.387	0.282	0.994	0.024	0.867	0.014

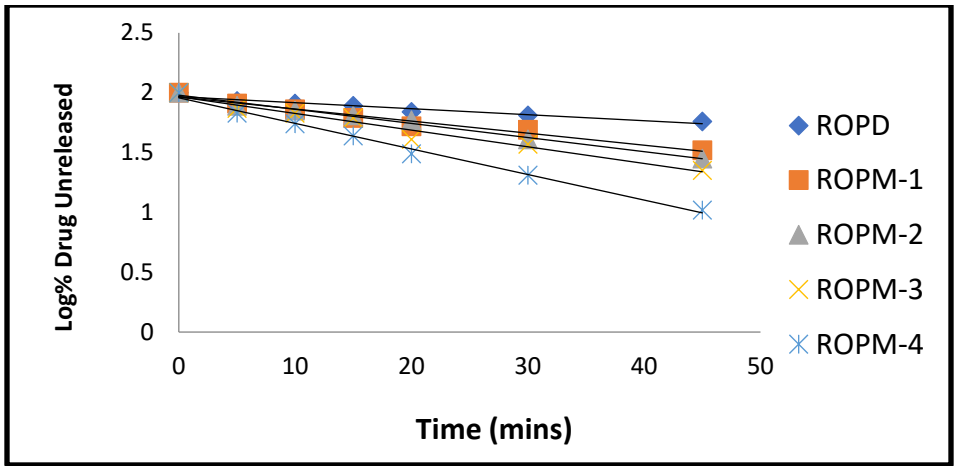


Figure 5: First Order Plots of Rosuvastatin Solid Dispersions Prepared by Physical Mixing Method

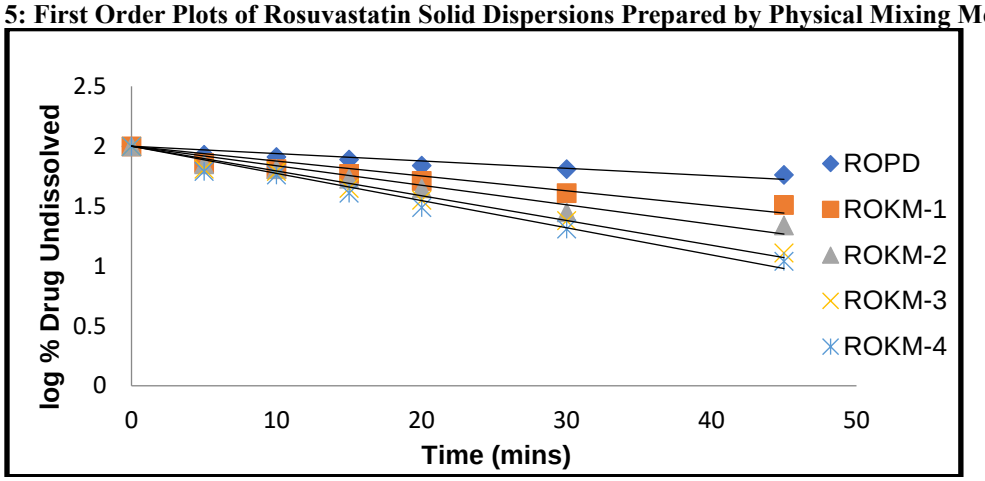


Figure 6: First Order Plots of Rosuvastatin Solid Dispersions Prepared by Kneading Method

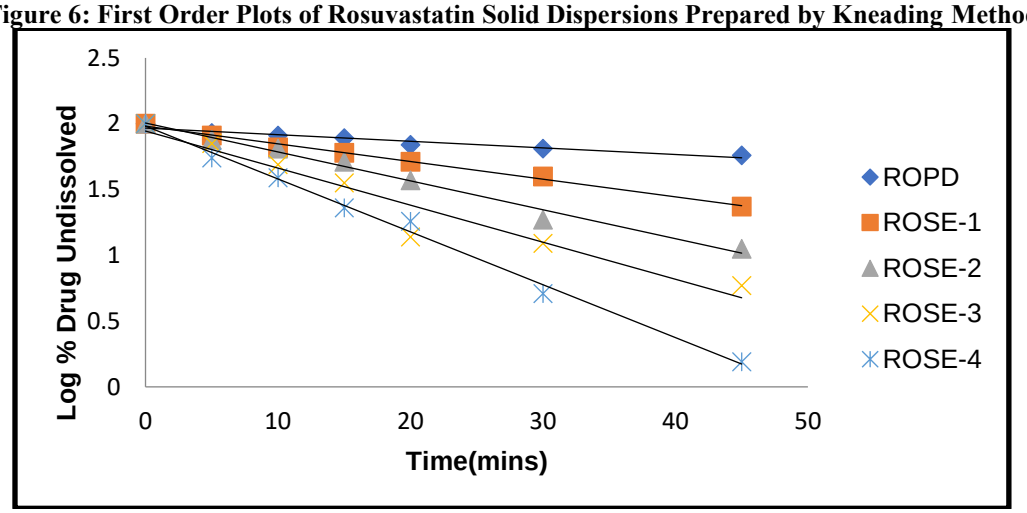


Figure 7: First Order Plots of Rosuvastatin Solid Dispersions Prepared by Solvent Evaporation Method

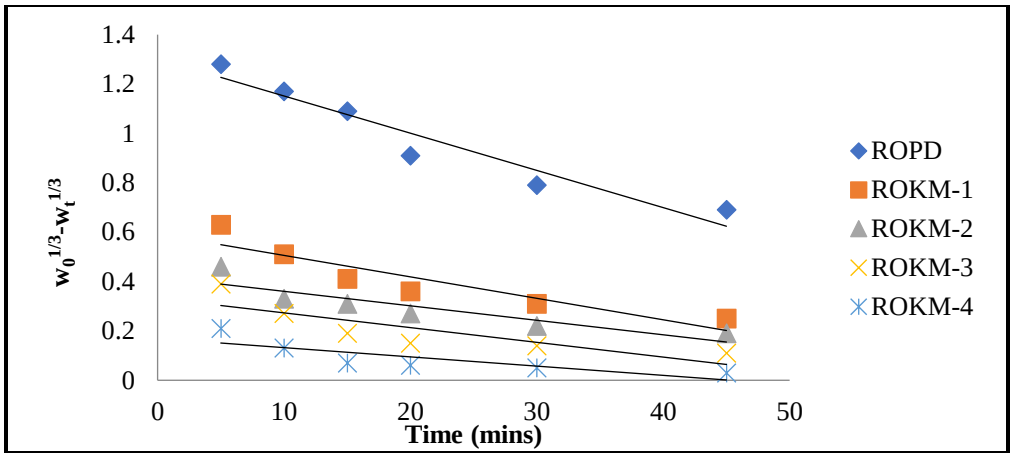


Figure 8: Hixson Crowell Plots of Rosuvastatin Solid Dispersions Prepared by Physical Mixing Method

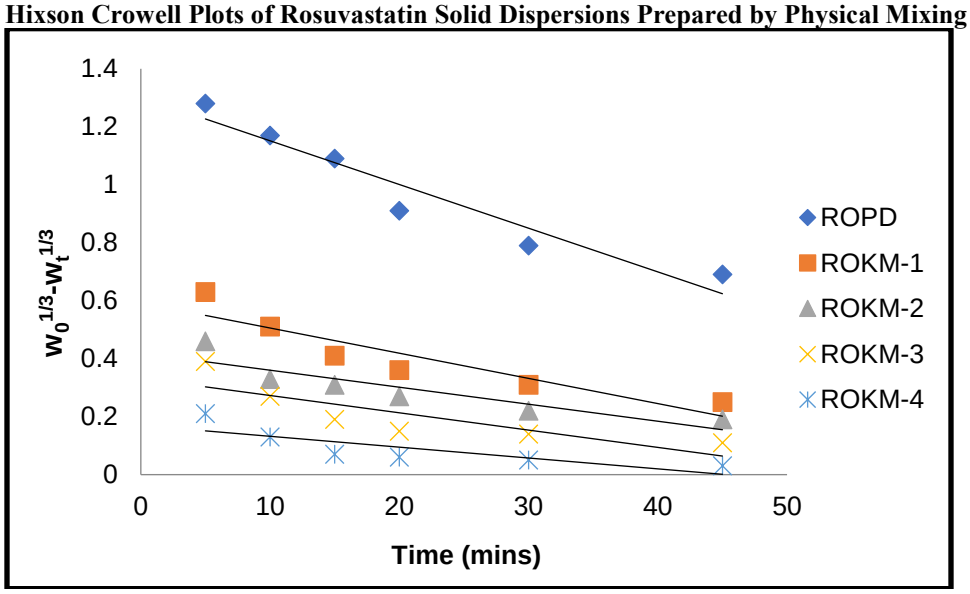


Figure 9: Hixson Crowell Plots of Rosuvastatin Solid Dispersions Prepared by Kneading Method

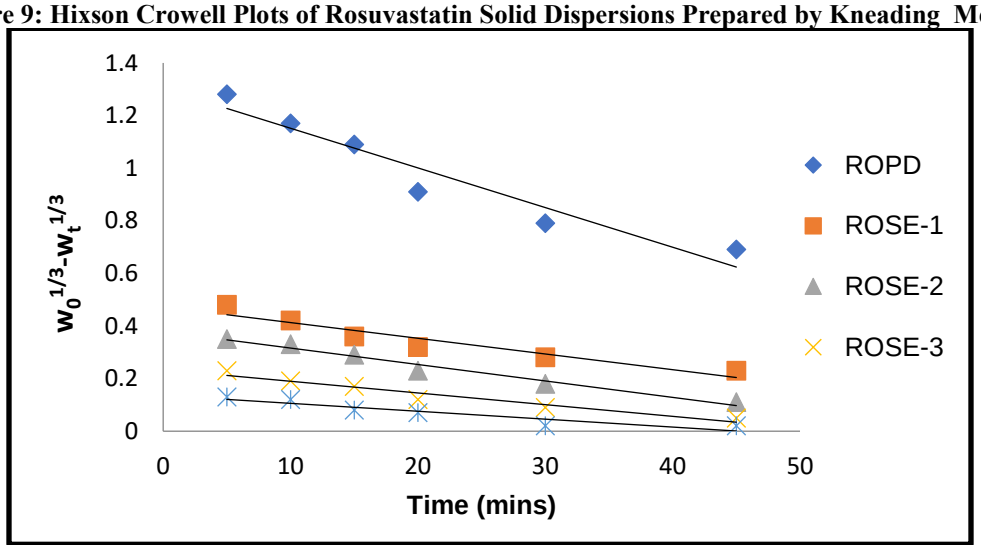


Figure 10: Hixson Crowell Plots of Rosuvastatin Solid Dispersions Prepared by Solvent Evaporation Method

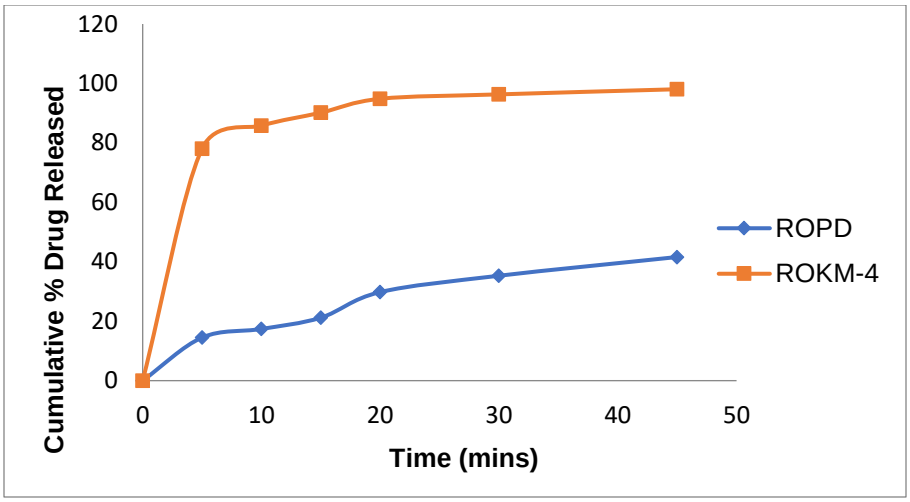


Figure 11: Comparison of Drug Release Profiles of Rosuvastatin Pure Drug to Solid Dispersion Prepared by Kneading Method

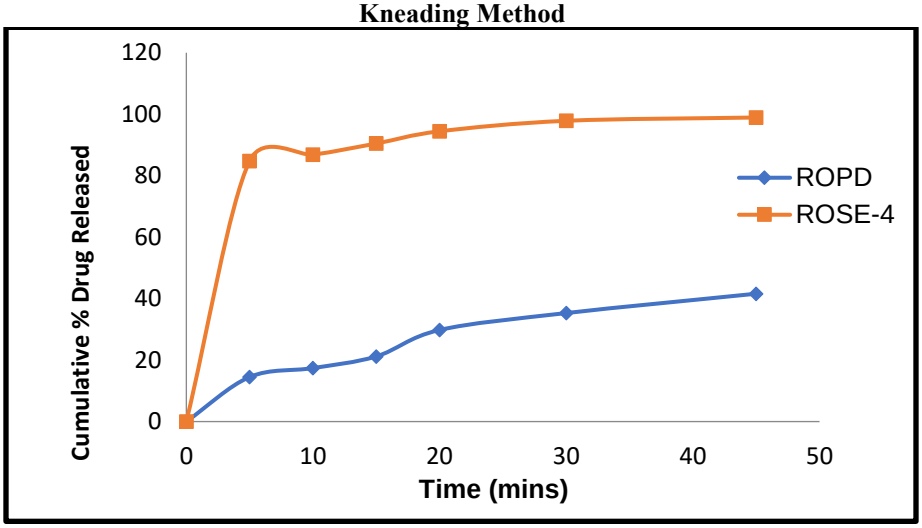
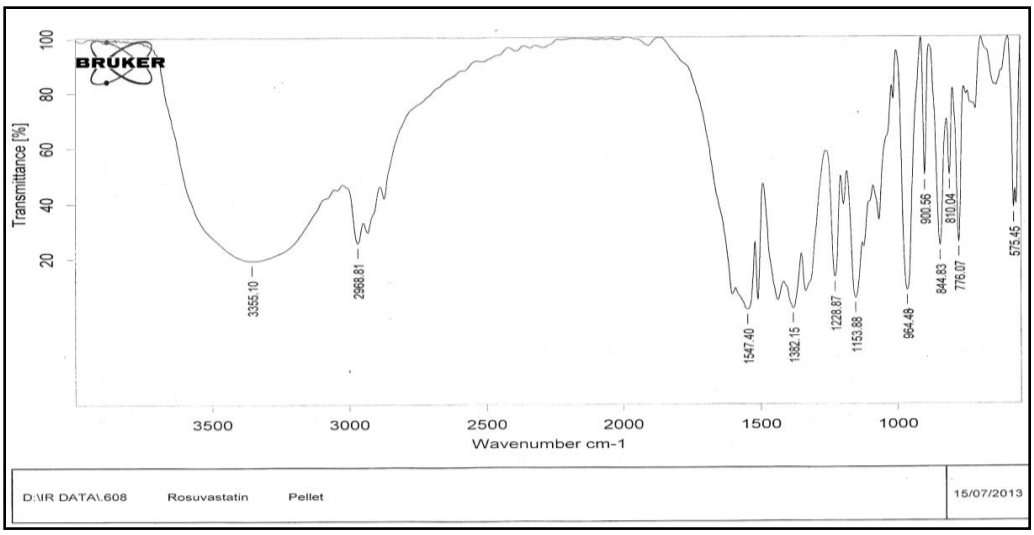


Figure 12: Comparison of Drug Release Profiles of Rosuvastatin Pure Drug to Solid Dispersion Prepared by Solvent Evaporation Method

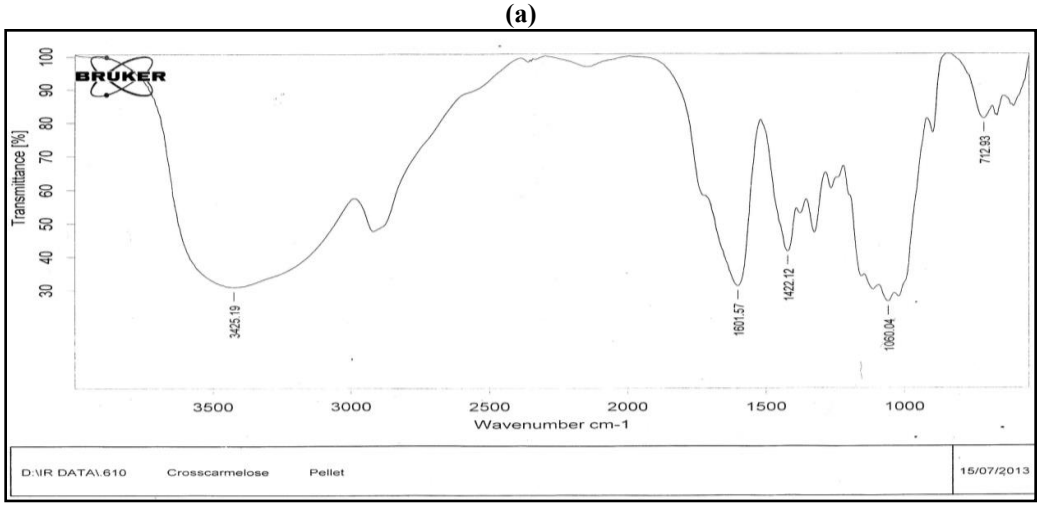
CHARACTERIZATION

FTIR Studies:

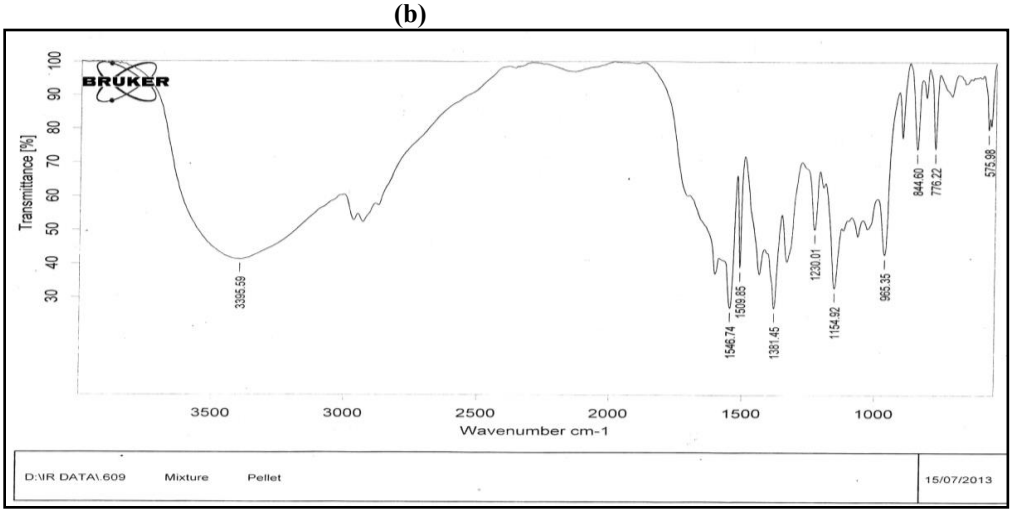
FTIR studies of Rosuvastatin calcium and optimized formulations were carried out to study the interaction between the drug and excipients used. C-H stretching, O-H stretching, C-F stretching, C=C bending of pure Rosuvastatin and the optimized formulations were almost in the same region of wave number ranging from 3395.59 cm^{-1} to 776.07 cm^{-1}. It showed that IR spectrum of Rosuvastatin calcium and optimized formulations were having similar fundamental peaks and pattern. This indicated that there were no drug excipient interactions in the formulations. The results were shown in table 5 & Figure 13.



(a)



(b)



(c)

Figure 13: IR Interpretations of (a) Rosuvastatin Calcium (b) Poly Vinyl Pyrrolidone (c) Rosuvastatin Calcium + PVP Complex Prepared by Kneading Method

Table 5: IR INTERPRETATIONS

Functional Groups Present	ROS	PVP	ROS + PVP Complex Prepared by Kneading Method
O-H Stretching	3355.10	3425.19	3395.59
C-H Stretching	2968.81	2830.55	2971.22
C-F Stretching	1382.15	-	1381.45
C=C Bending	776.07	712.93	776.22

Accelerated Stability Studies:

Accelerated stability studies were performed for the optimised formulation (ROKM-4). Ther e were no significant changes observed in drug release from the tablets after storage at different conditions remained unaltered and found to be quite stable. the results were given in table 6 and shown in figure 14.

Table 6: Physical Parameters of Rosuvastatin Calcium Solid Dispersions (ROKM-4**) Before and After Storage at Different Conditions**

Formulation	Storage Condition	Angle of Repose (^{O})	Carr's Index (%)	Particle Size (microns)	Drug Content (%)
ROKM-4	Before Storage	13.32	09.47	174.04	97.65±0.6
	25±2^{0}C, 60±5% RH	12.89	09.83	172.15	97.07±0.3
	40±2^{0}C, 75±5% RH	10.53	09.45	170.16	96.96±0.5

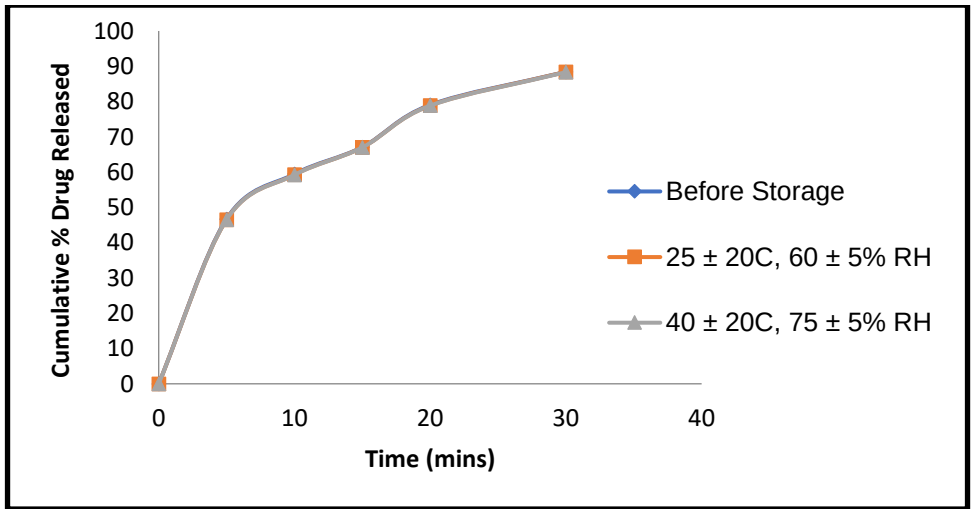


Figure 14: Drug Release Profiles of Rosuvastatin Calcium solid dispersion (ROKM-4) Before and After Storage at Different Conditions

CONCLUSION:

Solid dispersions were prepared by physical mixing, kneading and solvent evaporation methods using PVP as a superdisintegrant at different ratios were found to be stable and suitable for increasing the dissolution rate of Rosuvastatin. 1:2 ratio of drug and superdisintegrant respectively in every method showed highest rates of dissolution than the other ratios i.e., as superdisintegrant concentration increases the drug solubility increases. The flow properties such as Angle of repose and Carr's index evaluated for various solid dispersions were found to exhibit good flow characteristics. Particle sizes of prepared solid dispersions were found to be in the range. The *in vitro* dissolution studies were performed for various solid dispersions in 6.8p^{H} phosphate buffer. It was found that the solid dispersions ROSE-4, ROKM-4 in the drug: Superdisintegrants ratio 1:2 prepared by solvent evaporation and kneading methods respectively release the drug rapidly than the pure drug and other dispersions. Among ROKM-4, ROSE-4 solid dispersions ROKM-4 released the drug rapidly than the ROSE-4, pure drug, and other solid dispersions. The obtained results were found to be consistent with the preset ranges established for the dissolution parameters. Among the methods used for the preparation of solid dispersions drug: superdisintegrant in the ratio of 1:2 showed more drug release following the order kneading> solvent evaporation> physical mixing method. FTIR studies were performed for the pure drug, poly vinyl pyrrolidone, and complex prepared by Kneading method.

FUTURE SCOPE OF WORK:

Formulation prepared by different method was found to release the drug at faster rate and was suitable for preparing them as tablets. Further studies can be focused on the Rosuvastatin calcium by using newer polymers and even in the combinations of those polymers. Investigation can be extended by employing newer techniques for the preparation of tablets. *In vivo* pharmacokinetic and dynamic studies can be performed on a suitable animal model.

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