Preparation, Evaluation and Optimization of Nanosuspension of Poorly Water-Soluble Rosuvastatin Calcium

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ABSTRACT - Rosuvastatin calcium, an antihyperlipidemic agent with poor water solubility which comes under BCS Class II with 20% bioavailability, was chosen for the study. The present study focuses on Preparation, Evaluation and Optimization of Nanosuspension of Poorly Water-Soluble Rosuvastatin Calcium. Rosuvastatin Calcium Nanosuspension was prepared used Solvent-Anti-Solvent Precipitation method. Experimental Design was obtained using Design Expert Software. 10 Batches of Rosuvastatin Calcium Nanosuspension was prepared. Interaction between Independent factor and dependent factor was studied. Optimization was carried out and Batch B9 was selected as the Optimized Batch. Batch B9 was Prepared using 1:10 Ratio of Solvent-Anti-Solvent, 6 % Concentration of Stabilizer was used and it was sonicated for 10 minutes. The Results of the Formulation B9 was also satisfying. The Particle Size of the Optimized batch was found to be 351.64 nm, Zeta Potential was -20.21 mV and % Drug Release was 88.52 % in 60 minutes. Stability studies of B9 was carried out and no significant growth in Particle Size was found which indicates Good Stability. Comparison of *in-vitro* % Drug Release of Pure Drug and Optimized Batch of Rosuvastatin Calcium Nanosuspension was carried. Optimized Batch B9 showed 88.52% Drug Release in 60 min while Pure Drug Showed 26.29% drug Release in 60 min.

KEYWORDS – Nanosuspension; Solubility; Particle Size; Zeta Potential; Drug Release.

1. INTRODUCTION –

During development of new formulation, the parameters of the drug which are considered are aqueous solubility, stability, temperature, humidity, compatibility with solvent and excipient. The most important parameter is aqueous solubility (1). In 1995 to 2022, many drugs have been discovered and approved from which 46% of new drug belongs to class IV according to the BCS classification and only 9% of drug belongs to Class I of BCS classification. Drugs which belong to Class IV are poorly soluble in aqueous medium as well as in non – aqueous medium and have low permeability (2). As most of the drugs have low solubility, low bioavailability and low dissolution rate has been observed and the effectiveness of the drug is also affected (3).

As poorly soluble drug have major problem like poor bioavailability, lack of dose response proportional, suboptimal dosing, etc. When formulating such drugs, harsh excipient for solubility enhancement are used but they are not orally accepted. For minimizing this problem and to improve drugs property various

approaches have been made. In Last few decades, the main goal in drug development is to improve the bioavailability of the drug. There are many conventional techniques which were used to enhance the effectiveness of the drug[3]. Conventional methods includes Micronization, Solubilization using co-solvent, Salt formation technique, Precipitation technique, Oil solution, Solid dispersion, Emulsion, Milling technique, Complexation, Supercritical processing, etc.(1,2). The most common technique is Micronization (particle size reduction). Micronization is a technique where surface area of the drug particles is increased by particle size reduction. In which particle size ranges between 2 µm to 5 µm, but Dissolution rate and Absorption rate in the GI tract is not increase to excepted rate. The common disadvantage of Size reduction techniques is deterioration of the drug particles and their properties. Due to which electrostatic charge is enhanced and development of suspicious formulation may take place. Conventional methods also have other disadvantages too which are broad particle size distribution, contamination of formulation, crystal structure variation, uncontrolled particle morphology and many more. To tackle or to minimize the disadvantages of conventional technique use of advanced technique like Nanotechnology is being carried out from last few years (3).

Nano technique/technology is used to solve the problem which is arise due to poor solubility of the drug. Nano is a Greek word which means 'Small'. By the use of Nanotechnology, we can formulate drugs which belongs to Class II and Class IV which have major problems of solubility in both aqueous and non-aqueous medium. Nanotechnology is safe, simple and mostly importantly the advantages are more in compare to conventional methods (4). Nanosuspension is the formulation which contains submicron colloidal Nano sized drug particle which are stabilized by use of suitable surfactants. They are also defined as biphasic liquid dosage form in which pure drug are suspended or dispersed in aqueous medium intended for oral, topical or parental administration. Particle size distribution in Nanosuspension is less than 1micron and the average particle size is 1 µm. Furthermore, in Nanosuspension, the pure drug is maintained in its crystalline form with particle size less than 1 µm. Due to decrease in drug particle size, surface area increases which leads to enhancement in the dissolution rate and bioavailability (5). Nanotechnology also helps us to administer poorly soluble drug intravenously as the particle size is less, due to which there are minimum chances of blockage of blood capillaries (2). Most important advantage of Nanosuspension is it prevent Oswald ripening as there is absence of particles with large amount of difference in their sizes. In Oswald repining, there is movement of molecules from high concentration region (around small particle) to low concentration region (around large particle). When smaller particles in Nanosuspension moves towards larger particles super saturation occurs and due to aggregation of smaller particles and larger particles, large crystals (micro particles) formation takes place. Stability is an essential parameter which is considered in any type of biphasic liquid dosage form. The stability of Nanosuspension is high in comparison to Micro-Suspension due to uniform particle size. Nanosuspension also can be incorporated in the solid matrix by preforming lyophilization and spray drying techniques (5).

The attempt of this study was to prepare Nanosuspension of Poorly Water Soluble Rosuvastatin Calcium by Solvent-Antisolvent Precipitation technique which may enhance the Solubility and Dissolution Rate of the Drug and to find out suitable Ratio of Solvent: Anti-solvent, Concentration of Stabilizer and Sonication Time to prepare Nanosuspension of Rosuvastatin Calcium with Smaller Particle Size, perfect Zeta potential and faster Percentage Drug Release.

2. MATERIAL AND METHOD

2.1. MATERIALS –

Rosuvastatin calcium, an Antihyperlipidemic agent was received as gift sample from Lupin Limited, Pune. All other excipients and reagents such as Dimethyl Sulfoxide (DMSO) was used as an Organic Solvent, Polyvinylpyrrolidone (PVP K30) used as a Stabilizers and Distilled water used as an Anti-solvent all of these were obtained from COSMO CHEM, Pune.

2.2. METHOD OF PREPARATION OF NANOSUSPENSION OF ROSUVASTATIN CALCIUM

Nanosuspension was prepared using Anti-Solvent precipitation technique. 15 mg of API Rosuvastatin calcium was dissolved in 1 mL of Dimethyl Sulfoxide (DMSO) which results in formulation of drug Solution (Organic Phase). For Preparation of Stabiliser Solution (Aqueous Phase), Polyvinylpyrrolidone (PVP K30) of definite amount was dissolved in specific amount of distilled water. Rapid mixing of aqueous phase and organic phase was carried out. This mixture was then stirrer using magnetic stirrer at 1000 rpm for 5 minutes. After

Stirrer is completed, then the suspension was sonicated for sufficient period of time to obtain Nanosuspension (6-32).

2.3. EXPERIMENTAL DESIGN USING FACTORIAL DESIGN

The Experimental Design for formulating Nanosuspension of Rosuvastatin Calcium was done using Factorial design method. The three Level Factorial design was generated using Design expert stat ease software (Version 13.0). It is more advantageous because it required fewer experimental than a full factorial design. In this design, effect of independent factor on Dependant factor (Response) was studied. Ratio of Solvent: Anti-solvent (X1), Concentration of stabilizer (X2), and Sonication Time(X3) were selected as the three independent factor for study. Each factor was evenly set at low, medium, and high levels as shown in the Table 1. Particle Size (Y1), Zeta Potential (Y2), and % Drug Release (Y3) were selected as the Dependent Factors (Response) (33-44).

Table 1 – Independent Factors with their Level

Independent Factor	Unit	Variable Level		Actual Value
Ratio of S:AS (X1)	Ratio (mL)	Low	-1	1:10
		Medium	0	-
		High	+1	1:50
Concentration of stabilizer (X2)	% (w/v)	Low	-1	2
		Medium	0	6
		High	+1	10
Sonication Time (X3)	Minutes	Low	-1	8
		Medium	0	10
		High	+1	12

2.4. EVALUATION OF PREPARED ROSUVASTATIN CALCIUM NANOSUSPENSION

1. Appearance

The prepared Nanosuspension was inspected visually for clarity, colour and presence of any particulate matter.

2. Particle Size Determination

Particle size of the prepared Nanosuspension was determined using Dynamic Light Scattering (DLS) method. For DLS particle sizing, the sample needs to be crystal clear to very slightly hazy. If the solution is white or too hazy, it should be diluted further before attempting a DLS size measurement. When the solution is ready for analysis and transfer it in the cuvette, care should be taken to avoid bubbles which are formed on the walls of the cuvette. Slowly tilting or tapping the cuvette on a hard surface may help also. Once the solution was homogenous and ready for DLS measurement, the cuvette containing the solution was placed in the instrument. The instrument was run and solution was analysed for particle size (45-47).

3. Zeta Potential

Zeta Potential of the prepared Nanosuspension was determined using Light Scattering method. For Zeta Potential determination, the sample needs to be crystal clear. When the solution is ready for analysis and transfer it in the cuvette, care should be taken to avoid bubbles which are formed on the walls of the cuvette. Slowly tilting or tapping the cuvette on a hard surface may also help to remove the bubble formed. Then the electrode was dipped inside the cuvette containing sample solution. Care should be taken to avoid bubbles in between the electrodes. The cuvette containing the solution was be placed in the instrument. The instrument was run and solution was analysed for Zeta Potential (45, 48-49).

4. Polydispersity Index (PI)

Polydispersity Index (PI) of the prepared Nanosuspension was determined using Dynamic Light Scattering (DLS) method. For DLS method, the sample needs to be crystal clear to very slightly hazy. If the solution is white or too hazy, it should be diluted further before attempting a DLS size measurement. When the

solution was ready for analysis and transfer it in the cuvette, care should be taken to avoid bubbles which are formed on the walls of the cuvette. Slowly tilting or tapping the cuvette on a hard surface may also help to remove the bubbles formed. Once the solution was homogenous and ready for DLS measurement, the cuvette containing the solution was be placed in the instrument. The instrument was run and solution was analysed for Polydispersity Index (PI) (45-47).

5. Drug Content

About 1 ml of Rosuvastatin calcium Nanosuspension was taken and then it was diluted upto 10ml with 0.1N HCL. Sample prepared was analysed using UV spectroscopy. Absorbance was observed at 240 nm and drug content was calculated (25).

6. *in-vitro* Drug Release Studies

The *in- vitro* drug release study was carried out using paddle method (USP apparatus II). 900ml of 0.1N HCL was used as the dissolution medium. Temperature was set at 35ºC ± 0.5ºC. The Paddle was rotated at 50 rpm. The sample containing an equivalent 10 mg of Rosuvastatin Calcium was transfer to the Dissolution Medium. 10 ml of Sample was removed for the dissolution medium at 0, 10, 20, 30, 40, 50, 60 min of dissolution time. Sample removed at specific interval of time was analyzed using UV spectroscopy. Absorbance was observed at 240nm and Percentage Drug Release was calculated (32).

2.5. OPTIMIZATION OF ROSUVASTATIN CALCIUM NANOSUSPENSION

Optimization of the formulations was studied by Regular Level Factorial design. Ratio of Solvent: Anti-solvent (X1), Concentration of stabilizer (X2), and Sonication Time (X3) were selected as independent variables and the dependent variables were Particle Size (Y1), Zeta Potential (Y2), and % Drug Release (Y3). The data obtained were treated using Design expert stat ease software and analyzed statistically using Model Graph technique. Various graphs obtained from the Model Graph technique which indicates Interaction Between each independent Factor and dependent Factor (Response) were studied (33-44).

2.6. STABILITY STUDY

The Optimized Batch of Rosuvastatin Calcium Nanosuspension was selected for the stability Study. The Optimize Batch of Rosuvastatin Calcium Nanosuspension was kept at 2-4ºC in Refrigerator and at Room Temperature Physical Stability of the Nanosuspension was after 5 Months. Rosuvastatin Calcium Nanosuspension was inspected visually for clarity, any kind of colour change. The Nanosuspension was also evaluated for change in Particle size (25-30).

2.7. Comparison of *in-vitro* % Drug Release of Pure Drug and Optimized Batch of Rosuvastatin Calcium Nanosuspension

Comparison of *in-vitro* % Drug Release of Pure Drug and Optimized Batch of Rosuvastatin Calcium Nanosuspension was carried out using paddle method (USP apparatus II). 900ml of 0.1N HCL was used as the dissolution medium. Temperature was set at 35ºC ± 0.5ºC. The Paddle was rotated at 50 rpm. The sample containing an equivalent 10 mg of Rosuvastatin Calcium was transfer to the Dissolution Medium. 10 ml of Sample was removed for the dissolution medium at 0, 10, 20, 30, 40, 50, 60 min of dissolution time. Sample removed at specific interval of time was analyzed using UV spectroscopy. Absorbance was observed at 240nm and Percentage Drug Release was calculated (32).

3. RESULTS AND DISCUSSIONS

3.1 EXPERIMENTAL DESIGN USING FACTORIAL DESIGN

The Three level Factorial design was run using Design Expert Software and Experimental design Layout was obtained as shown in the Table 2, where X1 is the Solvent: Anti-Solvent Ratio (ml), X2 is the Concentration of Stabilizer (% w/v) and X3 is the Sonication time (min).

Table 2 - Experimental Design Layout

Batch Code	Independent Factor					
	Variable Level			Actual Value		
	X1	X2	X3	X1	X2	X3
B1	+1	0	0	1:50	6	10
B2	+1	+1	+1	1:50	10	12

B3	-1	-1	-1	1:10	2	8
B4	+1	+1	-1	1:50	10	8
B5	-1	+1	-1	1:10	10	8
B6	-1	-1	+1	1:10	2	12
B7	+1	-1	-1	1:50	2	8
B8	-1	+1	+1	1:10	10	12
B9	-1	0	0	1:10	6	10
B10	+1	-1	+1	1:50	2	12

Table 2 – Formulation Table

Ingredients	Rosuvastatin Calcium (in mg)	DMSO (in mL)	PVP K30 (in % w/v)	Distilled Water (in mL)
B1	15	1	6	50
B2	15	1	10	50
B3	15	1	2	10
B4	15	1	10	50
B5	15	1	10	10
B6	15	1	2	10
B7	15	1	2	50
B8	15	1	10	10
B9	15	1	6	10
B10	15	1	2	50

3.2. EVALUATION OF PREPARED ROSUVASTATIN CALCIUM NANOSUSPENSION

1. Appearance –

Appearance of the prepared Nanosuspension was inspected visually and all the batches of Rosuvastatin Calcium Nanosuspension were Clear, Colourless, and free from any particulate matters.

2. Particle Size Determination

Particle size of the prepared Rosuvastatin Calcium Nanosuspension was determined using Dynamic Light Scattering (DLS) method. Particle size determination results for all the prepared batches of Rosuvastatin Calcium Nanosuspension are presented in the Table 3 and all the Graph obtained are reported in the Figure 1.

3. Zeta Potential

Zeta Potential of the prepared Rosuvastatin Calcium Nanosuspension was determined using Light Scattering method. Zeta Potential results for all the prepared batches of Rosuvastatin Calcium Nanosuspension are presented in the Table 3 and all the Graph obtained are reported in the Figure 2.

Table 3 – Particle Size Analysis and Zeta Potential of Each Batch of Rosuvastatin Calcium Nanosuspension

Batch No	Particle size (nm)	Zeta Potential (mV)
B1	327.83 nm	-7.57 mV
B2	568.29 nm	-20.29 mV
B3	263.29 nm	-4.45 mV
B4	381.13 nm	-10.81 mV
B5	427.16 nm	-22.13 mV
B6	343.79 nm	-25.06 mV
B7	658.90 nm	-11.19 mV
B8	384.87 nm	-14.64 mV
B9	351.64 nm	-20.21 mV
B10	397.60 nm	-10.77 mV

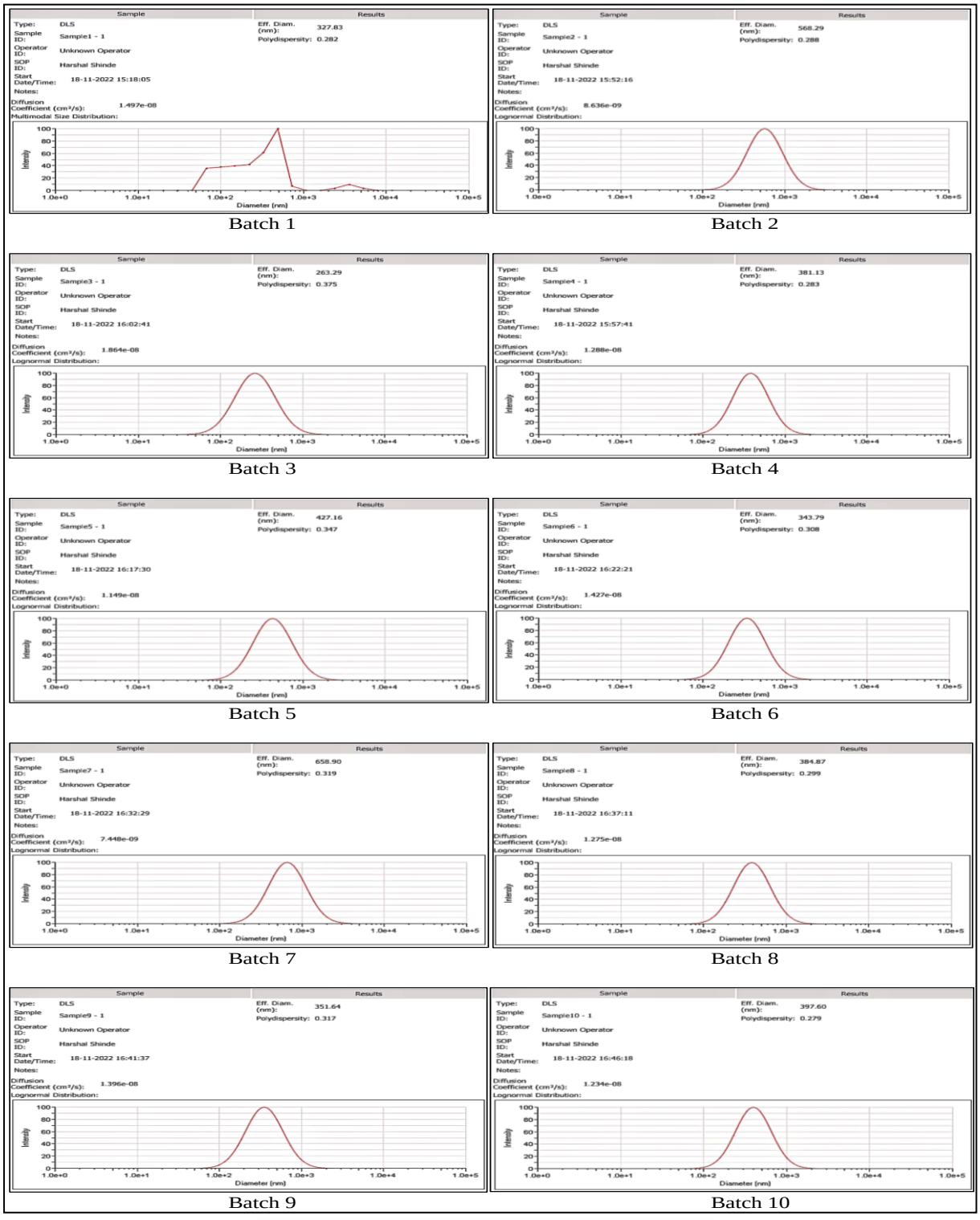

Figure 1 – Particle Size Analysis of all the Batches

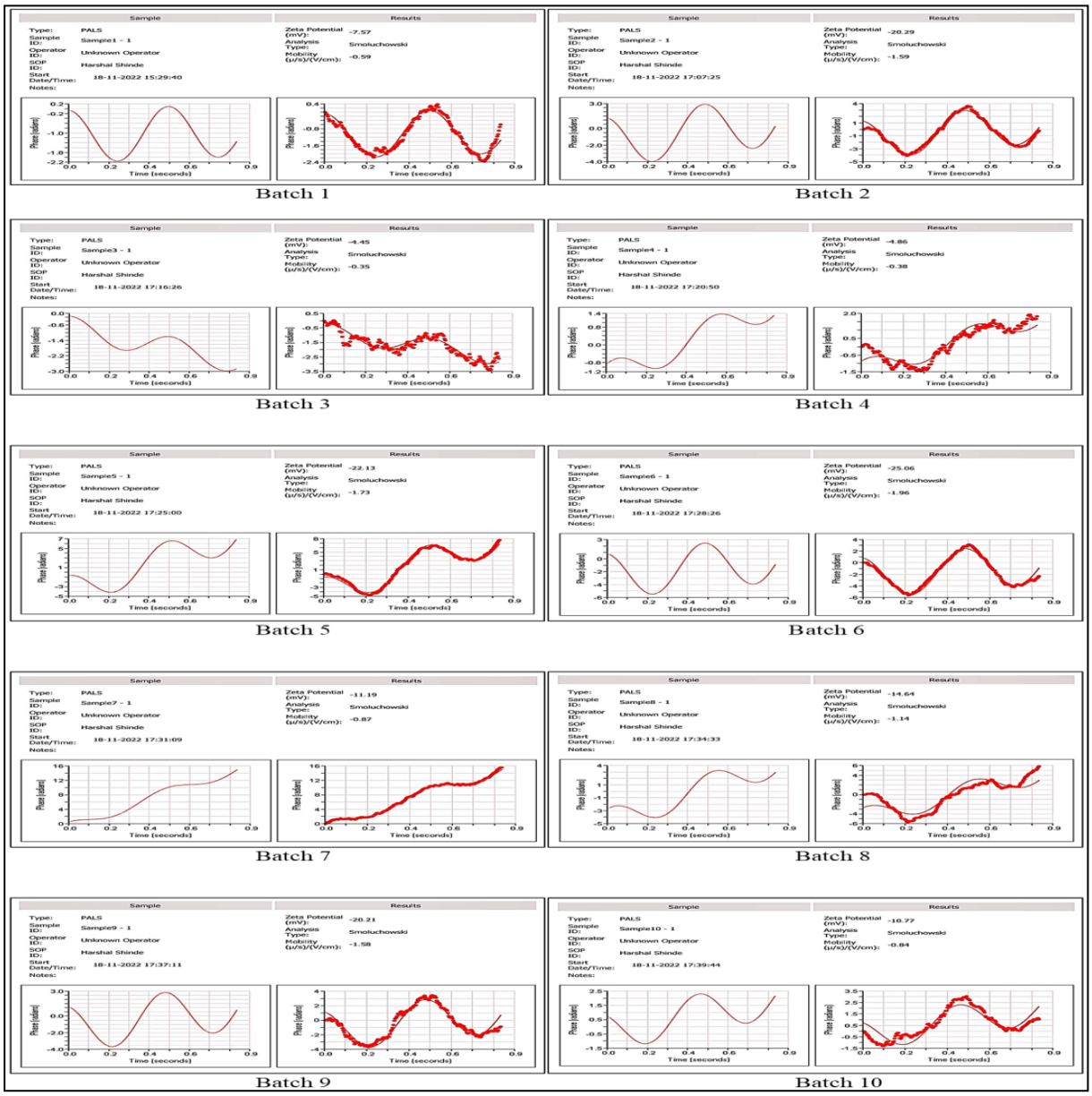

Figure 2 –Zeta Potential of all the Batches

4. Polydispersity Index (PI)

Polydispersity Index (PI) of the prepared Nanosuspension was determined using Dynamic Light Scattering (DLS) method. Result of Polydispersity Index (PI) is reported in the Table 4.

5. Drug Content

All the Batches of the Rosuvastatin Calcium Nanosuspension was evaluated for the Drug Content. Results of Drug Content are reported in the Table 4 and represented graphically in Figure 3.

Table 4 – Drug Content and Polydispersity Index of Each Batch of Nanosuspension

Batch No	Polydispersity Index	Drug Content (mg/ml)
B1	0.282	0.31
B2	0.288	0.26
B3	0.375	1.43
B4	0.283	0.46
B5	0.347	1.94
B6	0.308	1.73
B7	0.319	0.51

B8	0.299	1.11
B9	0.317	1.59
B10	0.279	0.39

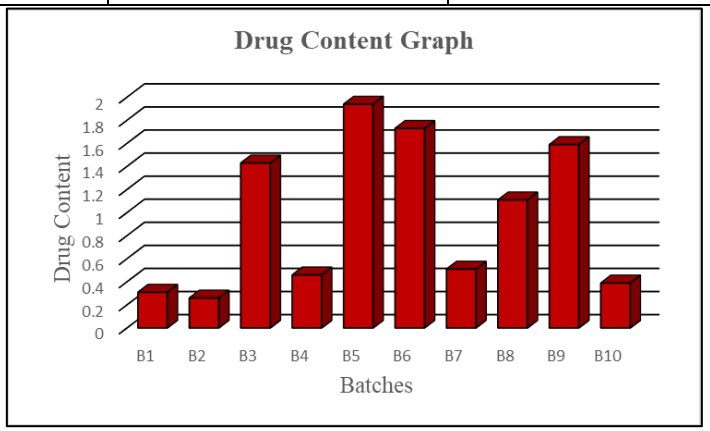

Figure 3 – Drug Content of each Batch

6. *in-vitro* drug release study

The *in-vitro* drug release study for all the batches for Rosuvastatin calcium Nanosuspension was carried out using paddle method (USP apparatus II). Data for in-vitro drug release study is presented in the following Table 5 and the graphical representation of Percentage Drug Release vs. Time graph is shown in the Figure 4.

Table 5 – Percentage Drug Release of Each Batch of Nanosuspension

Batch No	Percentage Drug Release (%)						
	0 min	**10 min**	**20 min**	**30 min**	**40 min**	**50 min**	**60 min**
B1	0	0.27	36.34	42.83	51.64	83.31	88.05
B2	0	4.91	24.43	26.69	27.34	41.16	54.06
B3	0	21.31	23.17	66.3	95.20	98.92	98.92
B4	0	11.76	29.38	41.60	71.45	82.51	84.50
B5	0	4.55	13.97	33.60	40.82	49.75	54.62
B6	0	6.89	20.13	46.86	63.69	74.19	86.26
B7	0	13.92	20.39	24.94	30.08	34.56	47.55
B8	0	5.99	24.81	36.62	50.19	69.93	79.23
B9	0	10.68	14.58	44.49	68.06	87.02	88.52
B10	0	11.94	27.40	32.21	51.85	55.85	65.29

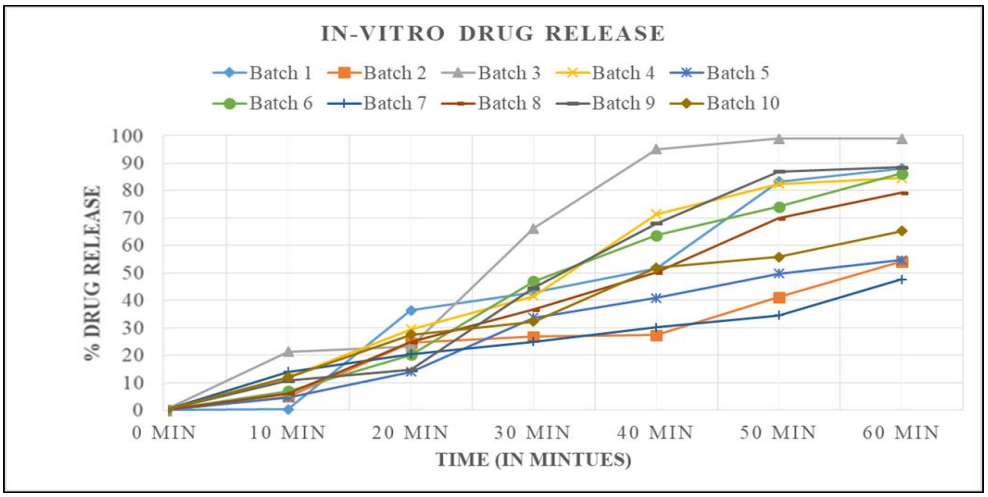

Figure 4 - Percentage Drug Release vs. Time Graph

3.3. OPTIMIZATION OF NANOSUSPENSION

Optimization of the formulations was studied by Regular Level Factorial design. The data obtained were treated using Design expert stat ease software and analysed statistically using Model Graph technique. Various graphs obtained from the Model Graph technique which indicates Interaction Between each independent Factor and dependent Factor (Response) were Studied. Graphical Optimization Technique was studied to select the Optimize Batch of Rosuvastatin Calcium Nanosuspension

Table 6 – Experimental Design and Data obtained of Particle Size, Zeta Potential and % drug Release

Batch No	Ratio of S:AS (ml)	Conc. of Stabilizer (% w/v)	Sonication Time (min)	Particle size (nm)	Zeta Potential (mV)	% Drug Release (%)
B1	1:50	6	10	327.83 nm	-7.57 mV	88.05 %
B2	1:50	10	12	568.29 nm	-20.29 mV	54.06 %
B3	1:10	2	8	263.29 nm	-4.45 mV	98.92 %
B4	1:50	10	8	381.13 nm	-10.81 mV	84.50 %
B5	1:10	10	8	427.16 nm	-22.13 mV	54.62 %
B6	1:10	2	12	343.79 nm	-25.06 mV	86.26 %
B7	1:50	2	8	658.90 nm	-11.19 mV	47.55 %
B8	1:10	10	12	384.87 nm	-14.64 mV	79.23 %
B9	1:10	6	10	351.64 nm	-20.21 mV	88.52 %
B10	1:50	2	12	397.60 nm	-10.77 mV	65.29 %

1. Analyses of Particle Size

The Graphs Obtained from Model Graph technique were studied.

From the Graphical representation in Figure 5, it was observed that as the Ratio of Solvent: Anti-solvent was kept 1:50 the Particle Size was found to be smaller in compare to the Formulation containing Ratio of Solvent: Anti-solvent as 1:10. But the Difference between Particle size of Formulation containing 1:10 and 1:50 was not so large we can say that Interaction between Ratio of S: AS (X1) and Particle Size (Y1) is minimum.

From the Graphical representation in Figure 5, it was observed that as the Concentration of Stabilizer Decreases the Particle Size also Decreases and vice versa. Three different concentration of Stabilizer were used for formulating Nanosuspension which is 2%, 6% and 10%. It was observed that when 2% of Stabilizer was used for formulating Nanosuspension the Particle Size was found to be the small. Therefore, it is concluded that Interaction between Concentration of Stabilizer (X2) and Particle Size (Y1) are directly proportional to each other.

From the Graphical representation in Figure 5, it is observed that Particle Size Decreases as Sonication Time goes on Increasing. Three different Sonication Time were used to prepare Nanosuspension and it was found that when Nanosuspension was sonicated for 12 minutes, Particle Size was found to be smaller in compared to Nanosuspension which was sonicated for 8 minutes. Therefore, it was concluded that Interaction between Sonication Time (X3) and Particle Size (Y1) is inversely proportional to each other and to Formulate Nanosuspension with smaller Particle Size, Sonication Time should be more as possible.

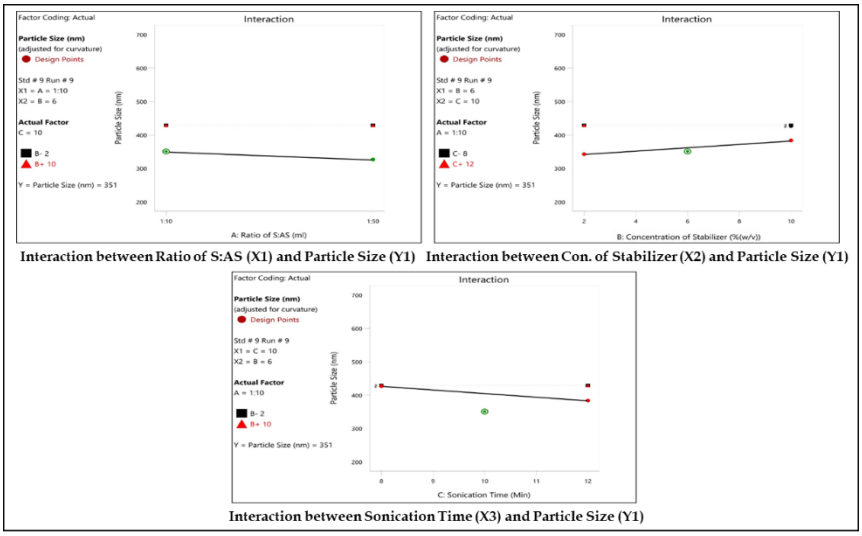

Figure 5 - Analyses of Particle Size (Y1)

2. Analyses of Zeta Potential

From the Graphical representation in Figure 6, it was observed that when the Nanosuspension was prepared using Ratio of Solvent: Anti-solvent as 1:10 then the prepared Nanosuspension is more stable in compared to Nanosuspension prepared using Ratio of Solvent: Anti-solvent as 1:50. To stabilize the Nanosuspension the Zeta Potential must be more that ±20 mV and it was observed from the Figure 5 that keeping the Ratio of Solvent: Anti-solvent 1:10, the Zeta Potential move close toward – 20 mV. Therefore, it was concluded that at low Ratio of Solvent: Anti-solvent (1:10) the prepared Nanosuspension is more stable and as the Ratio of Solvent: Anti-solvent increases the stability of Nanosuspension decreases.

From the Graphical representation in Figure 6, it was observed that when the Nanosuspension was prepared using 2% Concentration of Stabilizer than the prepared Nanosuspension is more stable than that of Nanosuspension prepared using 6% and 10% Concentration of Stabilizer. To stabilize the Nanosuspension the Zeta Potential must be more that ±20 mV. From Figure 5, we can say that at 2% Concentration of Stabilizer Zeta Potential was found to be more than -20mV. Therefore, it was concluded that to prepare stable Nanosuspension Concentration of the Stabilizer should be low (2%) in case of Rosuvastatin Calcium Nanosuspension.

From the Graphical representation in Figure 6, it was observed that as the Sonication Time is less than the Nanosuspension formed is more stable. When the Nanosuspension is sonicated for 8 minutes or for at least for 10 minutes, the Zeta Potential is found to be more than or close to -20mV which is sufficient for the Nanosuspension to be stable.

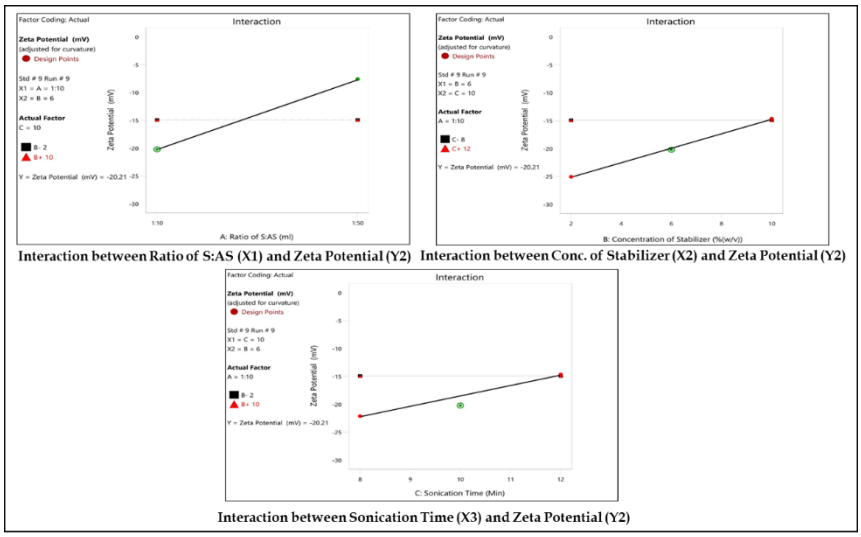

Figure 6 - Analyses of Zeta Potential (Y2)

3. Analysis of Percentage Drug Release

From the Graphical representation in Figure 7, it was observed that whether the Nanosuspension is prepared using Ratio of Solvent: Anti-Solvent as 1:10 or 1:50 the Percentage Drug Release is more than 80%. Formulation prepared using 1:10 Ratio of Solvent: Anti-Solvent, % Drug Release was found to be 88.52% and formulation prepared using 1:50 Ratio of Solvent: Anti-Solvent, % Drug Release was found to be 88.05 %. Therefore, to conclude any relation between Solvent: Anti-Solvent and Percentage Drug Release is difficult as there was no significant data observed.

From the Graphical representation in Figure 7, it was observed that as Concentration of Stabilizer Decrease the % Drug Release Increase. As Concentration of Stabilizer was 2% and 6% the formulation shows higher % Drug Release in compared to formulation containing 10% of concentration of Stabilizer. It was concluded that to achieve higher % Drug Release the Concentration of Stabilizer should be low as possible in the case of Rosuvastatin Calcium Nanosuspension.

From the Graphical representation in Figure 7, it was observed that as sonication Time increases the % Drug Release also Increases. These may due to Decrease in Particle Size with Increase i.e. at higher Sonication Time Particle Size is smaller and due to which there is Increase in % drug Release. Therefore, it was concluded that to Interaction between Sonication Time (X3) and % Drug Release (Y3) is directly proportional to each other and to achieve higher % Drug Release the sonication Time should be higher.

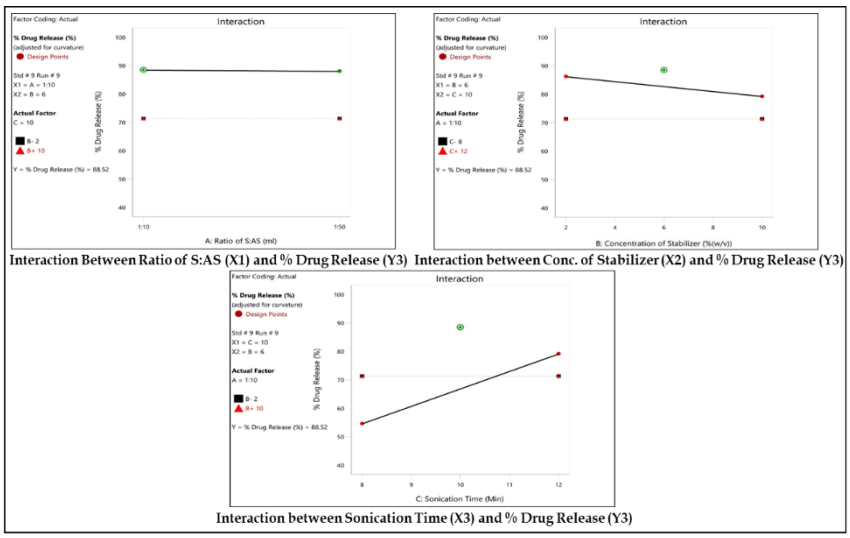

Figure 7 - Analyses of % Drug Release (Y3)

4. Optimization Analysis

The optimization module searches for a combination of factor levels that simultaneously satisfy the criteria and finds the best formulation. Graphical optimization was used for the Optimization Analysis. Graphical optimization uses the models to show the volume where acceptable response outcomes can be found. Data obtained from the Contour Plot and Overlay Plot shown in Figure 8 was studied and it showed that Formulation B9 lies in the Region were all the Criteria was satisfied and Formulation B9 was selected as the Optimized batch. Formulation B9 was prepared using 1:10 Ratio of Solvent: Anti-Solvent, 6% of Concentration of Stabilizer and it was sonicated for 10 minutes. The Results of the Formulation B9 was also satisfying and result is reported in the Table 7.

Table 7 – Results of Optimized Batch B9

Evaluations	Results of Batch B9
Particle Size (nm)	351.64 nm
Zeta Potential (mV)	- 20.21 mV
% Drug Release (%)	88.52 %

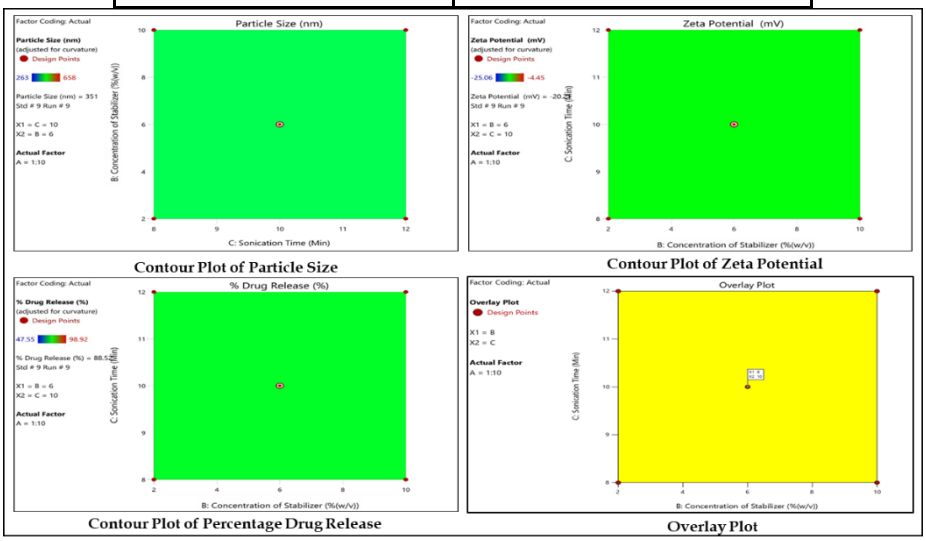

Figure 8 – Contour Plot and Overlay Plot

3.4. STABILITY STUDY

The Optimize Batch of Rosuvastatin Calcium Nanosuspension was selected for the stability Study. The Optimize Batch of Rosuvastatin Calcium Nanosuspension was kept at 2-4 ºC in Refrigerator and at Room Temperature Physical Stability of the Nanosuspension was after 5 Months. The Result of Stability Study is present in the following Table 8 and Figure 9 shows graph of Particle Size of Optimized Batch after 5 Months. It was found that Formulation kept at 4 ºC was stable in compared to Formulation kept in Room Temperature as no significant growth in Particle Size was found in the Optimized batch.

Table 8 - Stability Study of Optimized Batch

Batch No.	Initial Particle Size	Storage Condition (Temperature in ºC)	Particle Size After 5 Months
Batch 9 (Optimized Batch)	351.64 nm	4 ºC	354.12 nm
		Room Temperature	372.94 nm

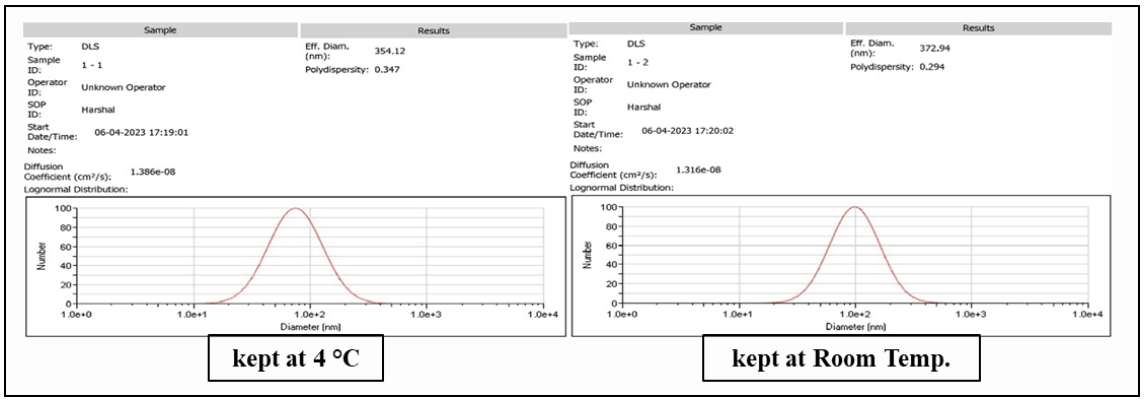

Figure 9 – Stability Study of Batch 9 (Optimized Batch) Using Particle Size Analysis

3.5. Comparison of *in-vitro* % Drug Release of Pure Drug and Optimized Batch of Rosuvastatin Calcium Nanosuspension

Comparison of *in-vitro* % Drug Release of Pure Drug and Optimized Batch of Rosuvastatin Calcium Nanosuspension was carried. Optimized Batch B9 showed 88.52% Drug Release in 60 min while Pure Drug Showed 26.29% drug Release in 60 min. Table 9 shows the Data Obtained in *in-vitro* Drug Release and Data is represented graphical in Figure 10.

Table 9 – Comparison of *in-vitro* Drug Release of Pure Drug and Optimized Batch of Rosuvastatin Calcium Nanosuspension

Time (in min)	Percentage Drug Release (%)	
	Batch B9	Pure Drug
0 min	0	0
10 min	10.68	4.37
20 min	14.58	10.79
30 min	44.49	13.49
40 min	68.09	19.84
50 min	87.02	22.57
60 min	88.52	26.29

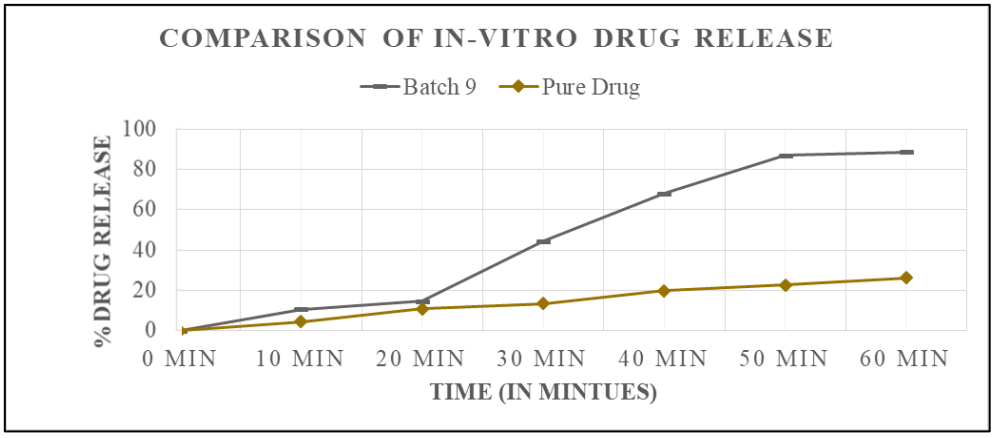

Figure 10- Comparison of *in-vitro* Drug Release of Pure Drug and Optimized Batch of Rosuvastatin Calcium Nanosuspension

CONCLUSION

In the present study, Rosuvastatin calcium, an antihyperlipidemic agent was successfully prepared in the form of Nanosuspension. Rosuvastatin Calcium Nanosuspension was prepared used Solvent-Anti-Solvent Precipitation method. Experimental Design was obtained using Design Expert Software. Ratio of Solvent: Anti-

solvent (X1), Concentration of stabilizer (X2), and Sonication Time (X3) were selected as the 3 independent factor and Particle Size (Y1), Zeta Potential (Y2), and % Drug Release (Y3) were selected as the Dependent Factors.

10 Batches of Rosuvastatin Calcium Nanosuspension was prepared. Interaction between Independent factor and dependent factor was studied. Optimization was carried out using Graphical Method and Batch B9 was selected as the Optimized Batch. Batch B9 was Prepared using 1:10 Ratio of Solvent-Anti-Solvent, 6 % (w/v) Concentration of Stabilizer was used and it was sonicated for 10 minutes. The Results of the Formulation B9 were was also satisfying. The Particle Size of the Optimized batch was found to be 351.64 nm with Zeta Potential of -20.21 mV and Percentage Drug Release was 88.52% in 60 minutes. Stability study was carried at 4 ºC and at room temperature for 5 Months. It was found that Formulation kept at 4 ºC was stable in compared to Formulation kept in Room Temperature as no significant growth in Particle Size was found in the Optimized batch. Comparison of *in-vitro* % Drug Release of Pure Drug and Optimized Batch of Rosuvastatin Calcium Nanosuspension was also carried out. Optimized Batch B9 showed 88.52% Drug Release in 60 min while Pure Drug Showed 26.29% drug Release in 60 min.

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CONFLICT OF INTEREST

All authors declared no conflicts of interest.

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