Chapter 5

Formulation Development of Saquinavir Nanosuspension

Materials and Methods

5.1 Materials:

Saquinavir was obtained as gift sample from Aurbindo Pvt Ltd, Mumbai. Poloxamer 407 (poly (oxyethylene) poly (oxypropylene) block copolymer) was obtained as gift samples from BASF, Mumbai. Tween- 80 was purchased from SD fine Chemicals. Chloroform. Methanol, Acetone AR grade were purchased from Spectrochem Labs.Ltd. Polyvinyl alcohol, Ammonium bicarbonate, Potassium dihydrogenphosphate, Disodium hydrogen phosphate, Sodium Lauryl Sulphate and Mannitol AR were purchased from S.D. fine chem. Pvt. ltd. Mumbai. Zirconium Oxide Beads were purchased from S.D. Fine chemicals, India. All other chemicals and solvents used were of AR grade. Double distilled water was used through out the study.

5.2 Methods

5.2.1 Preparation of Saquinavir Nanosuspension (SNS):

Saquinavir Nanosuspension (SNS) was prepared by Pearl milling technique (Sigfridsson K. et al 2007). Zirconium oxide beads were used as milling media while distilled water was used as an aqueous media. Nanosuspension was prepared by dissolving different types of surfactant (Poloxamer 407 or Tween 80 or Poloxamer 407: Tween 80) at varying concentration in distilled water (1% w/v to 3 % w/v). Zirconium oxide beads (40 % to 60% w/v of size – 0.4 - 0.7mm and 1.2 mm to 1.5 mm) were added. Saquinavir (SQ) was added to surfactant solution and milling was started by magnetic stirring at 500 rpm. The resulting nanosuspension was separated from the zirconium beads by decanting the suspension followed by washing of the beads with water. The process and formulation parameters were optimized to achieve minimum particle size. The obtained nanosuspension was lyophilized using suitable cryoprotectant such as sucrose and Mannitol.

5.2.2 Preparation of Saquinavir suspension (SQSV):

The aqueous suspension was prepared by mixing Saquinavir in ethanol with distilled water containing Tween 80 at the same proportion as was used for the nanosuspension formulations. The suspension was sonicated 5 min using the Probe sonicator (Vibra Cell VC 505 sonicator). Average particle size was measured using optical microscope and found to be 3.32 ± 1.09 µm.

5.2.3 Optimization of parameters:

Prior to the formulation step, the possible parameters influencing the formation of nanosuspension and size of nanosuspension were identified and optimized. The parameters studied were milling time, Ratio of beads and suitable surfactant.

5.2.3.1 Milling time:

To study the effect of milling time on nanosuspension formation, milling was continued for 12 hrs. Samples were taken at different intervals and studied for particle size and PDI. The surfactant used for the study was Tween 80 at 2 %w/v concentration.

Composition of batch:

Saquinavir	1 % w/v
Tween 80	2.0 % v/v
Vol of bead	60% w/v
Distilled water	10 ml

5.2.3.2 Ratio of beads:

Zirconium oxide beads of two different size ranges (i.e. small and large) were used for preparation of nanosuspension. Beads of small size range were in between 0.4 mm to 0.7 mm while large size ranges were between 1.2 mm to 1.5 mm. Ratio of bead was varied from 0:100 to 100:0 for small : large size range beads. Volume of beads maintained at 60 % w/v while milling time was kept at 12 hrs.

5.2.3.3 Selection of surfactant:

Batches were prepared with different surfactants (Tween 80, Poloxamer-407, polyvinyl alcohol and Poloxamer 407: Tween 80). Concentration of surfactant was kept at 1%.

5.2.4 Optimization by Factorial designs:

For the preparation of SNS process parameters were set as per preliminary optimization studies as described above. The optimization of parameters like Volume of milling media and Concentration of surfactant was carried out. Effect of these parameters on Initial Mean particle diameter and Mean particle diameter after 7 days was studied. A 3^2 randomized full factorial design was used in the study. In this design two factors were evaluated, each at 3 levels, and experimental trials were performed at all 9 possible combinations with two replicates. The replicate

experimental runs were carried out in complete randomized manner. The Volume of milling media (X_1) and concentration of surfactant (X_2) were selected as independent variables. Mean particle diameter - Day 0 (Y_1) and Mean particle diameter - Day 7 (Y_2) were chosen as dependent variable. A statistical model incorporating interactive and polynomial terms was used to evaluate the responses. The results of statistical analysis were tabulated. The response surface curves and contour plots were prepared to study the effects of independent variables. All the statistical operations were carried out using DESIGN EXPERT 7.1.4. **Table 5.1** summarizes 9 experimental runs studied, their factor combinations, and the translation of the coded levels to the experimental units employed during the study.

Table 5.1 Factor combinations as per 3^2 factorial design

Trial No.	Coded factor levels	
	Factor 1(X_1)	**Factor 2(X_2)**
1	-1	-1
2	-1	0
3	-1	1
4	0	-1
5	0	0
6	0	1
7	1	-1
8	1	0
9	1	1

Translation of coded levels in actual units			
Coded level	-1	0	+1
X_1: volume of milling media (% w/v)	40	50	60
X_2: Concentration of surfactant (% w /v)	1	2	3

5.2.5 Lyophilization of Nanosuspension

The optimized Nanosuspension formulation was lyophilized using lyophilizer (Drywinner Hetodryer). Sucrose was used as cryoprotectant. Ten milliliters of each sample was rapidly frozen to -80°C using liquid nitrogen, and lyophilized for 24hrs.

5.2.6 Characterization of Nanosuspension:

5.2.6.1 Particle Size and Zeta Potential Measurement:

Particle Size and Zeta Potential of nanosuspensions were investigated by using a Zetasizer Nano ZS 90 (Malvern Instruments ltd. UK). Samples were diluted with methanolic distilled water pre saturated with Saquinavir (in order to avoid reduction in particle size during dilution) (Lindfores et al, 2006). Each measurement was performed in triplicate and both the particle Z – average diameter and Polydispersity Index (PdI) were determined. The mean particle size and size distribution of the bulk Saquinavir powder (initial particle size before milling) was obtained by using Malvern Mastersizer 2000 (Malvern Instruments ltd. UK) .

5.2.6.2 Determination of Saturation solubility:

The saturation solubility of SQ and SNS was determined by adding excess material in distilled water and mechanical shaking for 24 hr. The dispersion was centrifuged at 15000 rpm for 15 mins in a centrifuge (Sigma, Osterode, Germany) to sediment the undissolved drug. The absorbance of the supernatant was determined at 239 nm after suitable dilution with methanol using a UV – Visible spectrophotometer (Hitachi U2000, Japan).

5.2.6.3 Assay: SNS was taken (weight equivalent to 5 mg of drug) in 10 ml of methanol: THF (1:1) mixture. The mixture was shaken for 5 mins and centrifugation was carried out at 8000 rpm for 10 min. Supernatant was taken and diluted with methanol and analyzed at 239 nm using UV – Visible spectrophotometer (Hitachi U2000, Japan). Assay was calculated using calibration curve of SQ in methanol.

5.2.6.4 Differential Scanning Calorimetry

Thermograms were taken for Saquinavir and Saquinavir nanosuspension on a Differential Scanning Calorimeter (Mettler-Toledo, Switzerland) at a heating rate of 10 °C/min in nitrogen atmosphere.

5.2.6.5 X Ray Diffractometry

The instrument was operated over the 2θ range from 10° to 40°. The XRD patterns of solid-state forms were measured with Philips PW 1729 X-ray diffractometer (Philips,

Holland) using an online recorder. XRD study of Saquinavir, Physical mixture and Saquinavir nanosuspension was carried out.

5.2.6.6 Transmission Electron Microscopy: Transmission Electron microscopy [Zeiuss TEM 109 (Germany)] study was carried out by operating at an acceleration voltage of 60 kV. Approximately 2 min after sample deposition, the grid was tapped with filter paper to remove surface water and air-dried. Negative staining was performed using a droplet of 2 %wt. aqueous uranyl acetate.

5.2.6.7 In vitro release: SNS (equivalent to 200 µg of drug in 1 ml of 0.1 N HCl) was placed in a dialysis bag (Mol. Wt. cut off 12000 Daltons, Himedia, India) and sealed at both ends. The dialysis bag was dipped into the receptor compartment containing 40 ml of diffusion medium (pH 7.2 phosphate buffer). The release of SQ from SQSV (as control) through dialysis bag was also studied in pH 7.2 phosphate buffer. The diffusion medium was continuously stirred at 100 rpm and maintained at 37 ± 2 ^{0}C. Samples were collected at regular intervals (5, 10, 20,40,60,80 and 100 mins) and equal volume of fresh diffusion media was added to receptor compartment. Collected samples were analyzed spectrophotometrically at 239 nm against diffusion medium as blank. All experiments were repeated thrice and the average values were taken.

5.2.6. 8 Optimization Data Analysis

Various RSM (Response Surface Methodology) computations for the current optimization study were performed employing Design Expert® software (version 7.1.4, Stat-Ease Inc, Minneapolis, MN). Polynomial models including interaction and quadratic terms were generated for all the response variables using multiple regression analysis (MLRA) approach (Section 2.8). The general form of MLRA model is represented as equation 4.5.

$$Y=B_0+B_1X_1+B_2X_2+B_3X_1^2+B_4X_2^2+B_5X_1X_2+B_6\,X_1^2X_2+B_7\,X_1\,X_2^2 \quad ...(4.5)$$

Where B_0 is the intercept representing the arithmetic average of all quantitative outcomes of 9 runs; B_1 to B_7 are the coefficients computed from the observed experimental values of Y; and X_1 and X_2 are the coded levels of the independent variable(s). The terms X_1X_2 and X_i^2 (i=1to2) represents the interaction and quadratic terms, respectively. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed. The

polynomial terms (X_1^2 and X_2^2) are included to investigate nonlinearity. The polynomial equation was used to draw conclusions after considering the magnitude of coefficients and the mathematical sign it carries, i.e., positive or negative. A positive sign signifies a synergistic effect, whereas a negative sign stands for an antagonistic effect.

Statistical validity of the polynomials was established on the basis of ANOVA provision in the Design Expert ®software. Level of significance was considered at $P<0.05$. The best fitting mathematical model was selected based on the comparisons of several statistical parameters including the coefficient of variation (CV), the multiple correlation coefficient (R^2), adjusted multiple correlation coefficient (adjusted R^2) , and the predicted residual sum of squares (PRESS), provided by software. Among them, PRESS indicates how well the model fits the data, and for the chosen model it should be small relative to the other models under consideration (Huang et al., 2005). Also, the 3-D response surface graphs and the 2-D contour plots were generated by the Design Expert® software.

5.3 Results and Discussion

5.3.1 Optimization of Parameters

5.3.1.1 Milling time : The Mean particle diameter of bulk Saquinavir was 117.75 µm with a broad particle size distribution (PI – 3.22) (Fig. 5.1). Pearl milling of 10h resulted in particles with mean particle diameter of 0.356 ± 0.01 µm (PI – 0.201). Further milling beyond 10 h did not result in significant reduction as mean particle diameter after 12 hrs was found to be 347 ± 0.01 µm. The results are tabulated in Table. 5.2.

Table 5.2 Effect of milling time on Mean particle diameter and PI

Sample No.*	Milling time (hours)	d (0.5) / Mean particle diameter ± S.D. (µm)	Polydispersity Index. (PI)
1	Initial	117.75 ± 16	3.22
2	0.5	51.22 ± 19	2.09
3	1	2.820 ± 0.079	1.032

4	2	1.460 ± 0.069	0.774
5	4	1.029 ± 0.048	0.639
6	8	0.555 ± 0.023	0.323
7	10	0.356 ± 0.010	0.201
8	12	0.347 ± 0.016	0.213

(* Sample No. 1 to 5 were measured by Malvern Mastersizer 2000 while sample number 6 to 8 were measured by Malvern Zeta Sizer Nano ZS 90)

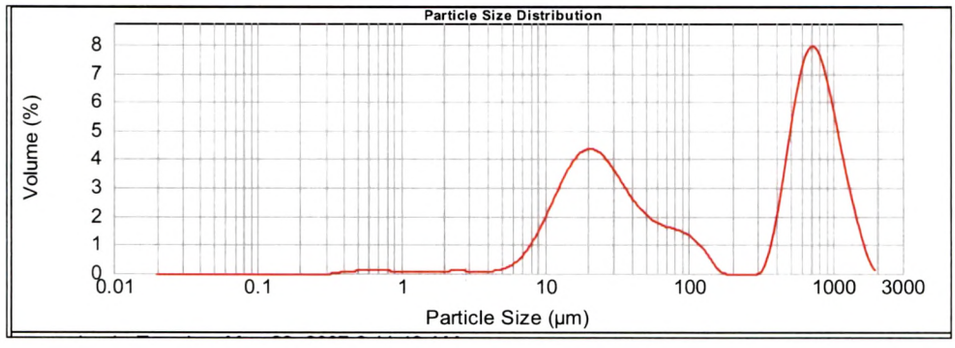

Fig 5.1 Particle size distribution of Bulk Saquinavir by Malvern Mastersizer

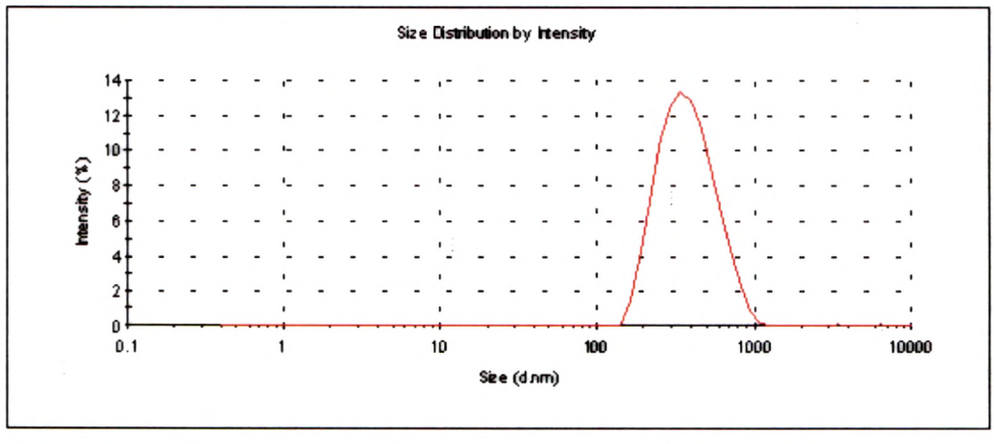

Fig.5.2 Particle size distribution of Saquinavir nanosuspension after 10 hrs

5.3.1.2 Ratio of beads

Increase in ratio of small size range beads (0.4 mm - 0.7 mm) resulted in significant decrease in mean particle diameter of nanosuspension to 367 ± 15 nm. When only large size beads were used , increase in particle size (570 ± 22 nm) was observed

while increasing ratio of small : large bead resulted in decrease in particle diameter. When only small size beads were used , particle size (432 ± 21 nm) was more than the minimun particle diameter (367 ± 15 nm). Hence, combination of small : large beads were tried at 25:75, 50:50 and 75:25 concentration.. Minimum particle size was obtained by 75: 25 concentration (367 ± 15 nm). Hence, ratio of bead was selected as 75 : 25 (small :large).The results are given in table no. 5.3

Table 5.3 Effect of ratio of beads on Particle diameter and Polydispersity Index

Ratio of beads (small : large)	**Mean Particle diameter ± S.D. (nm)**	**Polydispersity Index (PI)**
0:100	570 ± 22	0.386
25:75	496 ± 17	0.411
50:50	404 ± 26	0.309
75:25	367 ± 15	0.196
100:0	432 ± 21	0.350

5.3.1.3 Selection of surfactant:

During the course of optimization, the type of surfactant was chosen between Poloxamer 407, Tween 80, PVP K30 and Poloxamer 407: Tween 80. All other parameters were kept constant during the process of milling. Concentration of surfactant was kept at 1 %. Formulation prepared with Tween 80 showed smallest particle diameter (429 ± 14 nm) compared to other surfactants (Table 5.4). Hence, Tween 80 was used as a surfactant for further studies.

The main reason for efficient formation of droplets and stabilization of the nanosuspension appears to be the surfactant nature. The effectiveness of polymeric materials such as PVA, and Poloxamer 407 is significantly smaller than Tween 80 in terms of particle size. Nonionic nonpolymeric surfactants (e.g. Tween 80) offer an advantage over polymers in that they have a higher adsorption potential than an equal-chain-length polymer (Palla and Shah, 2002). Similar results were obtained by Kristl J. et al during preparation of Ibuprofen nanosuspension (Kristl J. et al 2006). Combination of Tween 80 and PVP K25 or combination of Tween 80 and poloxamer 188 produced Ibuprofen nanosuspensions. It was found that particle size was not significantly smaller than nanosuspension prepared with Tween 80 alone.

Table 5.4 Effect of surfactants on Particle diameter

Sr.No	Surfactant	Zavg In nm ± SD	PI
01	Tween 80	429 ± 14	0.201
02	Poloxamer-407	548 ± 27	0.241
03	Polyvinyl alcohol	523 ± 24	0.251
04	Poloxamer 407: Tween 80 (1:2)	495 ± 16	0.205
05	Poloxamer 407: Tween 80 (1:4)	479 ± 19	0.197

5.3.2 Optimization by Factorial design:

Nine formulations were prepared as per 3^2 Factorial Design. Table 5.5 enlists the response parameters of all the nine formulations.

Table 5.5 Response parameters for formulations of Saquinavir nanosuspension prepared as per 3^2 factorial design.

Formulation code	Factors		Particle Mean Diameter – Day 0 (nm) [Y_1]	Particle Mean Diameter – Day 7 (nm) [Y_2]
	Volume of milling media – X_1 (%w/v)	Concentration of surfactant - X_2 (%w/v)		
SQ1 (-1,-1)	40	1	660	850
SQ2 (0,-1)	50	1	517	835
SQ3 (1,-1)	60	1	434	682
SQ 4 (-1, 0)	40	2	598	707
SQ 5 (0, 0)	50	2	479	583
SQ 6 (1,0)	60	2	342	422
SQ7 (-1,1)	40	3	532	591
SQ 8 (0, 1)	50	3	442	495
SQ 9 (1, 1)	60	3	344	386

Table 5.6 Observed and Predicted values of response parameters

Batch	Response parameters					
	Y1			Y2		
	Observed	**Predicted**	**%RE**	**Observed**	**Predicted**	**%RE**
SQ1	660	643.61	2.483	850	872.06	3.012
SQ2	517	531.94	2.889	835	773.06	8.27
SQ3	434	420.28	3.161	682	674.06	3.771
SQ4	598	594.78	3.220	707	722.89	2.749
SQ5	479	483.11	0.858	583	623.89	5.794
SQ6	342	371.44	8.60	486	524.89	8.002
SQ7	532	545.94	2.62	591	573.72	2.92
SQ8	442	434.28	1.746	495	474.72	4.096
SQ9	344	322.61	6.218	386	375.72	2.663

% RE= % Relative Error

CALCULATED % RE = OBSERVED (ACTUAL) – PREDICTED / PREDICTED * 100

5.3.2.1 Effect of formulation variables on the response parameters:

On analyzing the data of all the 9 formulations prepared as per 3^2 Factorial design using Design Expert® software, various polynomial equations, response surface and contour plots were generated. The information obtained from the software is discussed in the following sections, depicting the effects of variables on the respective response parameters (Y_1 and Y_2).

Mean particle diameter - Day 0:

The polynomial equation and regression coefficient for Y_1 (**Mean particle diameter - Day 0**) are as follows:

$$Y1 = 1139.11 - 11.16 X_1 - 48.83 X_2 \quad \text{..... 5.1}$$

R-Squared = 0.9750

The linear model (Eq 5.1) was found to be significant with an F value of 116.96 ($p< 0.0001$). The value of correlation coefficient (R^2) was found to be 0.9750. The R^2 value is a measure of total variability explained by the model. The R^2 value of 0.9750 for model indicates that the model was significant. Value of probability was less than 0.0001 which indicated that model terms $X_1$and X_2 are significant.Value of probability less than 0.05 indicated model terms were significant. Negative values of X_1 and X_2 in Eq.5.1 indicate antagonistic effect on Y_1 of Saquinavir Nanosuspension i.e. any increase in X_1 and X_2 reduces value of Y_1. Effect of X_2 is found to be 4 fold higher than the effect of X_1 on Y_1.

The combined effect of factors X_1 and X_2 can further be elucidated with the help of response surface and contour plots (Fig. 5.2a and 5.2b respectively) which demonstrated that Y_1 varies in a reverse fashion with both the factors. Increase in X_1 and X_2 resulted in corresponding decrease in Mean particle diameter of Nanosuspension. High level of X_1 gave lower particle diameter at all the 3 levels of X_2 which indicates that X_1 has significantly positive effect on Y_1. Contour plot (Fig 5.2b) reveals that Y_1 varies in somewhat linear fashion with increase in X_1 and X_2. However, the effect of X_1 seems to be more pronounced as compared with that of X_2. Effect of medium (0) to high (+1) level of X_1 and X_2 is less significant than effect of low (-1) to medium (0) level on mean particle diameter. The predicted and observed values of response parameters are shown in table 5.6. Low values of the relative error showed that there was a reasonable agreement of predicted values and experimental values.

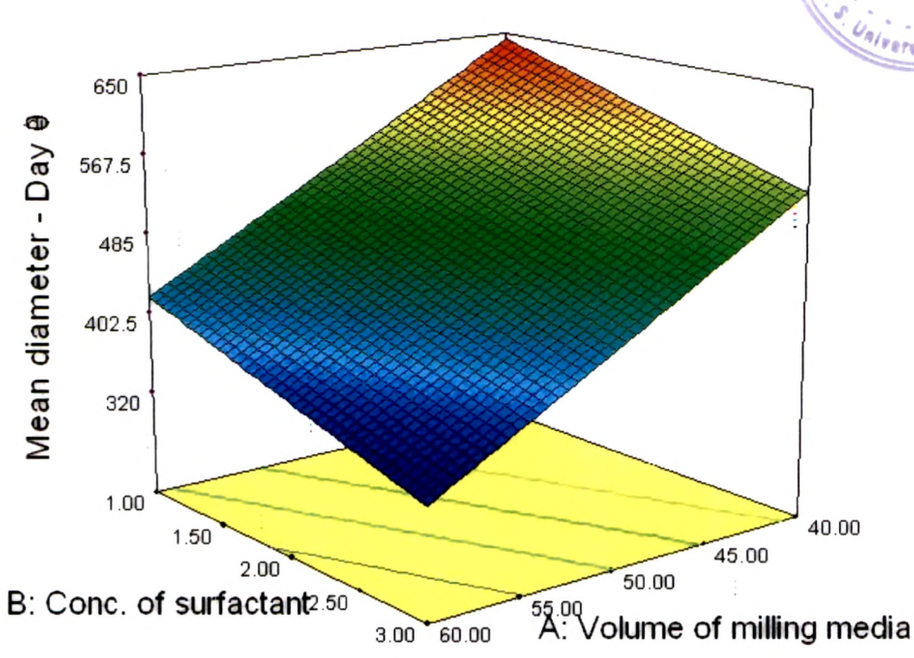

Fig. 5.2a: Response surface plot showing the influence of Volume of milling media and concentration of surfactant on Mean particle diameter on Day 0.

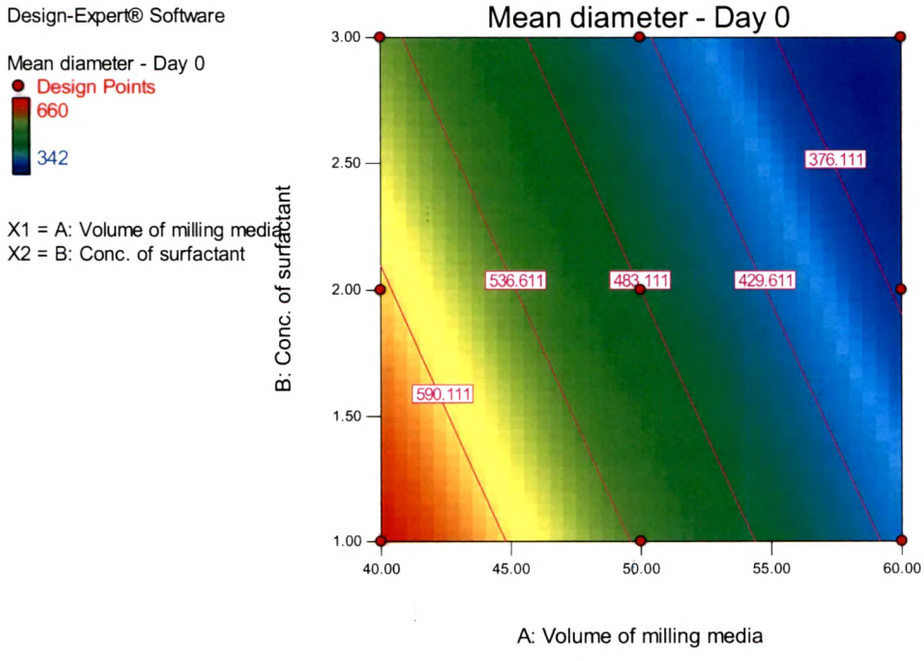

Fig 5.2b: Corresponding contour plot showing the effect of factors on Mean particle diameter - Day 0.

Mean particle diameter - Day 7 (Y_2):

The linear model for Y_2 was found to be significant (*p=0.0004*) with an F value of 37.23. Thus, model becomes:

$$Y_2 = 1463.44 - 10.96 X_1 - 149.16 X_2 \quad \ldots\ldots\ldots\ldots\ldots (5.2)$$

$$R^2 = 0.9254$$

The value of correlation coefficient (R^2) was found to be 0.9254. The R^2 value is a measure of total variability explained by the model. The R^2 value of 0.9254 indicates that the model was significant. That means the model can explain 92.54 % of varibility around the mean. Value of probability was found to be 0.0004 which indicates that model terms X_1 and X_2 are significant.Value of probability less than 0.05 indicate model terms are significant .

The value of Predicted Residual Sum of Squares (PRESS) for the linear model was 34066.27 whereas, for quadratic model it was found to be 56527.01. The PRESS value indicates how well the model fits the data, and for the chosen model it should be small relative to the other models under consideration (Huang et al., 2005). The linear model with the lower PRESS value was selected. Negative values of X_1 and X_2 in Eq.5.2 indicate antagonistic effect on Mean particle diameter – Day 7 (Y_2). According to Eq. 5.2, there is significant difference in value of X_1 (-10.96) and X_2 (-149.16) which indicates that effect of concentration of surfactant (X_2) on Mean Particle diameter - Day 7 is more pronounced than effect of volume of milling media (X_1).

Response surface and contour plots for effect of X_1 and X_2 on Y_2 are shown in Fig. 5.3a and Fig. 5.4b. Reduction in value of Y_2 was observed with consequent increase in Volume of milling media (X_1) and Concentration of surfactant (X_2). Increase in value of X_1 from low (-1) to high (+1) level while keeping value of X_2 constant at low level (-1) did not result in significant decrease in mean particle diameter whereas increase in value of X_2 from low (-1) to high (+1) level while keeping value of X_1 constant at low level (-1) resulted in significant decrease in value of mean particle diameter. High level of X_2 gave minimum value of Mean particle diameter – Day 7 at all the 3 levels of X_1 which indicates that X_2 has significantly positive effect on Y_1. Contour plot (Fig 5.3b) reveals that Y_1 varies in somewhat linear fashion with X_1 and X_2. However, the effect of X_2 seems to be more pronounced as compared with that of X_1. The predicted and observed values of response parameters are shown in Table 5.6.

Low values of the relative error showed that there was a reasonable agreement of predicted values and experimental values.

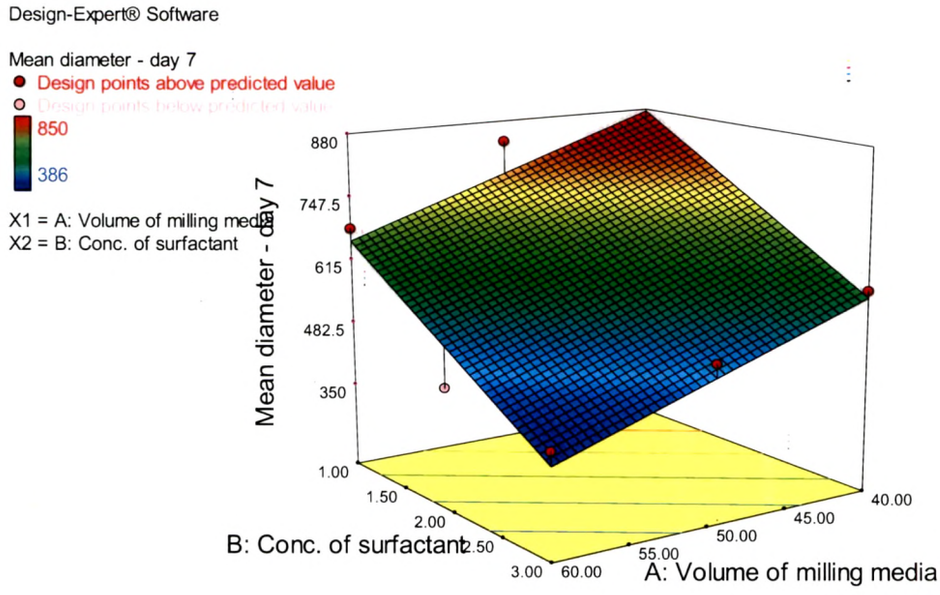

Fig. 5.3a: Response surface plot showing influence of Volume of milling media & concentration of surfactant on Mean particle diameter on day 7.

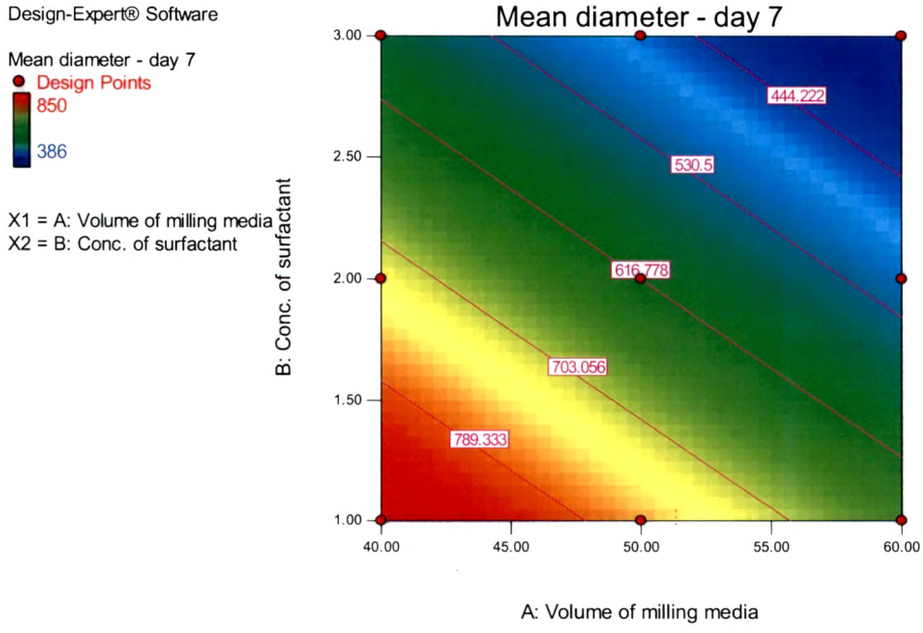

Fig 5.3b: Corresponding contour plot showing the effect of factors on Mean particle diameter on Day 7.

Table 5.7. Multiple Regression Output for Dependent Variables*

Coefficient of regression parameters					
Parameters	b_0	b_1	b_2	r^2	*P*
Mean particle Diameter - Day 0	1139.11	11.16	48.83	0.975	< 0.0001
Mean particle Diameter - Day 7	1463.44	10.96	149.16	0.9254	0.0004

Table 5.8 Results of Analysis of Variance (ANOVA) for measured responses

Parameters	df	SS	MS	F	Significance F
		Mean Particle Diameter – Day 0 (Y_1)			
Model	2	89124.83	44562.42	116.96	< 0.0001
Residual	6	2286.06	381.01	--	--
Total	8	91410.89	--	--	--
		Mean Particle Diameter – Day 7 (Y_2)			
Model	2	2.057E +0.05	1.028E +005	37.23	0.0004
Residual	6	16574.72	2762.45	--	--
Total	8	2.222E+005	--	--	--

5.3.2.2 Optimum Formulation:

A numerical optimization technique by the desirability approach was used to generate the optimum settings for the formulation. The process was optimized for the dependent (response) variables Y1–Y2 and the optimized formula was arrived by keeping the Mean particle diameter – Day 0 in range of 300 to 400 nm. Another dependant variable Mean particle diameter – Day 7 was kept at minimum level. Formulation SQ9 (containing high (+1) levels of variables, X_1 and X_2) fulfilled all the criteria set from desirability search (Narendra et al, 2005). To gainsay the reliability of the response surface model, new optimized formulation (as per formula SQ9) was

prepared according to the predicted model and evaluated for the responses (Y_1, and Y_2). The result in Table 5.10 illustrates a good relationship between the experimental and predicted values, which confirms the practicability and validity of the model. The predicted error of all the response variables was below 8 % indicating that the Response Surface Methodology (RSM) optimization technique was appropriate for optimizing Saquinavir Nanosuspension. The optimized formulation of SNS is shown in Table 5.9

Table No. 5.9 Optimized Saquinavir Nanosuspension

Parameters	Value
Milling time	10 hours
Ratio of Beads (Small : Large)	75:25
Selection of surfactant	Tween 80
Volume of milling media	60 % w/v
Concentration of surfactant	3 % w/w

Table 5.10. The predicted and observed response variables of the optimal Saquinavir Nanosuspension

	Y_1 (nm)	Y_2 (nm)
Predicted	322.61	357.94
Observed	344 ± 16	386 ±11
Predicted Error (%)	6.63	7.83

Predicted Error (%) = (Observed value – Predicted value)/ Predicted value x 100%

5.3.3 Saturation solubility:

The saturation solubility of bulk SQ in distilled water was found to be 29 ± 1.30 µg/ml at room temperature. The saturation solubility of SQ increased significantly after formulating as nanosuspension. The saturation solubility of SNS was 111 ±

2.27µg/ml. The increase in solubility in case of SNS was almost 4 folds higher than the bulk SQ.

5.3.4 Assay: Assay of SNS was found to be 97.23 ± 1.65 % w/w.

5.3.5 Differential Scanning Calorimetry

The crystalline structure of nanosuspension can be assessed by differential scanning Calorimetry (DSC). Because of its liquid existence at ambient conditions, DSC studies were not performed with Tween 80 (Chen Yajun et al, 2005). The DSC curves for bulk SQ and SQ lyophilized nanosuspension (SNS) are shown in fig 5.4.

The DSC curve of SQ (Fig 5.4 A) exhibited melting endotherm of the drug at 250.02^0 C. In case of SNS, a melting endotherm was observed at lower temperature i.e. 231.23^0 C (Fig 5.4 B). Furthermore, the peak was much broader compared to SQ peak (Fig 5.4 A). This indicated that SQ might be converted to an amorphous state. This may be attributed to increased lattice defects in the drug crystal, which in turn reflects reduced degree of crystallinity as a result of pearl milling (Otsuka M and Kaneniwa N., 1986). Another peak was observed in the thermogram of SNS at 191.09^0 C and it can be attributed to sucrose which is used as cryoprotectant during lyophilization.

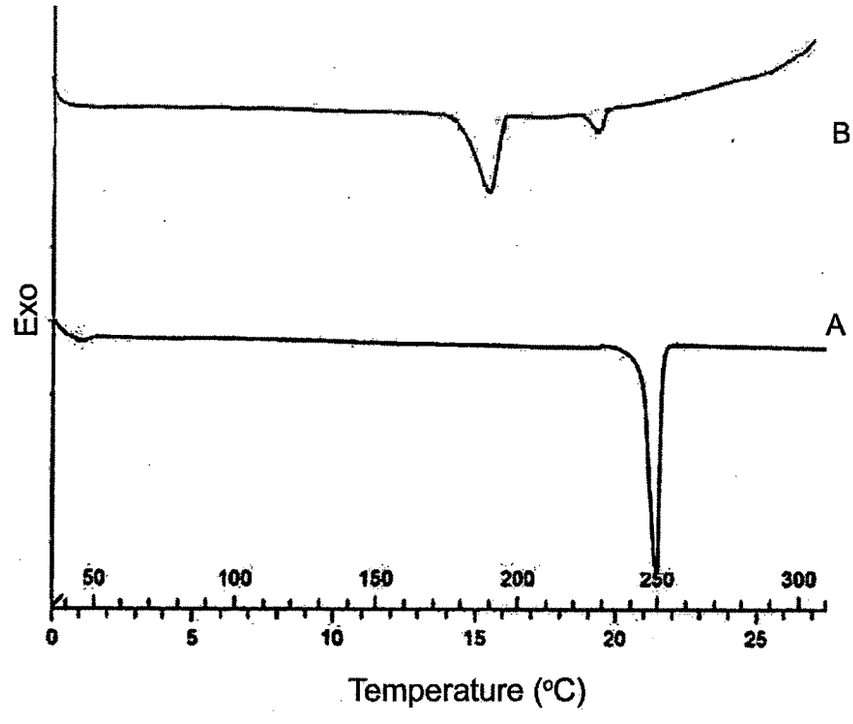

Fig. 5.4 DSC thermograms of Saquinavir (A) and Saquinavir lyophilized Nanosuspension (B).

5.3.6 X Ray Diffractometry:

Principal peaks of saquinavir (fig 5.5. A) were observed at 2θ angle 16.179, 18.716 and 19.881. In case of SNS, reduction in intensity of peak was observed at these 2θ angle values which may be due to small particle size (nanometer range), high specific surface area and presence of surfactant in nanosuspension. Because of its liquid existence at ambient conditions, XRD studies were not performed with Tween 80 (Chen Yajun et al, 2005).

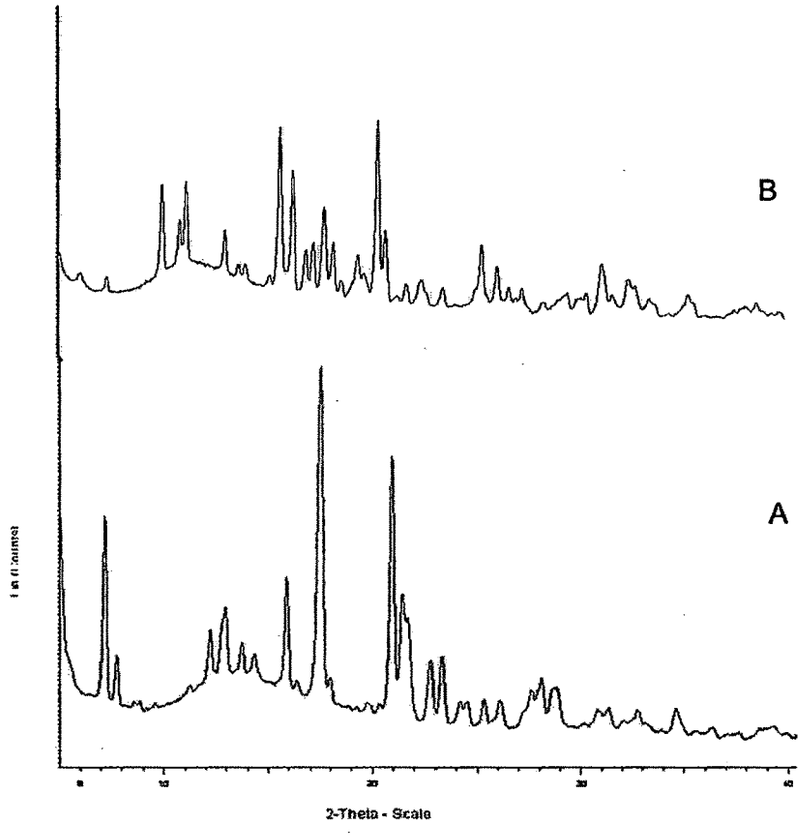

Fig. 5.5. XRD of Bulk Saquinavir (A) and SNS (B)

5.3.7 Transmission Electron Microscopy (TEM):

Optical microscope images of Saquinavir bulk (Fig 5.6) revealed that particles are aggregated and are not homogenous in the sample. TEM image reveals that particles are homogenously dispersed and are almost speherical in shape. Also, particles were discrete and non aggregated.

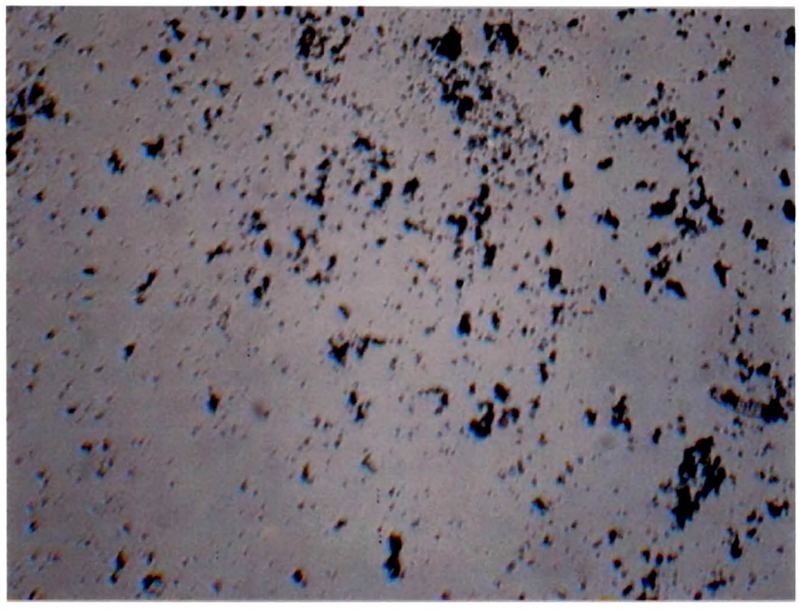

Fig. 5.6 Optical microscope image of Saquinavir Bulk

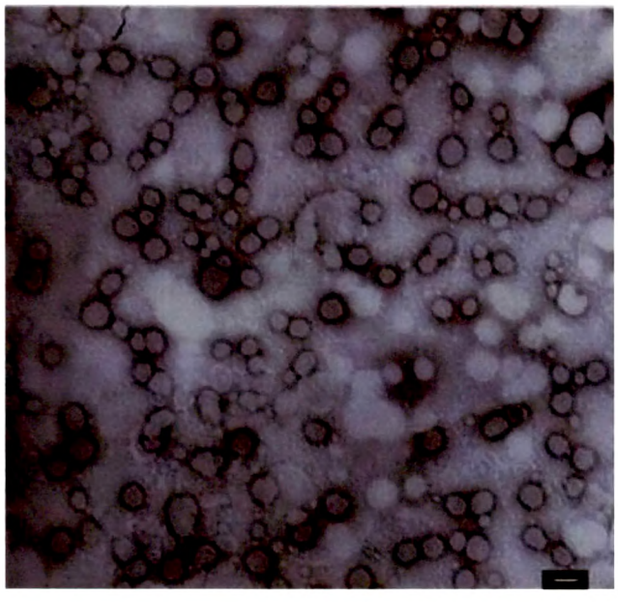

Fig. 5.7 TEM image of Saquinavir Nanosuspension (Bar indicates 0.4 µm)

5.3.8 In vitro release:

SQ from SNS showed 96.66 ± 1.98 % release in 20 minutes in pH 7.2 phosphate buffer while SQ from Saquinavir suspension (SMS) showed 97.21 ± 1.12 % in about 90 minutes. The release profiles are given in fig 5.8.The release profiles clearly indicated the faster release rate of SQ in nanosuspension form. Increase in release rate can be attributed to increase in the surface area after nanosizing the crystals.

According to Ostwald – Freundlich and the Kelvin equations (Grant DJW et al., 1995), the dissolution pressure increases due to the strong curvature of the particles leading to increase in saturation solubility and as per Noyes-Whitney, increase in surface area in turn increases dissolution rate.

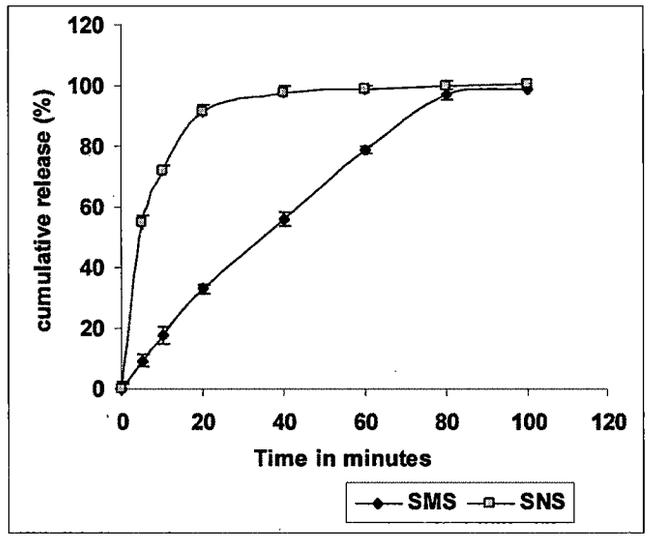

Fig 5.8 In vitro release of Saquinavir suspension and Saquinavir Nanosuspension in 0.1 N HCl

The release profiles were then fitted into different exponential equations such as zero order, first order, higuchi, and Peppas- Korsmeyer to characterize the release. It was found that drug release from SNS followed Peppas – Korsmeyer (r^2=0.940) more than Higuchi (r^2=0.749), Zero order (r^2=0.604) and First order (r^2=0.563). Release from SMS was found to follow Peppas Korsmeyer (r^2=0.993) better than Higuchi (r^2=0.989) model. Value of *'n'* indicates that SMS followed Super case II transport while SNS followed Case II transport (Costa P et al .2001, Venkatraju M.P. et al 2008).

Table No. 5.10 In vitro release kinetics of SMS and SNS.

Kinetic models	Zero order	First order	Higuchi		Peppas	
	r^2	r^2	r^2	h^{-1}	r^2	n
SMS	0.9598	0.8247	0.989	12.50	0.993	3.1803
SNS	0.604	0.563	0.749	5.17	0.940	0.920

Reference:

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