Chapter 8

Formulation Development of Adefovir Dipivoxil Nanosuspension

Materials and Methods

8.1 Materials:

Adefovir Dipivoxil was obtained as gift sample from Cipla Ltd, Vadodara. Poloxamer 407 [(poly (oxyethylene) poly (oxypropylene) block copolymer)] was obtained as gift sample from BASF, Mumbai. Sodium cholate was purchased from SD fine Chemicals. Chloroform. Methanol, Acetone AR grade were purchased from Spectrochem Labs.Ltd. Ammonium bicarbonate, Potassium dihydrogenphospate, 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.

8.2 Methods

8.2.1 Preparation of Nanosuspension:

Adefovir Dipivoxil Nanosuspension (ANS) was prepared by Pearl milling technique (Liversidge et. al. 1992). 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 sodium cholate or Poloxamer 407: sodium cholate) 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. Adefovir Dipivoxil (ADF) was added to milling chamber (containing surfactant solution & Zirconium oxide beads) and milling was started by magnetic stirring at 500 rpm for 20 hrs. 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 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, lyophilized for 24hrs. Lyophilized particles were reconstituted with distilled water prior to use by manual shaking with distilled water for 5 mins.

8.2.2 Preparation of Adefovir Dipivoxil Suspension (AMS):

The aqueous suspension was prepared by mixing Adefovir dipivoxil in distilled water containing Poloxamer 407: Sodium Cholate at the same proportion as was used for the nanosuspension formulation. The suspension was sonicated for 5 min using Probe sonicator (Vibra Cell VC 505 sonicator). Particle size was measured using Malvern Mastersizer and d (0.5) value was found to be 3.71 ± 0.71 µm.

8.2.3 Optimization of process 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 volume of milling media.

8.2.3.1 Milling time:

To study the effect of milling time on nanosuspension formation, milling was continued for 20 hrs. Samples were taken at different intervals and studied for particle size and PDI. The surfactant used for the study was Poloxamer 407: Sodium Cholate (1:3) at 3 %w/v concentration.

Composition of batch:

ADF ..... 1 % w/v

Poloxamer 407: Sod. Cholate (1:3)... 2.5 % v/v

Vol of bead..... 60% w/v

Distilled water..... 10 ml

8.2.3.2 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 20 hrs. Composition of batch was same as mentioned above.

8.2.3.3 Selection of surfactant:

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

8.2.4 Optimization of formulation parameters:

For the preparation of ANS, process parameters were set as per preliminary optimization studies as described above. The optimization of formulation parameters like Type of surfactant 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 type of surfactant *(X_1)* and concentration of surfactant *(X_2)* were selected as independent variables. Initial Mean particle diameter (Y_1) and Mean particle diameter after 7 days (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 8.1** summarizes an account of the 9 experimental runs studied, their factor combinations, and the translation of the coded levels to the experimental units employed during the study.

Table 8.1 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: Type of surfactant	Poloxamer 407	Sodium cholate	Pol 407 : Sodium cholate
X_2: Concentration of surfactant (% v/v)	2	3	4

8.2.5 Characterization of Formulation :

8.2.5.1 Particle Size and Zeta Potential Measurement: Procedure same as 5.2.6.1

8.2.5.2 Determination of Saturation solubility:

The Saturation solubility of ADF and ANS was determined by adding excess ADF in distilled water and mechanical shaking for 24 hr. The dispersion was centrifuged at 15000 rpm for 15 mins in a cooling centrifuge (Sigma, Osterode, Germany) to sediment the undissolved drug. The absorbance of the supernatant was determined at 261 nm using a UV – Visible spectrophotometer (Hitachi U2000, Japan).

8.2.5.3 Assay: ANS equivalent to 5 mg of drug was added in methanol. It was shaken for 5 mins and analyzed using UV – Visible spectrophotometer (Hitachi U2000, Japan) at 261nm. Assay was calculated using calibration curve of ADF in methanol.

8.2.5.4 Differential Scanning Calorimetry Study: Thermograms were taken for Adefovir, Physical mixture and Adefovir Nanosuspension (ANS) on a Differential Scanning Calorimeter (Mettler-Toledo, Switzerland) at a heating rate of 10°C/min in nitrogen atmosphere.

8.2.5.5 XRD Studies: 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 Adefovir, Physical mixture and Adefovir nanosuspension was carried out.

8.2.5.6 Transmission Electron Microscopy

Size and shape of the particles in formulation were investigated using Transmission Electron microscopy (TEM) [Zeiuss TEM 109 (Germany)]. It was carried out by operating at an acceleration voltage of 60 kV. After 2 min of sample deposition, grid was tapped with filter paper to remove surface water and air-dried. If necessary, negative staining would be performed using a droplet of 2 wt. % aqueous uranyl acetate.

8.2.5.7 In vitro release:

ANS in 0.5 ml of o.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 receptor compartment containing diffusion medium (pH 7.2 buffer). The diffusion media was continuously stirred at 100 rpm and maintained at 37 ± 2 ^{0}C. Samples were withdrawn at regular intervals and equal volume of fresh diffusion media was added to receptor compartment. Samples were analyzed at 261 nm against diffusion media as blank using UV spectrophotometer. The release of ADF from ADF suspension (as control) through dialysis bag was studied in pH 7.2 buffer. All the experiments were repeated thrice and average values were taken.

8.2.5.8 Optimization Data Analysis

Various RSM (Response Surface Methodology) computations for the current optimization study were performed employing Design Expert® software (version7.1.2, 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.

8.3 Results and Discussion :

8.3.1 Optimization of Process parameters :

8.3.1.1 Milling time : The Mean particle diameter [d (0.5)] of bulk ADF was 710.05 ± 70 µm with Polydispersity Index of 0.451 (Figure 8.1). Pearl milling of 18 h resulted in particles with mean particle diameter of 0.422 ± 0.026µm (Fig.8.2). Further milling beyond 18 hours did not result in significant reduction as mean particle diameter was found to be 0.410 ± 0.019 µm after 20 h. Hence, milling time was fixed as 18 hrs. The results are tabulated in table 8.2.

Table 8.2 Effect of milling time on Mean particle diameter and PI

Milling time (hours)	Mean particle diameter ± S.D. (µm)	Polydispersity Index (PI)
Initial	710.05 ± 70	0.451
0.5	441.22 ± 23	0.912
1	182 ± 31	0.732
2	56 ± 7	0.686
4	17 ± 0.99	0.514
8	2.09 ± 0.28	0.419
12	1.155 ± 0.43	0.512
16	0.756 ± 0.020	0.396
18	0.422 ± 0.026	0.343
20	0.410 ± 0.019	0.312

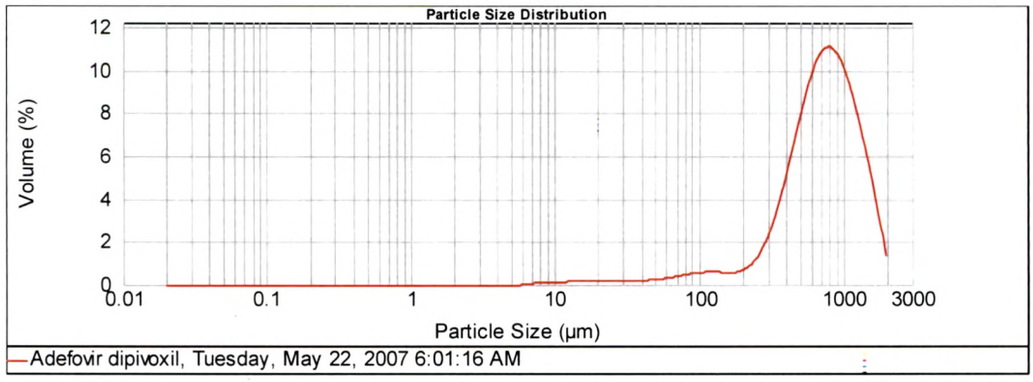

Fig 8.1 Particle size distribution of Bulk Adefovir by Malvern Mastersizer

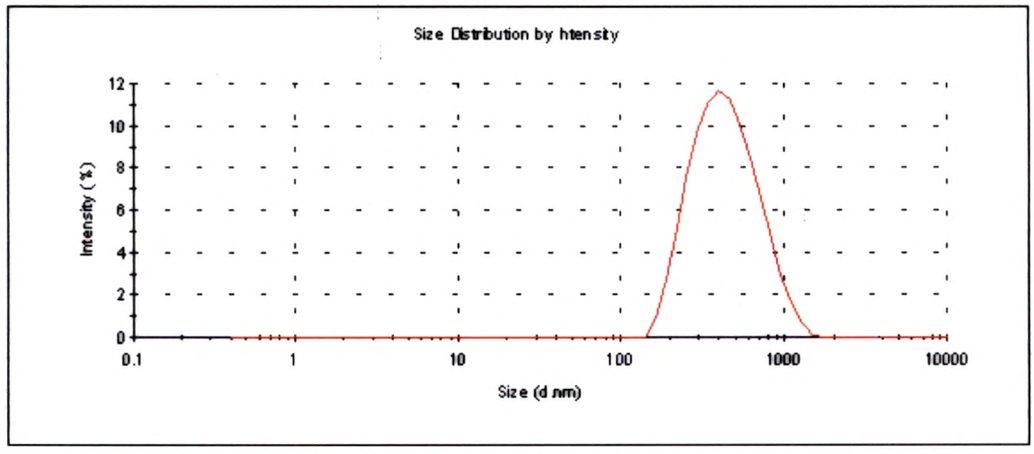

Fig. 8.2 Particle size distribution of Adefovir nanosuspension after 18 hrs of milling

8.3.1.2 Selection of surfactant:

During the course of optimization, the type of surfactant was chosen between Poloxamer 407, Sodium cholate, Tween 80 and Poloxamer 407: Sodium cholate. All other parameters were kept constant during the process of milling. Concentration of surfactant was kept at 3 %. Formulation prepared with Combination of Poloxamer 407: Sodium cholate has exhibited smallest particle diameter (395 ± 15 nm) compared to other surfactants. Hence, this combination was used as surfactant for further studies.

Table 8.3 Effect of surfactants on Particle diameter and polydispersity index

Sr.No	Surfactant	Zavg In nm ± SD	PI
01	Tween 80	511 ± 19	0.214
02	Poloxamer- 407	449 ± 21	0.229
03	Sodium cholate	472 ± 29	0.251
04	Poloxamer 407: Sodium cholate (1:2)	425 ± 16	0.204
05	Poloxamer 407: Sodium cholate (1:4)	395 ± 15	0.205

8.3.1.3 Ratio of beads

Minimum particle size i.e. 434 ± 17 nm was obtained when 50:50 ratio of small (0.4 mm - 0.7 mm) : large (1.2 mm -1.5 mm) size range beads were used (Table 8.4). Maximum particle size was observed when large size beads were used while increasing ratio of small : large bead resulted in decrease in particle diameter till 50:50 ratio was used. When small size range beads were used ,mean particle diameter was found to be 473 ± 11 nm with PI 0.411 which was more than the minimun particle diameter 434 ± 17 nm. Concentration of surfactant was kept at 3 % w/v and volume of media was 50 5 w/v.The results shows that equal proportion of small and large beads was suitable for obtaining minimum particle diameter.

Table 8. 4 Effect of ratio of beads on Particle diameter and Polydispersity Index

Ratio of beads (0.4 mm - 0.7 mm: 1.2 mm - 1.5 mm)	**Mean Particle diameter ± S.D. (nm)**	**Polydispersity Index (PI)**
0:100	612 ± 17	0.488
25:75	553 ± 22	0.402
50:50	434 ± 17	0.383
75:25	497 ± 20	0.489
100:0	473 ± 11	0.411

8.3.2 Optimization of formulation parameters :

Nine formulations were prepared as per 3^2 Factorial Design. Table 8.5 enlists the response parameters of all the nine formulations.

Table 8.5 Response parameters for formulations of Adefovir Nanosuspension prepared as per 3^2 factorial designs.

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)		
ADF1 (-1,-1)	40	2	834	1164
ADF2 (0,-1)	50	2	797	1109
ADF3 (1,-1)	60	2	696	1061
ADF4 (-1, 0)	40	3	689	803
ADF 5 (0, 0)	50	3	546	701
ADF 6 (1,0)	60	3	457	590
ADF7 (-1,1)	40	4	479	552
ADF 8 (0, 1)	50	4	393	413
ADF 9 (1, 1)	60	4	356	401

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

Table 8.6 Observed and Predicted values of response parameters

Batch	Response parameters					
	Y1			Y2		
	Observed	**Predicted**	**%RE**	**Observed**	**Predicted**	**%RE**
ADF1	834	841.94	0.943	1164	1144.61	1.69
ADF2	797	762.28	4.55	1109	1072.11	3.44
ADF3	696	682.61	1.96	1061	999.61	6.14
ADF4	689	658.78	4.58	803	821.94	2.30
ADF5	546	583	9.17	701	758.44	7.57
ADF6	457	503.61	9.22	590	685.94	13.98
ADF7	479	475.61	0.712	552	499.28	10.55
ADF8	393	395.94	0.74	413	426.78	3.22
ADF9	356	316.28	12.55	401	363.28	10.38

% RE= % Relative Error

8.3.2.1 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:

$Y1 = 1543.33 - 8.21 X_1 - 183.16 X_2$ 8.1

R-Squared = 0.973

The linear model (Eq 8.1) was found to be significant with an F value of 108.54 ($p< 0.0001$). The value of correlation coefficient (R^2) was found to be 0.973. The R^2 value is a measure of total variability explained by the model. The R^2 value of 0.973 for model indicates that the model is significant. Value of probability was less than 0.0001 which indicates that model terms X_1 and X_2 are significant. Negative values of X_1 and X_2 in Eq.8.1 indicate antagonistic effect on Y_1 of Adefovir Nanosuspension i.e. any increase in volume of milling media (X_1) and concentration of surfactant (X_2) reduces particle diameter. Effect of concentration of surfactant (X_2) was found to be higher than the effect of volume of media (X_1) on Mean particle diameter Day 0.

The combined effect of factors X_1 and X_2 can further be elucidated with the help of response surface and contour plots (Fig. 8.3a and 8.3b respectively) which demonstrates that Y_1 varies in a reverse fashion with both the factors. Increase in volume of milling media (X_1) and Concentration of surfactant (X_2) resulted in consequent 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 8.2b) reveals that Y_1 varies in somewhat linear fashion with increase in X_1 and X_2. However, the effect of X_2 seems to be more pronounced as compared with that of X_1. Effect of low (-1) to medium (0) level of X_1 and X_2 is more significant than effect of medium (0) to high (+1) level on mean particle diameter. The predicted and observed values of response parameters are shown in Table 8.6. Low values of the relative error showed that there was a reasonable agreement of predicted values and experimental values.

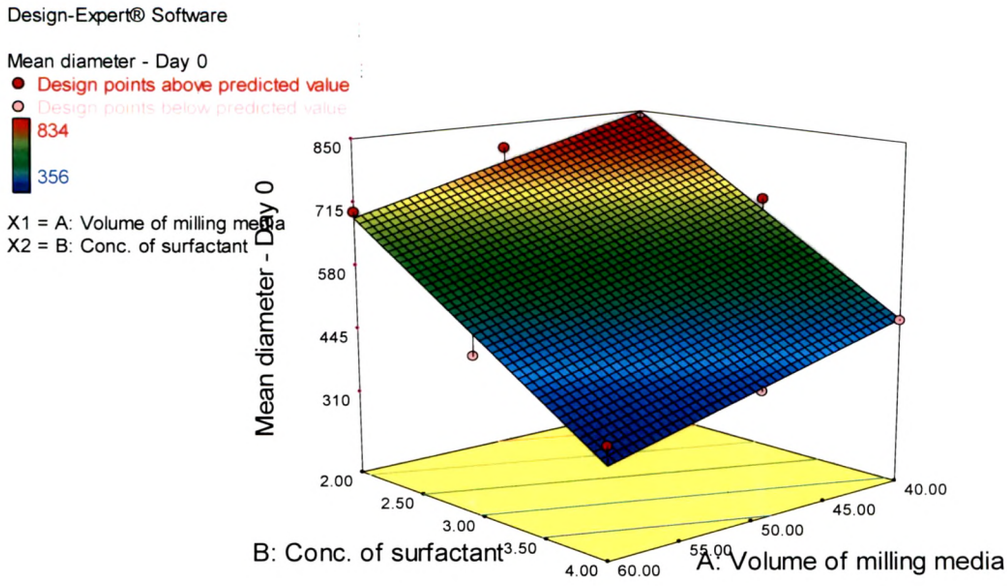

Fig. 8.3a: Response surface plot showing the influence of Volume of milling media and concentration of surfactant on Mean particle diameter on Day 0.

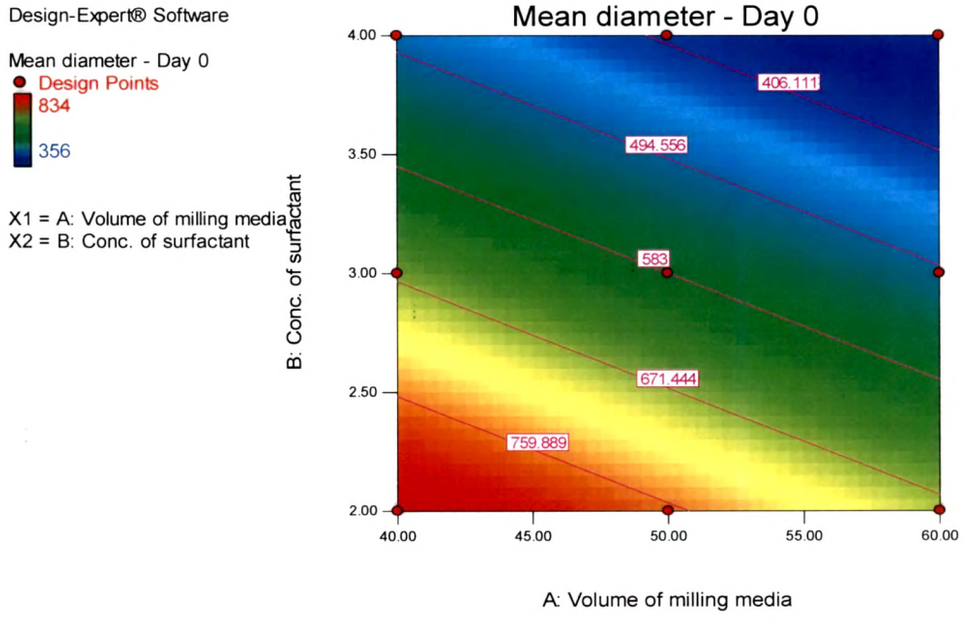

Fig 8.3b: Corresponding contour plot showing the effect of factors on Mean particle diameter - Day 0.

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:

$$Y_2 = 2128.05 - 7.78 X_1 - 328.00 X_2 \quad \ldots\ldots\ldots\ldots (8.2)$$

$$R^2 = 0.971$$

The value of correlation coefficient (R^2) was found to be 0.971. The R^2 value of 0.971 indicates that the model was significant. Negative values of X_1 and X_2 in Eq.8.2 indicate antagonistic effect on Mean particle diameter – Day 7 (Y_2). According to Eq. 8.2, there is significant difference in value of X_1 (-7.78) and X_2 (-328.00) 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. 8.3a and Fig. 8.4b. 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_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 compared to increase in value of X_1 from low (-1) to high (+1) level while keeping value of X_2 constant at low level (-1) . High level of X_2 gave minimum value of Mean particle diameter – Day 7 at all the 3 levels of X_1 although there was no significant difference in mean particle diameter day 7 at level 0 (413 nm) and level 1 (461 nm).It indicates that X_2 has significantly positive effect on Y_1. Contour plot (Fig 8.3b) 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 8.6. Low values of the relative error showed that there was a reasonable agreement of predicted values and experimental values

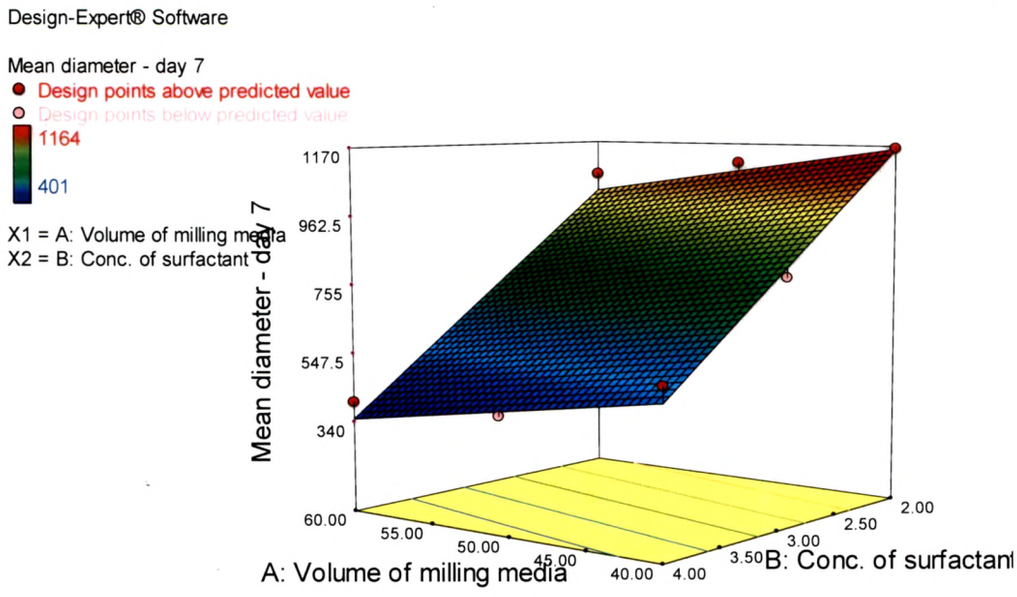

Fig. 8.4a: Response surface plot showing the influence of Volume of milling media and concentration of surfactant on Mean particle diameter on Day 7.

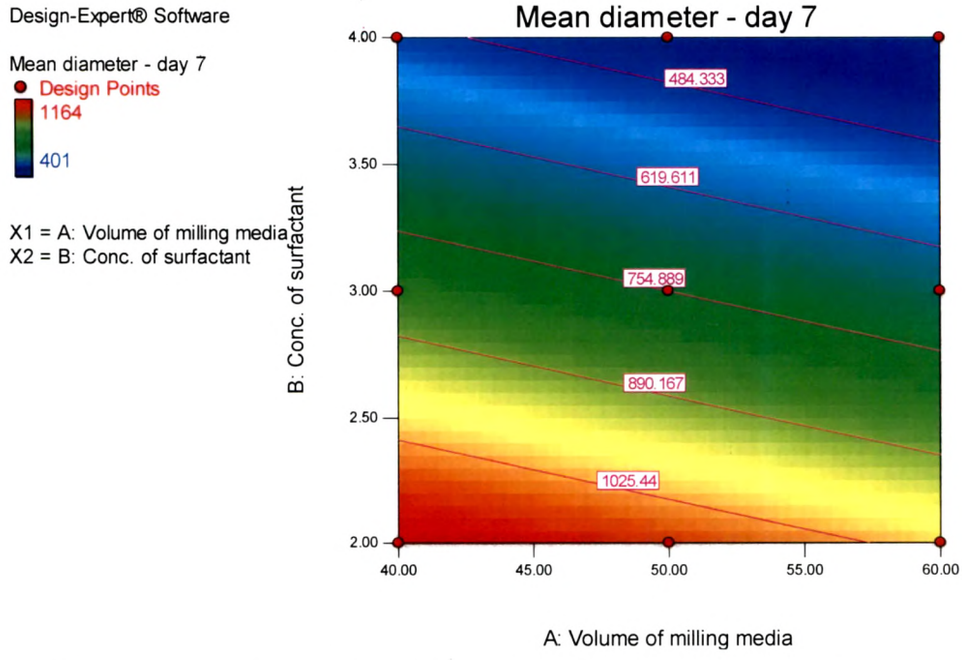

Fig 8.4b: Corresponding contour plot showing the effect of factors on Mean particle diameter on Day 7.

Table 8.7. 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	1543.33	8.21	183.16	0.973	< 0.0001
Mean particle Diameter - Day 7	2128.05	7.78	328	0.971	< 0.0001

Table 8.8 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	2.418E + 5	1.209E +005	108.54	< 0.0001
Residual	6	6683.67	1113.94	--	--
Total	8	2.485E + 005	--	--	--
		Mean Particle Diameter – Day 7 (Y_2)			
Model	2	6.81E +005	3.409E +005	100.69	< 0.0001
Residual	6	20314.72	3385.79	--	--
Total	8	7.022E+005	--	--	--

8.3.2.2 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 in range of 400 to 450. Formulation ADF 8 containing medium (0) level of X_1 and high (1) level of X_2 fulfilled all the criteria set from desirability search. To gainsay the reliability of the response surface model, new optimized formulation (as per formula ADF 8) was

prepared according to the predicted model and evaluated for the responses (Y_1, and Y_2). The result in Table 8.9 illustrates a good relationship between the experimental and predicted values, which confirms the practicability and validity of the model. The optimized formulation of ANS is shown in Table 8.10

Table 8.9 The predicted and observed response variables of the optimal Adefovir Nanosuspension

	Y_1 (nm)	Y_2 (nm)
Predicted	371.96	433.08
Observed	393	413
Predicted Error (%)	5.65	4.61

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

Table No. 8.10 Optimized Adefovir Nanosuspension

Parameters	**Value**
Milling time	18 hours
Selection of surfactant	Poloxamer 407: Sodium cholate (1:4)
Ratio of Beads (Small : Large)	50: 50
Volume of milling media	50 % w/v
Concentration of surfactant	3 % w/w

8.3.3 Characterization study of optimized formulation : Optimizd formulation was studied for Zeta potential, saturation solubility and assay. Results are given in table 8.11.

Sr.No.	Parameter	Value
01	Zeta potential	-30.55 ± 2.22 mV
02	Saturation solubility	1.12 ± 0.11 mg/ml
03	Assay	98.11 ± 1.05 %

Table No. 8.11 Zeta potential, saturation solubility and assay of optimized formulation of Saquinavir Nanosuspension

8.3.4 Differential Scanning Calorimetry Study:

The DSC curves for bulk ADF and ADF loaded lyophilized nanosuspension (ANS) are shown in fig 8.5.

The DSC curve of ADF showed melting endotherm at 101.2 ^{0}C. In case of ANS, melting endotherm was obtained at 91.08 ^{0}C and 192.15 ^{0}C. The melting endotherm peak at 91.08 ^{0}C was much broader which indicates that ADF might have been converted to an amorphous state. This may also 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). Melting endotherm at 192.15 ^{0}C may be attributed to sucrose.

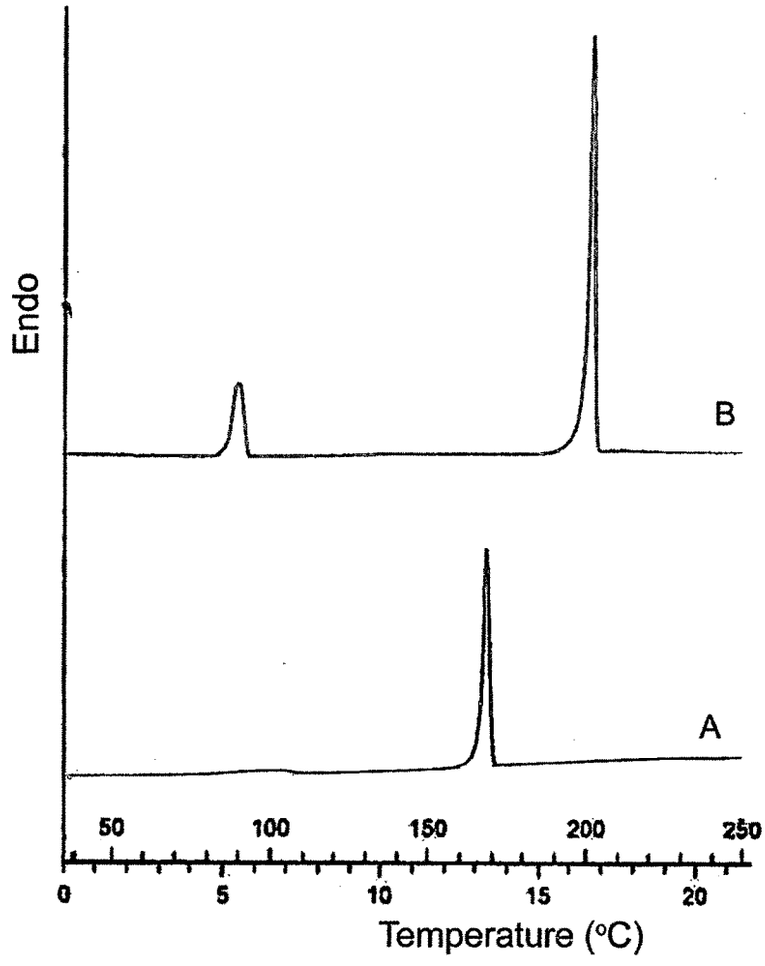

Fig. 8.5 DSC thermograms of ADF (A) and lyophilized Nanosuspension (B).

8.3.5 X Ray Diffractometry study:

XRD spectra of Adefovir dipivoxil and Adefovir nanosuspension are given in Fig.8.6 Principal peaks of Adefovir dipivoxil were observed at 2 θ angle 7.143, 17.486 and 20.934. In case of Adefovir Nanosuspension, reduction in intensity of peak was observed at these angles. It indicates reduction in crystallinity of Adefovir in nanosuspension compared to bulk adefovir. Peak broadening was observed in case of adefovir nanosuspension sample which may be due to small particle size (nanometer range), high specific surface area and presence of surfactant.

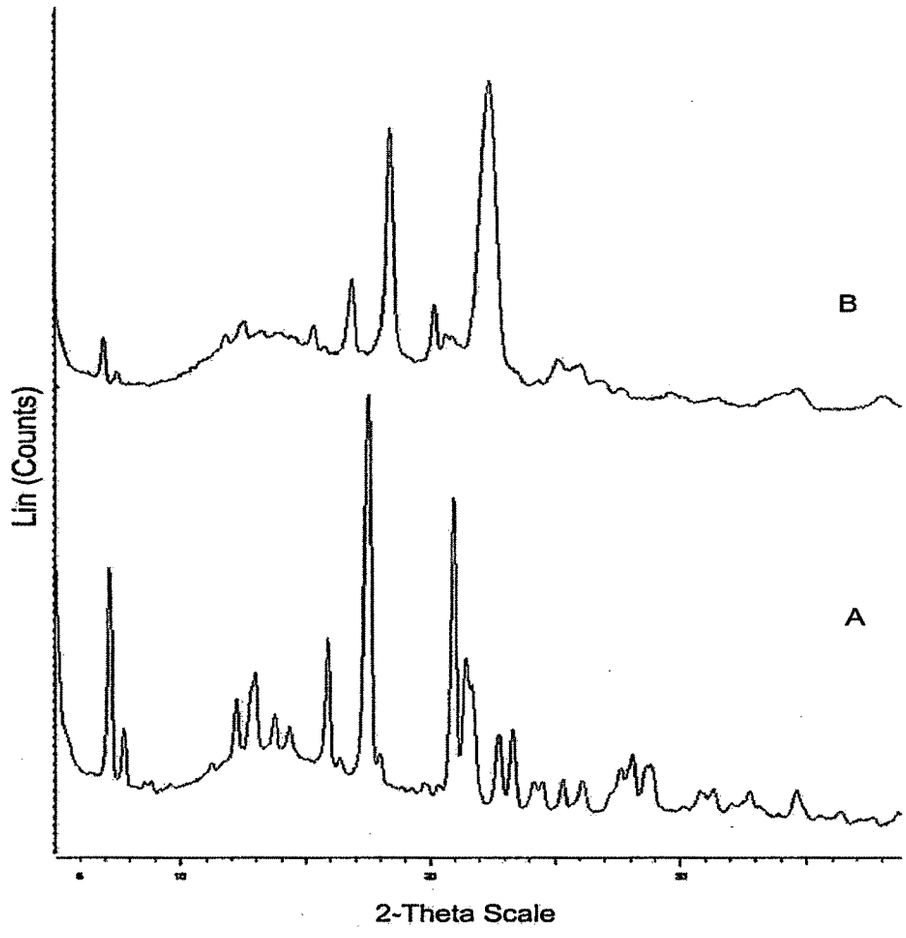

Fig. 8.6 X Ray diffractogram of ADF and ARDX

8.3.6 Transmission Electron Microscopy (TEM):

TEM image of ANS showed irregular shape, discrete and non aggregated particles (Fig.8.7). Optical microscopic image of ADF bulk sample revealed closely packed powder clusters (Fig 8.8). It can be confirmed with the difference in particle size of ADF bulk (d (0.5) was 710.05 ± 70 μm) and ADF nanosuspension after 18 hours of milling (0.422 ± 0.026μm).

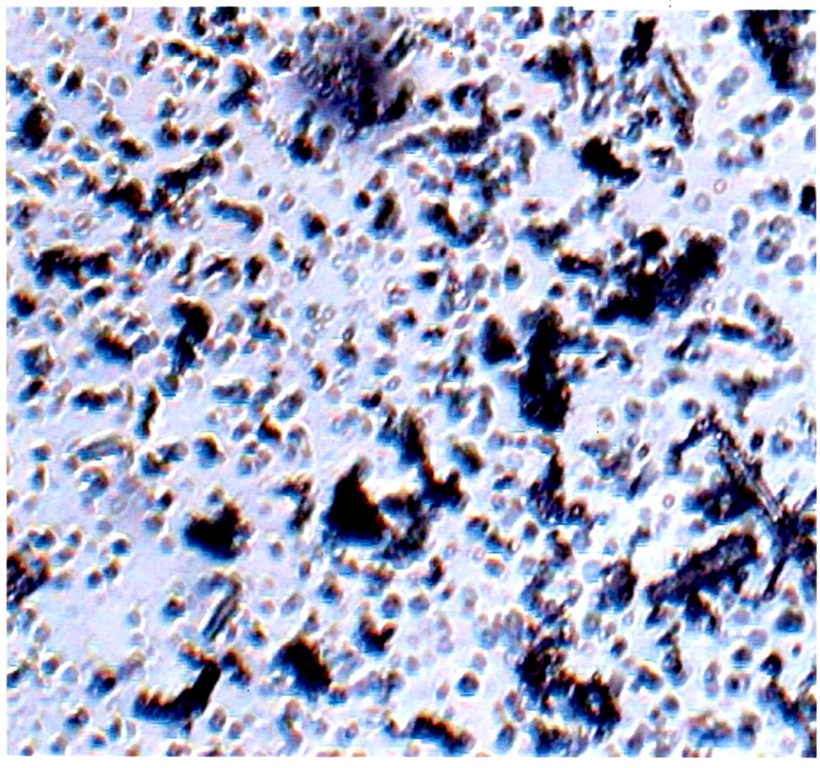

Fig 8.7 Optical image of Adefovir bulk sample

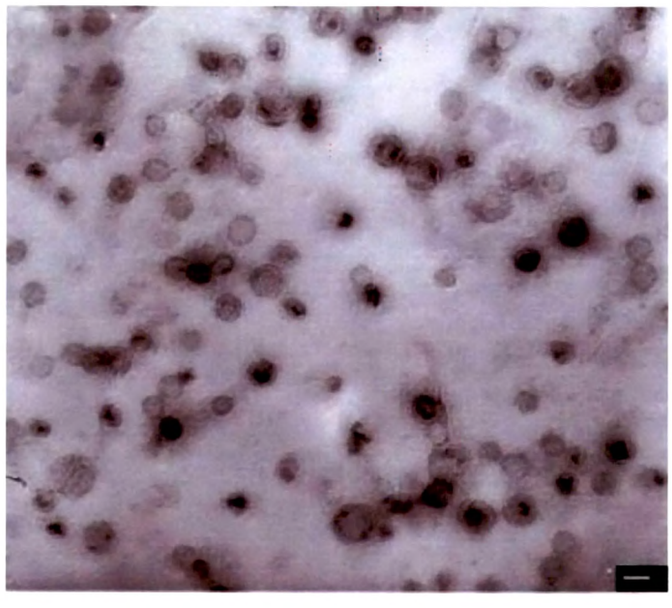

Fig. 8.8 TEM image of Adefovir Nanosuspension (Bar indicates 0.4 μm)

8.3.7 In vitro release:

In vitro diffusion profiles of ADF from suspension and nanosuspension were compared (fig. 8.9). ADF showed 97.36 ± 1.71 % diffusion in about 5 minutes from ANS in pH 7.2 phosphate buffer while from Adefovir Suspension (AMS), it showed 96.66 ± 1.33 % release after 25 minutes. The release profiles clearly indicated the faster diffusion rate of ADF from nanosuspension compared to suspension due to increase in surface area after nanosizing.

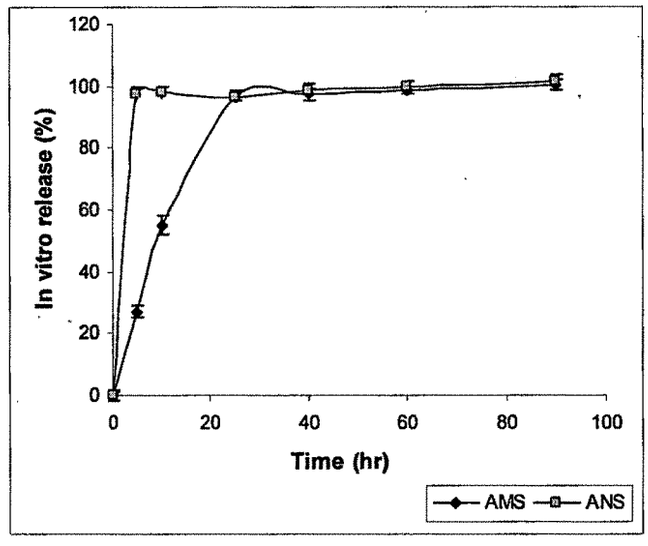

Fig. 8.9 In vitro release ADF from AMS and ANS

The release profiles were then fitted into different exponential equations such as zero order, first order, Higuchi, and Peppas Korsmeyer to characterize the release.

Table 8.12 In vitro release kinetics of AMS and ANS.

Kinetic models	Zero order	First order	Higuchi		Peppas
	r^2	r^2	r^2	h^{-1}	r^2
AMS	0.9598	0.8247	0.9892	10.7	0.993
ANS	0.604	0.563	0.721	9.51	0.933

It was found that drug release in AMS followed Peppas – Korsemeyer (r^2=0.860) more than Higuchi (r^2=0.749), Zero order (r^2=0.604) and First order (r^2=0.563) while dissolution of ANS was found to follow Higuchi (r^2=0.989) better then Peppas Korsmeyer model (r^2=0.940).

Reference:

- Chen Y, Jie L, Xiangliang Y et al. Oleanolic acid nanosuspensions: preparation, in-vitro characterization and enhanced hepatoprotective effect. Jour. of Pharm. Pharma. 2005; 57: 259–264.
- Huang YB, Tsai YH, Lee SH, et al. Optimization of pH-independent release of nicardipine hydrochloride extended-release matrix tablets using response surface methodology. Int. J. Pharm. 2005; 289: 87-95.
- Liversidge GG, Cundy KC, Bishop JF. Czekai DA. Surface modified drug nanoparticles. US Patent 5,145,684, 1992.
- Mooter VD, Van E, Froyen L, et al. Characterization of physico-chemical properties and pharmaceutical performance of sucrose co-freeze–dried solid nanoparticulate powders of the anti-HIV agent loviride prepared by media milling. 2007; 338(1-2):198-206.
- Otsuka M, Kaneniwa N. Effect of seed crystals on solid state transformation of polymorphs of chloramphenicol palmitate during grinding. J. Pharm. Sci. 1986; 75: 506-511.