Chapter 7
Formulation Development of Adefovir Dipivoxil Solid Lipid Nanoparticles

MATERIALS AND METHODS

7.1 Materials

Adefovir was obtained as gift sample from Cipla Ltd, Mumbai. Dyanasan 114 (Trimyristin), Dyanasan 116 (tripalmitin), and Dyanasan 118 (Tristearin) were obtained as kind gift sample from Ms. S. Zhaveri and Co., Mumbai. Precirol (Glyceryl palmitostearate) and Compritol (Glyceryl behenate) were obtained as gift sample from Gattefosse Ltd, Germany. Imwitor (Glyceryl monostearate) and Cutina CP (Cetyl palmitate) were obtained as gift samples from Torrent Research Centre, Gandhinagar. Soya Phosphatidylcholine was obtained as gift sample from Lipoid, Germany. Poloxamer 188 and Poloxamer 407 (poly (oxyethylene) poly (oxypropylene) block copolymer) were 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. Ammonium bicarbonate, Potassium dihydrogenphospate, Disodium hydrogen phosphate, Sodium Lauryl Sulphate and Mannitol AR were purchased from S.D. fine chem. Pvt. ltd. Mumbai. All other chemicals and solvents used were of AR grade. Double distilled water was used through out the study.

7.1.1 Equipments:

1. Remi high speed magnetic stirrer (Remi, MS500, Remi equipments, Mumbai)
2. High speed Centrifuge (Sigma 3K30, Germany)
3. Particle size Analyzer (Zeta Sizer Nano series, Malvern Instruments, UK)
4. UV-VIS spectrophotometer (Shimadzu UV-1601, Japan)
5. Lyophilizer DW1, 0-60E (Heto, Vaccubrand Denmark)
6. Differential Scanning Calorimeter (Mettler Toledo DSC 822e, Japan)
7. Laser diffraction particle size analyzer (Malvern Mastersizer, 2000, UK)
8. Ultra turrex (IKA werke, Germany)
9. High Pressure Homogenizer (Emulsiflex C5, Avestin, Canada).
10. Analytical balance (Precisa 205A SCS, Switzerland)
11. pH meter (Systronics 335, India)
12. Bath sonicator (INCO, Ambala)

7.2 Partition coefficient study:

1000 mg of lipid was taken in a glass vial and heated to about 60^0-70^0C on a magnetic stirrer cum hot plate. To the melted lipid, 10mg of Adefovir (ADF) was added. The drug was allowed to dissolve in the melted lipid. Then 5ml of distilled water, previously heated to the same temperature, was added to it. The mixture was stirred with the help of magnetic bead for approximately 30 minutes while temperature was maintained at 60^0 to 70^0C. Then it was cooled to room temperature and aqueous phase was separated. After suitable dilution of aqueous phase with methanol, absorbance was taken at 261 nm for ADF. The procedure was carried out in triplicate for each lipid. The partition coefficient value of ADF in lipid/Distilled water was calculated as following:

$$\text{Partition coefficient} = ADF_I - ADF_{DW} / ADF_{DW}$$

Where,

ADF_I – The initial amount of Adefovir added (10 mg)

ADF_{DW} – The concentration of Adefovir in Distilled water

7.3 Preparation of Adefovir Dipivoxil loaded SLN

Adefovir loaded SLNs were prepared by solvent injection method (Schubert MA, Muller-Goymann CC, 2003). Briefly, aqueous Poloxamer 407 solution was heated to 60 to 65^0C and kept for stirring. Optimized concentration of Adefovir Dipivoxil, lipid and Soya PC were added in Isopropyl alcohol and heated to 5-10 ^{0} C above melting point of Trimyristin. The resultant organic solution was rapidly injected through an injection needle into aqueous Poloxamer solution at 60 to 70 ^{0}C under continuous stirring. The resultant dispersion was then cooled down to room temperature while stirring was continued. The dispersion was transferred to a round bottom flask and solvents were evaporated under reduced pressure using Rota evaporator at 70^0C. Lipid content of SLN dispersion varied between 0.5 to 1.5 % w/v and was stabilized by 0.5 – 1.5 % w/v surfactant. Surfactant was chosen from the group of Soya PC, Poloxamer 407, Transcutol P and combination of Poloxamer 407 and Soya PC. ADF loading was varied from 2 % to 8 % w/w with respect to the lipid matrix. The SLN dispersion was lyophilized using lyophilizer (Drywinner Hetodryer). Sucrose was added as cryoprotectant in two parts by weight of total lipid content of the formulation. Ten ml of SLN dispersion was rapidly frozen to -80°C using liquid nitrogen, and lyophilized

for 24hrs. Degassing was carried out in between to prevent explosion. The resultant products were reconstituted using distilled water by manual shaking.

7.4 Optimization of Parameters:

The possible parameters influencing the formation of nanoparticles and their size and polydispersity were identified and optimized. The parameters divided into Process parameters and Formulation parameters.

7.4.1 Process parameters:

7.4.1.1 Stirring Speed:

Blank batches of SLNs were prepared to study the effect of stirring speed (400- 1000 R.P.M.) on nanoparticle formation. The response parameters measured were particle size and polydispersity index of the nanoparticle dispersion formed. Isopropyl alcohol was used as the water miscible solvent with distilled water. The surfactant used for the study was Poloxamer 407 in 0.8 % w/v concentrations. Stirring time was kept at 15 mins.

7.4.1.2 Stirring Time

To study the effect of stirring time on the nanoparticle formation, blank batches of SLNs were prepared. Stirring time was varied from 5, 15 and 25 mins by keeping stirring speed at 600 R.P.M. The response parameters measured were particle size and polydispersity index of the nanoparticle dispersion formed. The surfactant used for the study was Poloxamer 407 in 0.8 % w/v concentrations. Isopropyl alcohol was used as the water miscible solvent with distilled water. Batch composition for studying effect of stirring speed and stirring time is given in table 7.1.

Table 7.1. Composition of Blank batch of SLN for studying effect of stirring speed and stirring time

Sr.No	Class of Excipient	Name of Excipient	Quantity
01	Lipid	Trimyristin (Dynasan 114)	250 mg
02	Solvent	Isopropyl alcohol	6 ml
03	Surfactant	Poloxamer 407	0.8 % w/v
04	Distilled water	---	50 ml

7.4.2 Formulation parameters:

The formulation parameters optimized were type and concentration of surfactants and drug loading with respect to amount of lipid. The response parameters observed were particle size (Zavg), size distribution (pdI), Zeta potential (ξ) and percent drug entrapped. For the optimization of each parameter, it was varied between different levels keeping the other parameters fixed and its effect on the response was observed. The particle size, pdI and Zeta potential were measured by Zetasizer nanoseries (Malvern instruments, UK).

7.4.2.1 Organic Solvent:

Different solvents (Acetone, Isopropyl alcohol) and solvent combination (Dichloromethane: Isopropyl alcohol) were tried at three different levels i.e. 4, 6 and 10ml and the effect on the formation of nanoparticle were observed. All the other factors like Lipid concentration and surfactant concentration were kept constant. The dispersions formed were checked for presence of aggregation. The particle size and the polydispersity index were measured wherever there was least or no aggregation in the prepared batches. The ratio of lipid phase to solvent was fixed (250mg lipid in 6 ml of solvent mixture) in all the observations.

7.4.2.2 Lipid loading:

The optimum amount of lipid (one that gives maximum partition coefficient) that can yield stable SLN dispersion without visible aggregation was determined. The lipid load (0.5 to 1.5 % w/v) for a fixed level of solvent amount was used for the preparation of SLNs. Concentration of surfactant and the volume ratio between solvent and external medium of the dispersion was kept constant.

7.4.2.3 Selection of surfactant:

Batches were prepared with different surfactants (Soya PC, Poloxamer-407, Transcutol P and Poloxamer 407: Soya PC). Concentration of surfactant was kept at 1%.

7.4.2.4 Concentration of surfactant:

The concentration of Poloxamer: Soya PC was varied from 0.5%w/v to 1.5 %w/v and the effect on particle size, PDI, Zeta and entrapment were observed.

7.4.2.5 Drug loading:

Three different levels of drug loading (2%, 4% & 8% w/w) with respect to total lipid content were examined. The effect on particle size and % entrapment was determined.

7.5 Characterization of Formulations:

7.5.1 Particle size analysis: The size analysis of nanoparticulate dispersion and lyophilized nanoparticles was performed using a Malvern Zeta Sizer Nano ZS 90 (Malvern Instru, UK). Both the particle Z – average diameter and Polydispersity Index (PdI) were determined. SLN formulation (0.5 – 1ml) was kept in sample holding chamber of Malvern Zeta Sizer. Each measurement was performed in triplicate.

7.5.2 Zeta Potential: The charges acquired by the colloidal systems (Zeta Potential) were measured by Malvern Zeta Sizer Nano ZS 90 (Malvern Instruments, UK). SLN formulation (0.5 -1 ml) was kept in sample holding chamber of Malvern Zeta Sizer after appropriate dilution with water. Each measurement was performed in triplicate.

7.5.3 Entrapment efficiency: 1 ml SLN dispersion was centrifuged in a centrifuge (SIGMA 3K30, Germany) at 20000 rpm for 80 mins. The supernatant was diluted with 10 ml of IPA and analyzed at 261 nm using Shimadzu UV1610 Spectrophotometer, for unentrapped drug content. Dicholoromethane: Isopropyl alcohol (1:4) was added to sediment to solublise lipid matrices containing entrapped drug. The solution thus obtained was diluted and analyzed at 261 nm by UV spectrometer.

7.5.4 Solid State Studies:

7.5.4.1 DSC Thermograms: Thermograms were taken for Adefovir, lipid and Adefovir loaded SLNs on a Differential Scanning Calorimeter (Mettler-Toledo, Switzerland) at a heating rate of 10°C/min in nitrogen atmosphere.

7.5.4.2 XRD Studies: The instrument was operated over the 2θ range from 10° to 80°. The XRD patterns of Adefovir, Dynasan 114, Physical mixture, ADF SLN were measured with Philips PW 1729 X-ray diffractometer (Philips, Holland) using an online recorder.

7.5.5 Transmission electron microscopy:

Morphology of the particles in formulation was investigated using Transmission Electron Microscopy (TEM) [Zeiuss TEM 109 (Germany)]. Procedure same as 4.5.4.3.

7.5.5 In vitro release study: Dialysis bags with a molecular weight cut off of 12000 (Hi-media, Germany) were filled with 1 ml of SLN formulation and immersed in 40 ml of 0.1 N HCl , pH 4.5 phosphate buffer and pH 7.2 phosphate buffer respectively.

Aliquots were withdrawn periodically, replaced with same volume of fresh diffusion medium at regular intervals and estimated spectrophotometrically at 261 nm using UV spectrophotometer. The release profiles were then fitted into different exponential equations such as zero order, first order, Higuchi and Peppas- Korsemeyer to characterize the release.

7.5.6 Stability Study: Stability study was carried out as per section 5.5.4.5.

7.5.7 GI stability study (acid stability study): 1 ml of 0.1 N HCl was added to 1 ml of SLN dispersion. The samples were investigated for the particle size and zeta potential immediately and after 2 hour of incubation with 0.1 N HCl.

7.6 Results and Discussion

7.6.1 Partition coefficient study

The initial study of the partitioning nature of the drug between the melted lipid and aqueous media gives idea about the entrapment of drug in SLN. The partition coefficient values obtained between the different melted lipids and distilled water is given in Table 7.2. The partition coefficient was in the order of TM> TS > SA >GMS> GPS> CP. Adefovir Dipivoxil has log P value of 1.91. It has shown highest partition coefficient value of 2.48 in Trimyristin.

Table 7.2. Partition coefficient values of Adefovir in different lipids.

Sr. No.	Lipid	Ratio of ADF in lipid/ D.W.
01	Trimyristin (TM)	2.48 ± 0.11
02	Tristearin (TS)	2.05 ± 0.15
04	Glyceryl monostearate (GMS)	1.79± 0.26
05	Glyceryl palmitostearate (GPS)	1.68 ± 0.21
07	Stearic acid (SA)	2.01 ± 0.18
08	Cetyl palmitate (CP)	1.75 ± 0.16

7.6.2 Process parameters:

7.6.2.1 Stirring speed:

The rate of dispersion of the amphiphile into the aqueous phase has been shown to affect the preparation of polymeric nanoparticles prepared by the emulsification–diffusion method.

Table 7.3 shows the Average particle diameter and Polydispersity Index (PDI) at various stirring speed. Results indicate that at a mild stirring rate of 600 rpm (ADF 2), the particles showed minimum size (381± 17nm) and lower polydispersity index (0.255).

Table 7.3. Effect of stirring speed on Mean particle diameter and PI.

Batch No	RPM	Mean particle diameter (nm)	Poly dispersity index (PDI)
ADF 1	400	527 ± 25	0.409
ADF 2	600	381 ± 17	0.255
ADF 3	1000	412 ± 19	0.269

Stirring speed at 400 rpm showed particle size upto 527 ± 25 nm while increase in stirring speed to 600 rpm reduced particle diameter to 381 ± 17 nm. However further increase in stirring speed to 1000 rpm could not reduce particle size significantly (412 ± 19 nm). Hence for further studies the stirrer speed was kept at 600 rpm.

7.6.2.2 Stirring Time

Effect of stirring time was studied by increasing it from 5 mins to 30 mins. After 5 mins, mean particle diameter was found to be 670 ± 23 nm which reduced significantly to 387 ± 21 nm after 20 mins. Further increase in stirring time did not result in significant decrease in mean particle diameter. Hence stirring time was optimized to be 20 mins.

Table 7.4. Effect of stirring time on Mean particle diameter and PI.

Batch No	**Stirring time (mins)**	**Mean particle diameter (nm) ± S.D.**	**Poly dispersity index (PDI)**
ADF 04	5	670 ± 23	0.398
ADF 05	10	552 ± 30	0.402
ADF 06	15	426 ± 18	0.333
ADF 07	20	387 ± 21	0.202
ADF 08	25	371± 15	0.199
ADF 09	30	355 ± 18	0.205

7.6.3 Formulation parameters

7.6.3.1 Organic Solvent

Effect of various solvents and solvent combination on the formation of SLNs is shown in Table 7.5. Increase in volume of acetone from 4 ml to 10 ml increased particle size and particle size distribution. This could be due to the low solubility of Trimyristin in acetone. Trimysritin is soluble in DCM and hot alcohol (Merck index). Hence, combination of DCM: IPA was tried from 1:1 to 1:3 ratios at 4, 6 and 10 ml. At 1:1 ratio, aggregates were observed at all volumes. Increasing proportion of IPA in the mixture (1:3) produced better dispersion (Z Avg. diam. – 310 nm) but aggregates were visible.

Table 7.5. Effect of organic solvent composition on Mean particle diameter and PI

Batch no	Solvent / Solvent combination	Ratio	Volume In ml	Visible Aggregation	Z avg In nm ± S.D.	Size distribution (pdI)
ADF10	Acetone	-	4	present	-	-
ADF11	Acetone	-	6	present	787 ± 29	0.349
ADF12	Acetone	-	10	present	960 ± 32	0.482
ADF13	DCM : IPA	1:1	4	present	-	-
ADF14	DCM : IPA	1:1	6	very little	477 ± 25	0.393
ADF15	DCM : IPA	1:1	10	present	-	-
ADF16	DCM : IPA	1:2	4	present	-	-
ADF17	DCM : IPA	1:3	4	very little	310 ± 22	0.433
ADF18	IPA	-	4	least	298 ± 22	0.362
ADF19	IPA	-	6	no	260 ± 15	0.272
ADF20	IPA	-	10	present	-	-

Results show that increasing the concentration of IPA in combination with DCM reduced the aggregation (ADF16, ADF21). Dynasan is soluble in hot alcohol (The Merck index). As reported by Fessi et al., a prerequisite for the solvent diffusion

technique is miscibility between solvent and aqueous phases in all ratios [Fessi et al 1992]. At lowest volume of IPA (4 ml), particle size was 298 nm with particle size distribution 0.362 but there was presence of small aggregates. Increase in volume of IPA from 4 ml to 6 ml resulted in SLNs dispersion with particle size of 260 nm, particle size distribution of 0.272 and without any aggregates. These results are in agreement with those reported by Guerrero et al. They observed that higher the water miscibility of the solvent, the smaller the Lipid Nanoparticles (LN) obtained. This behavior might be attributed to different diffusion rate of organic solvents in water. Increase in volume of IPA to 10 ml, showed presence of small aggregates. Hence, 6 ml of IPA was selected as suitable solvent for preparation of SLNs.

7.6.3.2 Lipid loading:

At 0.5%w/v concentration of the lipid and 6ml of IPA, (mean particle diameter was 255 ± 21 nm with PI of 0.212) no aggregates formed (Table 7.6). Increase in concentration of lipid to 1 %w/v without changing volume of IPA, led to formation of aggregates. Schubert and Muller-Goymann observed that increase in lipid concentration leads to a concentration dependant increase in particle size. Rising concentration of lipid increases viscosity of solvent phase which in turn may reduce diffusion rate of lipid molecules in the outer phase. (Schubert M.A., C.C.Muller Goymann, 2003). Therefore, to reduce viscosity of lipid solvent phase, we increased volume of IPA from 6 ml to 10 ml. Nanoparticles with mean particle diameter of 432 ± 41 nm with PI of 0.398 were obtained without any aggregates. Further increase in lipid and solvent concentration did not result in reduction of particle diameter and PI. Based on these results, 0.5 % w/v of lipid concentration was taken for further studies.

Table 7.6. Effect of lipid load on nanoparticle formation

Batch No	Conc. of lipid (% w/v)	Volume of IPA in ml	Vol of external medium	Presence of Aggregates	Zavg in nm ± SD	Polydispersity Index (PI)
ADF 25	0.5	6	50	No	255 ± 21	0.212
ADF26	1.00	6	50	Yes	-	-
ADF27	1.00	8	50	Very less	432 ± 41	0.398
ADF28	1.5	8	50	Yes	-	-
ADF29	1.5	12	50	less	546 ± 49	0.416

7.6.3.3 Selection of surfactant:

Batches were prepared with different surfactants (Soya PC, Poloxamer-407, Transcutol P and Poloxamer 407 + Soya PC). The responses measured were particle size, size distribution, and zeta potential. All the other parameters like lipid loading (0.5 % w/v), volume of IPA (6 ml), stirring speed (600 R.P.M.) and stirring time (15mins) , Concentration of surfactant (1%w/v) , were kept constant . The results are tabulated in table 7.7.

Table 7.7. Effect of surfactant on particle size, size distribution and zeta potential.

Batch No	Surfactant	Zavg In nm ± SD	PI	Zeta Potential in mV ± SD
ADF 30	Soya PC	464 ± 24	0.340	-14.8 ± 3.2
ADF 31	Poloxamer-407	385± 29	0.312	-18.84 ± 2.1
ADF 32	Transuctol P	545 ± 36	0.543	-9.22 ± 3.2
ADF 33	Soya PC: Pol. 407 (1:2)	311 ± 15	0.35	- 21.00 ± 2.7
ADF 34	Soya PC: Pol. 407 (1:4)	267 ± 18	0.222	-26.90 ± 1.8

Average particle diameter and PI was less in Poloxamer 407 stabilized SLNs (385 ± 29 nm - 0.312) compared to Soya PC (464 ± 24 nm - 0.340) and Transcutol P stabilized SLNs (545 ± 36 -0.543). The use of 1% of a single emulsifier gave coarse emulsions with high coalescence rate of the solvent droplets (Trotta M. et al, 2003). In some rare cases a single emulsifier can yield the desired emulsion. More often, in the case of oil-in-water emulsions, mixed surfactants have been reported to have a synergistic effect on emulsion stability in term of coalescence rate. The combined use of two or more emulsifying agents appear to produce mixed surfactant films at the interface having high surfactant coverage as well as sufficient viscosity to promote stability (Tadros, 1983). Therefore, combination of Soya PC and Poloxamer 107 was tried at 1:2 and 1:4 ratio. At 1:4 ratio, average particle diameter was reduced significantly to 267 ± 18 nm with PI of 0.222.

Poloxamer stabilized SLNs showed higher zeta potential values compared to Soya PC and Transcutol stabilized SLNs but less than combination of Soya PC and poloxamer 407. The highest zeta values (-26.9 mv) were observed with Soya PC: Poloxamer 407 at 1:4 ratio which indicates less likely occurrence of particle aggregation.

7.6.3.4 Concentration of surfactant:

The concentration of Soya PC: Poloxamer 407 (1:4) was varied from 0.5%w/v to 1.5 %w/v and the effect on the particle size, PI and zeta potential was observed. Effect of emulsifier concentration on mean particle diameter is shown in Fig. 7.1.

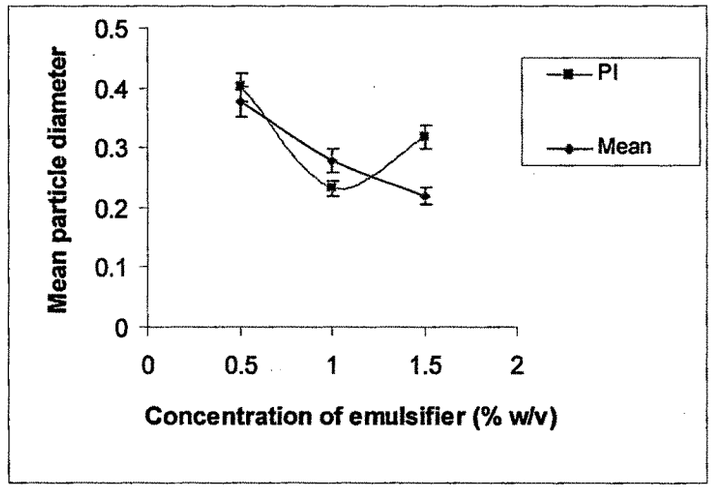

Fig 7.1 Effect of emulsifier concentration on mean particle diameter

The amount of the emulsifier should be optimum to cover the surface of the nanoparticles. Lesser amounts of emulsifier result in particle aggregation and lead to increase in particle size. However, use of excess amount of emulsifier should be avoided to prevent decrease in entrapment efficiency, burst release as observed in case of release studies of SLNs and also toxic effects associated with surfactants. (Muller, R.H et al, 2000)

Increase in concentration of Soya PC : Poloxamer 407 from 0. 5 to 1 % w/v resulted in decrease in particle size from 376 nm to 278 nm and particle size distribution from 0.401 to 0.232. Further addition of surfactant led to a further decrease of the particle size down to 219 nm, though PDI was increased. Hence, 1% w/v concentration of surfactant was selected for further studies.

7.6.3.5 Drug loading:

Different levels of drug loading (2%, 4%, 6% and 8 % w/w) with respect to total lipid content were examined. The effect on % entrapment was determined and shown in Fig. 7.2

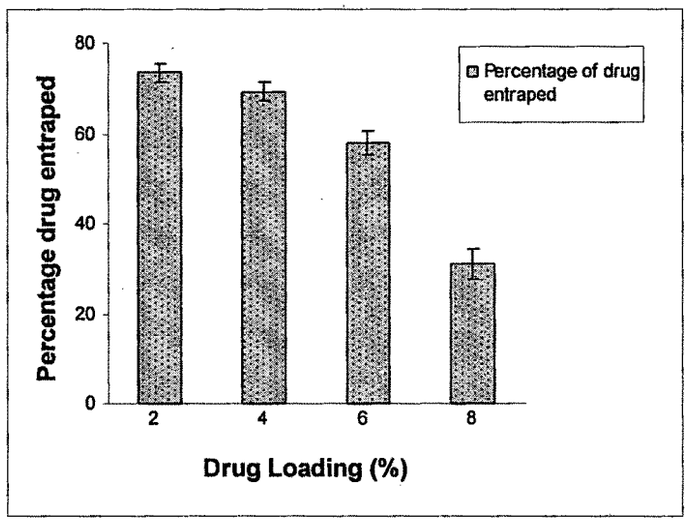

Fig. 7.2. Effect of drug loading on entrapment efficiency

The results indicate increase in drug loading from 2% w/w to 8% w/w resulted in decrease in entrapment from 73.5 ± 2.12 % to 31.15 ± 3.35 %. At 8 % drug loading, the decrease in entrapment can be due to insufficient lipid concentration (0.5 % w/v) to accommodate drug. There was no significant difference in entrapment at 2% (73.5 ± 2.12) and 4 % (69.25 ± 1.97) drug loading. Entrapment of drug at 2% loading is slightly higher which may be due to sufficient lipid concentration to accommodate drug in lipid matrix. So the optimum drug loading was chosen at 2 % w/w of lipid content.

7.6.4 Differential Scanning Calorimetry: DSC curves of bulk ADF, ADF loaded lyophilized SLNs (ADSLN) and physical mixture of Dynasan 114 & ADF is given in Fig 7.6.4.

The crystalline structure of SLNs can be assessed by Differential Scanning Calorimetry (DSC). The DSC curve of ADF showed melting endotherm at 101.2 ^{0}C. In case of physical mixture of ADF and Trimyristin, small endothermic peak was observed at 57.30^{0}C, which corresponds to M.P. of Trimyristin and sharp peak observed at 100.32 ^{0}C corresponding to ADF. In case of thermogram of ADF SLNs, small peak observed at 54.24 ^{0}C and a sharp peak at 179.17 ^{0}C, which corresponds to sucrose (melting point range 160 – 170^{0}C).

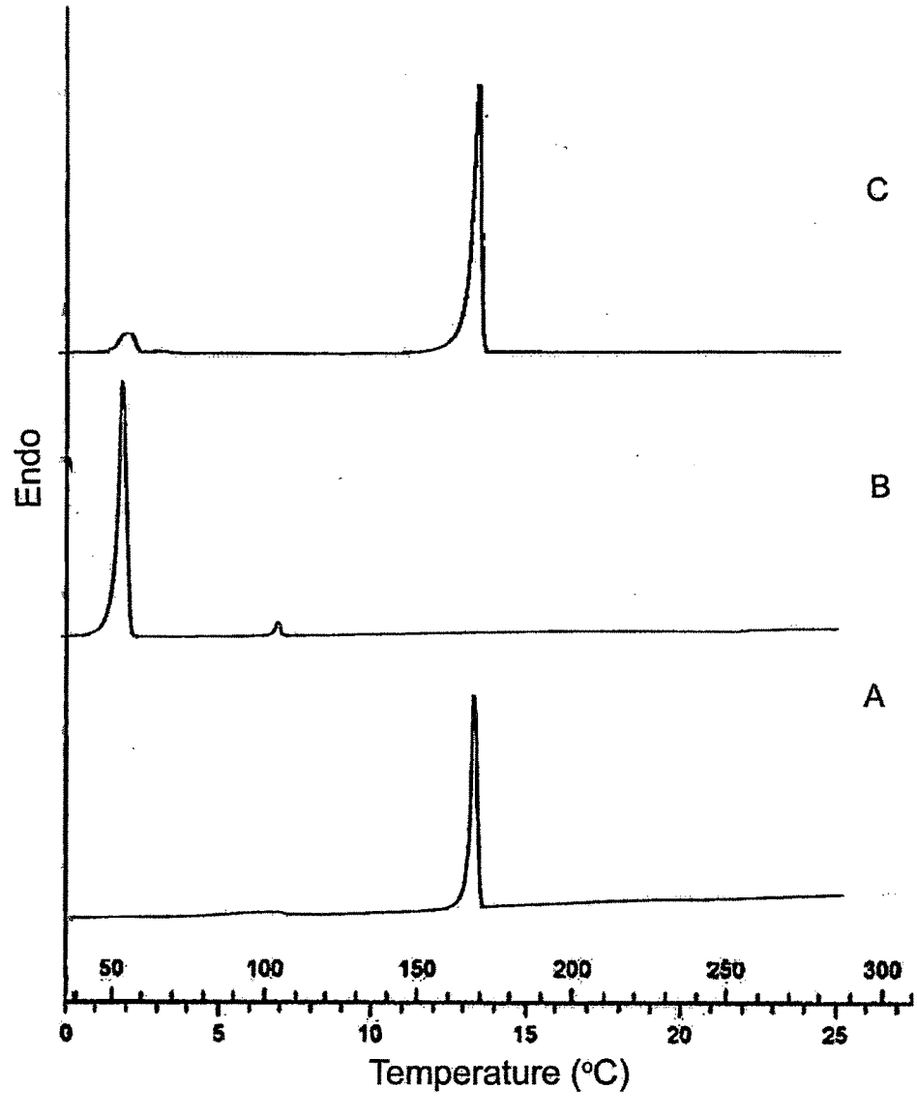

Fig. 7.3. DSC thermograms of, Adefovir (A) Physical mixture (B) and Adefovir loaded SLNs(C)

Incorporation of ADF inside the lipid matrix might have lead to an increase in the number of defects in the lipid crystal lattice. In case of DSC curve of ADF SLN, absence of ADF peak near its m.p. indicates that drug might have entrapped in lipid matrix. Reduction in the melting point (m.p.) of lipid was observed when formulated as SLN. The possible reason might be the change in crystal lattice of the lipid after the incorporation of ADF and formulation as nanoparticles. These results were in

agreement with those observed by Freitas and co workers (Freitas C and Muller R H., 1999). They observed that the crystallization behaviour of Compritol SLN differed distinctly from that of the bulk lipid. These changes were due to the increased number of lattice defects in the lipid crystal. Small particle size of SLN generates an energetically high suboptimal state which leads to decrease in the melting point.

7.6.5 X ray diffraction:

Comparison of XRD patterns are tabulated in Table 7.8. The XRD pattern of ADF (Fig.7.4) exhibited peaks at 2θ angle 17.483 and 20.98. The lipid (Dynasan 114) showed peaks at 2θ angle 19.185 and 23.82.

In case of ADF SLN, the characteristic peaks of ADF at 2θ angle 17.483 and 20.98 were not observed. This shows change in the crystallinity of ADF after incorporation into the lipid matrix.

Also, the characteristic peaks of lipid did not shift. However, there was a slight reduction in the intensity of the peaks which may be attributed to the incorporation of ADF in between the crystal lattice of the lipid leading to decrease in its crystallinity in the ADF loaded SLN.

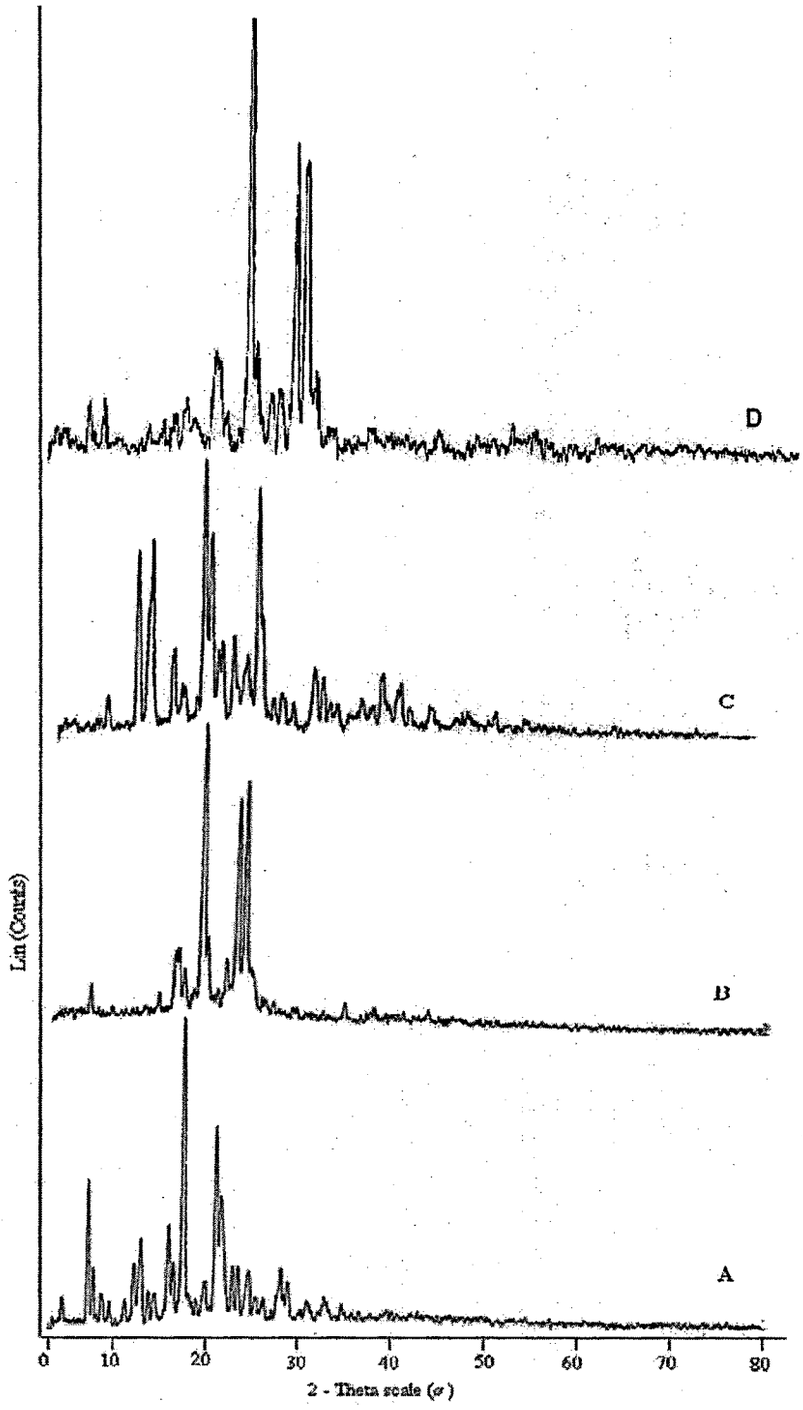

Fig 7.4 X ray diffractograms of ADF (A), ADF SLN (B), Physical mixture (C) and Dynasan 114 (D)

7.6.6 Transmission Electron Microscopy (TEM):

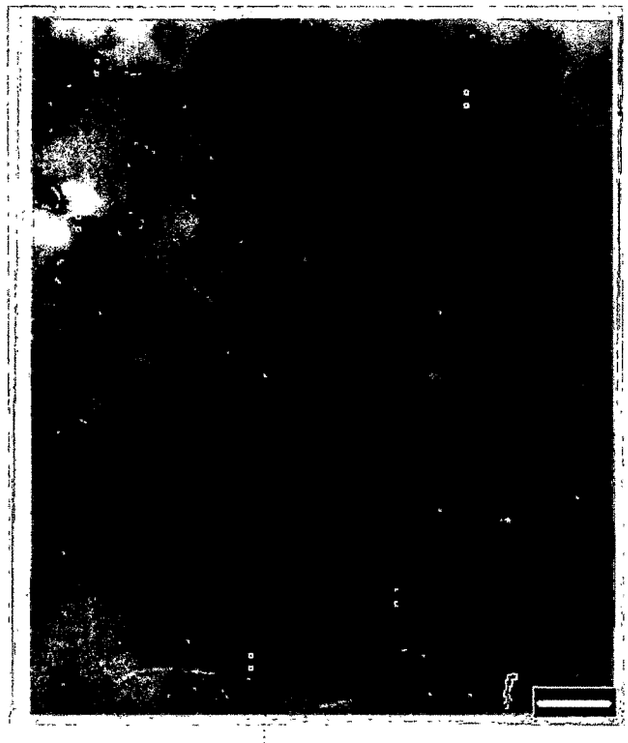

Fig 7.5 TEM image of Adefovir loaded SLNs sample (Bar indicates 500 nm)

TEM image revealed that most of the SLNs had round shape with smooth surface (fig 7.5). However, some of the particles were found to be of irregular shape.

7.6.7 In vitro release:

In vitro release study was carried out in three media i.e. 0.1 N HCl, pH 4.5 buffer and pH 7.2 buffer. In case of 0.1 N HCl, 96.63 ± 3.23 % of drug released in 36 hrs whereas in case of pH 4.5 and pH 7.2 buffer, 96.5 ± 2.01 % and 94.99 ± 3.99 % of drug released in 48 hrs ,respectively. The results are shown in Fig.7.6.

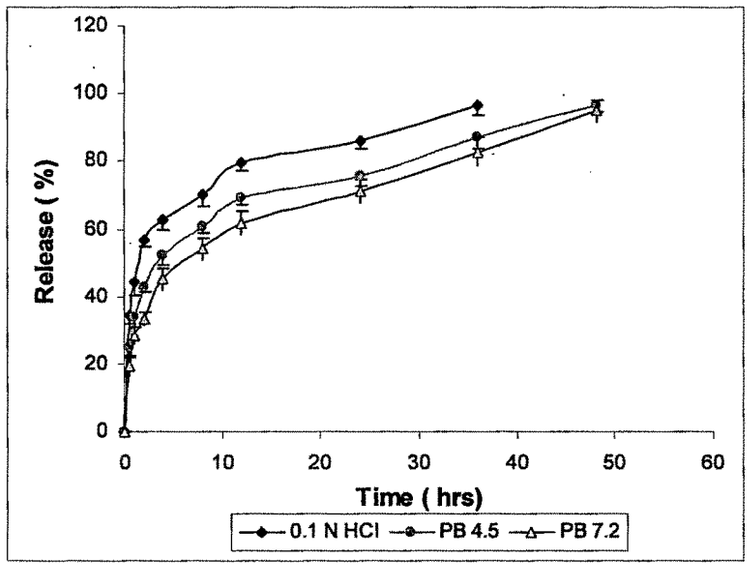

Fig. 7.6 In vitro release of Adefovir loaded SLNs in pH 1.2, PB 4.5 and PB 7.2

The release profiles indicated that SLN showed a retarded release of drug from lipid matrix. SLN exhibited initial burst release followed by sustained release in all media. The initial burst release is probably caused by the drug adsorbed on the nanoparticle surface (Muhlen A. Z. et al 1998.) or precipitated from the superficial lipid matrix (Reddy LH and Murthy RSR., 2005). The sustained release following the initial burst release is probably due to the diffusion of drug from the lipid matrix. pH of the dissolution media seems to affect the release rate of ADF. It was found that release was faster in the case of acidic media (pH 2), which may be attributed to the acid facilitated lysis of lipid matrix. After 2 hours, 56.64 ± 2.88 % of ADF released in 0.1 N HCl compared to 42.99 ± 2.76% in pH 4.5 buffer and 33.34 ± 1.96 % in pH 7.2 buffer . Also solubility of ADF is more in 0.1 N HCl (19 mg/mL) compared to pH 7.2 (0.4 mg/mL)

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 7.8 enlists the regression parameters obtained after fitting various release kinetic models to the in vitro dissolution data. It was found that drug release in all media followed Peppas – Korsmeyer model more than Higuchi, Zero order and First order. The values of diffusional exponent 'n', obtained from the slopes of the fitted

Korsemeyer–Peppas model, ranged between 0.226 and 0.326. In vitro release in all media exhibited 'n' value less than 0.5, indicating Fickian diffusion.

Table 7.8. In vitro release kinetics of Adefovir SLNs in different media.

Kinetic models	Zero order	First order	Higuchi		Peppas	
	r^2	r^2	r^2	h^{-1}	r^2	n
0.1 N HCl	0.794	0.667	0.926	10.7	0.972	0.226
pH 4.5 buffer	0.853	0.703	0.959	10.48	0.984	0.275
pH 7.2 buffer	0.889	0.725	0.977	11.21	0.988	0.326

7.7 Stability study

7.7.1 Short term

Initially for SLN dispersion, a Short-term stability study was carried out at Room Temperature (RT) for 15 days. Samples were taken at different time points like 1, 7 and 15 days and their particle size and PDI were determined. The results are shown in fig. 7.7.

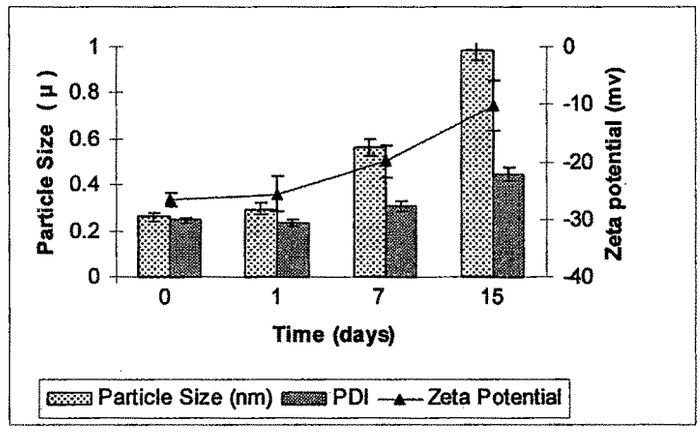

Fig. 7.7. Short term stability study of SLN dispersion

SLN dispersion kept at room temp. for 15 days showed increase in particle size from 260 ± 21 nm to 982 ± 22 nm (PDI – 0.442) while Zeta potential reduced from -26.6 mv to -10.22 mv. It indicates that prepared SLNs dispersion was not stable enough at RT. This may be due to destabilization process which is generally induced by high temperatures. Temperature might have modified packing arrangements of emulsifier on the surface of SLN as observed by Heiati et al (1996). Hence, further stability

study was carried out by storing SLN dispersion (at 2- 8 ^{0}C) and Lyophilized SLN (at 2-8 ^{0}C and RT) for 3 months.

7.7.2 Long term:

SLN dispersion were kept at 2-8 ^{0}C while lyophilized SLN were kept at 2-8 ^{0}C and RT for 3 months. Samples were studied after 1, 2 and 3 months for Particle Size, Drug content, Polydispersity index, and Zeta Potential.

7.7.2.1 Samples stored at 2 – 8 ^{0}C:

After 3 month of storage at 2 – 8 ^{0}C, particle size of **SLN dispersion** increased to 0.664 ± 0.041 μ (PDI – 0.455) while zeta potential decreased to -7.33 ± 4.1 mv (Fig 7.8) . In case of **Lyophilized SLNs,** particle size was 0.442 ± 0.036 μ (PDI -0.39) while zeta potential was -15.99 ± 4.01 mv (Fig 7.9). IT shows that lyophilized SLNs are more stable than SLN dispersion at 2 – 8 ^{0}C.

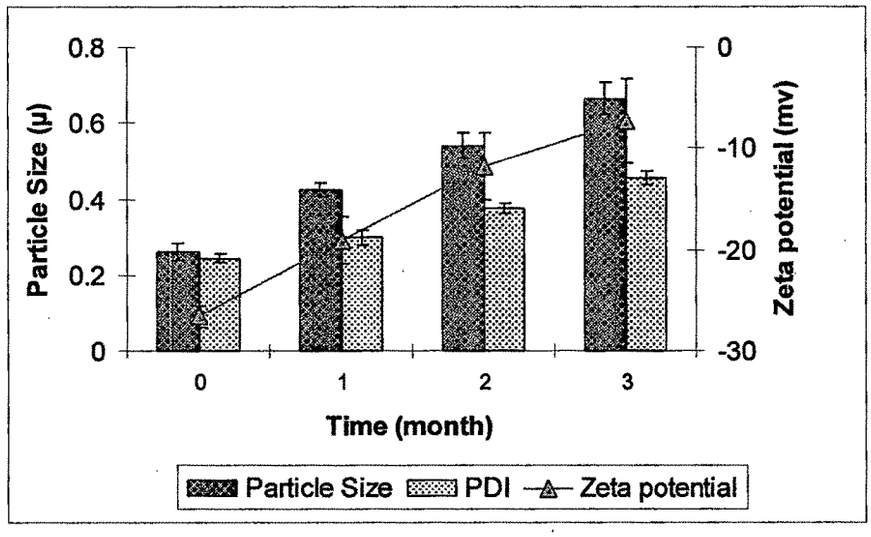

Fig. 7.8 Particle Size, PDI & Zeta potential of Adefovir SLNs dispersion stored at 2-8 ^{0}C

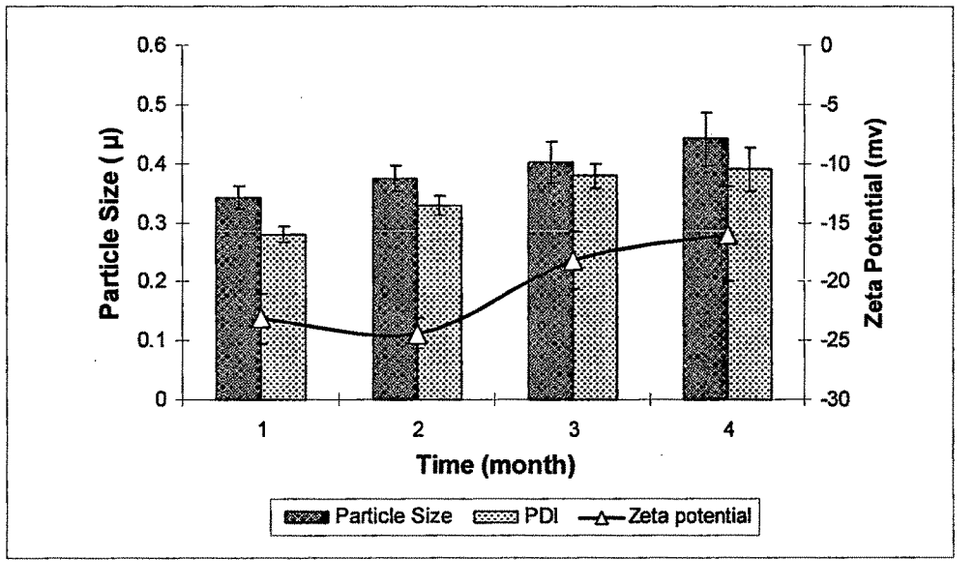

Fig. 7.9. P.size, PDI and Zeta potential of Adefovir lyophilized SLNs stored at 2 - 8^{0}C

After storage at 2- 8 ^{0}C for 3 months, drug content of SLN dispersion decreased from 66.24 % ± 2.79 to 36.24 ± 3.21 while that of lyophilized SLNs decreased from 63.66 ± 2.66 to 53.24 ± 2.31 (Fig 7.10). This may be due to expulsion of the drug from the particle matrix due to polymorphic transition of lipid (Westesen K. et al 2000). Drug expulsion during was explained by a reduction of amorphous regions in the carrier lattice due to polymorphic transition (Gohla SH et al 2000).

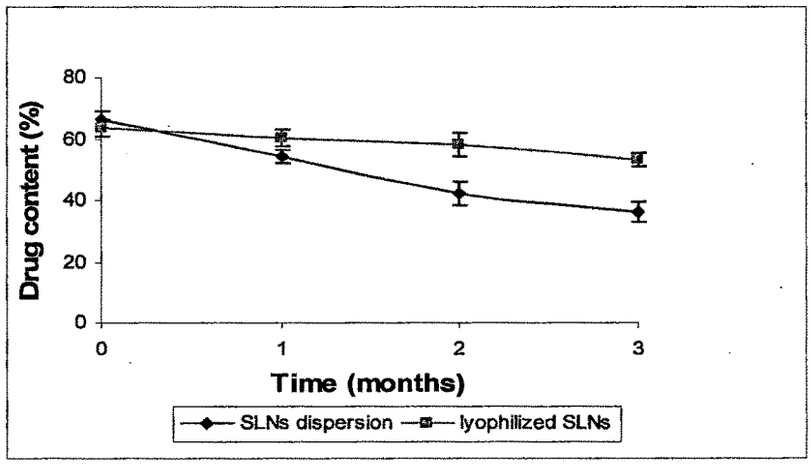

Fig. 7.10. Drug content of Adefovir SLN dispersion & Lyophilized SLNs stored at 2- 8^{0}C

7.7.2.2 Samples stored at RT:

After 3 month of storage at RT, particle size of **Lyophilized SLNs** increased to 577 ± 34 nm (PDI – 0.389) while zeta potential decreased to -11.01± 4.21 mv and drug content was 45.23 ± 1.91 % (Fig 7.11) . After 3 months of storage, mean particle diameter of lyophilized SLNs was more at RT (577 ± 34 nm) compared to 2- 8^0C (442 ± 39 nm). Also zeta potential reduced significantly in samples stored at RT (-11.01± 4.21 mv) compared to samples stored at 2- 8^0C (-15.99 ± 4.01 mv). This may be due to decrease in microviscosity (a high level of film rigidity of the emulsifier). Microviscosity is a temperature-dependent factor. When temperature increases, microviscosity decreases, leading to destabilization of the particles (Freitas C, Muller RH 1998).

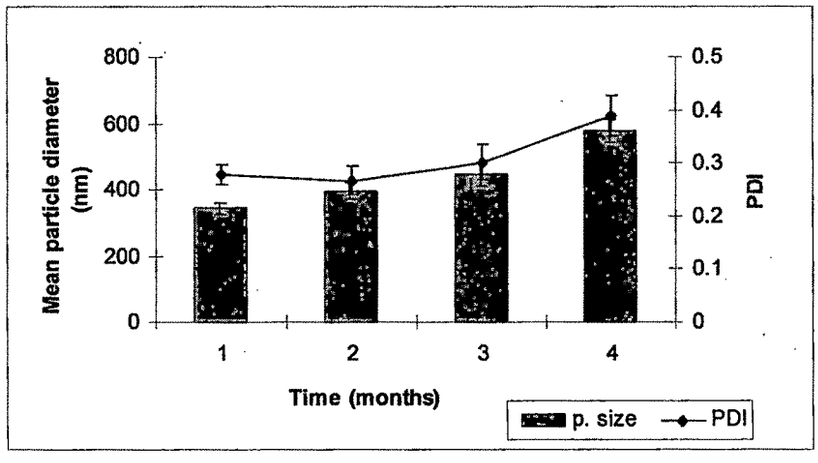

Fig. 7.11. Mean Particle diameter and PDI of Adefovir lyophilized SLNs stored at room temp

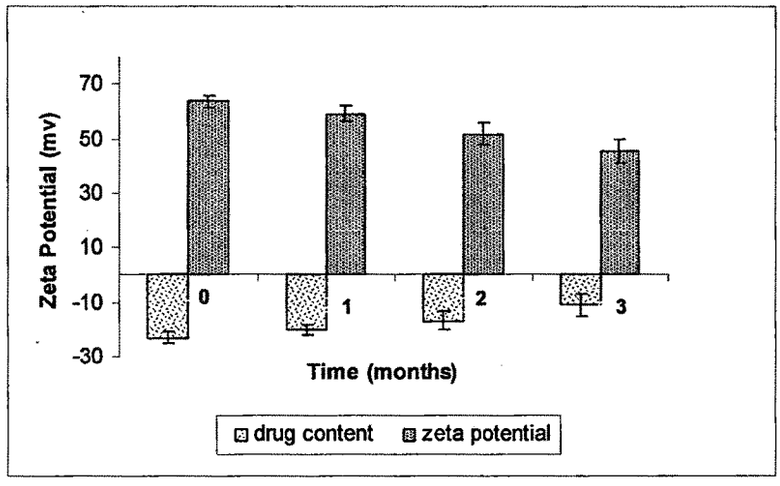

Fig. 7.12. Zeta potential & Drug content of Adefovir SLNs lyophilized and stored at room temp

7.8 GI stability study (acid stability study):

Adefovir loaded SLN dispersion was incubated with 0.1 N HCl for 2 hours. Samples were investigated for the Particle size & Zeta potential immediately and after 2 hour of incubation. Particle size was found to be increased to 312 ± 21 nm immediately from initial particle size of 257 ± 11 nm. After 2 hour incubation, particle size was increased to 451 ± 32 nm, Zeta potential was decreased to -13.45 ± 3.21 mv from initial value of - 26.6 ± 1.11 mv.

Sampling Interval		**Initial Mean particle diameter ± S.D. (nm)**	**Zeta potential (mv)**
Before incubation		257 ± 11	- 26.6 ± 1.11
After incubation	**Immediately**	312 ± 21	-21.11 ± 2.09
	After 2 hours	451 ± 32	-13.45 ± 3.21

Table 7.9. Mean particle diameter and Zeta potential of Adefovir loaded SLN after incubation with 0.1 N HCl.

The Adefovir loaded SLN formulation stabilized with Poloxamer 407 and Soya PC was found to be unstable in terms of particle size after 2 hrs of incubation. Aggregation of nanoparticles was prevented which might be due to steric stabilization of surfactant. The coating layer of Poloxamer 407 and Soya PC might have sufficient thickness and density on the surface in combination with complete coverage of particle surface to

prevent aggregation of particles. The contact of these uncovered lipid crystalline surfaces can lead to crystal growth between the particles.

Conclusion:

In the present investigation, preliminary partition studies of Adefovir between molten lipid (Trimyristin) and distilled water gave an idea about the achievable drug entrapment during formulation of lipid nanoparticles. Formulation and process parameters were optimized to get Adefovir loaded solid nanoparticles with suitable particle size, surface charge and entrapment efficiency. DSC study revealed that ADF was incorporated in lipid matrix whereas XRD showed reduced crystallinity of ADF after incorporation in lipid. SLN were found to be round shape with smooth surface. In vitro release study showed sustained release behaviour of ADF from SLN. Stability study revealed that lyophilized SLN are more stable than SLN dispersion at room temperature and 2 – 8^0 C. Thus characterization studies coupled with stability study highlighted the potential of this formulation for further in vivo study.

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