Chapter 4

Formulation Development of Saquinavir Solid Lipid Nanoparticles

Materials and Methods

4.1 Materials:

Saquinavir was obtained as gift sample from Aurbindo Pvt Ltd, Mumbai. Dynasan 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 PC 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.

4.1.1 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, Japan)
5. Lyophilizer, vacuum Pump (Heto, Vaccubrand, Denmark)
6. Differential Scanning Calorimeter. (Mettler Toledo DSC 822e, Japan)
7. Laser diffraction particle size analyzer (Malvern Mastersizer, 2000, UK)
8. Ultra turrax (IKA werke, Germany)
9. High Pressure Homogenizer (Emulsiflex C5, Avestin, Canada).

4.2 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 Saquinavir (SQ) was added and 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 by centrifugation at 20000 rpm for 25 min. The clear supernatant was diluted suitably with methanol and SQ content was determined at 239 nm using UV spectrophotometer (Shimadzu UV1610, Japan). The procedure was carried out in triplicate for each lipid and mean values are reported. The partition coefficient of Saquinavir in lipid/Distilled water was calculated as following:

$$\text{Partition coefficient} = SQ_I - SQ_{DW} / SQ_{DW}$$

Where

SQ_I – The initial amount of Saquinavir added (10 mg)

SQ_{DW} – The concentration of Saquinavir in Distilled water

4.3 Preparation of Saquinavir loaded SLNs (SQSLN):

SQ loaded SLNs were prepared by Hot High Pressure Homogenization (HPH) method (Lai Francesco, 2006). Lipid was melted at 5-10^0 C above its melting point and SQ (0.5 - 1.5% w/w) was added in it. This hot dispersion was added to hot aqueous solution of Poloxamer 407 : Tween 80 under stirring at 6500 RPM for 3 mins by ultra turrax (IKA werke, Germany). This primary O/W emulsion was then passed through high pressure homogenizer (Emulsiflex C – 5, Avestin, Canada) at optimized number of homogenization cycles at 10000 psi pressure. Process was carried out by maintaining temp between 60^0 C - 70^0 C. Various formulation parameters like type of emulsifier, Concentration of emulsifier and concentration of SQ were studied and optimized. Process parameter like number of homogenization cycles (1 to 5) was optimized at homogenization pressure 10,000 psi. To convert SLN dispersion into stable dry form, lyophilization was carried out using lyophilizer (Drywinner Hetodryer, Denmark). Ten milliliters of SLN dispersion was rapidly frozen to -80°C using liquid nitrogen, and lyophilized for 24hrs. The cryoprotectants tried were

mannitol and sucrose. Three different ratios of total solid content to cryoprotectant (1:1, 1:2 and 1:3) were tried. The lyophilized SLNs were reconstituted using distilled water by manual shaking and mean particle diameter and PI were determined..

4.4 Optimization of Parameters:

4.4.1 Process parameter:

4.4.1.1 Homogenization Cycles:

Prior to the formulation step, the possible process parameters influencing the formation of nanoparticles, size and polydispersity of nanoparticles were identified and optimized. The parameter studied was number of homogenization cycles. For each composition of emulsifier, 20 ml of nanodispersion was prepared, homogenization carried out for different period of time in terms of one, two, three and four cycles at 10000 psi for achieving desired particle size.

4.4.2 Formulation parameters:

4.4.2.1 Type of emulsifier

Different ratios of emulsifiers: Coemulsifiers were used to optimize the nanodispersion. Concentration of emulsifier was kept constant at 2% w/v. Poloxamer 407 was used as emulsifier while Tween 80 was used as co-emulsifier in different proportion with poloxamer 407. Combination of Poloxamer 407 and Tween 80 was studied at 1:1, 1:3 and 1:5 ratios.

4.4.2.2 Concentration of emulsifier

Besides type of emulsifier, their concentration has great impact on the quality of SLN dispersion. Total emulsifier concentration was varied from 1% w/v to 5% w/v to study its effect on particle size and Polydispersity index.

4.4.2.3 Concentration of Saquinavir (w.r.t. lipid):

Concentration of Saquinavir was studied at 2% , 3 % , 4 % and 5 % w/w with respect to lipid. Four different batches were prepared by keeping the lipid concentration constant at 5 % w/v.

4.5 Characterization:

4.5.1 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.

4.5.2 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.

4.5.3. Entrapment efficiency: 1 ml SLN dispersion was centrifuged in a centrifuge (Sigma 3K30, Germany) at 20000 rpm for 25 mins. The supernatant was diluted suitably with methanol and analyzed at 239 nm using Shimadzu UV1610 Spectrophotometer, for **unentrapped** drug content. Isopropyl alcohol was added to the sediment to solublise lipid matrices containing entrapped drug. The solution thus obtained was diluted and analyzed at 239 nm.

4.5.4. Solid State Studies:

4.5.4.1 Differential Scanning Calorimetry (DSC) study: Thermograms were taken for Saquinavir, lipid and Saquinavir loaded SLNs (2 – 3 mg) on a Differential Scanning Calorimeter (Mettler-Toledo, Switzerland) at a heating rate of 10°C/min in nitrogen atmosphere. An empty aluminium pan was used as the reference for all measurements.

4.5.4.2 XRD Studies: The instrument was operated over the 2θ range from 10° to 40°.The XRD patterns of Bulk Saquinavir, Stearic acid, SQSLN and sucrose were measured with Philips PW 1729 X-ray diffractometer (Philips, Holland) using an online recorder.

4.5.4.3 Transmission electron microscopy:

Morphology of the particles in formulation was investigated using Transmission Electron Microscopy (TEM) [Zeiuss TEM 109 (Germany)]. Briefly, it is carried out by operating at an acceleration voltage of 200 kV. Approximately 2 min after sample deposition (1- 2 µl), the grid was tapped with filter paper to remove surface water and air-dried. If necessary, negative staining is performed using a droplet of 2 wt % aqueous uranyl acetate.

4.5.4.4 In vitro release study: Dialysis bags with a molecular weight cut off of 12000 (Hi-media) 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 and estimated spectrophotometrically at 239 nm using UV spectrophotometer. The in vitro release media was continuously stirred at 100 rpm and maintained at 37 ± 2^0 C. The release profiles were then fitted into different exponential equations such as zero order, first order, Higuchi and Peppas-Korsemeyer to characterize the release.

4.5.4.5 Stability Study: Initially for SLN dispersion, a Short-term stability study was carried out at room temp 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 optimized SLNs dispersion and lyophilized SLNs were subjected to stability studies at 2-8 ^{0} C for 3 months while lyophilized SLNs were also kept at room temp for 3 months. All samples studied were stored in brown glass vials in dark. Particle size, Polydispersity Index (PDI), Zeta potential and drug content of these formulations were studied at different time intervals like 1, 2 and 3 months.

4.5.4.6 GI stability study (acid stability study): 1 ml of 0.1 N HCl was added to 1 ml of SLN dispersion. Temperature was maintained at 37 ± 2^0 C. The samples were investigated for the determination of particle size and zeta potential immediately and after 2 hour incubation.

4.6 Results and Discussion:

4.6.1 Partition coefficient study:

Study of partition coefficient of drug in lipids would provide an idea about the entrapment of drug in the SLN. The partition coefficient values obtained between the different melted lipids and distilled water is given in Table 5.1.

Table 4.1. Partition coefficient values of Saquinavir in different lipids.

Sr. No.	Lipid	Ratio of SQ in lipid/ D.W. ($K_{l/w}$)
01	Tristearin (TS)	2.43 ± 0.23
02	Glyceryl Monostearate (GMS)	3.09 ± 0.11
03	Glyceryl behenate (GB)	1.88 ± 0.29
04	Stearic acid (SA)	3.42 ± 0.15
05	Witepsol E 85 (WE 85)	2.95 ± 0.33

The partition coefficient was in the order of SA > GMS > WE > TS > GB. Saquinavir is a highly lipophilic drug with log P value of 4.1. It had shown highest partition coefficient value of 3.42± 0.15 in Stearic acid. Lowest partition coefficient value of Saquinavir was observed in Glyceryl Behenate (1.88 ± 0.29). Witepsol E 85 (WE 85) showed partition coefficient value of 2.95 ± 0.33. WE 85 is a mixture of mono, di and triglycerides and the presence of emulsifiers in WE 85 may have played role in the solublization of SQ in the lipid matrix leading to good partition. The prerequisite to obtain a sufficient loading capacity is a sufficiently high solubility of the drug in the lipid melt. Further, the solubility decreases after cooling down the lipid melt and might even be lower in the solid lipid (Muller R.H et al., 2000). Lipids that form highly crystalline particles with a perfect lattice (e.g. monoacid triglycerides) cause drug expulsion (Westsen K et al.1997). More complex lipids (mixtures of mono, di, and triglycerides containing fatty acids of different chain length) form less perfect crystals with many imperfections. These imperfections provide space to accommodate the drugs. Lipophilicity of the glyceride increases as the hydrocarbon chain length increases. Lipophilic drugs are better soluble in lipid melts of longer fatty acid chain lengths (Manjunath K et al, 2005). Stearic acid (SA) and Glyceryl Monostearate (GMS) showed almost similar partition coefficient values i.e. 3.42 ± 0.15 and 3.09 ± 0.11 respectively. Hence they were selected for further study.

4.6.2 Optimization of Parameters:

4.6.2.1 Process parameter:

4.6.2.1.1 Homogenization Cycles:

The effect of number of homogenization cycles on particle size of SLN is shown in Table 4.2. It was observed that increase in number of homogenization cycles from 1st to 3rd cycle resulted in decrease in mean particle diameter from 1.61 ± 0.054 µ to 0.229 ± 0.031µ for GMS SLN and 1.12 ± 0.039µ to 0.175 ± 0.044 µ for SA SLN. Further increase in number of cycle (i.e. after 4th cycle) resulted in increase in mean particle diameter. The reason may be that for each system, there is an optimum pressure and homogenization time until which the lipid nanoparticles undergo a decrease in size and above which the excess cavitation force and exposure of particles to these conditions for longer time leads to particle aggregation (Mehnert W, Mader K, 2001). Different trend was observed for Polydispersity Index (PDI) values. In case of GMS SLN, PDI was decreased from 1st to 3rd cycle but slightly increased after 4th

cycle while in case of SA SLN increase in number of cycles decreased PDI. Hence, optimum number of homogenization cycles resulting in smaller particle size with narrow size distribution was 3 cycles.

Table 4.2 Effect of homogenization cycles on Mean particle diameter and PDI

Sr. No.	No of Homogenization cycles	Particle mean diameter in μ ± S.D.		Polydispersity index (PDI) ± S.D.	
		GMS	SA	GMS	SA
1	1	1.61 ± 0.054	1.12 ± 0.039	0.249 ± 0.028	0.296 ± 0.025
2	2	0.789 ± 0.046	0.684 ± 0.044	0.243 ± 0.022	0.212 ± 0.028
3	3	0.229 ± 0.031	0.175 ± 0.025	0.152 ± 0.016	0.133 ± 0.018
4	4	0.263 ± 33	0.215 ± 0.027	0.191 ± 0.021	0.11 ± 0.02

4.6.2.2 Formulation parameters:

4.6.2.2.1 Selection of emulsifier

The choice of emulsifier and their concentration is of great impact on the quality of SLN dispersion (Muller R. H. et al 2000). The particle size of SQ loaded SLN dispersions was studied with Poloxamer 407, Tween 80 and mixture of Poloxamer 407: Tween 80 at 1:1, 1:3 and 1:5 ratios.

It was observed that Poloxamer 407 and Tween 80 alone could not reduce mean particle diameter below 300 nm. Hence, combination of Poloxamer 407 and Tween 80 at 1: 1 ratio could not reduce particle size significantly. However as the ratio of Poloxamer 407: Tween 80 increased to 1:3, mean particle diameter of GMS SLN reduced to 215 ± 9 nm while that of SA SLN reduced to 180 ± 10 nm. Further increase in ratio to 1:5 led to slight increase in particle size of GMS SLN (261 ± 21 nm) while it was not reduced significantly in case of SA SLN (171 ± 14). Similar to particle size, the PDI value of SLNs was lowered at 1:3 ratio of Tween 80 and Poloxamer 407 (0.198), suggesting narrow size distribution (Table 4.3).

Poloxamer 407 and Tween 80 alone could not reduce mean particle diameter below 300 nm. This may be because of limited mobility of high molecular weight surfactant molecules like Poloxamer and lecithin. Therefore, they are not able to immediately cover the newly created particle surfaces during high pressure homogenization

process. This sudden lack of emulsifier on the surface of the particle leads to particle aggregation and increase in the particle size of SLN [Mehnert W, 2001 and Manjunath K. et al, 2005]. In addition, it has been reported that SLN stabilized with surfactant mixtures - lipoid S75/poloxamer 188 [Zur, Muhlen A. 1996] or tyloxapol /lecithin [Siekmann, B. 1994] have lower particle sizes compared to formulation with only one surfactant.

Kim et al prepared all trans retinol loaded SLNs with surfactant mixture composed of varying ratios of Tween 80 and Egg PC, and investigated the effect of surfactant composition on the size and zeta potential of resultant SLNs. The combination of Tween 80 and Egg PC in 33:67 reduced particle diameter (228 nm) and PDI (0.198) significantly compared to 100% Tween 80 (554 nm) or 100% Egg PC (364 nm). Hence combination of Poloxamer 407 and Tween 80 was tried at different ratio. The combined use of two or more emulsifying agents appears to produce mixed surfactant films at the interface. These mixed surfactants cover the surface efficiently as well as produce sufficient viscosity to promote the stability (Cavalli R. et al 1998).

Table 4.3. Effect of type of emulsifier on Mean particle diameter and PDI

Emulsifier	Mean particle diameter (nm ± S.D.)		Polydispersity index (PDI)	
	GMS SLN	**SA SLN**	**GMS SLN**	**SA SLN**
Poloxamer 407	487 ± 41	415 ± 37	0.326 ± 0.029	0.373 ± 0.033
Tween 80	398 ± 27	351 ± 22	0.251 ± 0.021	0.291± 0.029
Pol 407 : tween 80 (1:1)	359 ± 16	312 ± 21	0.221 ± 0.029	0.210± 0.021
Pol 407 : tween 80 (1:3)	215 ± 9	180 ± 10	0.142 ± 0.017	0.123± 0.011
Pol 407 : tween 80 (1:5)	261 ± 21	171 ± 14	0.193 ± 0.014	0.213± 0.019

4.6.2.2.2 Concentration of emulsifier

The amount of the emulsifier should be optimum to cover the surface of the nanoparticles. Lesser amount of emulsifier results 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 and also toxic effects associated with surfactants.

The mean diameter of SQ loaded SLN dispersion stabilized with 1%, 2 %, 3% and 4 % w/v of Poloxamer 407: Tween 80 (1:3) was studied. As emulsifier concentration

increased from 1% w/v to 3 % w/v, the mean particle size of GMS SLNs decreased from

600 ± 15 nm to 291± 19 nm and mean particle size of SA SLNs decreased from 551± 19 nm to 194 ± 12 nm. Further increase in Poloxamer 407 : Tween 80 ratio did not result in significant improvement in particle size in case of GMS SLNs while in case of SA SLNs a slight increase in particle size was observed (Fig 4.1).

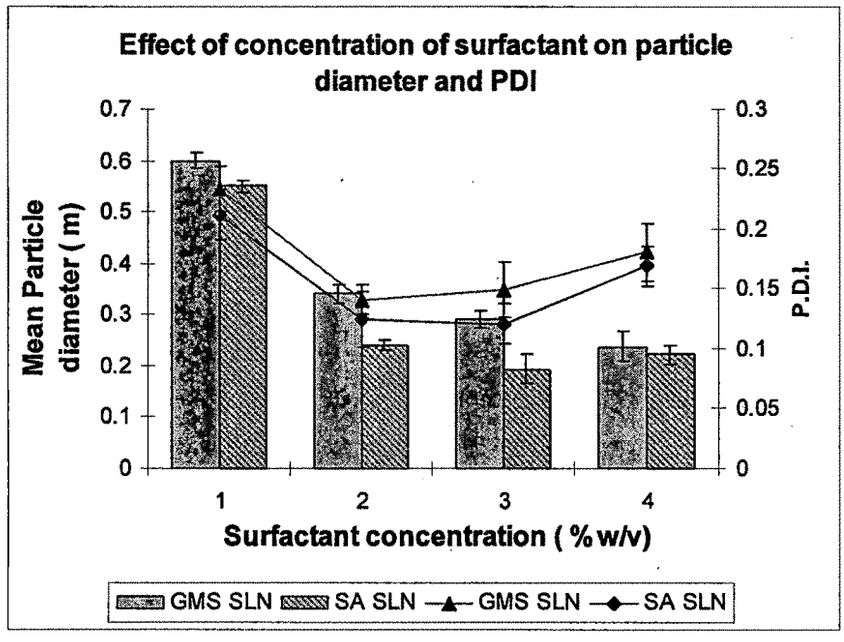


Fig. 4.1. Effect of emulsifier concentration on mean particle diameter and PDI

These results are in agreement with those reported by Rathapon (Rathapon Asasutjarit, 2007) who studied effect of formulation composition on size, zeta potential and potential for in vitro pHIS-HIV-hugag transfection. At the lower level of dimethyl dioctadecyl ammonium bromide (DDAB), the size of SLN slightly decreased with the increase in surfactant (Tween 80: Span 85) content to about 5%. However, at surfactant content above 5%, the size of SLN was increased which could be attributed to the accumulation of excess surfactant molecules at nanoparticle surface.

Hence, the optimum concentration of Poloxamer 407: Tween 80 ratio needed to produce smallest particle size (194 ± 12 nm) of SA SLNs was 3% w/v while that of GMS SLNs was 4% w/v (237 ± 21 nm).

4.6.2.2.3 Concentration of Saquinavir (w.r.t. lipid):

The concentration of SQ was varied between 0.5 %, 1 % and 2 %w/v while keeping the lipid concentration constant at 5 % w/v. The results showed that increase in loading of drug resulted in increased drug entrapment efficiency of the SLN dispersions. The entrapment efficiencies obtained with the different drug loadings are shown in Table 5.4. The maximum drug loading possible was 1 % w.r.t. the lipid. Entrapment efficiency was found to be 79.24 % ± 1.53 in case of SA SLNs and 72 % ± 1.23 in case of GMS SLN. Increase in loading upto 2% led to decrease in entrapment efficiency (SA SLNs- 68 ± 1.46 and GMS SLNs - 77 ± 1.23). This can be attributed to the fact that 1 % drug loading leads to saturation of lipid matrix and higher loading levels resulted in more of free drug rather than drug encapsulated inside the lipid matrix.

The difference in entrapment efficiency may be because of difference in physicochemical properties between these two lipids. GMS consists not less than 90% of monoglycerides while SA is a mixture of Stearic acid and Palmitic acid. Compared with SA, GMS owns more hydrophilic groups that would not allow the lipophilic drug to be accommodated in its bulk. Also, SQ had shown highest partition coefficient value of 3.42± 0.15 in Stearic acid compared to 3.09 ± 0.11 in GMS.

Table 4.4 Effect of drug loading on entrapment efficiency

Sr.No.	Drug loading (% w/w)	Percentage drug entrapped (%) ± S.D.	
		GMS	SA
01	0.5	59.21± 2.98	63 ± 1.88
02	1	72 ± 1.23	79.24 ± 1.53
03	2	68 ± 1.46	77 ± 1.23

Optimized formulation:

Based on the results of partition coefficient study, process and formulation optimization was carried out with SA and GMS. Based on the effect of these parameters on mean particle diameter and entrapment efficiency, Stearic acid was selected as suitable lipid matrix for Saquinavir SLN. Optimized parameters for Saquinavir loaded Stearic acid SLNs are as follows:

Parameters	**Value**
Partition coefficient	Stearic acid and Glyceryl monostearate
Homogenization cycles	3
Selection of emulsifier	Poloxamer 407: Tween 80 (1:3)
Concentration of emulsifier	3 % w/v
Concentration of Saquinavir (w.r.t. lipid)	79.24 ± 1.53

4.6.3 Differential Scanning Calorimetry (DSC):

The crystalline structure of SLNs can be assessed by Differential Scanning Calorimetry (DSC). DSC curves of Saquinavir, Bulk SQ, Bulk Glyceryl Monostearate, SQ loaded Stearic acid SLNs (SQSLN), SQ loaded GMS SLNs (SGMLN) are given in Fig 4.2. The DSC curve of SQ showed melting endotherm at 250.02 ^{0}C. Thermogram of Stearic acid showed a small endothermic peak at 56.36^{0}C. In case of thermogram of SQ loaded Stearic acid SLNs (SQSLN), small peak was observed at 49.37^{0}C which was slightly lower than peak obtained in thermogram of Stearic acid. The DSC curve of GMS bulk showed endothermic peak at 66.5 ^{0}C while it was lowered to 59.23 ^{0}C in case of SQ loaded GMS SLNs (D). The possible reason behind the reduction in melting point (m.p.) and enthalpy of lipid might be the change in crystal lattice of the lipid after incorporation of SQ and formulation as nanoparticles. Freitas et al observed that crystallization behaviour of Compritol SLN differed distinctly from that of the bulk lipid (Freitas C. and Muller R.H. 1999). These changes were due to the increased number of lattice defects in the lipid crystal. Small particle size of SLN created an energetically high suboptimal state which leads to decrease in the melting point. Absence of SQ peak near its m.p. in thermogram of SQSLN indicates that drug might be entrapped in the lipid matrix.

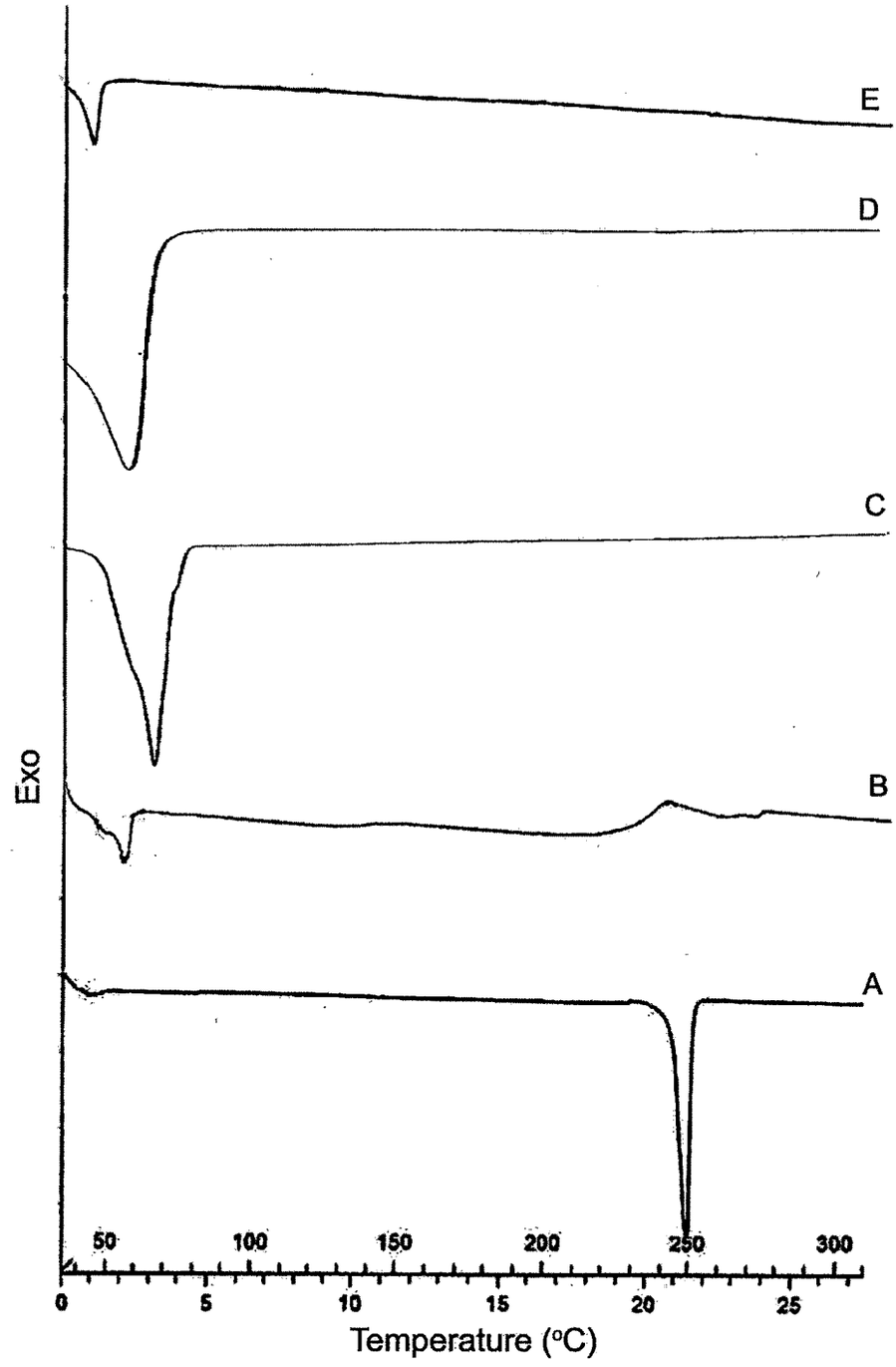


Fig. 4.2 DSC thermogram of Saquinavir (A), Stearic acid (B), Glyceryl monostearate (C), SQ loaded GMS SLNs (D), SQ loaded lyophilized SLNs - SQSLN (E)

5.6.4 X ray diffraction study:

X ray diffraction has wide applications in the study of crystal forms such as polymorphs, solvates and salts. In the present study, comparison of XRD patterns was done by considering the relative intensities of the diffracted peaks at a particular θ and positioning of relative peaks in different samples. X ray diffractogram of Bulk Saquinavir, Stearic acid, SQSLN and sucrose are shown in Fig. 4.3. The XRD pattern of SQ (Fig.4.3A) exhibited peaks at 2θ angle 6.061, 16.179, 18.716 and 19.881. The lipid (Stearic acid) showed peaks at 2θ angle 21.5 and 23.8 (Fig 5.3 B). In case of SQSLN (Fig.5.3 C), the characteristic peaks were observed at 2θ angle 18.873, 19.619, 24.774 and 20.98. XRD spectra of sucrose revealed principal peaks at 2θ angle 18.8, 24.74, and 25.22.

In XRD spectra of SQSLN, reduction in the intensity of characteristic peaks of SQ was observed which may be attributed to the incorporation of SQ in between the crystal lattice of the lipid leading to decrease in the crystallinity of SLN. This shows change in the crvstallinity of SQ after incorporation into the lipid matrix.

Also, the characteristic peaks of lipid did not shift in XRD spectra of SQSLN. One of the characteristic peak in SQSLN (at 2θ angle 24.774) which resemblance to characteristic peak obtained in XRD spectra of sucrose (at 2θ angle 24.74) which was used as cryoprotectant for lyophilization of SQSLN.

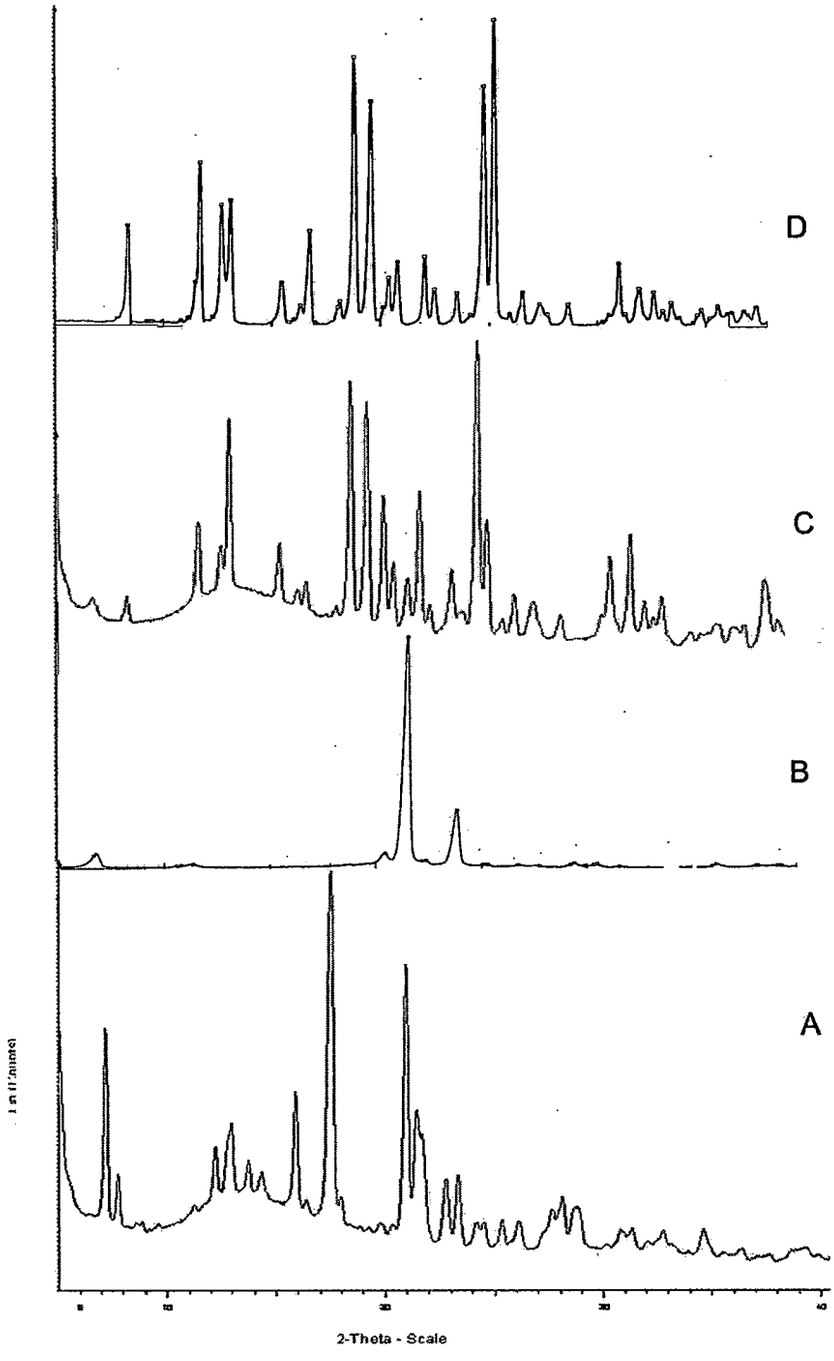
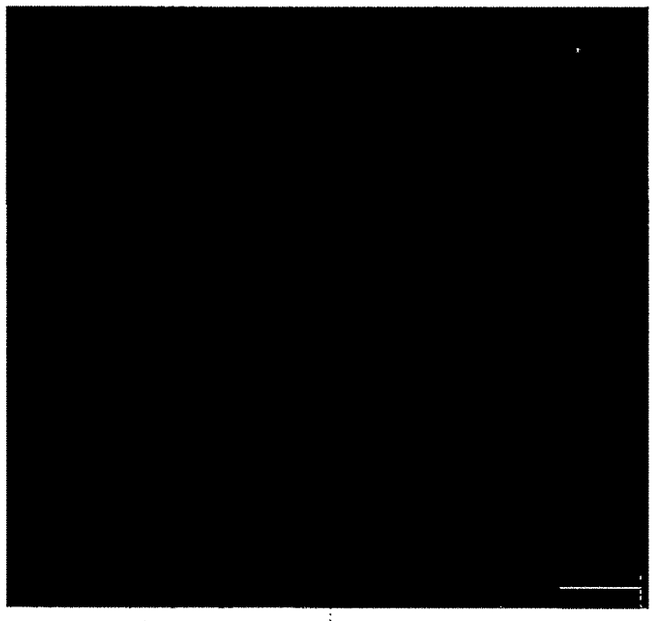


Fig. 4.3 X ray diffraction spectra of SQ (A), Stearic acid (B) and SQ loaded SLN (C), sucrose (D)

4.6.5 Transmission electron microscopy (TEM)

TEM image reveals that particles are of spherical shape. Surface of particle is found to be smooth and nonporous. Particles are homogenously distributed and aggregation was not observed.



A)

B)

Fig. 4.4 A) TEM image of SQ loaded SA SLN (bar indicates 500 nm);
B) TEM image of a single particle (bar indicates 100 nm)

4.6.6 In vitro release:

The release of active substance from SLN is influenced by different factors such as method of preparation, solubility of drug in the lipid, drug/lipid interactions, temperature employed during the preparation, surfactant used, composition of lipid matrix and particle size (Almeida AJ, Souto E, 2007 , Haynes CA, Norde W. 1994). In the present study, in vitro release of SQ loaded Glyceryl Monostearate SLN (SGMLN) and SQ loaded Stearic Acid SLN (SQSLN) was carried out in three media i.e. 0.1 N HCl, Phosphate buffer pH 4.5 and pH 7.2.

pH of the medium was found to have drastic influence on the release of the drug. The release rate was found to be in the decreasing order of 0.1 N HCl < pH 4.5 < pH 7.2. It was observed that release was faster in the case of acidic media (pH 2), which may be attributed to the acid facilitated lysis of lipid matrix. 93.01 % ± 3.02 released from SQSLN in 48 hrs and 96.69 % ± 4.51 released from SGMLN in 24 hrs. Eventually, the neutral media (pH 7.2) was found to retard the drug release maximally (97.23 % in 72 hrs from SQSLN and 98.21 % in 36 hours from SGMLN). The results are given in Fig. 4.5. and 4.6.

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). Slow diffusion of drug from the lipid matrix might be attributed to the prolonged release in the later stage. Release of SQ from SGMLN was found to be faster than SQSLN in all the media. The difference in release pattern may be because of difference in physicochemical properties between these two lipids. GMS consists not less than 90% of monoglycerides while SA is a mixture of Stearic acid and Palmitic acid. Compared with SA, GMS owns more hydrophilic groups that would not allow the lipophilic drug to remain in its bulk during in vitro release process.

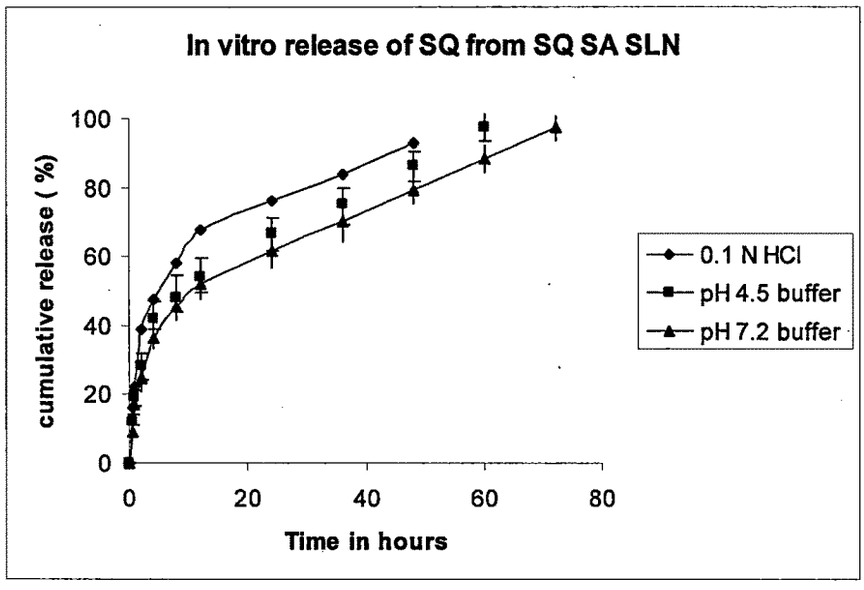


Fig. 4.5 In vitro release of SQ from SQSLN

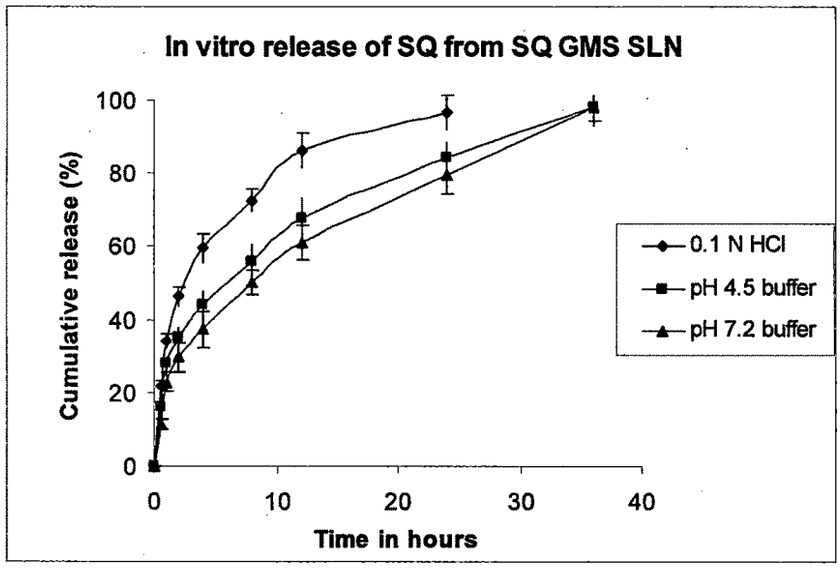


Fig. 4.6 In vitro release of SQ from SQMLN

The release profiles were then fitted into different exponential equations such as zero order, first order, Higuchi and Peppas-Korsemeyer and best fit was determined. The values of R^2, k and n are listed in Table 4.5.

When n approximates to 0.5, a Fickian/diffusion-controlled release is implied, where $0.5 < n < 1.0$, non-Fickian transport and $n = 1$ for zero order (case II transport). When the value of n approaches 1.0, phenomenologically one may conclude that the release is approaching zero order. The goodness of fit for various models investigated for SQSLN and SGMLN ranked in the order of Higuchi ≈ Peppas > zero order > first-order. All the kinetic models, other than the first order, fitted well. The values of diffusional exponent 'n', obtained from the slopes of the fitted Korsemeyer–Peppas model, ranged between 0.368 to 0.424 in case of SQSLN and 0.378 to 0.462 for SGMLN indicating fickian behaviour.i.e drug release is controlled by diffusion.

Table 4.5. In vitro release kinetics of SQSLN and SGMLN

Sample	Media	Kinetic Models					
		Zero order	First order	Higuchi		Peppas	
		r^2	r^2	r^2	h^{-1}	r^2	n
SQ SA SLN	0.1 N HCl	0.797	0.604	0.956	9.314	0.954	0.368
	pH 4.5 buffer	0.890	0.668	0.973	11.03	0.972	0.401
	pH 7.2 buffer	0.894	0.654	0.976	10.29	0.968	0.424
SQ GMS SLN	0.1 N HCl	0.802	0.646	0.943	17.75	0.971	0.378
	pH 4.5 buffer	0.894	0.705	0.984	14.82	0.979	0.394
	pH 7.2 buffer	0.926	0.697	0.992	15.5	0.973	0.462

4.6.7 Stability Study:

4.6.7.1 Short term stability study:

Initially for optimized 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 given in fig. 4.7.

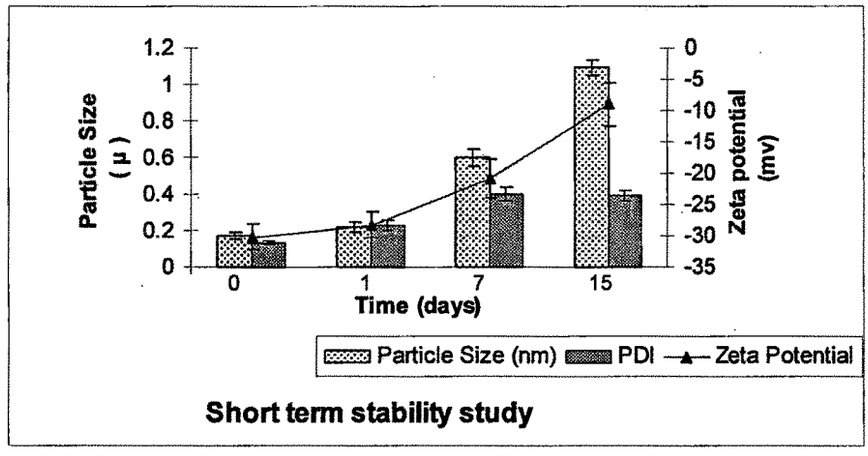


Fig. 4.7. Short term stability study of SQ Stearic acid SLN dispersion stored at

SLN dispersion kept at room temp. for 15 days showed increase in particle size upto 1.091 ± 27 nm (PDI – 0.391) while Zeta potential reduced from -30.21 mv to -9.02 mv. It indicates that prepared SLNs dispersion was not stable enough at R.T. Hence, further stability study was carried out by keeping SLN dispersion and Lyophilized SLN for 3 months.

4.6.7.2 Long term

Temperature corresponds to energy input and can lead to changes in the crystalline structure of lipids (Beatrice H. et al 2003). Effect of temperature on characteristics of SLN dispersion and lyophilized SLNs was studied. SLN dispersion was kept at 2-8 ^{0}C while lyophilized SLNs were kept at 2-8 ^{0}C and RT for 3 months. Samples were studied after 1, 2 and 3 months and studied for Particle Size, drug content, Polydispersity index, and Zeta Potential.

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

After 3 month of storage at 2 – 8 ^{0}C, particle size of SLN dispersion increased to 0.629 μ (PDI – 0.131) while zeta potential decreased to -10.33 mv (fig 4.8) . In case of Lyophilized SLNs, particle size was 0.409 μ (PDI -0.342) while zeta potential was 19.99 mv (Fig 4.9). The increase in particle size after 3 months storage was less pronounced in lyophilized SLNs (0.409 μ) compared to SLN dispersion (0.629 μ). Also, zeta potential decreased to lower level in SLN dispersion (-10.33 mv) compared to lyophilized SLNs (-19.99 mv).

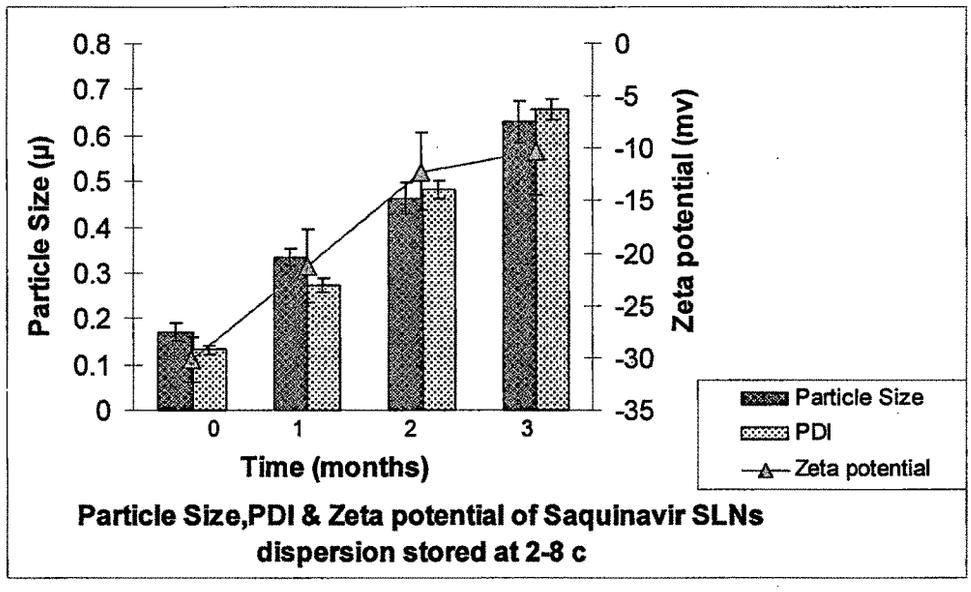


Fig. 4.8 SQ SLN dispersion stored at 2 – 8 ^{0}C

Freitas et al observed effects of the temperature on compritol SLNs by storing them at 8^{0}C, 20^{0}C, and 50^{0}C. Dispersions stored at 20^{0}C showed improved stability than those stored at 50^{0}C (Freitas, C., and Muller, R.H., 1998). Increase in temperature causes a decrease in microviscosity (less rigidity of emulsifier film), leading to destabilization.

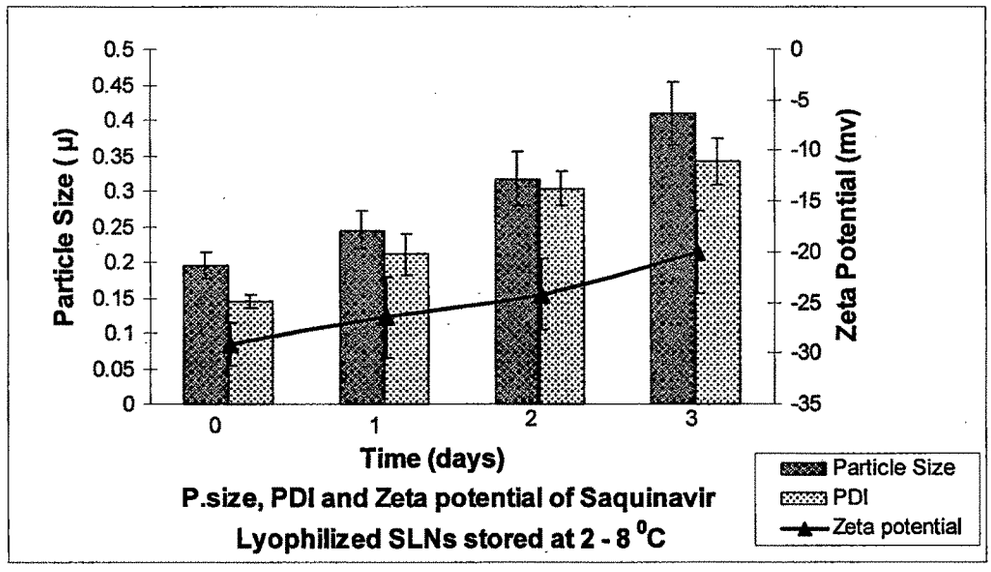


Fig. 4.9 Saquinavir lyophilized SLNs stored at 2 – 8 ^{0}C

Drug content of SLN dispersion decreased from 79.24 % to 44.33 % while that of lyophilized SLNs decreased from 76.24 % to 51 % after storage at 2- 8 ^{0}C for 3 months. This may be due to expulsion of the drug from particle matrix because of recrystallization of lipid (Westesen K. et al 2000). Drug expulsion during polymorph transition was explained by a reduction of amorphous regions in the carrier lattice due to polymorphic transition (Gohla SH et al 2000).

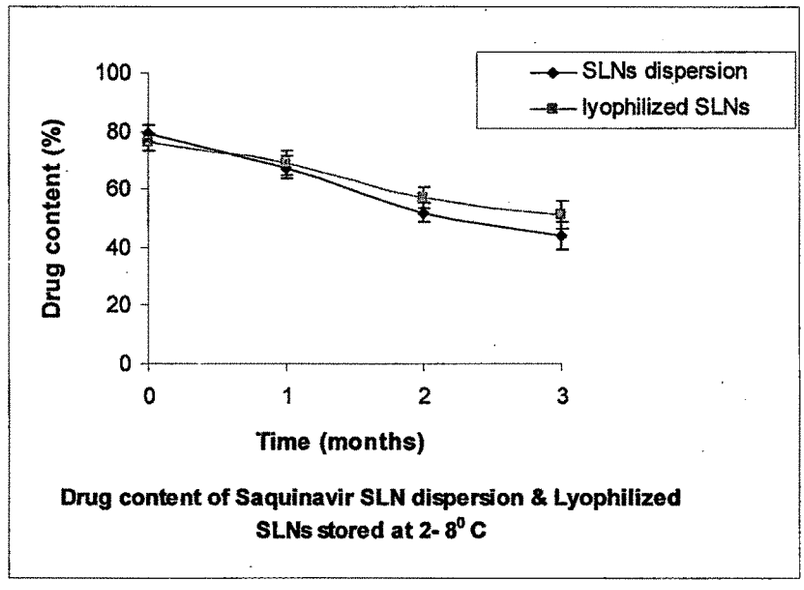


Fig. 4.10 Drug content of Saquinavir SLNs dispersion and lyophilized SLNs stored at 2 – 8 ^{0}C

4.6.7.2.2 Samples stored at RT:

After 3 month of storage at RT, particle size of **Lyophilized SLNs** increased to 0.665 μ (PDI – 0.333) while zeta potential decreased to -13.01 and drug content was 50.77 % (fig 5.11 and 5.12). As the particle size of SLN dispersion stored at room temperature increased above 1 μ, further study was discontinued.

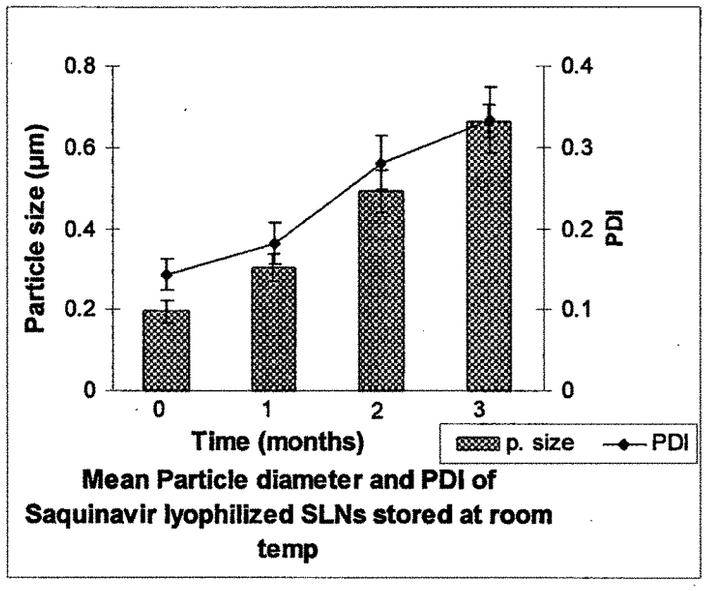


Fig. 4.11 Mean Particle diameter and PDI of Saquinavir lyophilized SLNs stored at room temp.

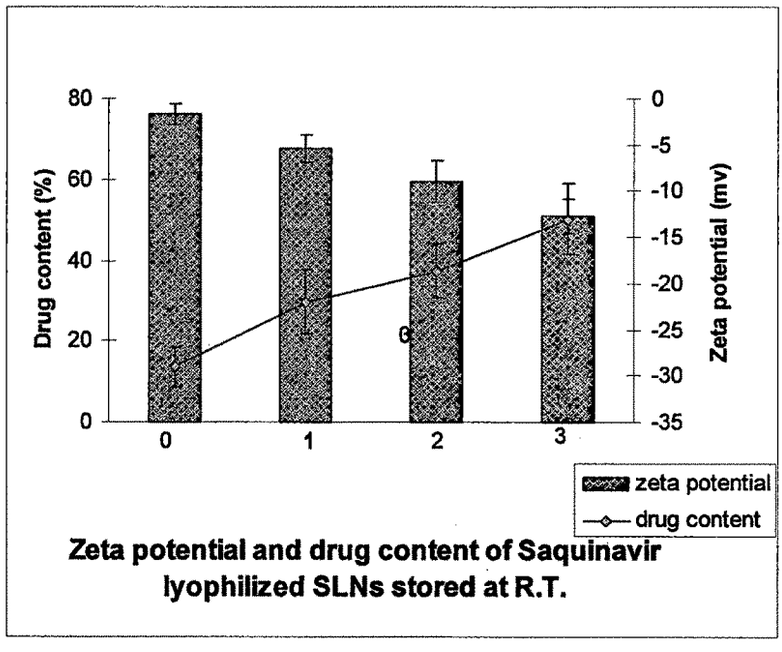


Fig. 4.12 Zeta potential and drug content of Saquinavir lyophilized SLNs stored at R.T.

Conclusion of stability study:

Effect of temperature on SLN dispersion and lyophilized SLN was studied. Lyophilized SLNs can be stored at 2 – 8 ^{0}C and room temperature while SLN dispersion should be stored at 2 – 8 ^{0}C and not on room temperature.

4.6.8 GI stability study (acid stability study):

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 281 ± 23 nm immediately from initial particle size of 173 ± 13 nm. After 2 hour incubation, particle size was increased to 434 ± 32 nm. Zeta potential was decreased to -10.22 ± 2.5 mv from initial value of -28.09 ± 0.93 mv.

Sampling Interval		**Mean particle diameter ± S.D. (nm)**	**Zeta potential (mv)**
Before incubation		173 ± 13	- 28.09 ± 0.93
After incubation	**Immediately**	281 ± 23	-22.34 ± 2.32
	After 2 hours	434 ± 32	-10.22 ± 2.5

Table 4.6. Effect of Gastric media (0.1 N HCl) on Mean particle diameter and Zeta potential

Saquinavir loaded SLN formulation was found to be unstable in terms of particle size after 2 hrs of incubation although formation of microparticles or aggregates was not observed. Tween 80 might have played a role in preventing aggregation. Exposure to acidic media led to an increase in size and reduction in zeta potential of SLNs.This may be due to compression of the electric double layer by the ionic medium which in turn increased the particle size. However, the particles were still in nanorange and remained nonaggregated.

Reference:

- Almeida AJ, Souto E. Solid lipid nanoparticles as a drug delivery system for peptides and proteins. Adv Drug Del .Rev. 2007; 59: 478-490.
- Cavalli R, Caputo O, Marengo E, et al. The effect of components of microemulsions on both size and crystalline structure of solid lipid nanoparticles (SLN) containing a series of model molecules. Pharmazie .1998; 53:392-396.
- Freitas C, Muller RH. Co-relation between long-term stability of solid lipid nanoparticles (SLNs) and crystallinity of the lipid phase European Journal of Pharmaceutics and Biopharmaceutics .1999; 47: 125–132.
- Freitas C, Muller RH. Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticle (SLNTM) dispersions. Int. J Pharm. 1998; 168: 221-229.
- Haynes C.A, Norde W. Globular proteins at solid/liquid interfaces. Colloids Surf B Biointerfaces .1994; 2517-2566.
- Lai Francesco, Wissing SA, et al. Artemisia arborescens L Essential oil–loaded solid lipid nanoparticles for potential agricultural application: preparation and characterization. AAPS Pharm Sci Tech 2006; 7 (1): E1-E9.
- Manjunath K, Reddy J, Venkateswarlu V. Solid Lipid Nanoparticles as Drug Delivery Systems. Methods Find Exp Clin . Pharmacol. 2005; 27(2): 1–20.
- Mehnert W, Mader K. Solid lipid nanoparticles: production, characterization and Applications. Adv Drug Deli .Rev . 2001;47(2-3): 165-196.
- Muller R.H, Mehnert W, et al. Solid lipid nanoparticles (SLN) - An alternative colloidal carrier system for controlled drug delivery. Eur. J. Pharm. Biopharm.1995; 41: 62–69.
- Muller RH, Mader KS, Gohla. Solid lipid nanoparticles (SLN) for controlled drug delivery-a review of the state of the art. Eur J Pharm Biopharm. 2000; 50:161-177.
- Rathapon A, Sven-Iver L, Sunee S, et al. Effect of Solid Lipid Nanoparticles Formulation Compositions on Their Size, Zeta Potential and Potential for In Vitro pHIS-HIV-Hugag Transfection. Pharmaceutical Res. 2007; 24(6), 1098 - 1107.
- Siekmann B, Westesen K. Pharma. and Pharmaco. Letters. 1994; 3: 94-197.
- Westesen K, Bunjes H, Koch MHJ. Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential. J Control Release. 1997; 48: 223-236.