6. DEVELOPMENT OF PRAZIQUANTEL LOADED BINARY SOLID LIPID NANOPARTICLES (PZQ-BSLN)

6.1. Preparation of PZQ-loaded SLN

Praziquantel loaded binary solid lipid nanoparticles (PZQ-BSLN) were prepared by hot homogenization followed by ultrasonication method in the same way as single lipid nanoparticle discussed in previous chapter.

6.2. Formulation design

In SLN, drug molecules stay in between the fatty acid chains or as amorphous clusters in crystal imperfections within SLN matrix. But, when lipid transform to low-energetic form, it forms a perfect crystalline lattice that allows very small space for the drug molecules. Therefore, expulsion of encapsulated drug molecules may be observed during storage, especially when SLN matrix is composed of a highly purified lipid, which leads to limited drug-loading capacity of SLNs (Das *et al.*, 2011; Muller *et al*., 2011; Westesen *et al*., 1997). Therefore, the EE, DL, physical stability and drug release kinetics of SLNs may change with storage time.

6.2.1. Optimization of binary lipid composition

To study the effect of lipid matrix composition on the characteristics of SLN, the binary lipid nanoparticles (BSLN) (containing combination of two lipids of different type as matrix material) were formulated. The binary lipid matrix was prepared with addition of parts of monoglyceride (25-100 % w/w) by replacing same parts (w/w) of triglycerides (Tabl6.1). The combination of two lipids may result in deformation in

crystal order of lipids. The GMS (monoglyceride) is a polar lipid and also exhibits surfactant property. Moreover, the solubilising capacity of GMS was more than the diglyceride (GB). The other formulation conditions were similar to the conditions to prepare single lipid SLN in previous chapter and were as follows: HT = 5 min, ST = 10 min, VF = 50ml, LSC = 2.5% (w/v), HSC = 2.5% (w/v) LC = 5% (w/v), DC = 0.05% (w/v), lipophilic surfactant = lecithin granular and hydrophilic surfactant = pol.188.

Table 6.1: Optimization of binary lipid composition for PZQ-BSLN

Code	**TP (%w/w of total lipid)**	**GMS (%w/w of total lipid)**	**LG (%w/v)**	**P188 (%w/v)**	**DC (%w/v)**
$TP_{75}GM_{25}SLN$	75	25	2.5	2.5	0.05
$TP_{50}GM_{50}SLN$	50	50	2.5	2.5	0.05
$TP_{25}GM_{75}SLN$	25	75	2.5	2.5	0.05
$TP_{0}GM_{100}SLN$	0	100	2.5	2.5	0.05

6.2.2. Effect of binary lipid composition on characterizing parameters of BSLN

Increasing the GMS amount by replacing the triglyceride within lipid matrix increased PS and PI of the SLN. Replacing the TP with 25% GMS resulted in enormous increase in EE.

Table 6.2: Effect of binary lipid composition on characterizing parameters of BSLN

Formulation code	**Lipid (%w/w) (TP:GMS)**	**Particle Size(nm)**	**Polydispersity index (PI)**	**Zeta potential (-mV)**	**Entrapment Efficiency (%EE)**
$TP_{75}GM_{25}SLN$	75:25	102.5 ± 4.07	0.263 ±0.038	12.0 ±0.71	92.4 ±0.64
$TP_{50}GM_{50}SLN$	50:50	134.6 ±5.18	0.382 ±0.041	12.2 ±0.58	88.3 ±0.72
$TP_{25}GM_{75}SLN$	25:75	148.9 ±5.83	0.417 ±0.053	12.4 ±0.43	85.7 ±0.68
$TP_{0}GM_{100}SLN$	0:100	169.7 ±6.24	0.494 ±0.054	12.5 ±0.54	80.3 ±0.53

(mean ±SD; n=4**)**

However, further increasing the GMS amount in lipid matrix resulted in decreased EE (Table 6.2). This may be due to presence of excess surfactant concentration above the critical concentration as GMS also exhibit surfactant property. The zeta potential of all the formulation was around -12 mV.

From the results obtained, the optimized lipid composition TP: GMS= 75:25(%w/w) was found suitable for further optimization of BSLN.

6.2.3. Optimization of drug concentration

After deciding the binary lipid composition, the drug concentration was optimized to produce SLN of desired particle size and entrapment efficiency according to following formulation design (Table 6.3).

Table 6.3: Optimization of drug concentration for PZQ-BSLN

Code	**TP (%w/w of total lipid)**	**GMS (%w/w of total lipid)**	**LG (%w/v)**	**P188 (%w/v)**	**DC (%w/v)**
BSLN(0.05)	75	25	2.5	2.5	0.05
BSLN(1.0)	75	25	2.5	2.5	0.1
BSLN(2.0)	75	25	2.5	2.5	0.2

6.2.4. Effect of drug concentration

The PS and PI of the SLN significantly increased with increasing drug concentration (0.05–0.2% w/v). EE significantly decreased with increasing DC whereas ZP was around -12 mV in all cases (Table 6.4). The decrease in EE could be due to increase in drug to lipid ratio with increasing DC at fixed amount of lipid, which resulted in higher un-encapsulated drug and lowered EE.

Table 6.4: The effect of drug concentration on characterizing parameters of BSLN

DC (%w/v)	Particle Size (nm)	Polydispersity index (PI)	Zeta potential (-mV)	Entrapment Efficiency (%EE)
0.05	102.5 ± 4.07	0.263 ±0.038	12.0 ±0.71	92.4 ±0.64
0.1	108.9 ±7.63	0.383±0.051	12.8±0.57	86.8±1.14
0.2	161.7± 8.43	0.391±0.067	12.9±0.65	79.6±1.26

mean ±SD; n=4

From the results discussed above, the optimized formulation conditions for the preparation of PZQ BSLN were decided as follows: HT = 5 min, ST = 10 min, VF = 50ml, LSC = 2.5% (w/v), HSC = 2.5% (w/v) LC = 5% (w/v), DC = 0.1% (w/v), lipophilic surfactant = lecithin granular, hydrophilic surfactant = pol.188 and the lipid matrix composition (TP:GMS) = 75:25(% w/w).

6.2.5. Storage stability

The ability of the BSLN to keep its physicochemical properties during storage was assessed at refrigerated conditions (5±3°C) as well as at 25 °C/65% RH for six months. The stability was evaluated in terms of mean particle size, surface charge (ZP) and entrapment efficiency.

After six months storage of BSLN at refrigerated conditions, there was insignificant difference in the PS for BSLN ($p<0.05$); while in case of six months storage at 25 °C/65% RH, the particle size was increased

significantly from the 108.9 ± 7.63 nm to 119.4 ± 4.92 nm for BSLN ($p<0.05$)(Table 6.5).

The EE (%) of the optimized BSLN was initially found to be 86.8 ± 1.14 which was significantly decreased to 82.7 ± 0.52 after six months of storage at refrigerated conditions ($p<0.05$). The significant decrease in EE (%) was also observed when stored at 25 °C/65% RH for 6 months and found to be 83.2 ± 0.49, which may be due to the expulsion of drug from lipid matrix during storage($p<0.05$) (Table 6.5).

Table 6.5: Effect of storage at refrigerated conditions (5±3°C) and at 25 °C /65% RH on characterizing parameters of BSLN

Particle Size(nm)			**Entrapment Efficiency (%)**		
(Initially	**After 6 months**		**Initially**	**After 6 months**	
	5±3°C	**25±1°C**		**5±3°C**	**25±1°C**
108.9 ±7.63 m	115.6 ± 5.71	119.4 ± 4.92	86.8±1.14	82.7±0.52	83.2±0.49

mean ±SD; n=4

6.2.6. The effect of pH on the stability

The effect of pH on the stability of PZQ BSLN was found to be remarkable (Table 6.6). The BSLN kept in SGF (pH 1.2) for 2 hours showed significant increase in PS along with decrease in EE($p<0.05$), where as BSLN kept for 6 hours in SIF (pH 6.8) were found to be stable. The effect of pH conditions on characterizing parameters was similar to single lipid SLN.

Table 6.6: Stability studies of various PZQ-BSLN at different pH conditions

Particle Size(nm)			**Entrapment Efficiency (%)**		
Initially (0hr)	pH 1.2 (2 hr)	pH 7.4 (6 hr)	Initially (0hr)	pH 1.2 (2 hr)	pH 7.4 (6 hr)
108.9 ±7.63	412.2±4.37	133.7±3.94	86.8±1.14	39.6±0.73	83.6±0.96

(mean ±SD; n=4)

6.2.7. The effect of sterilization on the stability of BSLN

The BSLN for parenteral administration should be sterile. The sterilization by filtration cannot be applied in this case as particle size may change due to pressure applied in the filtration processes. In the present study, the effect of moist heat sterilization on particle size and EE was compared with non sterilized formulation and the results are presented in Table 6.7.

In addition to variations in particle size, sterilization also resulted in reduction of less than 7% in PZQ content (Table 6.7). This feature of SLN may in turn be related to the higher resistance to temperature of the complex surfactant layer consisting of lecithin and poloxamer 188. Moreover, the presence of GMS further stabilized the complex surfactant layer. From the results, it can be seen that inclusion of GMS as lipid matrix along with lecithin and poloxamer 188-stabilized BSLN did not induce significant variations in nanoparticulate properties during sterilization by autoclaving.

Table 6.7: Effect of sterilization on characterizing parameters of PZQ-BSLN

Particle Size(nm)		**Entrapment Efficiency (%)**	
Before Sterilization	After Sterilization	Before Sterilization	After Sterilization
108.9 ±7.63	413.7 ±20.86	86.8±1.14	79.4 ±1.48

(mean ±SD; n=4)

6.2.8. *In-vitro* drug release studies

The *in vitro* release of the different SLN formulations was determined in 0.1 N HCl (for 2 hours) and in phosphate buffer (pH 6.8) (for 48 hours).

The results revealed that BSLN also released a high amount of drug during passage through the strong acidic environment of the stomach (as in 0.1 N HCl). BSLN released around 70.6% of PZQ in its stay for 2 hours in 0.1 N HCl (Figure 6.1). This indicated its susceptibility to with stand the strong acidic environment which has been also seen with single lipid SLN.

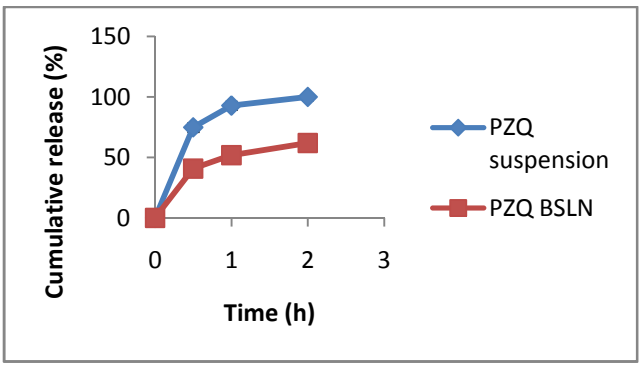

Figure 6.1: *In vitro* release studies at different time intervals of PZQ-loaded BSLN in 0.1N HCl (pH 1.2) as dialysis medium (mean ±SD; n=4).

PZQ-BSLN formulation was found to release the PZQ in a controlled manner in phosphate buffer (pH 6.8). The maximum release up to 4 hours was 29.2 % (Figure 6.2). It can be observed that the release from binary SLN was comparatively more in comparison to single lipid SLN. In case of BSLN, some amount of the drug is residing in the deformed crystal lattice and may be adsorbed on the SLN surface. It can also be observed that after initial burst release the BSLN showed the controlled release.

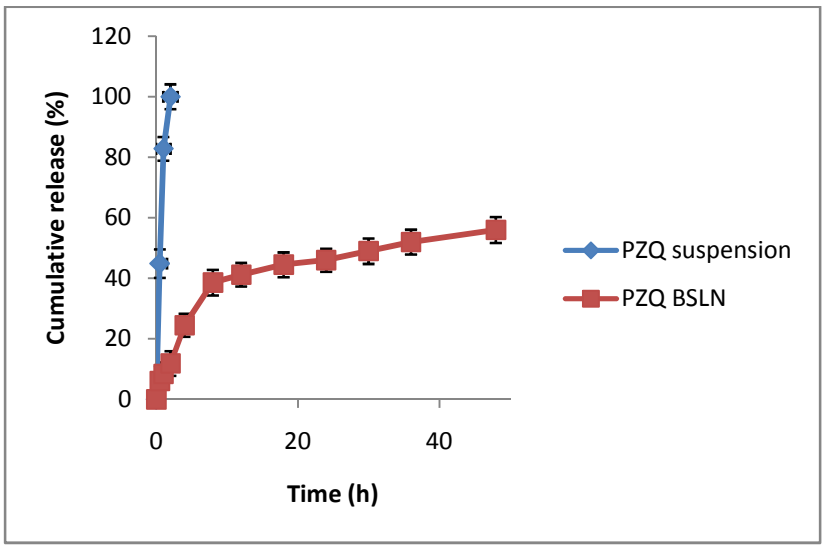

Figure 6.2: *In vitro* release studies at different time intervals of PZQ-BSLN in phosphate buffer (pH 6.8) as dialysis medium (mean ±SD; n=4).

6.2.9. Release kinetics study

Table 6.8: The *in-vitro* release kinetics model of PZQ-BSLN in phosphate buffer (pH 6.8) as dialysis medium.

Batch Code	First order (R^2)	Higuchi model (R^2)	Korsmeyer-Peppas Model (R^2)	Weibull (R^2)
BSLN	0.758	0.909	0.949	0.842

It can be seen from table 6.8 that best linearity was found in Korsmeyer-Peppas Model, indicating the drug release from lipid matrix is through diffusion and erosion. However, the value of 'n' obtained using Peppas equation indicates that it should follow non-fickian or analomous release pattern. It suggests that PZQ is released from BSLN by diffusion as well as through lipid erosion. The n value is near to 0.5, therefore drug release by diffusion is predominant release kinetic mechanism than erosion. The results are in agreement with the finding of previous studies (Hu *et al*., 2005; Costa and Lobo, 2001; Brigger *et al*., 2002)

6.2.10. Shape and morphology

The binary lipid nanoparticles were viewed through Transmission electron microscopy (TEM) for their shape and morphology. The TEM image of PZQ-BSLN is shown in Figure 6.3. The TEM revealed that particles were almost spherical with smooth surface morphology with

unimodal distribution. These morphology results are in agreement with the particle size data determined by PCS (Table 6.4).

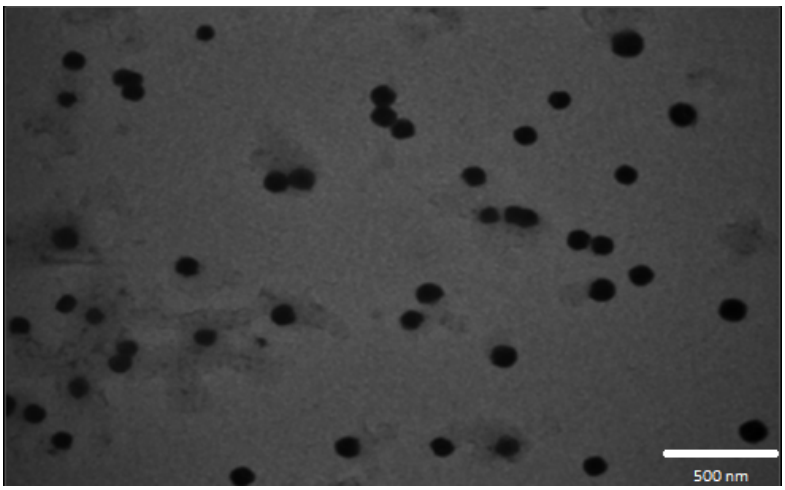

Figure 6.3: Transmission electron micrographs (TEM) of PZQ-BSLN

6.2.11. Differential scanning calorimetry

Figure 6.4 gives an overview of the melting process of pure PZQ, pure lipids and binary lipid nanoparticles. Addition of GMS in TP resulted in depression of melting point. This depression in melting point could be due to the small size of nanoparticles, which significantly increases the surface area or due to adsorption of an amphiphilic molecule (stabilizer) on particle surface (Bunjes and Koch, 2005; Muhlen *et al*., 1998; Schubert *et al*., 2005; Reddy *et al*., 2005). Nevertheless, the melting point reduction of the different formulations has no apparent relation to the particle size. In conclusion, this result indicates that apart from the particle size an additional process influences the SLN melting point of the dispersed binary lipid phase such as presence of drug molecules in the lipid matrix which influences lipid layer organization and crystal lattice.

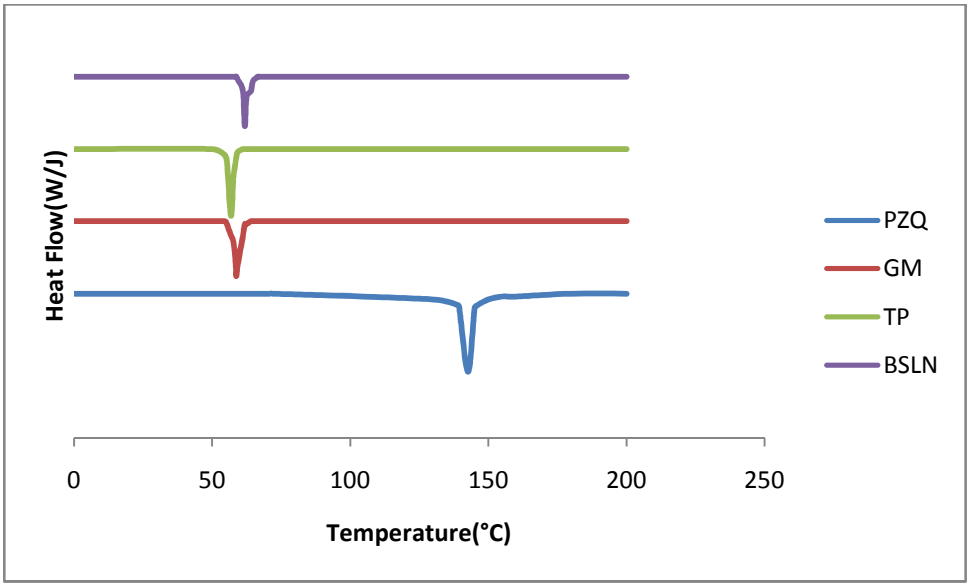

Figure 6.4: DSC thermograms of (A) Praziquantel, (B) Gleceryl monostearate, (C) Tripalmitin, (D) Praziquantel loaded BSLN.

6.2.12. X-Ray diffractometry (XRD) study

In case of binary SLN also, the principle peak of PZQ disappeared. The intensity of peaks also reduced with addition of GMS (Figure 6.5). This may be due to the incorporation of GMS into the crystal lattice of the single lipid (TP), leading to a change in the crystallinity of the lipid. In addition PXRD spectra obtained between 2 θ scattered angles=18–25◦, where the bulk lipids had sharp peaks that were almost absent in the diffractograms of the BSLN (Figure 6.5). This indicates lower crystallinity and hence, the less ordered crystal arrangements in the BSLN formulations compared to the bulk solid lipid, such an amorphous state, resulted in the higher drug loading capacity (Hau *et al.*, 2003, Chattopadhyay *et al.*, 2007).

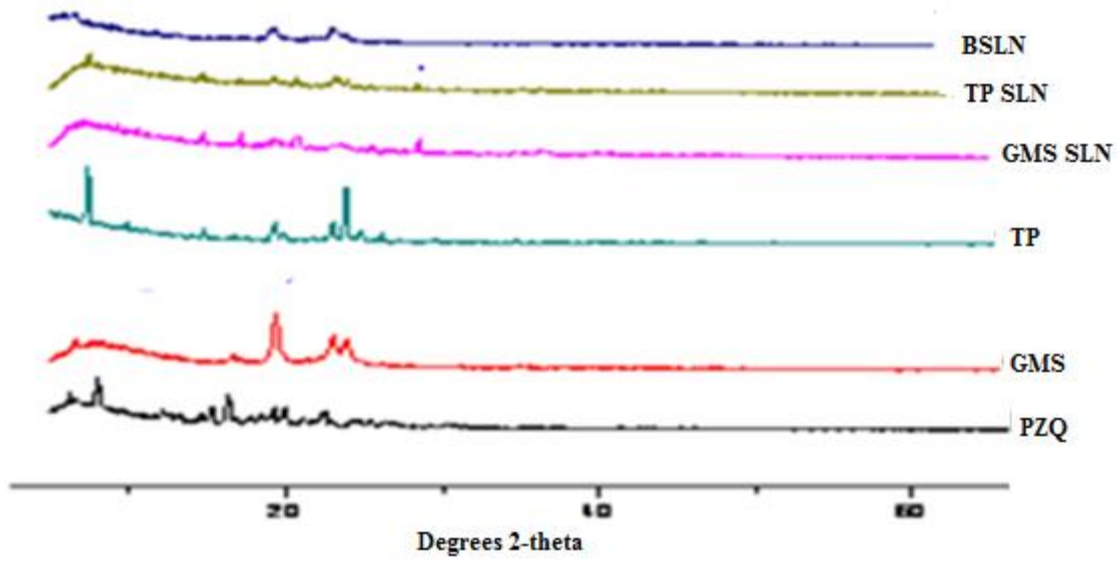

Figure 6.5: Overlapped XRD patterns of (A)Praziquantel(PZQ), (B)Glyceryl monostearate(GMS), (C)Tripalmitin (TP),(D) Praziquantel loaded GMS-SLN,(E) Praziquantel loaded TP-SLN, (F) Praziquantel loaded binary SLN(TP:GMS:75:25).