5. DEVELOPMENT OF PRAZIQUANTEL LOADED SOLID LIPID NANOPARTICLES (PZQ-SLN)

5.1. Objective

PZQ is practically insoluble in water and exhibits extensive hepatic first-pass metabolism. It also suffers with great pharmacokinetic and therapeutic variability after its oral administration**.** These shortcomings necessitate its frequent administration and limit its use. The objective of this work was to develop solid lipid nanoparticles or nanoparticulate syste suitable for targeting intestinal lymphatic system, which in turn could enhance its therapeutic efficacy as well as reduce dose and dosage regimen.

5.2. Materials

Praziquantel (PZQ) and Glyceryl monostearate (GMS) were kind gifts from Wockhardt Research Centre, India. Tripalmitin (TP) and tristearin (TS) were purchased from SRL, India. Poloxamer 188 (P188) and Poloxamer 407 (P407) were kindly gifted by BASF, India. Trimyristin (TM) and lecithin granular (LG) were purchased from Sigma-Aldrich, France and Acros Organics, USA respectively. Tween 80 (Tw80) and Dialysis membrane (molecular weight cut off -MWCO between 12,000 and 14,000) were purchased from HiMedia, India. Nanosep® centrifugal filter devices (Omega Membrane, MWCO 100 kD) were purchased from Pall Life Sciences, India. HPLC grade Acetonitrile and methanol were obtained from SRL, India. All other chemicals and reagents were of analytical grade.

The water used in all experiments was ultrapure, obtained from a Millipore–DirectQ UV® ultra pure water system of Millipore, France.

5.3. Methods

5.3.1. Partitioning behaviour of the drug between lipids and water

PZQ (20mg) was dispersed in a mixture of melted lipid (2 g) and hot water (2 ml). The mixture was kept on a hot water bath shaker maintained at temperature 10°C above the melting point (M.P.) of concerned lipid and shaken for 30 min. Aqueous phase was then separated after cooling by centrifugation with the help of Nanosep® centrifuge tubes and analyzed for PZQ content by HPLC (Venkateswarlu and Manjunath, 2004).

5.3.2. Preparation of PZQ-loaded SLN

SLN were prepared by hot homogenization followed by ultrasonication method (Manjunath and Venkateswarlu, 2004; Mishra *et al*., 2014). Briefly, the lipid phase consisting of PZQ (0.05-0.2 %w/v); lipid (1-10 %w/v) and lipophilic surfactant (lecithin granular, 0.5-2.5 %w/v) were weighed precisely using an electronic balance (Shimadzu AX100, Japan) and dissolved in a mixture of chloroform and methanol (2:1). The mixture was transferred to rota-evaporator (IKA RV 10 digital, Germany) and evaporated at 300 mbar, 50°C to obtain a thin lipid layer. Nitrogen was blown on the lipid layer to remove vapours of organic solvents, if any. The hot aqueous phase containing hydrophilic surfactant (1 - 3 % w/v) heated to same temperature of the molten lipid phase, was added to thin lipid

layer and hydrated for 30 min. A coarse hot o/w emulsion thus obtained was homogenized at 13,000 rpm with the help of Ultra-turrax (T 25 digital, IKA, Germany) for 1-10 min. The obtained pre-emulsion was sonicated with probe ultrasonicator (UP 200H, Hielscher Ultrasonics Gmbh, Germany) using 13 mm microprobe with amplitude of 55% at 200 W for 1-15 min. To prevent recrystallization during homogenization and ultrasonication, production temperature was kept at least 5°C above the melting point of lipid**.** The hot nano-emulsion (o/w) obtained was quickly poured into 200 ml cold water to obtain PZQ incorporated SLN. The SLN were collected by centrifugation (Cooling Centrifuge BL 24; Remi Instruments Ltd., India) at 15,000 g for 90 min at 4°C and washed three times with purified water. The SLN were suspended in purified water and pre-frozen under -40°C in deep freezer for 12 hrs. The samples were lyophilized for 48 h (Lypholizer, Decibel, India) under vacuum of 7mm Hg and a temperature of -40°C using sucrose (3% w/v) as a lyoprotectant to obtain SLN powders and stored at 4°C. The control SLN was prepared in the same way without adding PZQ. SLN were reconstituted to their original volume with distilled water using manual shaking and subsequent sonication when it's necessary, for further analysis.

5.3.3. Formulation design

5.3.3.1. Optimization of process parameters

The processing parameters such as homogenization time (HT), ultrasonication time (ST) and total volume of formulation (VF) were optimized as described previously (Gokce *et al*., 2008).

Table 5.1: Optimization of process variables

Formulati-on Code	HT (min)	ST (min)	VF (ml)	LSC (%w/v)	HSC (%w/v)	LC (%w/v)	DC (%w/v)
P1	2.5	10	50	2	2	5	0
P2	5	10	50	2	2	5	0
P3	10	10	50	2	2	5	0
P4	15	10	50	2	2	5	0
P5	5	2.5	50	2	2	5	0
P6	5	5	50	2	2	5	0
P7	5	10	50	2	2	5	0
P8	5	15	50	2	2	5	0
P9	5	10	50	2	2	5	0
P10	5	10	100	2	2	5	0
P11	5	10	200	2	2	5	0

To optimize the process parameters, the following formulation parameters were kept constant: Lipophilic Surfactant (LG) Concentration (LSC) = 2% w/v, Hydrophilic Surfactant (P188) Concentration (HSC) = 2% w/v, Lipid (TP) Concentration (LC) = 5% w/v, Drug Concentration (DC) = 0 % w/v. The scheme for optimization is mentioned below (Table 5.1):

a) HT: 2.5, 5, 10 and 15 min; while ST = 10 min, and VF = 50 ml

b) ST: 2.5, 10 and 15 min; while HT = 5 min, and VF = 50 ml

c) VF: 50,100 and 200 ml; while HT = 5 min, and ST= 10 min

5.3.3.2. Optimization of formulation variables

The formulation parameters such as lipid type and concentration, surfactant type and concentration and drug concentrations for preparing SLN were also optimized using formulation design (Table 5.2) as described previously (Das *et al*., 2011; Gokce *et al*., 2008)**,** after determination of optimum process parameters (i.e. HT = 5 min, ST = 10 min and VF = 50 ml.).

Table 5.2: Optimization of formulation Variables

Formulation Code	Lipid	Surfactant	LSC (%w/v)	HSC (%w/v)	LC (%w/v)	DC (%w/v)
F1	TP	P188	0.5	2.0	5.0	0.1
F2	TP	P188	1.0	2.0	5.0	0.1
F3	TP	P188	1.5	2.0	5.0	0.1
F4	TP	P188	2.0	2.0	5.0	0.1
F5	TP	P188	2.5	2.0	5.0	0.1
F6	TP	P188	1.5	1.0	5.0	0.1
F7	TP	P188	1.5	1.5	5.0	0.1
F8	TP	P188	1.5	2.0	5.0	0.1
F9	TP	P188	1.5	2.5	5.0	0.1
F10	TP	P188	1.5	3.0	5.0	0.1
F11	TP	P188	1.5	2.0	1.0	0.1
F12	TP	P188	1.5	2.0	2.5	0.1
F13	TP	P188	1.5	2.0	5.0	0.1
F14	TP	P188	1.5	2.0	5.0	0
F15	TP	P188	1.5	2.0	5.0	0.025
F16	TP	P188	1.5	2.0	5.0	0.05
F17	TP	P188	1.5	2.0	5.0	0.1
F18	TP	P188	1.5	2.0	5.0	0.2
F19	TP	P188	1.5	2.0	5.0	0.1

F20	TS	P188	1.5	2.0	5.0	0.1
F21	TM	P188	1.5	2.0	5.0	0.1
F22	GMS	P188	1.5	2.0	5.0	0.1
F23	GB	P188	1.5	2.0	5.0	0.1
F24	TP	Tw 80	1.5	2.0	5.0	0.1
F25	TP	P 407	1.5	2.0	5.0	0.1
F26	TP	P188	1.5	2.0	5.0	0.1
F27	TM	P188	2.0	2.5	5.0	0.05
F28	TP	P188	2.0	2.5	5.0	0.05
F29	TS	P188	2.0	2.5	5.0	0.05

5.3.4. Particle size and zeta potential

5.3.4.1. Particle size analysis

The mean particle size (z-average), size distribution, and polydispersity index (PI) were measured by photon correlation spectroscopy (Delsa Nano C Particle Analyzer; Beckman Coulter, USA). The samples were kept in polystyrene cuvettes. All measurements were performed at 25°C following a 1:20 dilution with Milli-Q water to derive the optimum kilo counts per second (Kcps) of 50 to 200 for measurements. The observations were made at 165° scattering angle. PI was determined based on the following equation:

PI = {D (0.9) – D (0.1)}/ D (0.5).

where, D (0.9) corresponds to particle size immediately above 90% of the sample and D (0.5) corresponds to particle size immediately above 50% of the sample and D (0.1) corresponds to particle size immediately above 10% of the sample.

5.3.4.2. Zeta potential analysis

Zeta potential (ZP) measurements were used to determine the surface charge of SLN which influence the stability and uptake of particulate carriers by intestinal lymphatics. ZP of SLN was measured by using Particle size analyzer; (Delsa™ Nano C Beckman-Coulter, USA) at 25°C. Samples were diluted appropriately with Milli-Q water for measurements.

ZP measurement is based on the Helmoltz-Smoluchowski equation:

$$\xi = Ue \times 4\pi\eta/\varepsilon$$

where, ξ is zeta potential, Ue is electrophoretic mobility, η is viscosity and ε is dielectric constant of the medium.

5.3.5. Assay and entrapment efficiency

PZQ loaded SLN (100 µl) were diluted to 10 ml with chloroform/methanol mixture (1:1) and vortexed to extract drug from lipid. The obtained solution was filtered through 0.45 µm PVDF membrane filter (Whatman), and total drug content (TDC) was determined by HPLC.

The entrapment efficiency (EE) of the PZQ-SLN was determined indirectly by calculating the amount of free PZQ (un-entrapped) in the aqueous phase of the SLN dispersion. After a suitable dilution, PZQ-SLN (500µl) was transferred to the upper chamber of Nanosep® centrifuge tubes fitted with an ultrafilter (MWCO 100KD, Pall Life Sciences, India). The Nanosep® tubes were centrifuged by using Centrifuge (Cooling Centrifuge BL 24; Remi, India) at 15000 g for 30 min at -10°C (Joshi *et al*., 2006)**.** The SLN along with encapsulated drug were retained in the upper chamber while the un-entrapped PZQ along with dispersion medium moved through the filter membrane into the lower chamber of Nanosep®. The amount of un-entrapped PZQ (A_{un}) in the aqueous phase after isolation of the system was detected by RP HPLC. The EE of SLN were calculated following equation (1) (Muller *et al*.,2011; Manjunath and Venkateshwarlu, 2005)**.**

$$\text{Percentage of Entrapment Efficiency (EE \%)} = [(A_t - A_{un})/ A_{t]} \times 100 \qquad (1)$$

where A_t, and A_{un} were the weight of total drug in the system and analyzed weight of un-entrapped drug in the system, respectively.

5.3.6. Accelerated stability studies

The lyophilized powder samples of optimized formulations were subjected to accelerated stability studies according to International Conference on Harmonisation (ICH) Q1A (R2) guidelines and previously reported method [Alex *et al*., 2011, Das *et al*., 2011]. The optimized PZQ-loaded SLN formulations under a sealed condition were kept at refrigerated temperature (5 ± 3°C) and in stability chamber maintained at 25 ± 2 °C/60 ± 5% RH and pH conditions (SGF, pH 1.2 for 2 hours, SIF, pH 7.5 for 6 hours)(Paliwal *et al*., 2009). The samples were analyzed periodically for any change in average particle size and drug content for a total period of six months.

5.3.7. Effect of sterilization

To observe the effect of sterilization on particle size and EE, PZQ-loaded SLN suspension was autoclaved at 121 °C for 15 min.

5.3.8. Solubility studies

5.3.8.1. Determination of PZQ solubility in 0.1 N HCl (pH 1.2)

PQZ saturation solubility (Cs) was obtained by dispersing 3 gm of drug in 100 ml of 0.1 N HCl (pH 1.2). The suspensions were stirred under constant magnetic stirring (100rpm), at 37 ° C ± 0.5 ° C for 24 hr

(adequate time for equilibration), filtered through a membrane (pore size 0.45µm), and then assayed by HPLC.

5.3.8.2. Determination of PZQ solubility in phosphate buffer (pH 6.8)

Praziquantel saturation solubility (Cs) was obtained by dispersing 3 gm of drug in 100 ml of Phosphate Buffer (pH 6.8). The suspensions were stirred under constant magnetic stirring (100 rpm), at 37 ° C ± 0.5 ° C for 24 hr (adequate time for equilibration), filtered through a membrane (pore size 0.45µm), and then assayed by RP HPLC.

5.3.9. *In vitro* drug release studies

In vitro drug release studies were performed by using dialysis bag diffusion technique [Rawat *et al*., 2011]. The dialysis bag retains the SLN but allows the transfer of the dissolved / released drug molecules into the release media (Das *et al*., 2011). The dialysis membrane (MWCO between 12 -14 kDa) was soaked in dissolution medium for 12 hours prior usage. The SLN dispersion (equivalent to 2 mg of PZQ) was placed in the dialysis bag, both ends were tightly sealed and immersed into the dialysis medium (75 ml, 0.1 M HCl for two hours and phosphate-buffer, pH 6.8 for 24 hours) kept at 37°C ± 1°C and stirred magnetically at 100 rpm using magnetic stirrer. At regular time intervals, aliquots of dialysate samples were withdrawn and an equal volume of dissolution medium was replaced by fresh medium to maintain a constant volume throughout the study. The aliquots were filtered through 0.1-µm filter and were analyzed for PZQ concentration by HPLC. During the release studies, sink condition

was maintained ($C_1 < C_S$ X 0.2) C_1=Final concentration of Praziquantel after the complete release of the drug in the dissolution medium, C_S=Saturation solubility of Praziquantel in the dissolution medium) (Moneghini *et al*., 2000).

Praziquantel aqueous dispersion in 0.5%w/v methyl cellulose was used as control.

The amount of PZQ was quantified as percentage using the following equation:

$$\% \text{ PZQ released} = \frac{\text{Amount of PZQ in dissolution medium}}{\text{Total amount of PZQ}} \times 100$$

5.3.10. Drug release mechanism

The mechanism of drug release from the SLN formulation was analyzed by fitting the *in vitro* release studies results following three kinetic models: first order [$\ln(100-Q)=\ln Q_0 - k_1 t$, which explains the drug release rate is proportional to its concentration; Higuchi equation ($Q=k_H t^{1/2}$) (Higuchi, 1962 and Mehta *et al*., 2007), which is dependent on diffusion under Fick's law; Korsmeyer–Peppas exponential model $Mt/M\infty=Kt^n$, where, $Mt/M\infty$ is fraction of drug released after time 't' and 'K' is kinetic constant and 'n' is release exponent which characterizes the drug transport mechanism (Korsmeyer *et al*., 1983). The determination coefficient (R^2) was used as an indicator of the best fitting of the data for each model.

5.3.11. Solid state characterisation by differential scanning calorimetry (DSC)

Thermograms of the different samples were obtained using a DSC (DSC 30; Mettler-Toledo, Viroflay, France). The instrument was calibrated with indium (calibration standard, purity >99.999%) for melting point and heat of fusion. A heating rate of 10 °C/min was employed in the temperature ranges between 20-200°C, under a constant nitrogen purge (80 ml/min). PZQ, lipids and lyophilized SLN samples (3–5 mg) were kept in standard aluminium sample pans. An empty aluminium pan was used as reference.

5.3.12. Solid state characterisation by X-ray diffractometry (XRD)

X-ray diffraction (XRD) studies were performed by powder X-ray diffractometer (Siemen's D-5000, Germany) equipped with a 2θ compensating slit, using Cu Kα radiation (1.54 Å) at 40 kV and 30 mA passing through nickel filter. Samples (PZQ, lipids and lyophilized SLN) were mounted on zero-background sample holder and subjected to a continuous scan over an angular range of 2° to 70°, 2θ at a step size of 0.045° and step time of 0.5 s.

5.3.13. External morphology by transmission electron microscopy (TEM)

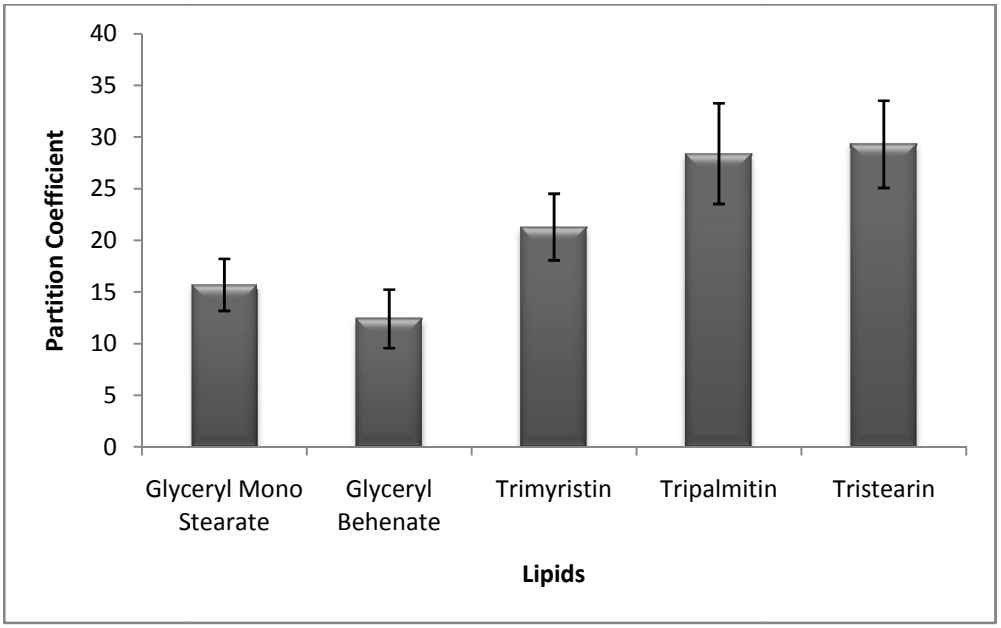
Transmission Electron Microscopy (TEM) is a method of probing the microstructure of rather delicate system such as micelles, liquid crystalline phase, vesicles, emulsions and also nanoparticles. Lyophilized

SLN were dispersed directly into the milli Q water without any surfactants. A drop of SLN dispersion was spread over a 200-mesh copper grid coated with carbon film and excess droplet was removed with a filter paper. After 5 min, a drop of a negative stain (phosphotungstic acid, 2% w/v) was placed on to the copper grid. The grid was dried at room temperature and observed by the TEM (TECNAI-20 G^2, FEI, Holland).

5.4. Result and discussion

5.4.1. Partitioning behavior of PZQ in triglyceride lipid matrix

Solubility of drug in lipid is one of the most important factors for determining drug loading capacity of the SLN (Chakraborty *et al*., 2011). Partitioning behaviour of PZQ was tested in monoglyceride (GMS), diglyceride (GB) and triglycerides with different chain lengths such as trimyristin (TM, C_{14}), tripalmitin (TP, C_{16}) and tristearin (TS, C_{18}). Partition coefficients (ratio of the amount of PZQ in lipid to the amount of PZQ in aqueous phase) obtained were 15.7± 2.52, 12.4 ± 2.84, 21.3± 3.22, 28.4± 4.87 and 29.3± 4.22 for GMS, GB, TM, TP and TS respectively. These results indicated that the triglycerides have highest solubilization efficiency for PZQ followed by monoglycerides and diglycerides (Figure 5.1). The GMS showed more solubilization efficiency than GB which, this might be due to surfactant property of GMS. Among the triglycerides, solubilization capacities of tristearin (TS) and tripalmitin (TP) were comparatively similar while trimyristin (TM) showed lowest solubilization capacity (Figure 5.1). This might be due to TS having higher chain length (C_{18}) in comparison to TP (C_{16}) and TM (C_{14}).



(mean ±SD; n=4)

Figure 5.1: Partition behaviour of Praziquantel in different lipids.

5.4.2. Fabrication of lipid nanoparticles

SLN have been prepared by various researchers using different methods including high-pressure homogenization (hot and cold) (Muller *et al*., 1995), microemulsion technique (Gasco *et al*., 1993), solvent emulsification technique (Sjostrom *et al*., 1992), solvent injection method (Rawat *et al*., 2010) and solvent emulsification diffusion (Hu *et al*., 2002) in aqueous medium. In the present study, a simple, economical, and reproducible method for the preparation of SLN, i.e. hot homogenization followed by ultrasonication (at above the melting point of the lipid) was used (Venkateswarlu and Manjunath, 2004). Solvent system chloroform / methanol (1:1) was used to disperse the praziquantel homogeneously in the lipid. Co-evaporation of the lipid and drug from chloroform in a round bottom flask was found to produce maximum PZQ entrapment in

liposomes (Akbraih *et al*., 1992). This is consistent with other work on SLN, suggesting that co-evaporation of lipids and lipophilic compounds from organic solvents generate practically the highest incorporation (Venkateswarlu and Manjunath, 2004). Lyophilization is a promising way to increase chemical and physical stability of SLN over extended period of time (Ohshima *et al*., 2009). Sucrose (3% w/v) was chosen as lyoprotectant in the present study because disaccharides have been shown to be advantageous in comparison to monosaccharides e.g. fructose, glucose, mannose, etc (Kamiya *et al*., 2006; Ohshima *et al*., 2009). The effect of various process and formulation parameters on the different characteristics of SLN were evaluated in detail and discussed below.

5.4.3. Optimization of process variables

Many researchers have reported that production parameters have a significant effect on the particle size of the SLN systems (Almeida *et al*., 1997, Das *et al*., 2011, Gokce *et al*., 2008). The hot homogenization–ultrasonication method (at above the melting point of the lipid) was found to be efficient and quick to produce SLN. Effect of different process parameters such as HT, ST and VF have a significant effect on the physicochemical properties of the SLN produced by hot homogenization followed by ultrasonication method. Effect of different process variables on PS, (PI), and ZPwere observed. The process parameters were optimized for preparing SLN with a small particle size (<200 nm) together with a low polydispersity (<0.3).

5.4.3.1. Effect of homogenization time

As shown in Table 5.3, PS and PI were not affected by increasing HT after 5 min. The homogenization step is considered to be an intermediate step and responsible mainly for primary emulsification of lipid in aqueous phase and do not produce final nanoparticles (Das *et al*., 2011). ZP is an important criterion for study of the storage stability of lipid particles and their cellular behaviour in drug release. The ZP indicates the degree of repulsion between close and similarly charged particles in the nanodispersion. Generally, high ZP (highly negative or positive) prevents aggregation of the particles due to electric repulsion and electrically stabilizes the nanoparticle dispersion. ZP either more than 30 mV or less than -30 mV indicates stable nanodispersion. However, ZP was around -10 mV in the present study due to the presence of non-ionic stearic stabilizer (Table 5.3). HT of 5 min was sufficient to obtain SLN with suitable PS and PI.

Table 5.3: Effect of HT on PS, PI, and ZP

Formulation	HT (min)	Size (nm)	PI	ZP (-mV)
P1	2.5	146.7 ±2.59	0.463±0.027	10.1±0.29
P2	5	132.3 ±1.43	0.289±0.024	9.8±0.38
P3	10	121.6±1.58	0.213±0.012	9.9±0.45
P4	15	129.2±1.72	0.392±0.019	9.8±0.52

mean ±SD; n=4

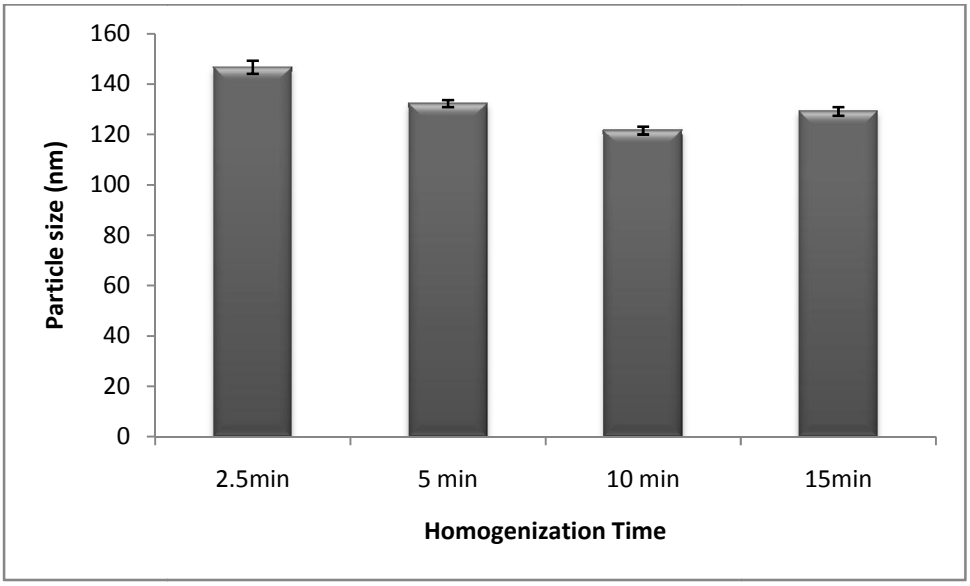


Figure 5.2: Effect of homogenization time on particle size of SLN

5.4.3.2. Effect of ultrasonication time

Ultrasonication time (ST) plays a major role in obtaining a formulation with narrow particle size distribution. Sonication breaks the coarse emulsion drops to nano-droplets and is responsible for final particle size of SLN (Das *et al*., 2011).

Table 5.4: Effect of ST on PS, PI, and ZP

Formulation	ST(min)	Size(nm)	PI	ZP(-mV)
P5	2.5	421.1±7.69	0.631±0.034	9.7±0.49
P6	5	218.7±7.14	0.512±0.031	9.6±0.42
P7	10	133.4±1.73	0.404±0.039	9.7±0.38
P8	15	130.3±1.82	0.293±0.044	9.7±0.47

mean ±SD; n=4

Longer sonication time put more sonication energy to the SLN dispersions and significantly decreased both PS and PI (Table 5.4). The sonication time of 10 min was considered suitable for SLN production. ZP was found to be around -9.7mV.

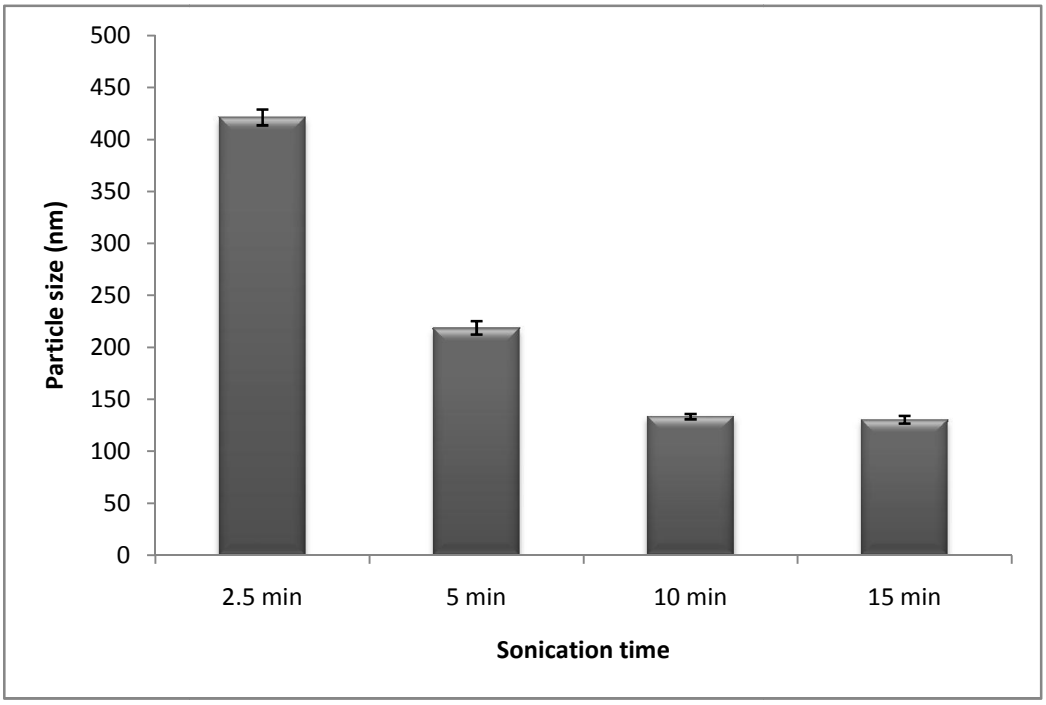


Figure 5.3: Effect of sonication time on particle size of SLN

5.4.3.3. Effect of VF

In case of high shear homogenization, the basic mechanism is the homogenizer mixing efficiency, which is mainly affected by the volume of the dispersion (Mehnert and Mäder, 2001). Increase in VF did not significantly increase the PS, however PI was increased. A total volume of 50 ml corresponded to the best results in terms of PS and PI of the SLN (Table 5.5).

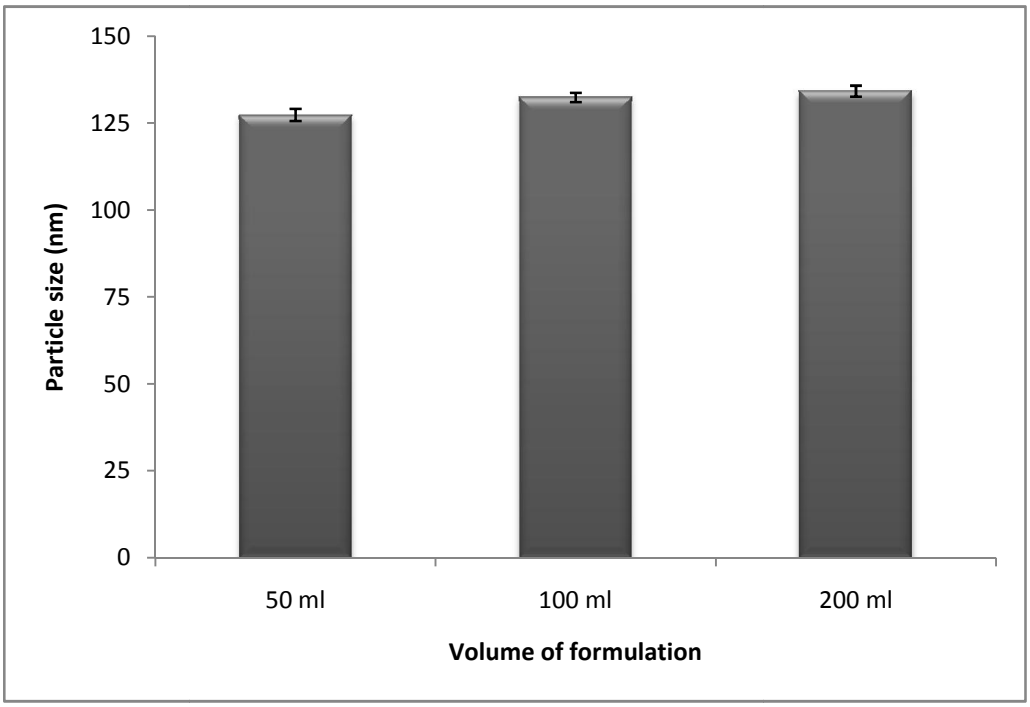


Figure 5.4: Effect of volume of formulation on particle size of SLN

Table 5.5: Effect of VF on PS, PI, and ZP

Formulation	VF	Size (nm)	PI	ZP (-mV)
P9	50	133.4±1.75	0.404±0.039	9.8±0.38
P10	100	132.4±1.32	0.314±0.038	9.7±0.38
P11	200	134.2±1.58	0.396±0.042	9.7±0.47

mean ±SD; n=4

From the results discussed above, the HT, ST and VF) were decided as 5 min, 10 min and 50ml respectively, for further optimization of the formulation conditions.

5.4.4. Optimization of formulation variables

Various lipids (matrix materials) have been used so far for the production of SLN includes monoglycerides, diglycerides and triglycerides (Schwarz and Mehnart, 1997; Freitas and Muller, 1998; Trotta *et al*., 2006; Windbergs *et al*., 2009). Each lipid varies in its physico-chemical characteristics. Due to these differences, an influence on the characterizing parameters of SLN has been found. Moreover, variety of surfactants has been used for the preparation of SLN including phospholipids, bile salts, poloxamers and other ionic /non-ionic surfactants. Nonionic or zwitterionic surfactants have been found to be favourable for pharmaceutical applications since they are less toxic and less affected by changes in pH and ionic strength (Strickley, 2004). Only a very limited number of surfactants can be used in parenteral formulations, due to the irritating, haemolytic, or sensitizing action of most of these substances. The surfactant selection was limited to those acceptable for pharmaceutical use in parenteral formulations, such as tweens, soya lecithin and sodium deoxycholate (Nema *et al*., 2007). The objective of the present study was to develop PZQ loaded SLN for oral as well as parenteral drug delivery. Hence, the components such as oil/lipid(s), surfactant(s) and co-surfactant(s) should be biocompatible and safe. Therefore, all formulation ingredients used in the study were selected from Generally Recognised as Safe (GRAS) list (www.usfda.gov). Stabilization of SLN with phospholipids and an additional surfactant rather than with a single surfactant frequently yields SLN with a more

homogeneous appearance and lower tendency to form macroscopic particles (Bunjes *et al*., 2003).

The type of surfactant used as a stabilizer in the hot homogenization–ultrasonication method is among the variables affecting emulsion droplet size, and probably the properties of the resultant SLN. Optimization of the surfactant system is of utmost importance for the preparation of SLN intended for parenteral administration in order to maintain the colloidal state of the formulation during storage and upon administration.

In the present study, a phospholipid (lecithin) was used as lipophilic surfactant along with a non-ionic surfactant to stabilize the nanoparticles. As the schistosomiasis parasite has a high affinity for phospholipids and it is reported that ingested lipids are found to be incorporated into stable parasite structures rather than utilized by the host for degradative energy-yielding metabolism (Rumjanek and Simpson, 1980).

Poloxamer series of surfactants e.g. poloxamer 188 (triblock copolymer of polyoxyethylene and polyoxypropylene, HLB>24) and poloxamer 407 were used as hydrophilic non-ionic surfactant. Non-ionic surfactants are commonly used in oral formulations to enhance the bioavailability of water insoluble pharmaceutical actives and offer several advantages, like they are more hydrophobic than ionic surfactants; possess a better capacity to dissolve water insoluble drugs; and in general, less toxic to biological membranes.

5.4.4.1. Effect of lipophilic surfactant (lecithin) concentration

The lipophilic surfactant used in present study was lecithin granular (LG) in combination with hydrophilic surfactant (P188) could effectively cover the SLN and thus, prevent agglomeration. The PS measurements of SLN revealed that the incorporation of lecithin up to 2.0% w/v led to a concentration dependent particle size reduction down to 117 nm. Further, increase of lecithin up to 2.5% w/v caused no further decrease in PS (Table 5.6).

Table 5.6: The effect of lipophilic surfactant concentration (LSC) on characterizing parameters of SLN

LSC (%w/v)	Particle Size (nm)	Polydispersity index s	Zeta potential (-mV)	Entrapment Efficiency (%EE)	Drug loading (%DL)
0.5	279.8± 29.7	0.541±0.038	08.2±0.59	62.7±0.59	1.2384
1	167.4 ± 15.8	0.497±0.042	10.9±0.46	68.9±1.21	1.3592
1.5	128.4 ± 12.3	0.383±0.039	12.8±0.57	75.6±1.32	1.4894
2	116.9 ± 12.8	0.367±0.041	18.2±0.43	79.5±0.93	1.5651
2.5	129.9 ± 12.8	0.389±0.041	20.7±0.43	84.7±0.78	1.6657

mean ±SD; n=4

This observation is related to the increased concentration of surfactant (lecithin) in formulations, which reduces surface tension and facilitates the uniform droplet division during homogenization (Noriega-Peláez *et al*., 2011**).**

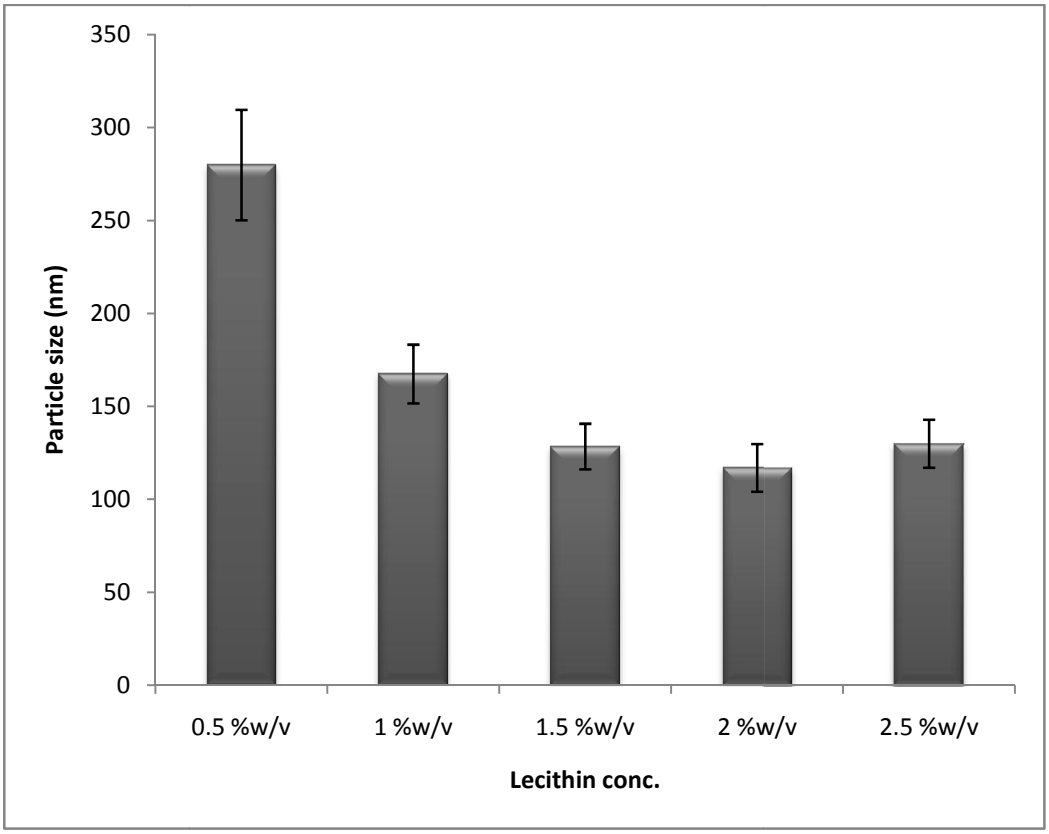


Figure 5.5: Effect of lipophilic surfactant concentration on particle size of SLN

A critical LG concentration was reached at 2.0 % w/v, addition of LG above this concentration did not cause a further decrease in PS and no additional surface area was provided to accommodate lecithin. An excess of lecithin might possibly form multi-layers around the particles and/or leak into the aqueous phase leading to the formation of liposomes, mixed micelles or other aggregates **(**Heiati *et al*., 1996; Westesen and Siekmann, 1994**)** and hence resulted in increased PS.

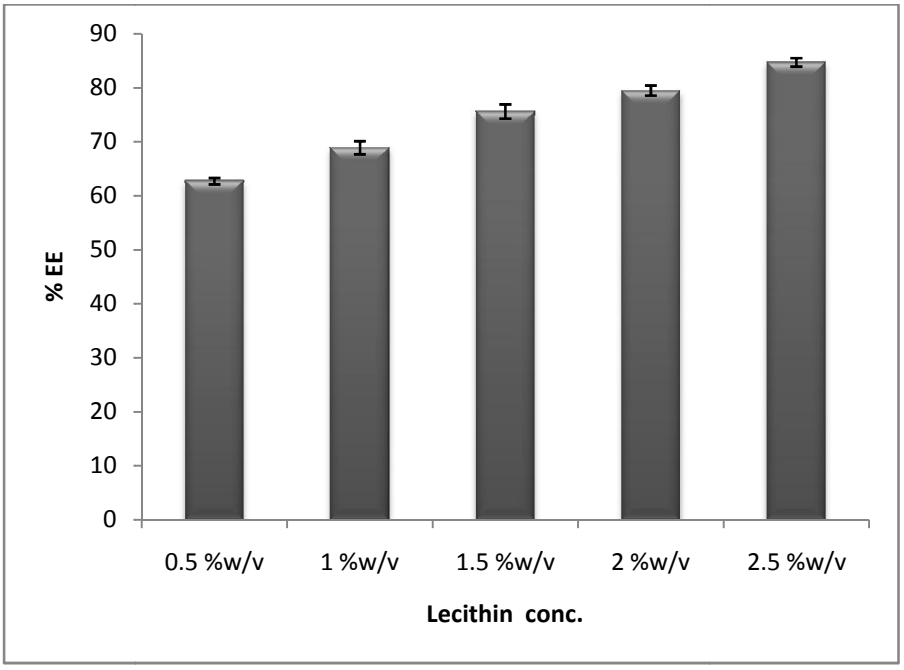


Figure 5.6: Effect of lipophilic surfactant concentration on Entrapment Efficiency of SLN

The combination of hydrophilic surfactant (P 188) and lipophilic surfactant (LG) as surfactant showed a significant effect on zeta potential of SLN. SLN prepared with higher LG concentration as a zwitterionic surfactant possessed the highest zeta potential of -20.7 mV (Table 5.6).

EE was increased from 62 %w/w to 84 %w/w as the lecithin content was increased from 0.5% to 2.5%w/v, this may be due to increase in lipid content as lecithin is a phospholipid (Table 5.6). It is clear that composition of surfactant mixture significantly affected the PZQ loading capacity of SLN, specifically the presence of lecithin. This fact suggests that part of the incorporated PZQ may be embedded in the surfactant layer as shown for other SLN formulations described elsewhere (Lim and Kim, 2002; Tiyaboonchai *et al*., 2007).

5.4.4.2. Effect of hydrophilic surfactant (poloxamer 188) concentration

Different hydrophilic surfactant (P188) concentrations enabled not only the control of the particle size, but in addition also allowed the formation of surface charge modified particles. PS and PI decreased with increasing P188 concentration up to 3.0% w/v (Table 5.7).

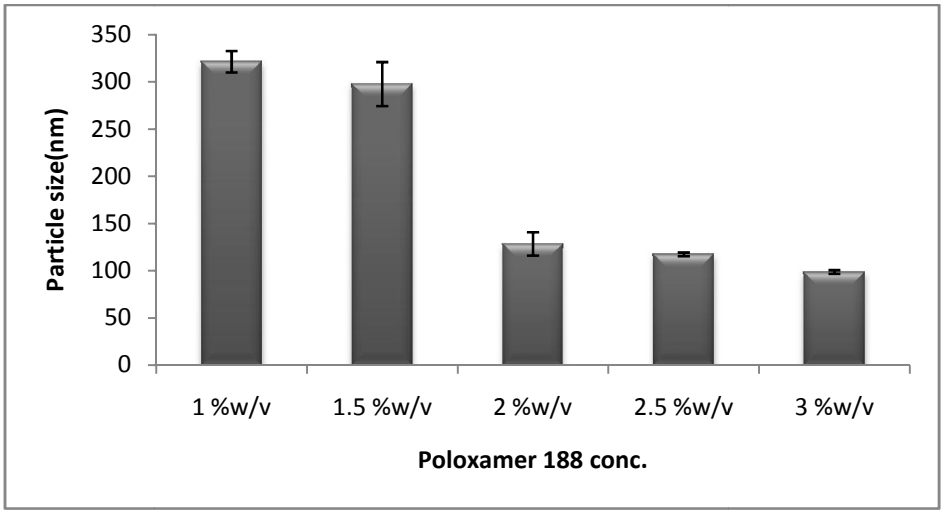


Figure 5.7: Effect of hydrophilic surfactant concentration on particle size of SLN

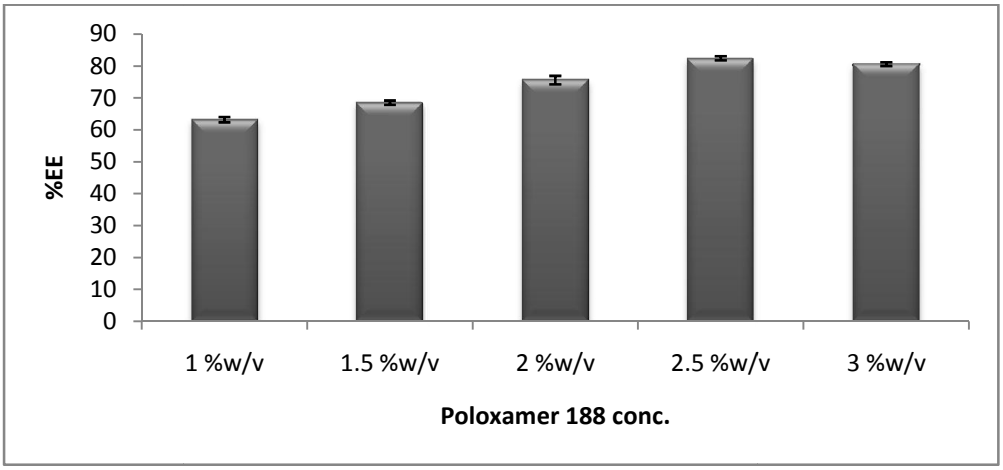


Figure 5.8: Effect of hydrophilic surfactant concentration on Entrapment Efficiency of SLN

Table 5.7: The effect of Hydrophilic surfactant concentration (HSC) on characterizing parameters of SLN

HSC(%w/v)	Particle Size (nm)	Polydispersity index	Zeta potential (-mV)	Entrapment Efficiency (%EE)
1	321.2 ± 11.4	0.482±0.058	19.8±0.68	63.2±0.83
1.5	297.5±23.3	0.341±0.027	17.3±0.73	68.5±0.69
2	128.4 ± 12.3	0.383±0.039	12.8±0.57	75.6±1.32
2.5	117.3 ± 0.93	0.348±0.043	12.6±0.65	82.4±0.65
3	098.6 ± 0.98	0.386±0.029	09.7±0.57	80.6±0.58

mean ±SD; n=4

These observations might be due to production and stabilization of smaller lipid droplets at higher HSC as enough amount of surfactant was present to stabilize the SLN. EE also increased with increasing HSC. This could be due to the presence of sufficient HSC which helped the drug to remain within the lipid particles and/or on the surface of the particles (Schubert *et al*., 2005). ZP decreased as the concentration of P188 increased. This could be attributed to the increased amounts of non ionic surfactant which counteract the negative charge of the particle surface,

which is believed to go along with a displacement of the plane of shear of the nanoparticles and a steric stabilization of the stern layer.

5.4.4.3. Effect of lipid concentration

The amount of lipid which solubilizes the drug in formulation has significant effect on the particle size of the SLN. The increase in lipid concentration from 1.0 to 5.0% w/v resulted in a significant increase in PS of the SLN (Table 5.8). The higher viscosity of the dispersed phase due to the high lipid content would negatively affect the homogenizer efficiency and distribution of sonication energy (Mehnert and Mader, 2001).

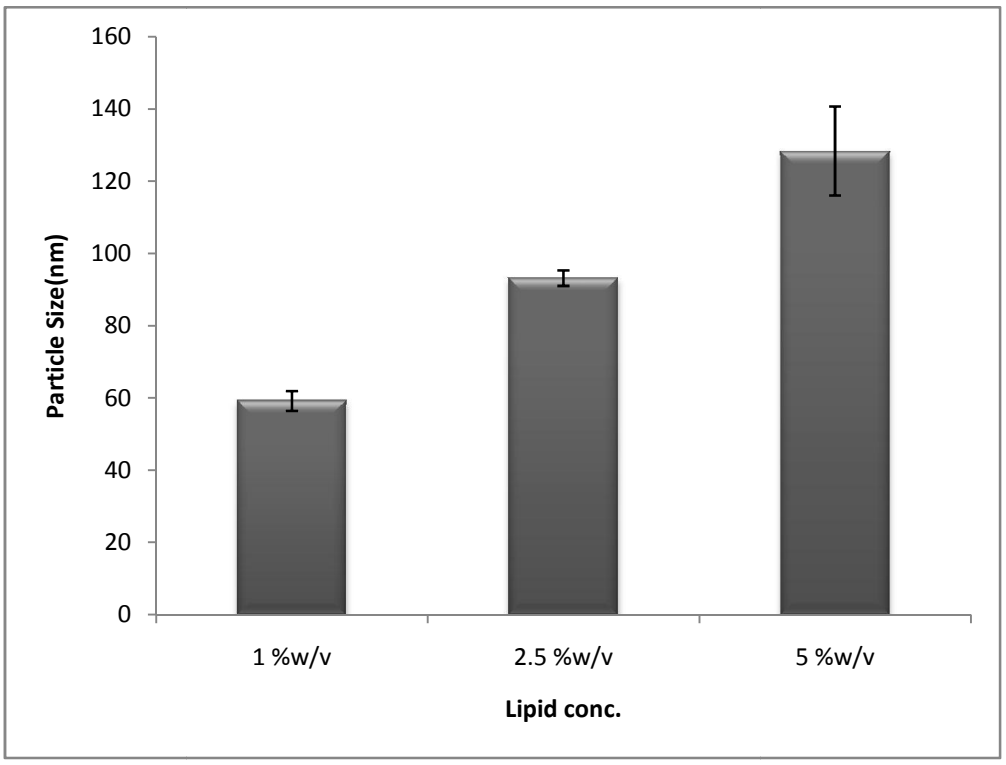


Figure 5.9: Effect of lipid concentration on particle size of SLN

At higher LC, the amount of surfactant available may not be sufficient to cover newer particle surfaces resulting in larger particles and more heterogeneous particle size distribution. The EE also increased with increasing LC. This may be explained on the fact that as there was increase in the lipid phase and more amount of the lipid was available for the PZQ to dissolve.

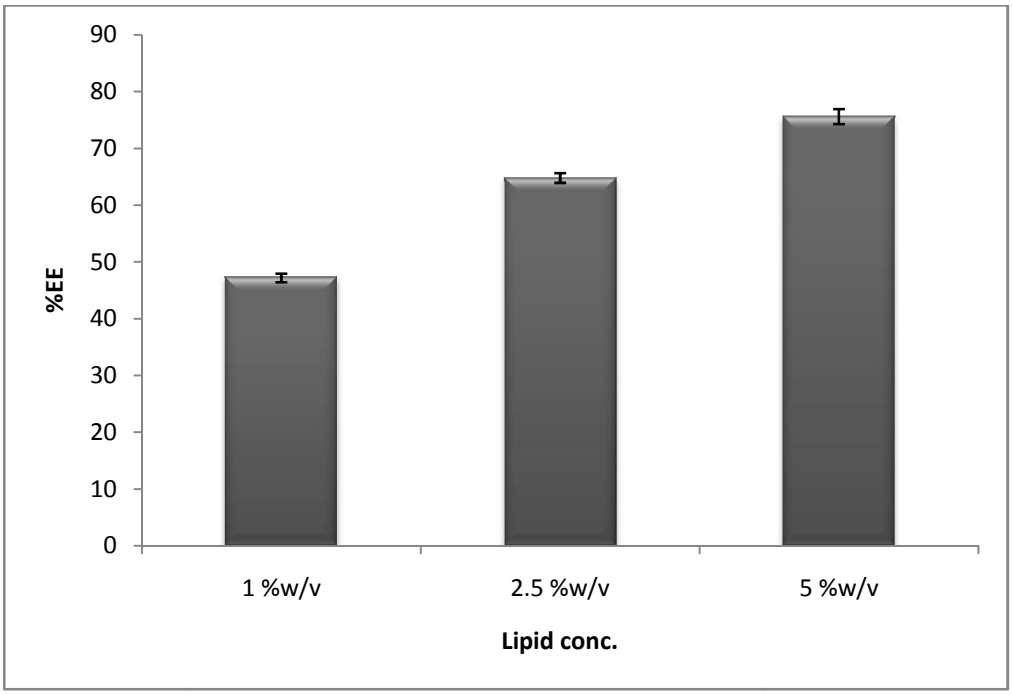


Figure 5.10: Effect of lipid concentration on EE of SLN

Table 5.8: The effect of lipid concentration on characterizing parameters of SLN

LC (%w/v)	Particle Size (nm)	Polydispersity index	Zeta potential (-mV)	Entrapment Efficiency (%EE)
1	59.2 ± 2.73	0.434±0.038	11.3±0.63	47.2±0.75
2.5	93.2 ± 2.16	0.289±0.041	12.5±0.84	64.8±0.87
5	128.4 ± 12.3	0.383±0.039	12.8±0.57	75.6±1.32

mean ±SD; n=4

5.4.4.4. Effect of drug concentration

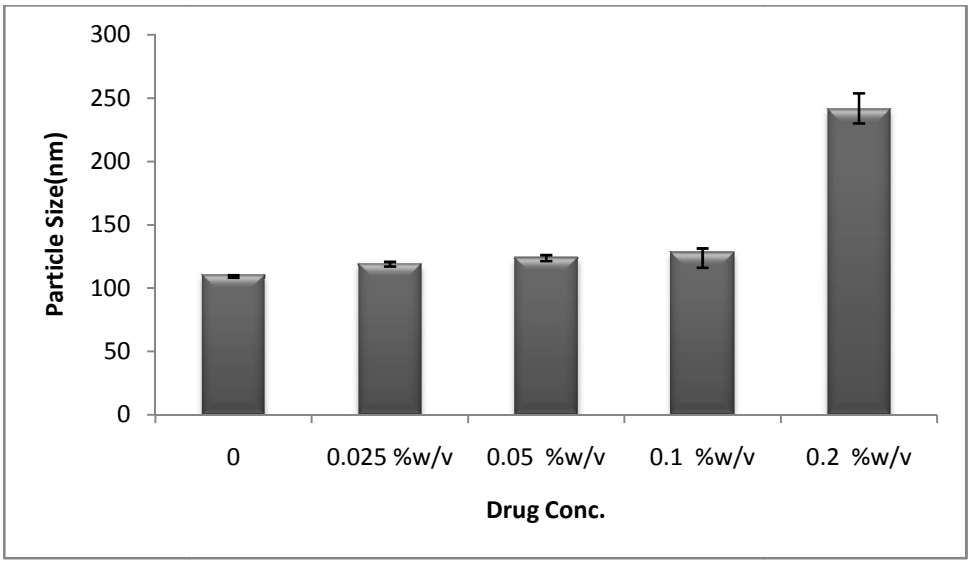


Figure 5.11: Effect of drug concentration on particle size of SLN

The PS and PI of the SLN significantly increased with increasing drug concentration (0.025–0.2% w/v). ZPs were around -10 mV in all cases whereas EE significantly decreased with increasing DC. This 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 lower EE.

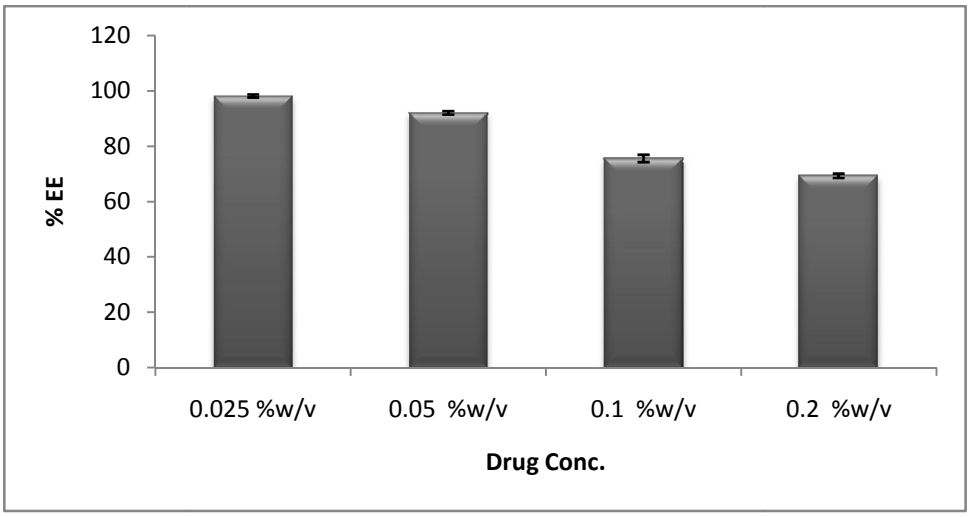


Figure 5.12: Effect of drug concentration on EE of SLN

Table 5.9: The effect of drug concentration on characterizing parameters of SLN

DC (%w/v)	Particle Size (nm)	Polydispersity index	Zeta potential (-mV)	Entrapment Efficiency (%EE)
0	110.2±1.89	0.211±0.039	9.8±0.73	0
0.025	118.9±1.73	0.276±0.047	9.9±0.69	98.2±0.56
0.05	124.3±2.98	0.312±0.057	9.9±0.75	92.1±0.63
0.1	128.4 ± 12.3	0.383±0.039	12.8±0.57	75.6±1.32
0.2	241.4±11.43	0.387±0.059	11.9±0.65	69.3±0.79

mean ±SD; n=4

5.4.4.5. Effect of lipid type

The lipid core material was found to affect the extent of PZQ entrapment in SLN. In case of TM- SLN, TP- SLN and TS- SLN, the EE was estimated to be 72.5 ± 0.44%, 75.6±1.32%, and 75.4 ± 0.14% respectively. However, among the triglycerides, SLN-TP and SLN-TS showed higher drug entrapment than SLN-TM. This might be due to the high solubility of PZQ in TP and TS in comparison to TM (Vivek *et al*., 2007). Moreover, the PS was found to be lower in case of TP in comparison to TS. The GMS-SLN and GB-SLN exhibited higher PS and PI in comparison to triglycerides. Moreover, the EE for GMS-SLN and GB-SLN was lower in comparison to triglycerides. This might be due to the solubilisation capacities of GMS and GB for PZQ were lower than the triglycerides.

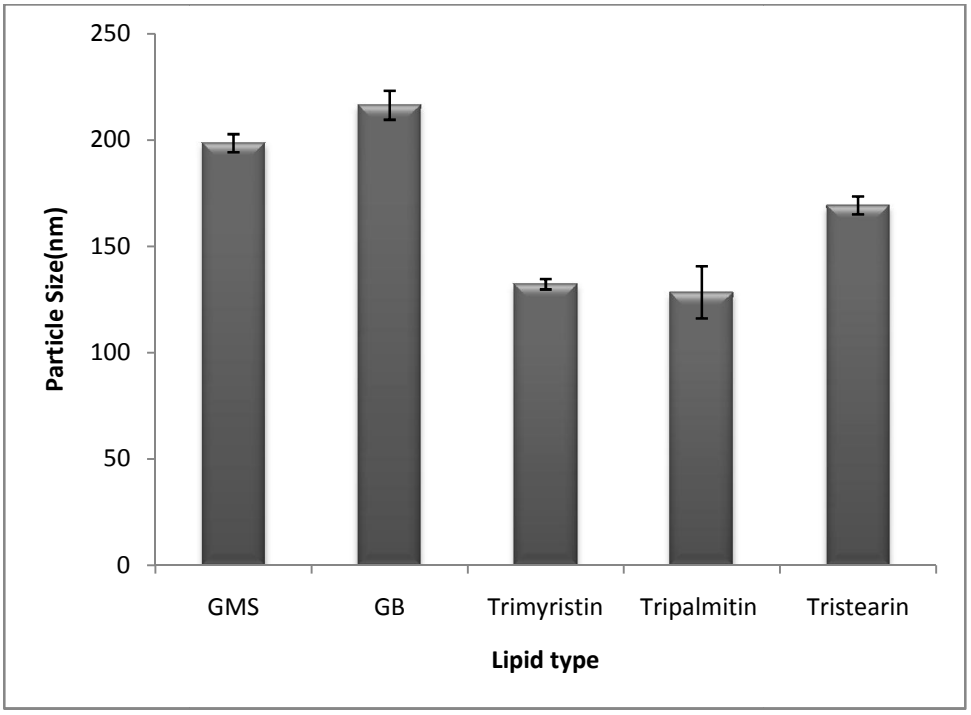


Figure 5.13: Effect of lipid type on particle size of SLN

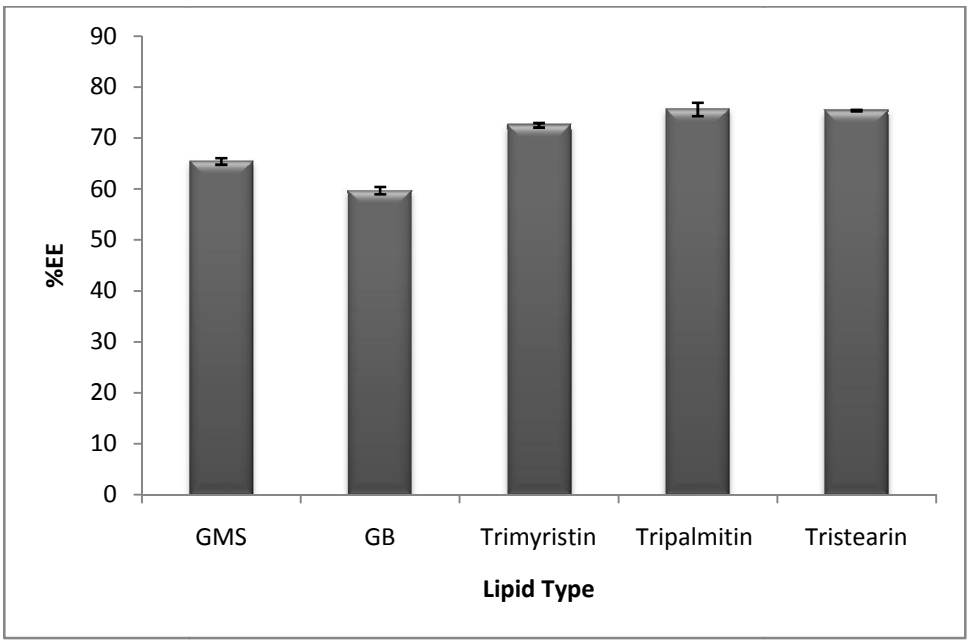


Figure 5.14: Effect of lipid type on EE of SLN

Table 5.10: The effect of lipid types on characterizing parameters of SLN

Lipid	**Particle size (nm)**	**Polydispersity index**	**Zeta potential (-mV)**	**Entrapment Efficiency (%EE)**
GMS	198.5 ± 4.27	0.441 ±0.035	11.5 ±0.71	65.4 ±0.64
GB	216.4 ±6.83	0.512 ±0.041	10.6 ±0.58	59.7 ±0.72
Trimyristin	132.2 ± 2.43	0.252±0.041	10.7±0.79	72.5 ± 0.44
Tripalmitin	128.4 ± 12.3	0.383±0.039	12.8±0.57	75.6 ± 1.32
Tristearin	169.3 ± 4.19	0.367±0.037	11.8±0.58	75.4 ± 0.14

mean ±SD; n=4

5.4.4.6. Effect of surfactant type

Poloxamer series of surfactant (Poloxamer 188 and poloxamer 407) produced particles of lower PS and higher EE in comparison to Tween 80. (Table 5.11). When tween 80 was used in combination with lecithin; there was a high tendency towards gelation under high shear homogenization. This gelation phenomenon was found to be reversible upon storage but resulted in microparticles after storage. The phenomenon seems to be correlated with specific interactions between lecithin and tween 80. The inclusion of P188 as hydrophilic surfactant produced SLN with particle size of about 132.3 ± 2.93 nm and PI of about 0.248 ± 0.043. EE were slightly higher when P188 was used than P407. The results indicated that

steric stabilizer (P188) in combination of zwitterionic lipophilic surfactant can be suitable for the production of desired SLN.

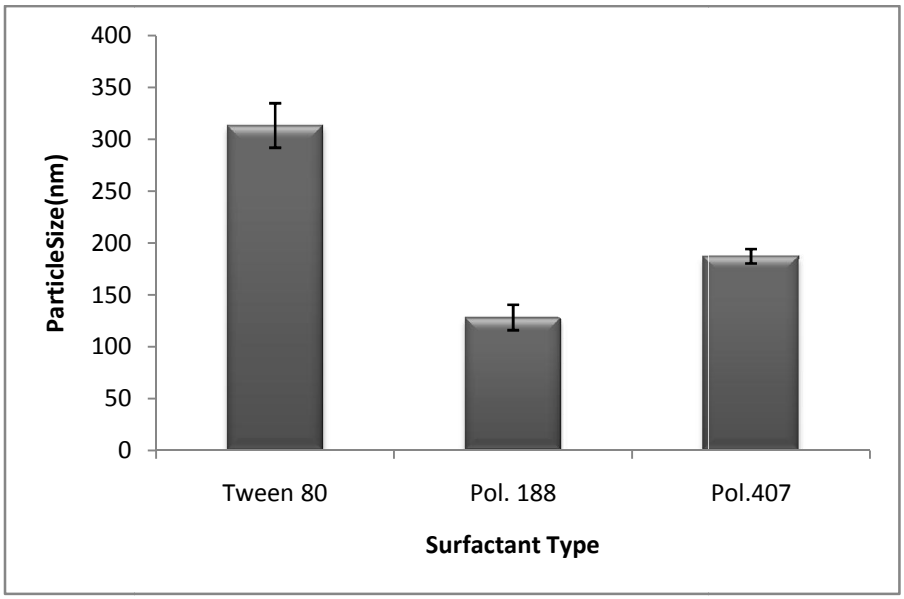


Figure 5.15: Effect of surfactant type on particle size of SLN

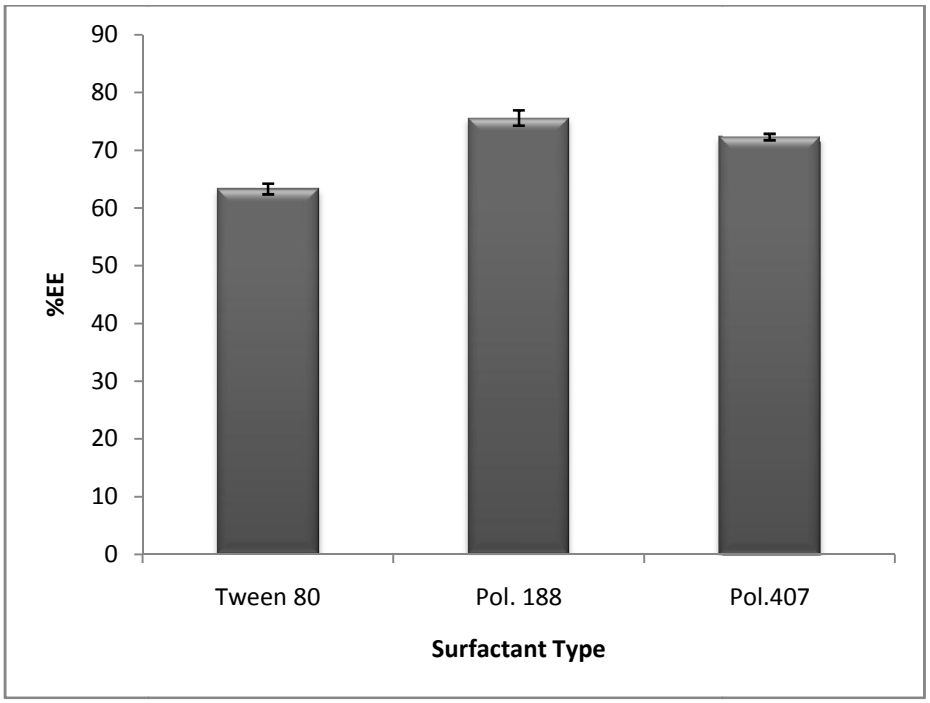


Figure 5.16: Effect of surfactant type on EE of SLN

Table 5.11: The effect of surfactant types on characterizing parameters of SLN

Surfactant	Particle Size (nm)	Polydispersity index	Zeta potential (-mV)	Entrapment Efficiency (%EE)
Tween 80	313.4±21.43	0.482±0.058	9.8±0.73	63.3±0.93
Pol. 188	128.4 ± 12.3	0.383±0.039	12.8±0.57	75.6±1.32
Pol.407	187.3 ± 6.89	0.229±0.028	14.9±0.65	72.3±0.56

mean ±SD; n=4

From the results discussed above, the optimized formulation conditions 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 = 0.05% (w/v), lipophilic surfactant = lecithin granular and hydrophilic surfactant = pol.188.

5.4.5. Optimized single lipid formulation:

The characterization parameters of optimized formulation for TM, TP and TS as lipid matrix are summarized in Table 5.12. The optimized SLN formulations having TP and TS as lipid matrix can be able to produce the SLN of PS 123 nm and 132 nm, respectively having PI less than 0.3 and EE around 85 %. It can be seen that by optimizing the process and formulation parameters, the SLN of desired PS, PI and EE can be obtained. These optimized SLN formulations conditions were subjected to the

morphological study, DSC study, XRD study, sterilization study, *in vitro* release study and storage stability studies.

Table 5.12: The characterization parameters of optimized formulation for TM, TP and TS as lipid matrix

Lipid	Particle Size(nm)	Polydispersity index	Zeta potential (-mV)	Entrapment Efficiency (%EE)
TM	139.2 ± 2.43	0.382±0.058	9.8±0.87	74.5 ± 0.44
TP	123.1 ± 3.41	0.283±0.039	10.8±0.65	86.6 ± 0.94
TS	132.6 ± 4.29	0.229±0.028	11.1±0.74	84.3 ± 0.81

mean ±SD; n=4

Drug stability during the study was analyzed by HPLC and no quantitative/qualitative changes were found. Therefore, SLN preparation method and incorporation of PZQ do not induce any chemical modifications or degradation in the drug molecule and maintains its activity throughout the study.

5.4.6. Storage stability studies

The ability of the SLN 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 of SLN was evaluated in terms of mean particle size, surface charge (ZP) and EE.

After six months storage of SLN at refrigerated conditions, there was insignificant difference in the PS for TM-SLN (F27), TP-SLN (F28) and TS-SLN (F29); while in case of six months storage at 25 °C/65% RH, the particle size was increased significantly from 123.1 ± 3.41nm to 139.3 ± 3.82 nm for TP-SLN and from 132.6 ± 4.29 nm to 153.6 ± 4.74 nm for TS-SLN ($p<0.05$)(Table 5.13).

The EE (%) of the optimized SLN batches TM-SLN, TP-SLN and TS-SLN was initially found to be 74.5 ± 0.44, 86.6 ± 0.94 and 84.6 ± 0.81, respectively which was significantly decreased to 67.9 ± 0.58, 82.6 ± 0.29; 79.3 ± 0.47, respectively after six months 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 68.3± 0.37, 83.9 ± 0.14 and 78.6 ± 0.43 for TM-SLN (F27), TP-SLN (F28) and TS-SLN (F29), respectively ($p<0.05$) (Table 5.13).

Lecithin presence not only provided higher EE but also improved formulation stability in terms of drug retention during storage (Lopes *et al*., 2012). Lecithin provides a less ordered arrangement in the lipid matrix by forming one or several bilayers surrounding the SLN , which in turn results high entrapment, physical stability and less drug expulsion during storage (Attama *et al*., 2007; Kheradmandnia *et al*., 2010, Lopes *et al*., 2012)

Transitions of dispersed lipid from metastable forms to stable form might occur slowly on storage due to small particle size and the presence of emulsifier that may lead to drug expulsion from solid lipid nanoparticles.

The decreased EE observed on storage could be attributed to drug expulsion during lipid modification. An absolute large negative or positive ZP (I30ImV) is required for stability of colloidal dispersion because the electrostatic repulsion could prevent the agglomeration. The ZP of SLN was about -10mV, which was not high enough to provide a strong electrical field around the particles, but measuring the PS of samples throughout storage period showed no significant changes in the PS of these samples. The stability studies confirmed that SLN stabilized with a combination of stabilizers (LG and P188) helped the SLN to remain stable over storage period. Storage at refrigerated conditions provided marginally better stability with regards to both PS and EE.

Table 5.13: Effect of storage at refrigerated conditions (5±3°C) and at 25 °C/65% RH on characterizing parameters of SLN

Formulation	**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**
TP-SLN	123.1 ± 3.41	128.1 ± 4.72	139.3 ± 3.82	86.6 ± 0.94	82.6 ± 0.29	83.9 ± 0.14
TS-SLN	132.6 ± 4.29	140.2 ± 5.11	153.6 ± 4.74	84.3 ± 0.81	79.3 ± 0.47	78.6 ± 0.43
TM-SLN	139.2 ± 2.43	141.2 ± 4.31	164.2 ± 3.77	74.5 ± 0.44	67.9 ± 0.58	68.3 ± 0.37

mean ±SD; n=4

5.4.7. The effect of pH on the stability

The effect of pH on the stability of PZQ loaded SLN was found to be remarkable (Table 5.14). The SLN kept in SGF (pH 1.2) for 2 hours showed increase in PS along with decrease in EE, where as SLN kept for 6 hours in SIF (pH 6.8) were found to be stable. The results are in accordance with other reports (Paliwal *et al*., 2009, Manjunath and Venkateswarlu, 2005). The effect of pH conditions on characterizing parameters was similar irrespective of the lipid matrix used.

Table 5.14: Stability studies of various PZQ loaded SLN at different pH conditions

Formulation	**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)
TP-SLN	123.1 ± 3.41	348.4 ± 6.93	169.2 ± 3.62	86.6 ± 0.94	34.4±0.81	77.3±0.89
TS-SLN	132.6 ± 4.29	364.6± 7.46	184.6 ±3.49	84.6 ± 0.81	36.1±0.72	74.5±0.95
TM-SLN	139.2 ± 2.43	259.3 ± 3.89	171.3±3.79	74.5 ± 0.44	22.3±1.54	50.6±1.73

mean ±SD; n=4

5.4.8. Effect of sterilization

The SLN for parenteral administration should be sterile. The sterilization by filtration cannot be applied in the SLN as particle size may change due to pressure applied in the filtration processes. Moist heat sterilization by autoclaving (121 °C/15 min) has been reported by many researchers as a reliable terminal sterilization process (Venkateswarlu and Manjunath, 2004; Gokce *et al*., 2007). In the present study, the effect of sterilization on particle size and EE was compared with non sterilized formulation and the results are presented in Table 5.15.

In addition to variations in Particle size, sterilization also resulted in reduction of less than 10% in PZQ content for SLN-TP and SLN-TS whereas it was almost 20% in SLN-TM (Table 5.15). The observed differences in PS and EE may be related with the higher stability of SLN during the sterilization process. 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. In fact, the mobility and hydrophilicity of all surfactants molecules were affected by temperature to a different extent (Mehnert and Mader, 2001). From the present study, it can be seen that lecithin and poloxamer 188-stabilized tripalmitin SLN did not induce significant variations in nanoparticulate properties during sterilization by autoclaving.

The variations were observed for SLN and may be related to the particle stability during sterilization. Triglycerides used in the present study had a melting range between 58–70° C, therefore, the SLN lipid core will first

melt during the autoclaving and will recrystallize during the cooling, resulting in particles with altered particle size.

Table 5.15: Effect of sterilisation on characterizing parameters of PZQ SLN

Formulation	**Particle Size(nm)**		**Entrapment Efficiency (%)**	
	Before Sterilization	After Sterilization	Before Sterilization	After Sterilization
TP-SLN	123.1 ± 3.41	434.3 ± 28.86	86.6 ± 0.94	68.3±1.23
TS-SLN	132.6 ± 4.29	448.9 ±24.02	84.3 ± 0.81	65.3±1.56
TM-SLN	139.2 ± 2.43	559.3 ±31.79	74.5 ± 0.44	53.6±1.37

mean ±SD; n=4

Therefore, the stability of SLN during moist heat sterilization mainly depends on the composition of the SLN, mainly the stabilizing surfactant layer that surround the lipid core (Lopes *et al*., 2012; Cavalli *et al*., 1997; Müller *et al*., 2000).

5.4.9. Solubility studies

Saturation solubility studies were carried out for determining saturation solubility of Praziquantel in different medias. Praziquantel saturation

solubility in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 6.8) was found to be 513 µg/ml and 438 µg/ml, respectively.

5.4.10. *In-vitro* drug release study

The saturation solubility (Cs) of PZQ in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 6.8) was found to be 513µg/ml and 438µg/ml, respectively at 37 ± 0.5°C. Sink conditions was maintained for *in vitro* release study: $C_1 < C_S$ X 0.2 where, C_1=Final concentration of Praziquantel after the complete release of the drug in the dissolution medium (Moneghini *et al*., 2000). Therefore, the final concentration of PZQ after the complete release in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 6.8) as dissolution medium was maintained less than 102.6 µg/ml and 87.6 µg/ml, respectively in compliance with the sink condition.

The *in vitro* release of the different SLN formulation was determined in 0.1 N HCl (for 2 hours) and in phosphate buffer (pH 6.8) (for 48 hours). During *in vitro* release studies, the drug release rate of the PZQ from the SLN was governed by the partition coefficient of the drug between the lipid phase and the aqueous environment in the dialysis bag and by diffusion of the drug through the dialysis membrane into the dissolution media. The dialysis bag retained the SLN and allowed diffusion of the drug immediately in to receiver compartment (Das *et al*., 2011). Hence, drug released from the SLN were measured by determining drug concentration in the dissolution media.

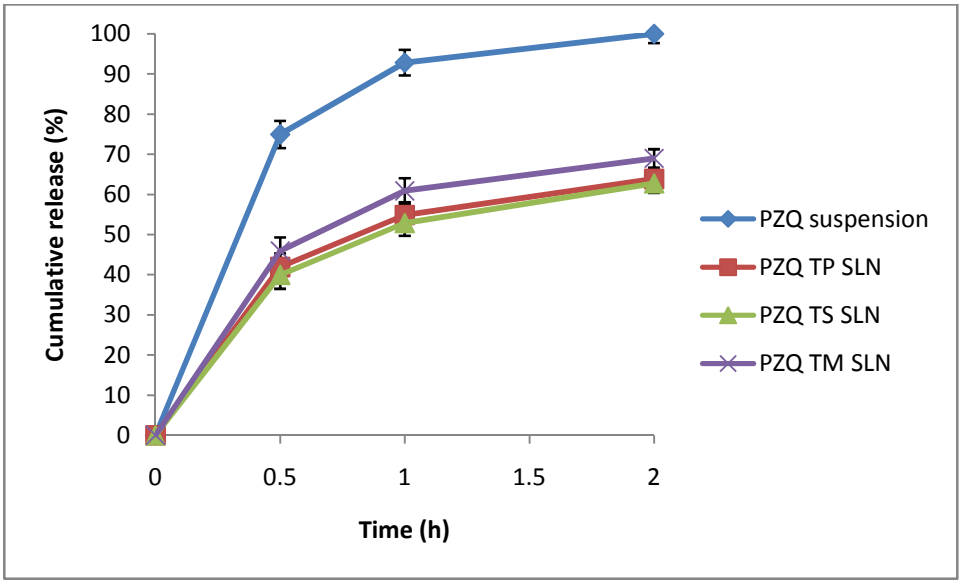


Figure 5.17: *In vitro* release studies in different time intervals of PZQ-loaded SLN in 0.1N HCl (pH 1.2) as dialysis medium.(mean ±SD; n=4)

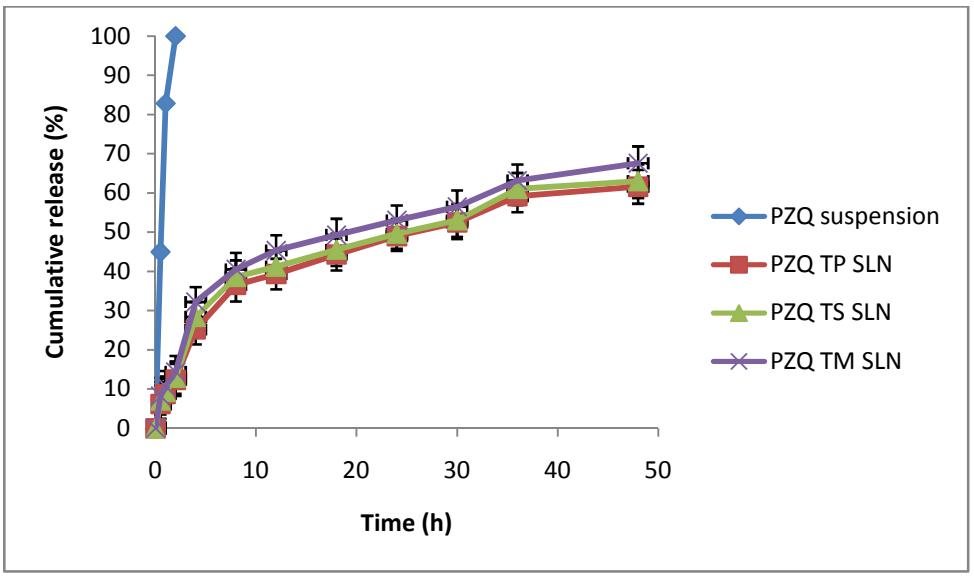


Figure 5.18: *In vitro* release studies in different time intervals of PZQ-loaded SLN in phosphate buffer (pH 6.8) as dialysis medium.(mean ±SD; n=4)

The complete drug release (100%) was achieved within 2h from the control formulation (PZQ suspension) across the dialysis bag indicates

rapid diffusion of the PZQ. The results revealed that all formulations passing through the strong acidic environment of the stomach (as in 0.1 N HCl) tend to release a high amount of drug (68.9% with TM-SLN, 62.7% with TP-SLN, and 63.8% with TS-SLN) (Figure 5.17). The higher drug release obtained in 0.1N HCl for different SLN formulation can be explained due to their susceptibility to with stand the strong acidic environment which has also been showed during stability studies.

In phosphate buffer (pH 6.8), almost all the optimized SLN formulations showed initial burst release with the 25-30% of drug release within first four hours followed by the sustained release from the SLN formulations (Figure 5.18). The slow release of the PZQ from all SLN formulations suggests homogeneous entrapment of drug throughout the systems. Thus, the loading dose of PZQ due to initial burst release can be obtained and followed by maintenance dose due to the sustained release by PZQ-SLN which in turn will prevent the fluctuations in the PZQ plasma level. Similar sustained/prolonged drug release from the SLN was also observed by other researchers (Paliwal *et al* 2010, Venkateswarlu and Manjunath, 2004,Vivek *et al*.,2007).

The drug release from SLN is influenced by the nature of the lipid matrix, surfactant concentration and production parameters (Muller *et al*., 2000). As the surfactant composition and concentration was same in all the formulation, the drug release was affected by other parameters such as lipid nature and its lipophilicity (carbon chain length). The controlled drug release may be attributed to the hydrophobic long carbon chain of

triglyceride that retains lipophilic drugs (Mehnert and Madar, 2001). The presence of longer carbon chain length in TP and TS resulted in increased drug entrapment inside the lipid core and hence, prolonged the release. The drug release can also be correlated with their EE. Higher the EE, lower is the amount of free drug available for burst release and this ultimately results in controlled release. As TP exhibited higher EE than TM and TS, therefore, it exhibited prolonged release.

5.4.11. Release kinetics study

The release from different optimized batches of PZQ- SLN in phosphate buffer (pH 6.8) was fitted in first order, Higuchi model Korsmeyer-Peppas model and Weibull kinetics. The regression coefficient of first order, Higuchi model, Korsmeyer-Peppas model and Weibull kinetics are shown in Table 5.16.

It can be seen from R^2 values 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 may be prominent 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)

Table 5.16: The *in-vitro* release kinetics model of optimized PZQ solid lipid nanoparticles 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)
TM- SLN	0.807	0.944	0.957	0.842
TP –SLN	0.832	0.960	0.970	0.842
TS –SLN	0.817	0.948	0.960	0. 842

5.4.12. Shape and morphology

The lipid nanoparticles were viewed through Transmission electron microscopy (TEM) for their shape and morphology.

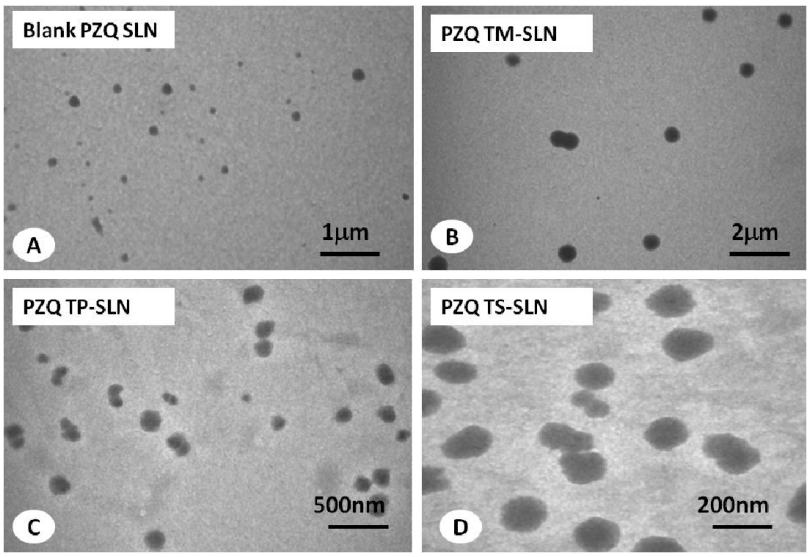


Figure 5.19: Transmission electron micrographs (TEM) of different solid lipid nanoparticles. (A) Blank PZQ SLN ,(B) PZQ TM-SLN,(C) PZQ TP-SLN and (D) PZQ TS-SLN

The TEM images of Blank SLN, PZQ TM-SLN, PZQ TP-SLN and PZQ TS-SLN are shown in Figure 5.19 (A–D). The TEM revealed that particles were almost spherical with smooth surface morphology and monodispersed. The blank SLN and the PZQ-SLN being similar in appearance reflects that encapsulation of PZQ did not affect the morphology of SLN. These results are in agreement with the particle size data determined by PCS (Table 5.12).

5.4.13. Differential scanning calorimetry

DSC is a useful technique to understand solid dispersions like solid solutions, simple eutectic mixtures, or, as in this case, drug and lipid interactions. It is a tool that gives an insight into the melting and recrystallization behaviour of crystalline materials like SLNs (Hou *et al*., 2003; Jenning *et al*., 2000).

Figure 5.20 shows DSC thermograms of pure Praziquantel, trimyristin, tripalmitin, tristearin and lyophilized PZQ-loaded SLN. DSC thermogram of pure Praziquantel demonstrated a sharp peak at 142 °C corresponds to melting temperature of Praziquantel (Passerini *et al*., 2006; Liu *et al*., 2004). The thermogram of trimyristin, tripalmitin and tristearin bulk lipids showed endothermic peaks at 58 °C, 63.4°C and 69.6°C, corresponding to their melting temperature, indicating crystalline nature respectively. The SLN were lyophilized to protect the physical state of lipid.

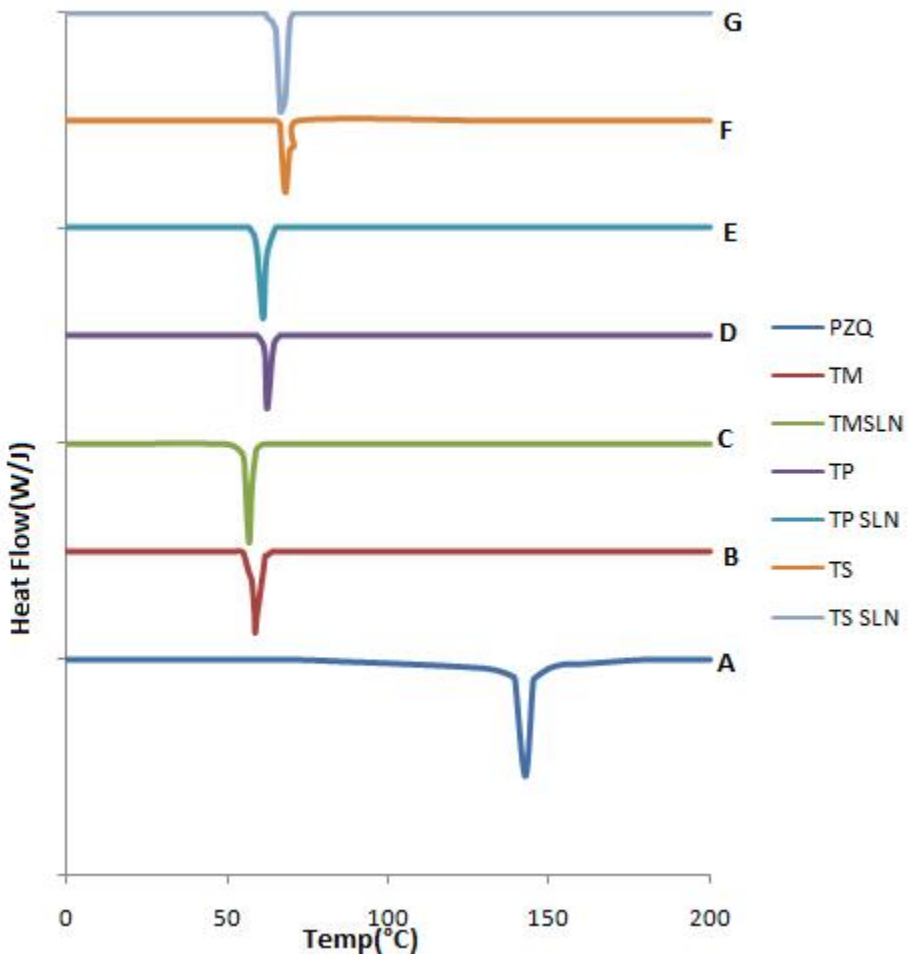


Figure 5.20: DSC thermograms of (A)-Praziquantel, (B)-Trimyristin, (C)-Praziquantel loaded TM -SLN, (D)-Tripalmitin, (E)-Praziquantel loaded TP-SLN, (F)-Tristearin, (G)-Praziquantel loaded TS-SLN .

The absence of PZQ melting peak at 142 °C in SLN indicates either formation of amorphous dispersion of PZQ in lipid matrix or solubilization of PZQ in lipid matrix upon heating.

In SLN formulations, the melting points of lipids were depressed in comparison to the melting point of the bulk lipid. This was attributed to the creation of lattice defects onto the lipid matrices following a decrease in their crystallinity in comparison to their bulk counterparts. For less

ordered crystals or amorphous solids, the melting of the substance requires much less energy than crystalline substances that need to overcome lattice forces (Hou *et al*., 2003). Despite the reduction on the melting point of triglycerides in the SLN, no significant effect was observed on thermal behaviour pattern of lipid matrix after incorporation of PZQ.

5.4.14. X-Ray diffractometry (XRD) study

X-Ray Diffraction techniques are a family of non-destructive analytical techniques used to reveal information about the crystallographic structure, chemical composition and physical properties of materials and thin films. Hence, XRD has become an important diagnostic and analytical tool with a broad range of applications. The technique is based on observing the scattered intensity of an X-Ray beam hitting a sample as a function of incident and scattered angle, polarization, and wavelength or energy.

The principle advantage of this technique lies in its capability to establish the chemical identity of crystalline materials rather than its elemental composition; the different polymorphic forms of the same compound can be easily identified in a mixture of two or more. However, it requires a library of diffractograms of standard pigment sample or a mixture of pigments in a sample.

The XRD patterns of pure praziquantel, trimyristin, tripalmitin, tristearin, lyophilized drug free SLN and lyophilized PZQ-loaded SLN are shown in

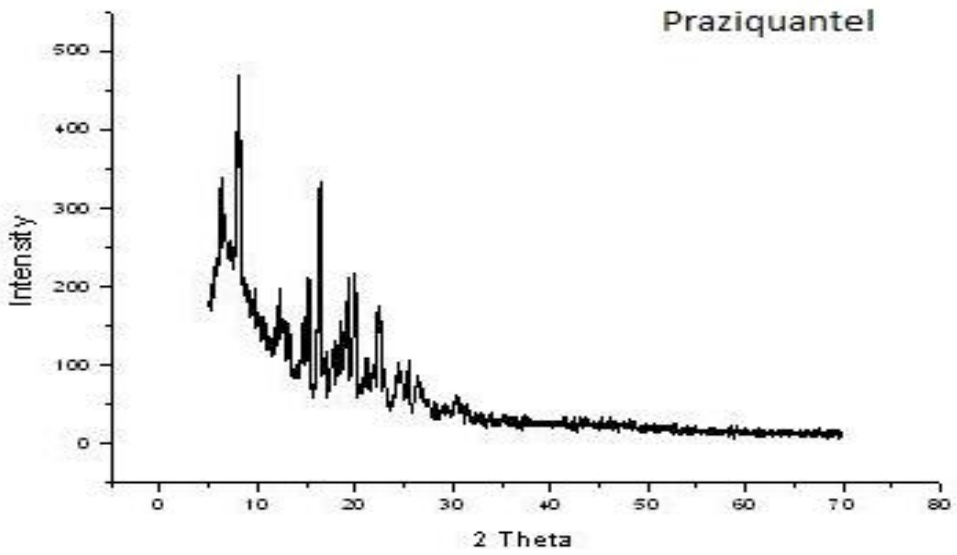
Figure 5.21. Powder XRD data confirmed the results demonstrated by DSC study. The diffraction pattern of PZQ indicates that the drug is crystalline and distinct sharp peaks were observed at 2 θ scattered angles 6.2°, 7.9° and a series of peaks above 10° in the diffractogram. The XRD pattern corresponded to that of PZQ racemate crystal, as reported in literatures (Li *et al*., 2010; Passerini *et al*., 2006; Liu *et al*., 2004). However, these peaks could not be detected in diffractogram of PZQ-SLN which indicates that praziquantel was solubilised within the lipid matrix of SLN and stabilized in amorphous form and was not in crystalline form in SLN.

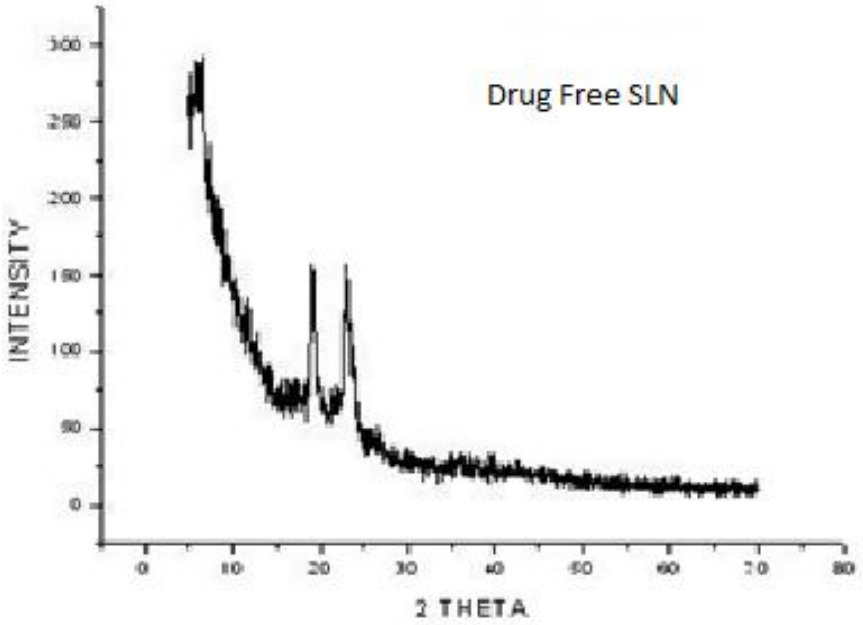
XRD pattern of TM shows sharp peaks at 2 θ scattered angles around 5, between 18-27°; these characteristic peaks were observed in lyophilized PZQ-TM indicating that TM was in crystalline state. XRD pattern of TP shows sharp peaks at 2θ scattered angles around 5° and between 18-27°; these characteristic peaks were observed in lyophilized PZQ-TP SLN indicating that TP remains in crystalline state. Similarly, TS (25.23°) were present in crystalline state in lyophilized SLN (Venkateswarlu and Manjunath, 2004).

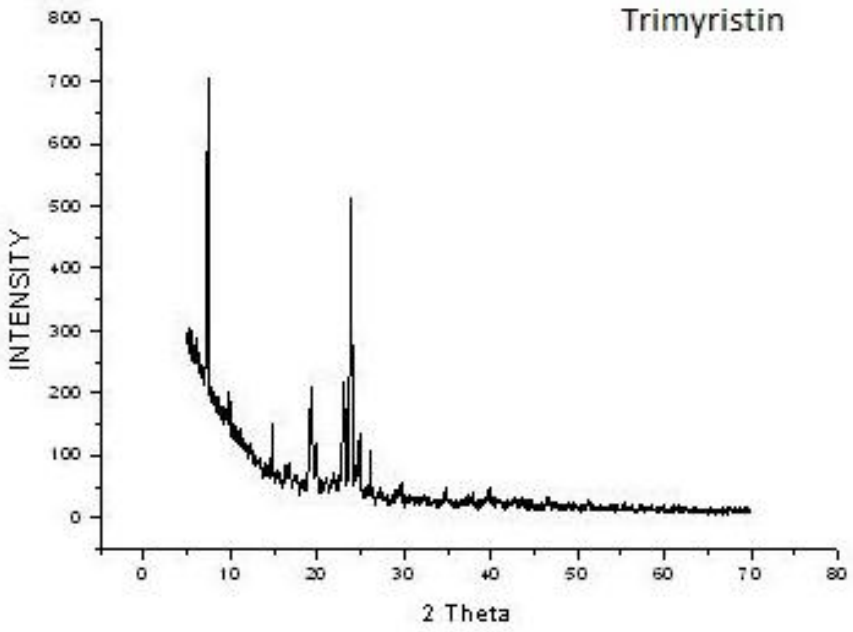
Due to its poor aqueous solubility, if praziquantel was located outside the lipid matrix, crystallization would occur which should have affected the diffraction patterns of drug-loaded SLN. This suggests that the drug was successfully incorporated into the lipid matrix of the developed SLN. Also, looking at the diffraction patterns of drug-free (control) SLN and drug loaded SLN, there was not much difference in the pattern, indicating that the addition of PZQ did not changed the nature of SLN. An explanation to

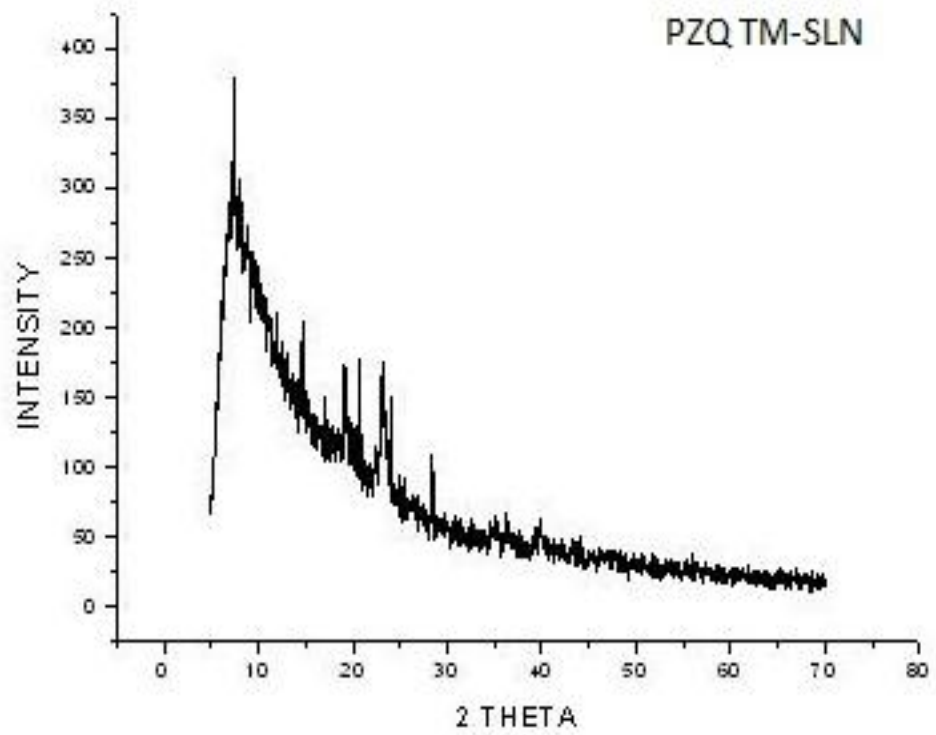
this observation is that the PZQ might be entrapped in the lipid core of SLN.

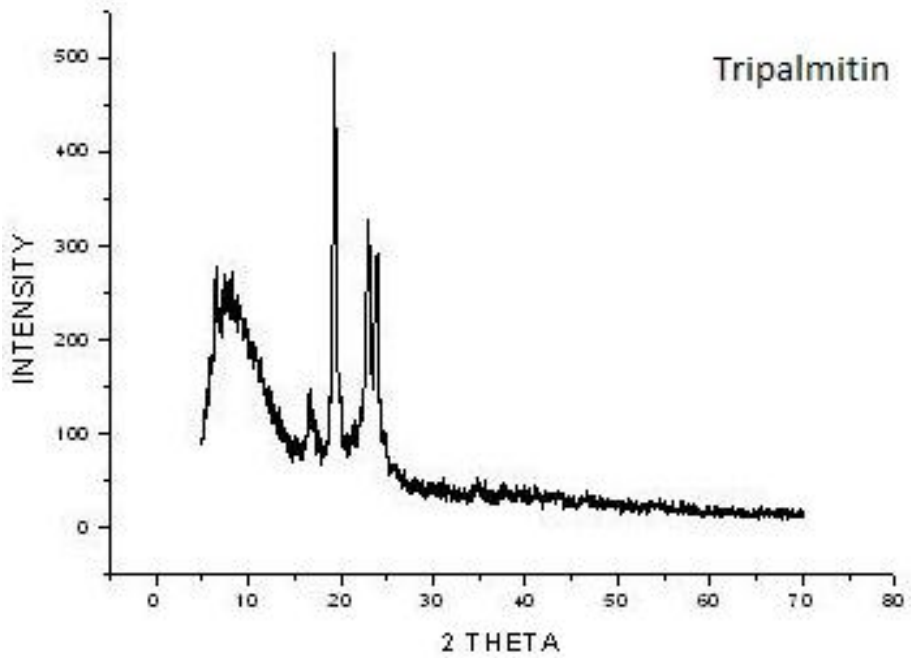
In addition PXRD spectra obtained between 2 θ scattered angles=18–25◦, where the bulk lipids had sharp peaks that are almost absent in the diffractograms of the SLN. This indicates lower crystallinity and hence, the less ordered crystal arrangements in the SLN formulations compared to the bulk solid lipid, such an amorphous state, would contribute to the higher drug loading capacity (Hau *et al*., 2003, Chattopadhyay *et al*., 2007).

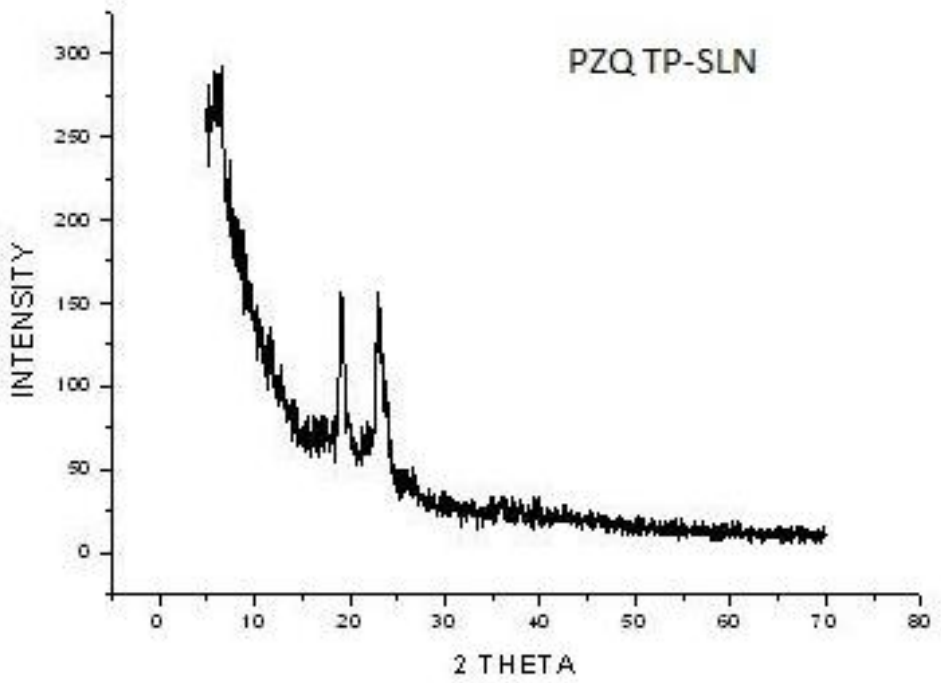


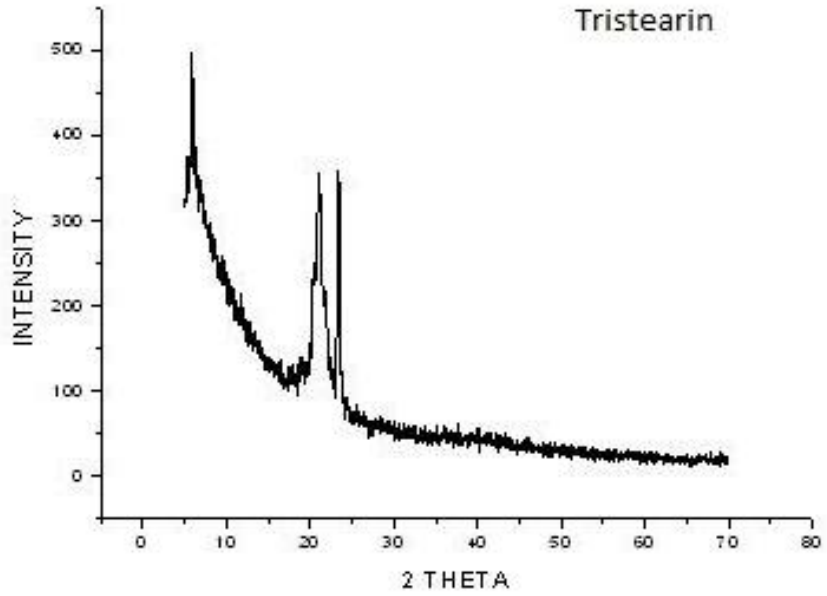

Drug Free SLN
INTENSITY
2 THETA


Trimyristin
INTENSITY
2 Theta


PZQ TM-SLN
INTENSITY
2 THETA
0
50
100
150
200
250
300
350
400
0
10
20
30
40
50
60
70
80


Tripalmitin
INTENSITY
2 THETA
0
100
200
300
400
500
0
10
20
30
40
50
60
70
80


PZQ TP-SLN
INTENSITY
2 THETA
0
50
100
150
200
250
300
0
10
20
30
40
50
60
70
80


Tristearin
INTENSITY
2 THETA
0
100
200
300
400
500
0
10
20
30
40
50
60
70
80

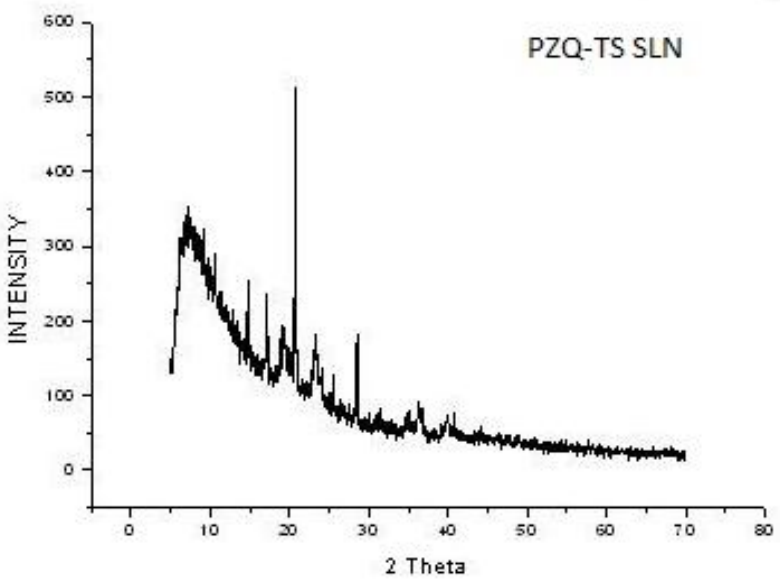


Figure 5.21: XRD patterns of (A)Praziquantel, (B)Trimyristin, (C)Praziquantel loaded TM -SLN, (D)Tripalmitin, (E) Praziquantel loaded TP-SLN (F)Tristearin (G) Praziquantel loaded TS-SLN and (H)Drug free SLN.