CHAPTER - 5

FORMULATION DEVELOPMENT

5.1 INTRODUCTION

In the beginning of 1990, apart from the alternative novel drug delivery system such as liposomes, polymeric nanoparticles and emulsions, Solid Lipid Nanoparticles (SLN) was introduced as an alternative approach. SLNs are manufactured by o/w emulsion techniques by using solid lipid or solid lipid blend for the replacing oily lipid. SLN has a numerous unique property such as larger surface area, smaller size, phases interaction at the interfaces for their ability to improve the performance of pharmaceuticals, nutraceuticals and other dosage forms (1). SLN particles have a capability to solubilize lipophilic molecules as it is composed of a solid lipid core matrix. Surfactants are used to stabilize lipid core matrix (2). For pharmaceutical purposes and applications, generally recognized as safe (GRAS) excipients are used in the formulation. For the administration of solid lipid particles, the melting point of lipid nano particles must exceed body temperature (37°C). Acylglycerols, fatty acids, waxes, triacylglycerols (triglycerides), steroids and combinations there of were investigated as high melting point lipids (3). Bile salts such as sodium taurocholate, membrane lipids such as lecithin, biocompatible nonionic such as sorbitan esters, fatty acid ethoxylates, ethylene oxide/propylene oxide copolymers and mixtures thereof were investigated as surfactants (4).The literature on solid lipid nano technology as a drug delivery, various reviews are available which explains the preparation techniques, types of SLN, characterization and structural properties investigation and its factors which affect the formation and stability storage including drug release characterization and drug loading principles (5).

In the present study, attempts were made to prepare and characterize Tapentadol (TAP) loaded SLN for improvement of T_{max} and attaining higher concentrations in the brain. Research efforts were primarily focused to encapsulate the free base in selected lipids along with different surfactants for particle stabilization. Lyophilization with suitable cryoprotectants (mannitol and sucrose) was carried out for steric stabilization of the particles as well as to facilitate ease of reconstitution.

Lyophilization also helps to stabilize the solid state of lipids. Selected formulation, based on in vitro evaluation results, were further used for in vivo pharmacokinetic and extrapolated brain studies on animal model and compared with oral solution.

Alternatively, another formulation approach was explored using *in situ* gelling systems. These systems are formulated with polymers which are in sol form at room temperature and undergoes phase transition when administered in the body into gel form. The gelation rate, strength depends on the nature of the polymer, pH of the microenvironment, ionic concentrations of the body fluids. Various natural polymers like alginic acid, pectin, Gellan gum and synthetic block polymers like polylactic-glycolic acid have been explored in different delivery systems like vaginal, nasal, injectable,ocular and intra-peritoneal.

Gellan gum is an anionic deacetylated, exocellular polysaccharide secreted by Pseudomonas elodea with a tetrasaccharide repeating unit of 1-l-rhamnose, 1-d-glucuronic acid and 2- d-glucose. The mechanism of gelation involves the formation of double-helical junction zones followed by aggregation of the double-helical segments to form a 3-D network by complexation with cations and hydrogen bonding with water.Since nasal mucosa is rich in Sodium, Pottasium and Calcium ions, it was hypothesized to dissolve tapentadol base in aqueous solution of Gellan gum and study the effect of altered mucoadhesive property on pharmacokinetic properties.

5.2 EXPERIMENTAL SECTION

5.2.1 Materials and Methods

Tapentadol (TAP) Base (assay 99.8 %) was synthesized from Tapentadol Hydrochloride obtained from Symed labs, India. Glyceryl behenate (Compritrol 888 ATO) was obtained from Gattefosse, Polysorbate 80 was obtained from Seppic, France, sucrose was obtained from Pfanstiehl, HPMC 5cps from FMC Biopolymers and Gellan gum was obtained from CP Kelco. Other chemicals were obtained as Analytical Reagent grade.

5.2.2 Equipment/Instruments

An overhead homogenizer (Polytron PT2000, GmbH) and Probe sonicator (Hielscher, GmbH) was used for the preparation of SLN and size reduction. Lyophilizer (Lyostar-II) was used for lyophilization of the SLN suspension. IKA simple anchor stirrer was used to prepare *in situ*Gellan gum formulation.

Raman spectrophotometer with a microscope (Morphologi G3 SE ID, Malvern UK) was used to characterize for the spectra and stability, Zetasizer (Malvern, UK, Nano-ZS) was

used for particle size, zeta-potential (ZP) analysis and poly-dispersity index (PDI). Zeiss polarized microscope (Zeiss) was used for initial characterization of formulations.

5.2.3 Solid lipid nanoparticle preparation

Development of Tapentadol SLN was initiated by selecting the suitable ingredients which include lipid, selected emulsifier, and water. The term lipid is used here in a broader sense and includes fatty acids (e.g. stearic acid), steroids (e.g. cholesterol), triglycerides (e.g. tristearin), waxes (e.g. cetyl palmitate) and partial glycerides (e.g. Imwitor). After studying the physical properties Glyceryl behenate (glyceride of 22 carbon chain fatty acids, behenic acid) was chosen due to its low toxicity (GRAS) and amenable physical properties to pharmaceutical processing like the melting point. Emulsifying agents were used during formulation to improve stability properties of the lipid dispersion. It is widely reported that the emulsifiers alone or in combination helps in steric stabilization of both lipids as well as SLNs

SLNs has been demonstrated to be relatively safe of potential acute and chronic toxicity (5) as most of the lipids used for the matrix formation is made from biocompatible lipids. A wide array of emulsifying agents have been approved for the intravenous route, in the present study emulsifiers listed in Inactive Ingredient Database for the parenteral route has been used. Polysorbate 80 was the preferred choice as an emulsifier as it has been widely used in various marketed nasal formulations.

In the present research work, hot homogenization method was selected because the drug has allowed melting range in tandem with low lipid melting point to improve entrapment efficiency. Lyophilization cycle was designed using Smart Cycle to optimize the primary drying cycle with an objective to stabilize the SLN particles with respect to shape and size.

Different formulation trials were executed to attain monodisperse SLN, with improved stability at room temperature. The design of Experiments was planned to optimize the concentration of surfactant, type of surfactant, type, and concentration of cryoprotectant.

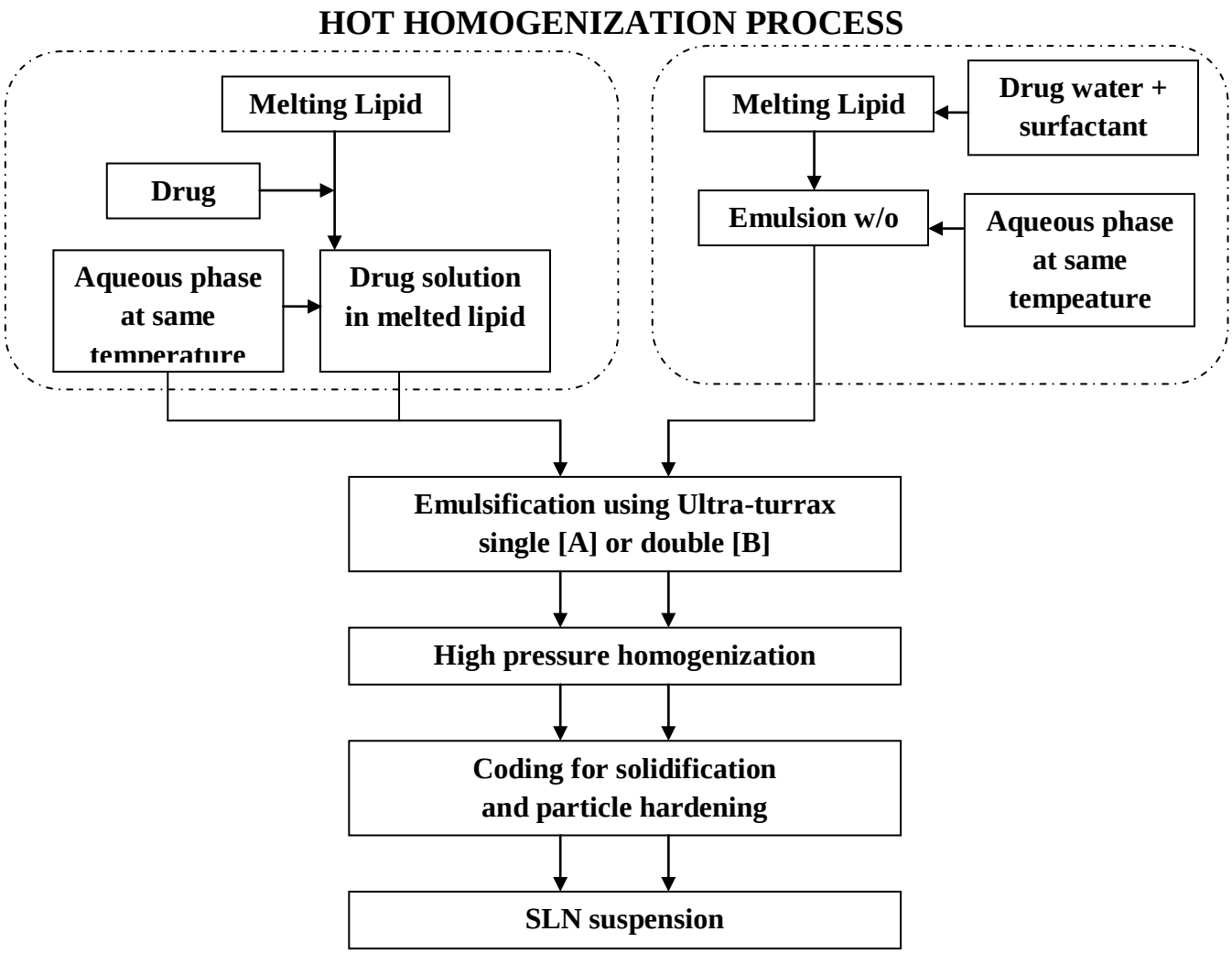

Figure5.1: Schematic presentation of Hot homogenization techniques for preparation of TAP loaded SLN

5.2.4 Freeze drying and storage

The prepared SLN, diluted by Sucrose solution and filled into vials, were immediately freeze dried using the lyophilization cycle as under:

Table 5.1: Optimized lyophilization cycle for Tapentadol SLN

Step	Shelf set point (°C)	Ramp rate (°C/min)	Time (min)
Freezing			
1	5	1	30
2	-5	1	30
3	-40	1	180
4	-20	1	10
5	-40	1	180
Primary/secondary drying			
6	-37	0.5	300
7	-26.6	0.5	540
8	-23.7	0.5	1200
9	0	0.3	960
10	20	0.3	480

5.2.5 Formulation and process variables effects

Various SLNs were prepared by Hot homogenization method containing Tapentadol and were tested for various critical attributes. The effect of formulation factors and process variables on characteristics of lipid (Glyceryl behenate) and SLNs were investigated using different polymeric stabilizers and emulsifiers. Different concentration of emulsifiers and type were tested on the final prepared suspension with respect to stability and homogeneity. Also, the effect of cryoprotectant type and concentration on the size and stability of the prepared SLN were evaluated.

5.2.6 Characterization of SLN

All the prepared SLN formulations were extensively characterized as per following procedures.

5.2.7 Estimation of Tapentadol in SLN

The estimation of entrapment efficiency and loading efficiency was carried out by measuring the drug content in the individual SLN formulations. Accurately weighed freeze-dried SLN were transferred to a freshly calibrated flask. In order to release entrapped drug, the SLN

wasdigested by dissolving in IPA with ultra-sonication for 30min at 25^0C. Then it was suitably diluted with buffer pH4.0 after filtering through a membrane filter (0.22µm, Millipore®) and then was used for analysis. The amount of drug was determined using the analytical method described in Chapter 3. Each determination was performed in triplicate and values are represented as average with standard deviations.

The encapsulation efficiency (EE) in percentage was calculated from the total drug amount entrapped by using following formula

$$EE\ (\%) = \frac{\text{Amount of Drug in SLN (mg)}}{\text{Initial amount of drug taken (mg)}} \quad X\ 100$$

The loading efficiency (LE) was calculated from the drug amount present per unit weight of the final product using following formula

$$LE\ (\%) = \frac{\text{Amount of Drug in SLN (mg)}}{\text{Amount of SLN (mg)}} \quad X\ 100$$

5.2.8 Particle size distribution (including particle size)

The particle distribution (including size) of the individual formulation were analyzed by photon correlation spectroscopy (PCS) using a Zetasizer. Freeze-dried SLN formulations were suitably dispersed in Milli-Q® water. For each measurement, sufficiently reconstituted and diluted SLN dispersions 1:4 were assessed for poly dispersity index (PDI) and average particle size.

5.2.9 Particle morphology and shape

The morphological characterization and direct visualization of the prepared SLN formulations were performed using polarized microscopy. The prepared SLN were dispersed in pure water by sonication for analysis. For polarized microscopy, SLN suspension was (drop) placed on a glass slide and observed under 50X objective lens and the same procedure was repeated after lyophilization of SLN followed by reconstitution with pure water.

5.2.10 In-vitro drug release studies

Different SLN formulations were investigated for in-vitro drug release study using dialysis bag diffusion technique. An accurately weighed amount of Tapentadol-loaded SLN dispersions containing the drug equivalent to 2.5 mg was transferred to a dialysis bag and

sealed. The sealed bag was then suspended in a beaker containing 250 ml of phosphate buffer saline pH 7.4 and stirred at a constant speed of 50 rpm at 37°C ± 0.5°C. Aliquots were withdrawn at pre-determined intervals from the receptor compartment up to 48 hours and the same was replaced with fresh buffer. Then the drug content was determined spectrophotometrically by measuring the absorbance at 207 nm (HPLC-dual absorbance) using the respective receptor medium as a blank, to calculate the amount of drug released from the nanoparticles.

5.2.11 Stability studies

The physical state of entrapped drug in the optimized SLN formulations was assessed by performing thermal studies using Raman spectroscopy. All measurements were carried out on G3 morphologi SE ID, as described earlier. Raman shift was observed in initial sample and stability sample kept at different temperature condition (25°±5°C/75%±5% RH). The Raman spectra of the optimized SLN formulations loaded with and without Tapentadol were compared with the pure drug, pure lipids and the physical mixture as mentioned in pre-formulation Chapter 4.

US-FDA has not framed any specific guidelines for solid lipid drug delivery systems (SLDDS), production, characterization, handling, and use. There is no protocol and limits available from any international bodies to conduct stability studies of these formulations. In the present work, the claimed stability of optimized SLN formulations, in the dispersed and freeze-dried state, was investigated over a period of time by exposing samples to three different conditions, ambient temperature (25°±5°C/75%±5% RH), refrigerator (5°±3°C) over a period of 4 months. The SLN was evaluated at 0, 1 and 4 months for the size, PDI, ZP, EE and in-vitro dissolution. In addition, any change in physical appearances was observed and the samples were characterized by their structure using polarized microscopy.

5.3 RESULTS AND DISCUSSION

5.3.1 Preparation of SLN

Composition and physical characters of prepared SLN are presented in Table 5.1-5.6. Hot homogenization technique followed by lyophilization method was found to be the most suitable technique for preparation of TAP loaded SLN with stable critical attributes. Hot homogenization was reproducible and scalable for encapsulating lipophilic drugs with high encapsulation efficiency. Based on the initial screening experiments, (melting point of lipid

and melting point of drug) lipid and drug composition ratios were fixed throughout the study when other formulation parameters were changed. The quality of pre-emulsion was greatly influenced by the type of surfactant and its concentration on the size, shape and size distribution of SLN. The surfactant not only stabilized the lipid drug droplets but stabilized the particles when precipitated into an aqueous medium and when high energy processes like ultra sonicationwere used for size reduction.

Temperature is the most critical process parameter, it plays an important role in the formation of emulsion and droplet size distribution, therefore during the homogenization, the temperature of the aqueous phase and the lipid drug phase kept constant (85-90°C). Gradual cooling was found beneficial in the hardening of SLN from emulsion stage.

Lyophilization was required for steric stabilization of the SLN to prevent agglomeration and aggregation, to prevent the leaching or premature release of drug from SLN during stability. During hardening step, some of the free drug gets deposited on the surface of SLN, therefore washing step was introduced to remove the free drug to prevent the burst effect.

Table 5.2 : Composition and characterization of TAP loaded SLN prepared by hot homogenization method

Material	TAP/SLN/02	TAP/SLN/03	TAP/SLN/04	TAP/SLN/05	TAP/SLN/06/A	TAP/SLN/06/B	TAP/SLN/07	TAP/SLN/09 (optimized)
	% composition							
Lipid (Glyceryl Behenate)	10%	10%	10%	10%	10%	10%	10%	10%
Drug (Tapentadol base)	1%	1%	1%	1%	1%	1%	1%	1%
Surfactant (Polysorbate 80)		2%	2%	4%	4%	4%	4%	4%
Poloxamer 188	0.60%	-	0.60%	-	-	-	-	-
Stabilizer (PVP K12 0.5%)	_	_	_	0.50%	-	-	-	-
Mili Q water	Q. S (100%)	Q. S (100%)	Q. S (100%)	Q. S (100%)	Q. S (100%)	Q. S (100%)	Q. S (100%)	Q. S (100%)
Morphology	spherical in shape with no uniformity in size	spherical in shape withnot uniformity in size	spherical in shape with no uniformity in size	spherical in shape with uniformity in size	spherical in shape with uniformity in size	spherical in shape with uniformity in size	spherical in shape with uniformity in size	spherical in shape with uniformity in size
Particle size (nm) (before lyo)[b]	897.2 ± 5.34	_	_	297.8 ± 4.15	238.2 ± 8.32	232.4 ± 5.26	290.4 ± 7.2	245.5 ± 6.12
PDI[a]	0.478	_	_	0.371	0.238	0.251	0.279	0.345
Zeta potential[c](before lyo)	-	-	-	-32.2	-34.5	-26.6	-38.0	-43.5
Stability	Gelation after formulation.	Gelation after formulation.	Gelation after formulation.	Gelation 24 hrs. after formulation.	Gelation and crystal growth after approx. 5 days.	Gelation and crystal growth after approx. 5 days.	Gelation and crystal growth after approx. 5 days.	No gelation and crystal growth

*Each data represents the average and standard deviation of three independent determinations, [a]polydispersity index, [b]particle size

5.3.2 Physiochemical characterization of SLN

a) Microscopic characteristic of SLN

The detailed microscopic characteristic properties of the prepared SLN were investigated and presented using the polarized microscopic techniques (Figure: 5.2). The simple microscopic image showed that all the prepared SLN were spherical in shape with homogeneous solid matrix structure and no evidence of aggregation (Figure: 5.3).

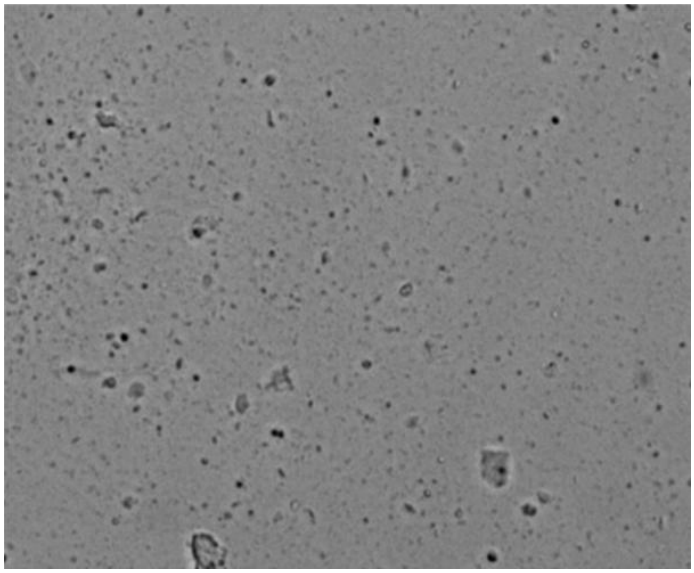

Figure 5.2: Microscopic View at 50X (Before Lyo)

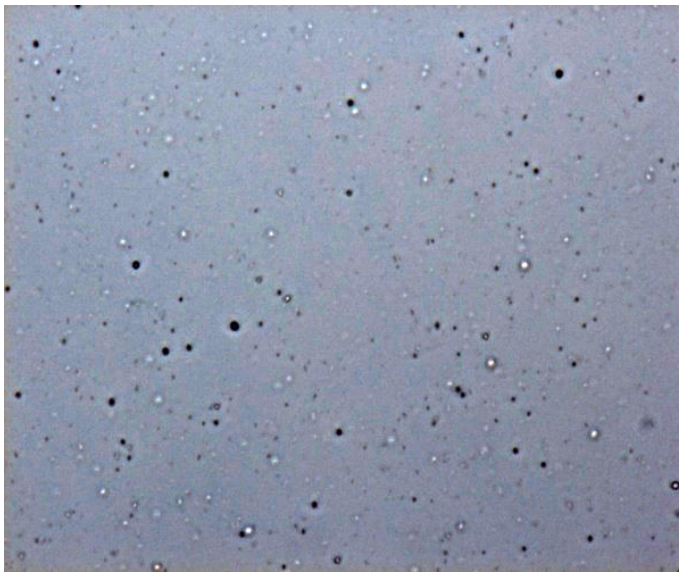

Figure 5.3: Microscopic View at 50X (After Lyo)

This examination was done before lyophilization and after lyophilization of the SLNs. It was observed that the size of SLN prepared using hot homogenization method is seen

smaller which is again re-confirmed with zetasizer. After lyophilization no aggregation was seen when reconstituted with pure water, thus reconfirming that sterically stabilized SLN particles were produced

5.3.3 Effect of formulation parameters and processing variables on SLN characterization.

Effect of amount of concentration and type of surfactant on SLN characteristics

The results indicated that the concentration and type of surfactant effects encapsulation efficiency and loading efficiency of prepared SLN to a greater extent (Table 5.1). However, the variation observed in particles size with respect to drug amount and surfactant was lesser as compared to loading efficiency and encapsulation efficiency. The results indicate that LE was highest (≈15%) in 1:10:4 (drug:lipid:surfactant) ratio but EE was lowest (≈1%).The average particle size was almost constant (≈230 nm) for drug. However, the average particle size was maximum (≈897 nm) with lowest surfactant concentration and type i.e. poloxamer188 at a concentration of 0.6%.

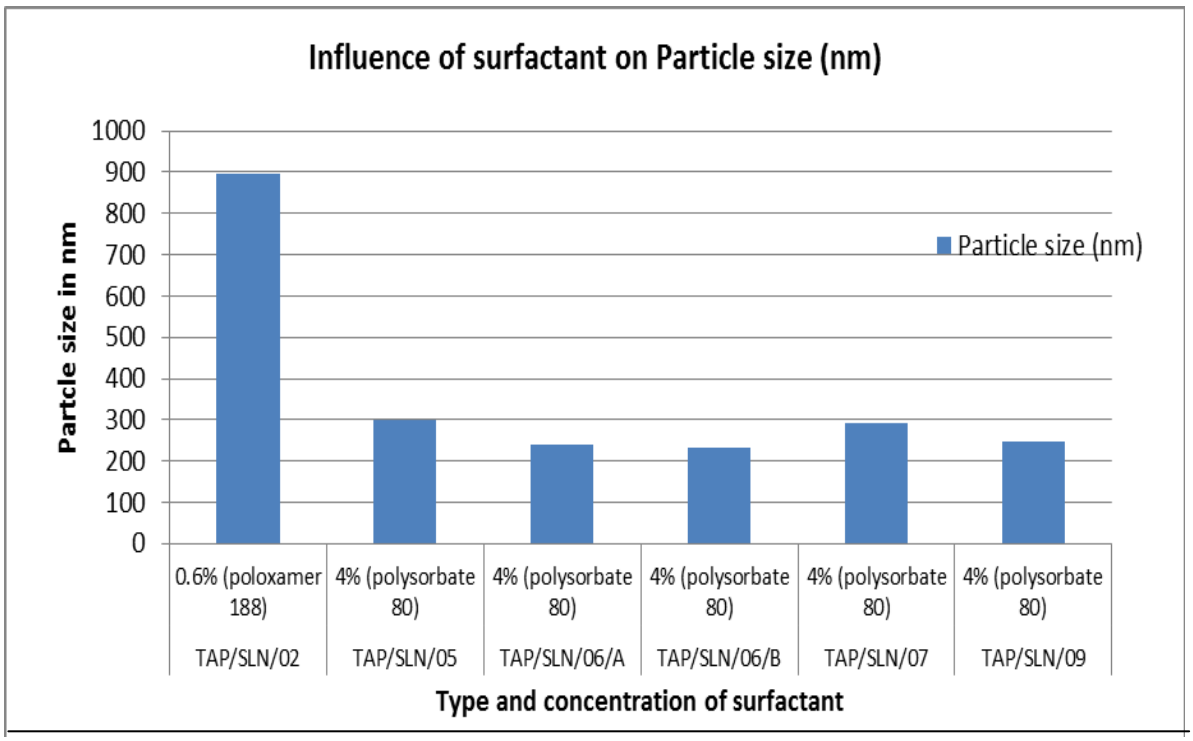

Figure 5.4: Effect of amount of type and concentration of surfactant on prepared TAPSLN particle size

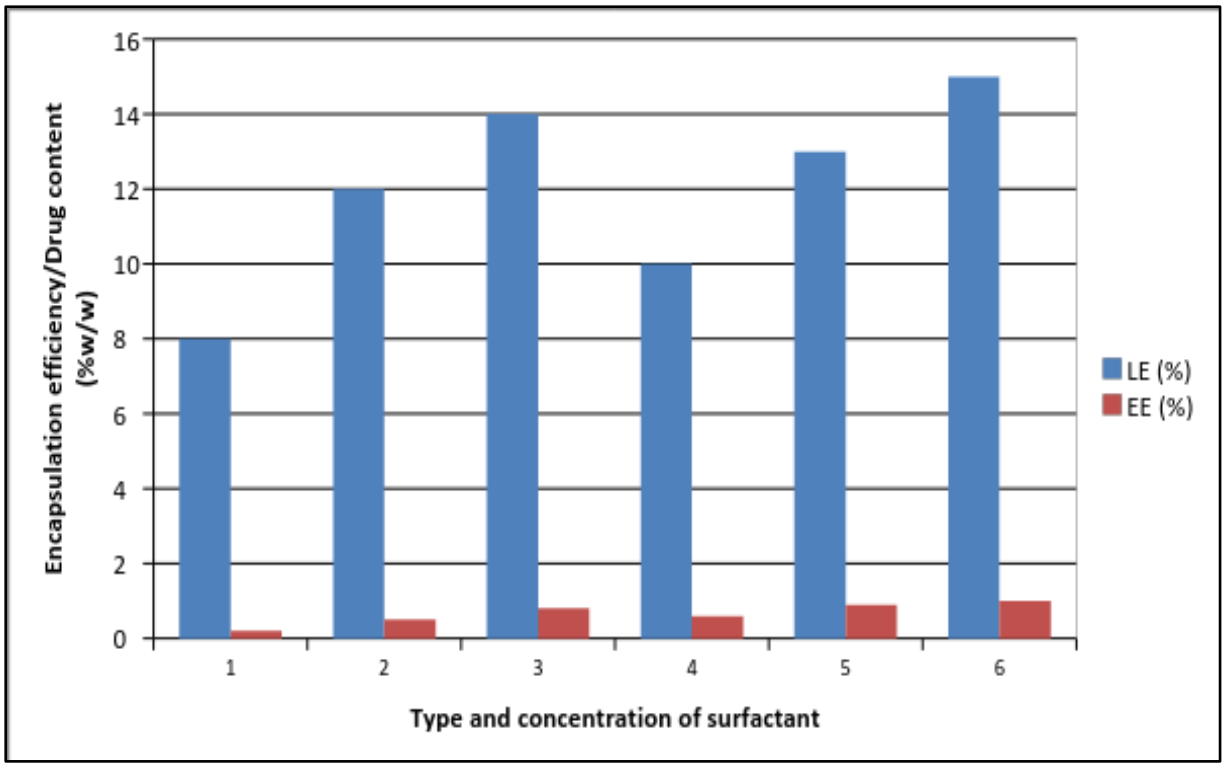

Figure 5.5: Effect of amount of type and concentration of surfactant on prepared TAP SLN Encapsulation efficiency and Loading efficiency

5.3.4 Effect of different cryoprotectant and concentration on SLN characteristics

Lyophilization process had a significant effect on the particle size and stability of the finished formulation and amount of TAP encapsulation, zeta potential, and particle size. Different type of cryoprotectant and concentration was used for the lyophilization and stabilization of SLN.

Mannitol was initially used which is a crystalline material to influence the freeze drying characteristics of the prepared SLN compared to sucrose which is amorphous material and having the glass transition temperature of ~-32°C. Compared to mannitol, sucrose gave more uniform and stabilized SLN, and during reconstitution sucrose helped in ease of uniform dispersion than mannitol as rate of solubilization of sucrose is higher than mannitol.

Table 5.3: Effect cryoprotectant on the characteristics of TAP SLN

Material	TAP/SLN/06/A	TAP/SLN/06/B	TAP/SLN/07	TAP/SLN/09
Cryoprotectant	**Sucrose 5%**(hot solution in water	**mannitol 5%** (hot solution in water	mannitol 10% (hot solution in water)	**Sucrose 7.5%**(hot solution in water
Morphology and stability	spherical in shape with uniformity in size, growth in particle size observed after lyo	spherical in shape with uniformity in size, does not reconstitute properly	spherical in shape with uniformity in size, does not reconstitute properly	Reconstitute easily, slight increase in particle size after lyo, spherical in shape
Particle size (nm)	650.3	450.1	420.2	367.9
PDI	0.824	0.562	0.423	0.390

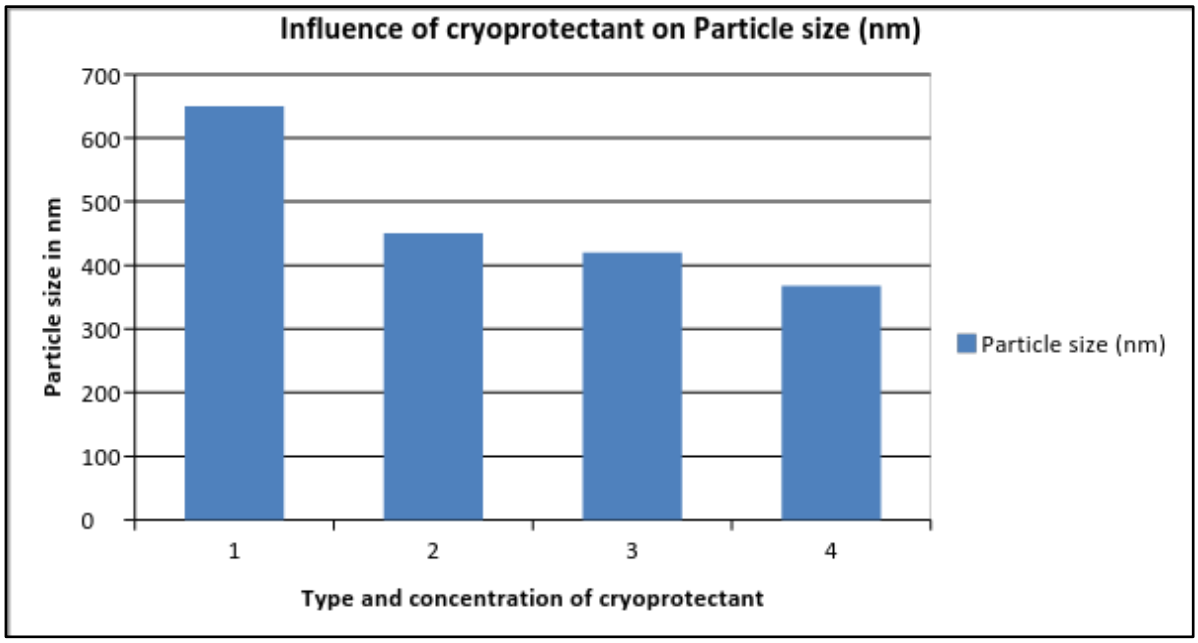

Figure 5.6: Effect cryoprotectant on the characteristics of TAP SLN

a) Effect of Processing Parameters on SLN characteristics

It is known that a large number of process parameters influence the critical quality attributesof SLNparticles.There was a prominent trend observed for average particle size distribution in relation to change in the intensity and duration of ultra-sonication. Sonication (probesonication) at a different intensity from 10 to 20W and different cycle time was studied to create smaller particle size and was compared with various speed and time of high shear homogenization. It was observed that the increased

emulsification energy associated with either increased duration or intensity of the ultra-sonication treatment helped in rapid emulsification of lipid phase, forming a uniform emulsion with narrow size distribution. Therefore, ultra-sonication was preferred choice over high shear homogenization. Although sonication was performed under controlled temperature conditions, higher rates and duration of sonication, resulted in an increase of temperature which was a major confounding factor to decrease in EE owing to decrease of particle size of the drug and solubility enhancement.

The other variable, responsible for determining SLN characteristics, was temperature. During hot homogenization, the high temperature is required for emulsification. Rapid cooling led to the formation of gelation of lipids due to the fusing of lipid emulsion droplets. Further, this was optimized by the controlled rate of cooling of emulsion and constant stirring at room temperature.

Another variable is lyophilization, which influenced the stability of the prepared SLN. Lyocycle was optimized with smart cycle recipe using Lyostar-II (SMART) cycle software. The optimized lyocycle was as follows:

Table. 5.4:Optimized Lyophillization Cycle

Step	Shelf set point (°C)	Ramp rate (°C/min)	Time (min)	Pressure (mTorr)
Freezing				
1	5	1	30	0
2	-5	1	30	0
3	-40	1	180	0
4	-20	1	10	0
5	-40	1	180	0
Primary/secondary drying				
6	-37	0.5	300	75
7	-26.6	0.5	540	75
8	-23.7	0.5	1200	75
9	0	0.3	960	75
10	20	0.3	480	75

These optimal conditions were proposed after systematic experimentation and observing the physical attributes of reconstitution time and quality with respect to the use of various surfactants and cryo-protectants.

5.4 IN-SITU GELLING SYSTEM

A certain amount of Gellan gum (0.2%, 0.4% ,0.5%, 1.0% w/v) was added to water for injection dissolved by gentle heating and moderate stirring using IKA anchor stirrer. Thereafter, the solution was cooled to room temperature, TAP (0.05%, w/v), HPMC 5cps (0.2%, w/v), was added to the gel base understirring till clear sol is obtained. The pH of formulation was kept between 4.0 and 6.0. 100 mcl of the prepared nasal solution and artificial nasal fluid (100mcl) were mixed (1:1, v/v) and gelation was observed by visual examination and spray pattern from Aptar spray pumps were evaluated using dye in a TLC plate. Formulation with 0.4% Gellan gum was further evaluated for solution stability based on the sol-gel transition and spray pattern as it had the optimum ovality ratio.

5.5 IN-VITRO DRUG RELEASE STUDIES

A range of SLN formulations was prepared to evaluate the effect of various formulation parameters on the in-vitro drug release profile. As per hypothesis, drugs encapsulated in SLN systems are released through two possible mechanisms: a) passive diffusion b) lipid erosion.

In-vitro drug release and its kinetics to optimize formulation

The particle size distribution, shape, morphology of TAP loaded SLN were dependent on nature of lipids and surfactant selected as well as the ratio of lipid: surfactant. This ration also had a direct effect on the release characteristics. In-vitro drug release studies were carried out to determine the effect of lipid to surfactant ratio on TAP release. In-vitro cumulative percent release profile of SLN along with physical dispersion was conducted on the optimized formulation prepared with lipid and surfactant ratio and is represented in Fig. 5.7 It was observed that pure TAP and its physical mixture (PD)with lipids and surfactant used in the same proportion as formulation got dissolved completely within 6 h (Fig. 5.7). However, the release rate from SLN was extended from 18 to 48 h on optimized formulations.

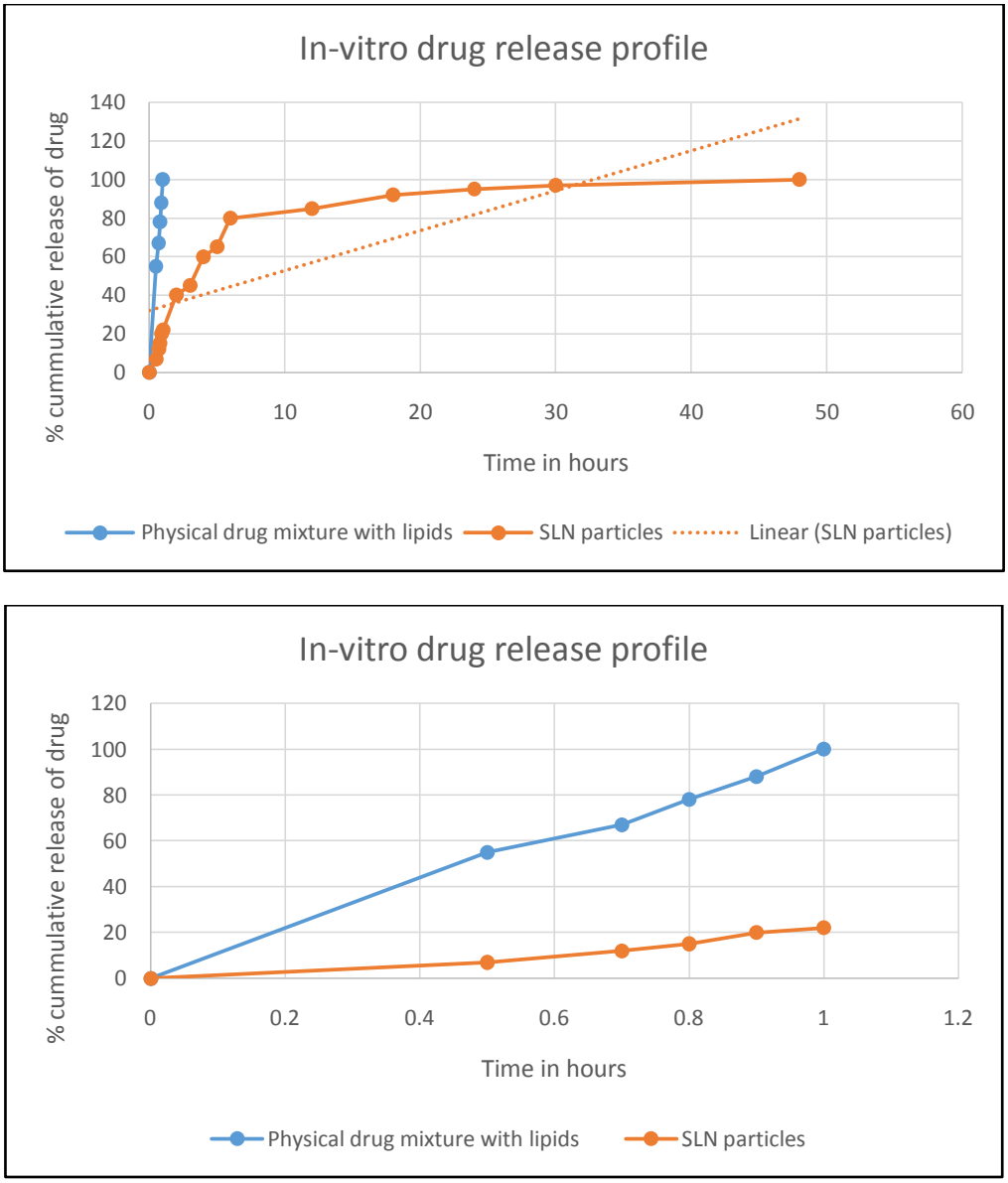

Figure 5.7: In-vitro cumulative percent release profile of SLN along with physical dispersion

The release profile indicates that 20% of the absorbed drug was instantaneously dissolved in media with constant stirring, which may be beneficial for getting an early T_{max} and may be attributed to drug embedded in the outer crust of the SLN.

Data fitting and trendline results indicate that the drug release data can be well described by first order kinetics with an R^2 value of 0.936.

Howsoever, when the same methodology was used for the in-vitro dissolution studies of the *in situ* gelling system, 100% of the drug was released within 10 mins as the drug is already in solution form unlike the physical drug mixture(PD) where in the drug

particles partially embedded in glyceryl behenate -surfactant. The Gellan gumsystem , by design is appropriate for in vivo evaluation as altered muco-adhesive as well as altered muco-cilliary clearance cannot be evaluated in-vitro.

5.6 STABILITY STUDIES

Stability studies were monitored by Raman spectroscopy where pure drug and lipid Raman spectra were compared with the lipid drug SLN particles at different temperature condition. Previously, several researchers have observed similar findings, which were attributed to Raman shift was observed in SLN resulting from molecular level dispersion of the drug within the lipid. The Raman spectra of physical mixtures (Chapter 4) further supported this fact as the Raman shift was found to be unaffected. Additionally, the drug present in the physical mixture had shown no change in the Raman spectra. These results indicated that the physical state of the drug remains unchanged with the treatments given during manufacturing process and there is less possibility of physical interactions between the drug and lipids or other excipients.

Relatively higher extent of room temperature stability and refrigerated condition was observed with SLN prepared using lyophilization stored for 4 months with the acceptable change in various product characteristics such as size, size distribution, drug entrapment, loading efficiency and in-vitro release. All the SLN stored at 5±5°C and 25°±5°C/75%±5% RH for more than 4 months showed some increase in size, due to aggregation of particles (Table 5.4). All lyophilized formulations showed better dispersibility even after 8 months of storage at 2-8^{0}C. The stability study result confirmed that the prepared SLN were stable in all the selected temperatures tested.

The *in situ* Gellan gum with HPMC 5cps formulation was tested for physical and chemical stability as per ICH Zone 4 real time and accelerated conditions and no significant changes were observed from initial owing to the chemical nature of the molecule.

Table 5.5: Stability study results of optimized Tapentadol loaded SLN formulations in three different condition[a]poly dispersity index, [b]zeta potential, [c]loading efficiency

Stability Conditions	Evaluation Parameters	Tapentadol SLN	
		Observation (months)	
		0	4
25°±5°C/75%±5% RH	Physical appearances	White	No change
	Size (nm)	367.9 ± 3.34	382.2 ± 4.35
	PDI[a]	0.390 ± 0.11	0.420 ± 0.18
	ZP[b]	(-)34.3 ± 0.49	(-)32.5 ± 0.48
	LE[c]	0.102 ± 0.22	0.956 ± 0.22
5 ± 2° C	Physical appearances	White	No change
	Size (nm)	367.9 ± 3.34	379 ± 2.35
	PDI[a]	0.390 ± 0.11	0.411 ± 0.20
	ZP[b]	(-)34.3 ± 0.49	(-)33.8 ± 0.27
	LE[c]	0.102 ± 0.22	0.921 ± 0.18

5.7 CONCLUSIONS

Tapentadol SLN were prepared successfully using Hot homogenization technique coupled with Lyophilization which helped in the stabilization of both particles and lipids used for encapsulation. The various formulation variables such as lipids, surfactant and drug amount along with other processing variables had confounding effects on the critical attributes of SLN including particle size, size distribution, entrapment efficiency, loading efficiency and in-vitro drug release profile.

The prepared SLN were characterized for the morphology using polarized microscopy. The classic microscopic examination using polarized microscopy revealed the absence of free drug. The developed formulations exhibited good entrapment within the lipid with excellent particle morphology. The in-vitro drug release studies revealed that the developed formulations extended the drug release over 37-48h depending on formulation construct, which could be useful for controlled drug delivery and explained the release profile by different drug release mechanisms.

The in-vitro dissolution curve of TAP loaded SLN was best fitted to First order release kinetics and it was extremely rapid in case of the *in situ* gelling system with 100% drug release in less than 10 mins.

Moreover, the Raman spectroscopy studies have confirmed uniform distribution of the drug, at the molecular level, within the lipid without any chemical and physical interactions between the drug and lipid or other excipients. Consequently, the prepared SLN by selected method was found to be stable with acceptable reproducibility.

The stability studies of the SLN as well *in situ* gelling system indicate both physical and chemical stability are acceptable as per global regulatory requirements.

The encouraging results motivated us to evaluate its in-vivo properties in a preclinical species which is most apt for intranasal delivery i.e., NZ rabbits to ascertain the advantages of these formulations.

REFERENCES

1. Paolo Blasi et.al, (2007) Solid lipid nanoparticles for targeted brain drug delivery.*Advanced Drug Delivery Reviews* 59; 454–477.
2. Jia-You Fang et.al. (2008) Lipid nanoparticles as vehicles for topical psoralen delivery: Solid lipid nanoparticles (SLN) versus nanostructured lipid carriers (NLC).*European Journal of Pharmaceutics and Biopharmaceutics* 70; 633–640.
3. Gasco MR(1993). Method for producing solid lipid microspheres having a narrow size distribution.*US Patent 5250236.*
4. Sjostrom B, Bergenståhl B. (1992). Preparation of submicron drug particles in lecithin-stabilised o/w emulsions I. Model studies of the precipitation of cholesterylacetate. *Int J Pharm*. 88; 53–62.
5. Mehnert W et.al. (2001) Solid lipid nanoparticles: production, characterization and applications.*Adv Drug Deliv Rev*. 25;47(2-3); 165-96.