Chapter 4

PREFORMULATION STUDIES

AND

PRELIMINARY OPTIMIZATION

4.1 Introduction

Preformulation study is performed to investigate the physical and chemical characteristics of a drug candidate alone and in combination with the excipients, which have to be used in formulating a complete dosage form. Preformulation study is the learning phase about the drug and excipients separately and combined. The principle behind preformulation studies is that it can thwart the errors arising in the process of a new product development stages. Thorough and adequate understanding of physicochemical properties of the drug and the excipients are needed to be assessed for fabricating an ideal drug delivery system. The drug molecule characterization is a critical step of preformulation study in the development of a formulation [1].

A formulation scientist utilizes maximum data from preformulation testing which significantly increases the ease of pharmaceutical product development. Adequate knowledge about the physicochemical characters such as drug solubility, stability, permeability and drug-excipient compatibility is a prerequisite for assessing the processing conditions and evaluation of final developed formulation. Inadequate preformulation studies may risk the formulation to fail in mid development stage. The data or information acquired from preformulation studies contributes to a certain extent in product development and selection of proposed route of administration [2]. Figure 4.1 shows the number of preformulation testings that can done for a drug molecule.

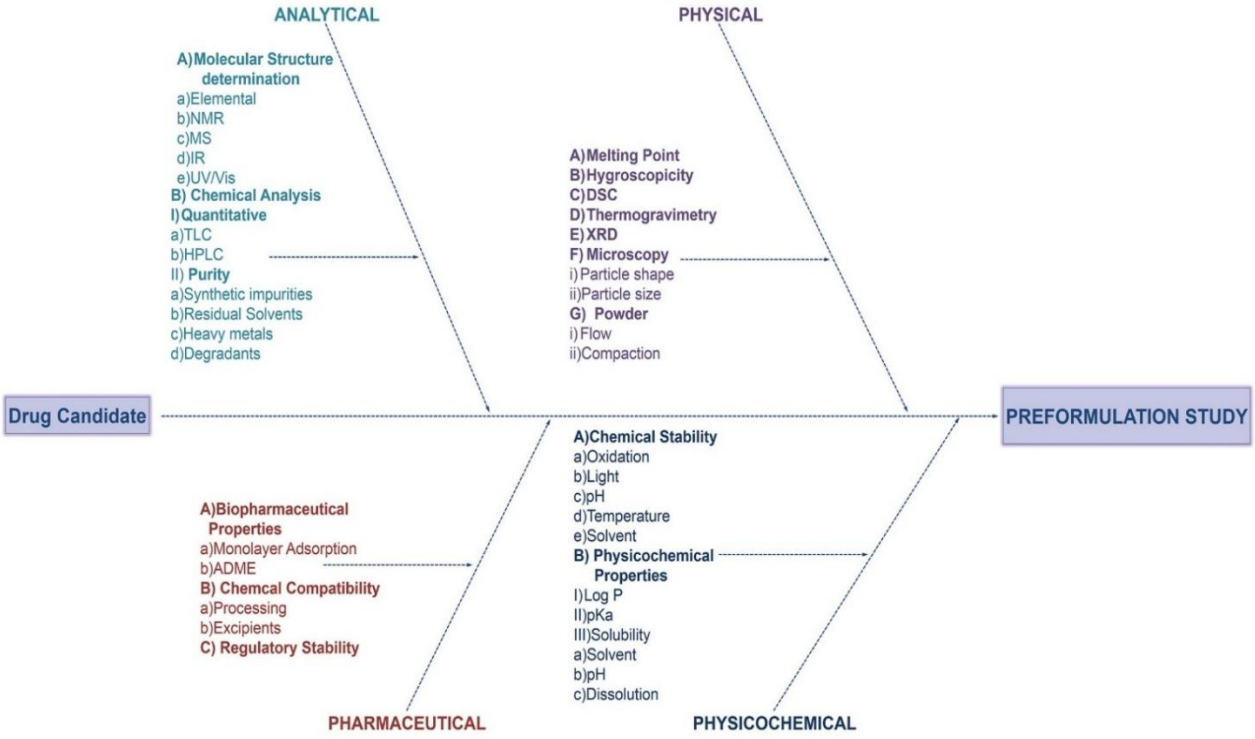

Figure 4.1: Various types of Preformulation studies.

4.2 Materials and Equipment

4.2.1 Materials

Gemcitabine HCl (GCH) was obtained as a gift sample from Sun Pharmaceutical Industries Ltd., Vadodara, India. Vinorelbine tartrate (VLB) was obtained as a gift sample from Cipla Ltd. Mumbai, India. Cholesterol, Mannitol and Potassium oleate were purchased from Sigma Aldrich (St. Louis, MO, USA). Soyabean Phosphatidylcholine (SPC) 95% (PhospholiponVR 90 G), 1,2-Dimyristoyl-sn-glycero-3-phosphorylcholine (DMPC), Dipalmitoylphosphatidylcholine (DPPC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino (polyethylene glycol)-2000] (DSPE-PEG 2000) were obtained as gift sample from Lipoid GmbH (Ludwigshafen, Germany). Dichloromethane, Methanol and Chloroform (A. R. grade) were purchased from S.D. Fine-chemicals limited (Vadodara, India). Distilled water was prepared using in-house distillation assembly. 0.22µ membrane filter was purchased from Pall Life Sciences (Mumbai, India). All other reagents were purchased from S.D. Fine-chemicals limited, Baroda, India and were of analytical reagent grade.

4.2.2 Equipments

- Analytical Weighing Balance (ATX 224, Shimadzu, Japan)
- Vortex Mixer (Spinix-Vortex Shaker, Tarsons, India)
- Ultrasonic Bath Sonicator (Ultrasonics Selec, Vetra, Italy)
- Differential Scanning Calorimeter (DSC) (Shimadzu, Japan)
- Fourier-transform infrared (FTIR) spectrometer (Bruker, Germany)
- Rotary evaporator (IKA RV10, Karnataka, India)
- Particle Size Analyzer 3000 HS (Zeta Sizer Nano Series, Malvern Instruments, UK)
- UV-Visible spectrophotometer (UV-1800, Shimadzu, Japan)
- Probe Sonicator (LabsonicM, Sartorius Ltd, Mumbai, India)
- Cooling Centrifuge (Remi Equipment, Mumbai, India)
- Nikon H600L Microscope (Nikon, Japan)

4.3 Preformulation Study

4.3.1 Methods

Identification and authentication of GCH, VLB and the assessment of drug-excipient compatibility was performed by following methods:

a) Organoleptic characters: Appearance, color and odor were determined by visual observation and smelling of drugs.

b) Melting point: Melting point of the drugs was determined using capillary tube method. A capillary tube with one end sealed was filled with the drug. The capillary was tied to the thermometer with the help of a thread. A Thiele tube was filled with liquid paraffin and the thermometer was immersed in it. Arm of Thiele tube was heated using a burner and the temperature range at which the drug starts to melt and when it gets completely melted were noted.

c) Solubility: Solubility of both the drugs in various solvents was determined. Briefly, 1-2 mg of drug was taken in pre-labeled test tubes and solvents like distilled water, methanol, dichloromethane and chloroform added drop wise. Visual observation was done to confirm whether the drug dissolves or leaves any sediments. Addition of solvent was continued upto the volume when the drug completely dissolves.

d) FTIR Spectroscopy: Drug sample were mixed thoroughly with IR grade anhydrous Potassium bromide in a ratio of 1:100. Pellets of this mixture were prepared using 10 metric ton pressure in a hydraulic press. FTIR instrument (Bruker, Germany) was used to scan the pellets over a range of 4000-400cm^{-1}. The obtained FTIR spectra was compared with the reference spectra. Purity of drug sample was judged by differences in the principle peaks of working standard and reference standard FTIR spectra.

e) DSC: 3-5 mg of drug was taken in an aluminum pan. Aluminum pan was sealed using an aluminum lid by pressing with the help of a hydraulic press. Sealed aluminum pan was placed in DSC instrument and thermograms were recorded over a temperature range of 30-300°C.

f) Drug-Excipient Compatibility: Presence of any possible incompatibilities between drug and excipient was also assessed. Drugs with different excipients to be used in formulation (Mannitol, Cholesterol, Potassium oleate and SPC) were subjected to DSC and FTIR studies. 1:1 ratio of drug:excipients was used for study. The obtained thermograms and spectra were compared with standard data of pure drugs.

4.3.2 Results and Discussion

The purpose of preformulation study was to establish a pathway of formulation development with maximizing the chances in formulating an acceptable delivery system.

4.3.2.1 Results of preformulation study of GCH

a) Organoleptic characters: GCH is a white to off white powder, without any odor

b) Melting point: Melting point of GCH was found to be in the range of 269-274ºC, which is in close proximity to the reported value [3].

c) Solubility: GCH was found to be freely soluble in water, where 1 mg drug required less than 1 m1 of water. Moreover, GCH was slightly soluble in organic solvent such as Methanol and insoluble in Ethanol. Reported solubility of GCH in water is ≥10 mg/ml [4].

d) FTIR spectra of GCH is shown in Figure 4.2. Table 4.1 shows the principle functional groups and their characteristic peaks. FTIR study confirms the drug was pure as it do not showed any additional peak. The obtained FTIR spectra is in compliance with the reference spectra of GCH [3, 5].

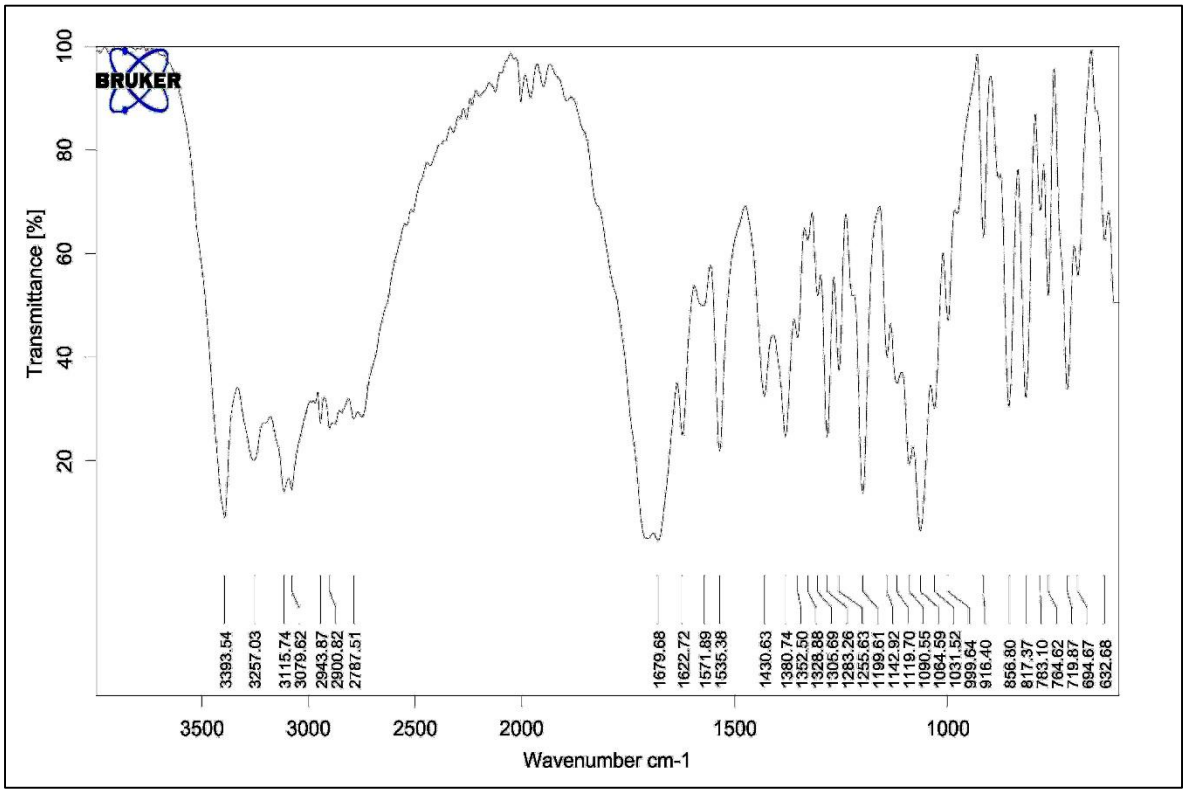

Figure 4.2: FTIR Spectra of GCH.

Table 4.1: Principle functional groups and their characteristic peaks in GCH FTIR spectra.

Functional group	Characteristic peak (cm^{-1}) (Reference peak [6])
C=O (Stretching)	1679.68 (1700-1725)
NH_2 (Stretching)	3393.54, 3115.74 (3300-3500)
NH (Bending)	1622.72 (1600)
Aliphatic C-H (Stretching)	2943.87, 2900.82, 2787.51 (2850-3000)
Aromatic C-H (Stretching)	3079.62 (3000-3100)
Aliphatic C-O (Stretching)	1119.70 (1050-1150)

e) DSC: The obtained DSC thermogram of GCH shown in Figure 4.3 depicts the presence of a single endothermic peak at its melting point i.e.273.49ºC. Single and sharp endothermic

peak at GCH melting point shows that the drug was pure and authentic. The endotherm matches with the melting point of GCH.

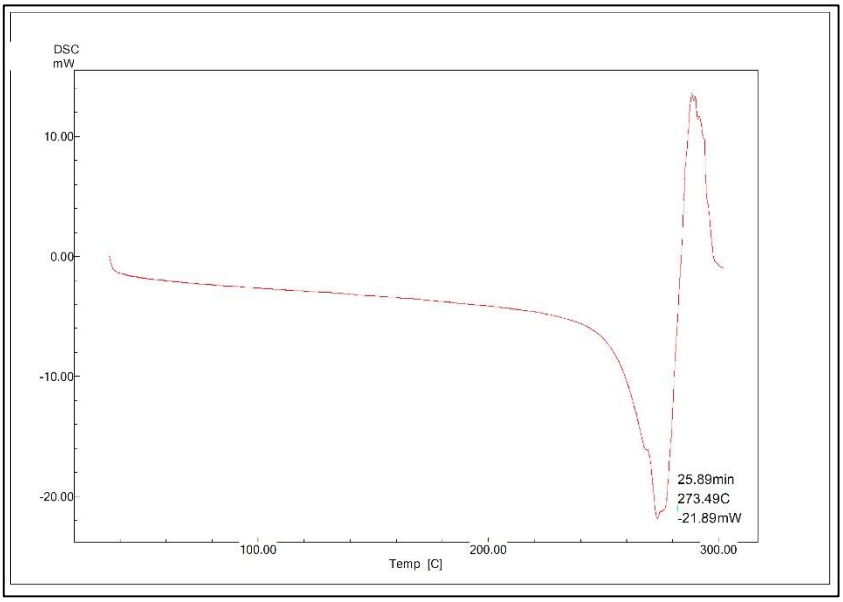

Figure 4.3: DSC thermogram of GCH.

f) Drug-Excipient Compatibility: To rule out any incompatibility between drug and excipients FTIR and DSC analysis were performed. Figure 4.2 and Table 4.1 shows the functional groups and their characteristic peaks present in pure GCH. Whereas, Figure 4.4 and Table 4.2 showing FTIR spectra of physical mixture of excipients (Mannitol, Potassium oleate, SPC and Cholesterol) with GCH indicate that the drug retained its principle peaks as shown in Table 4.1. This revealed that there is no chemical interaction between the drug and excipients.

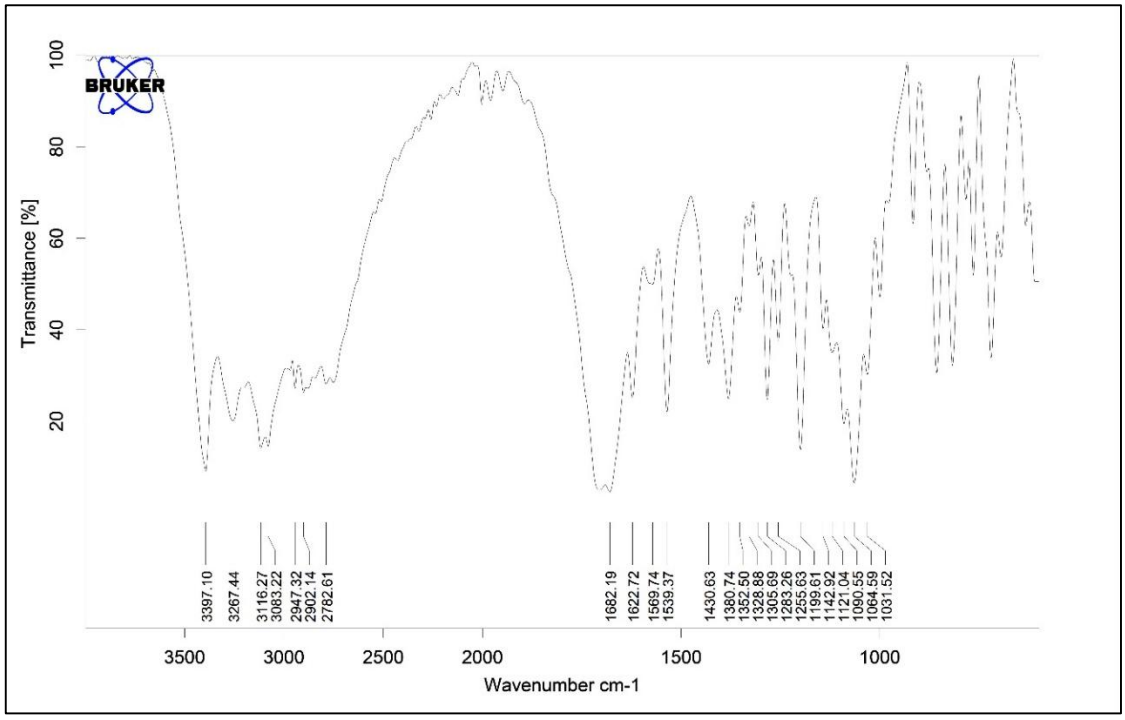

Figure 4.4: FTIR Spectra of physical mixture of GCH and excipients.

Table 4.2: Principle functional groups and their characteristic peaks in physical mixture of GCH and excipients FTIR spectra.

Functional group	**Characteristic peak (cm^{-1})**
C=O (Stretching)	1682.19
NH_2 (Stretching)	3397.10, 3116.27
NH (Bending)	1622.72
Aliphatic C-H (Stretching)	2947.32, 2902.14, 2782.61
Aromatic C-H (Stretching)	3083.22
Aliphatic C-O (Stretching)	1121.04

Similarly, DSC of physical mixture of GCH and excipients showed that drug retained its endothermic peak at its characteristic melting point i.e. 273.46ºC (Figure 4.5). This indicates that there was no incompatibility between drug and excipients.

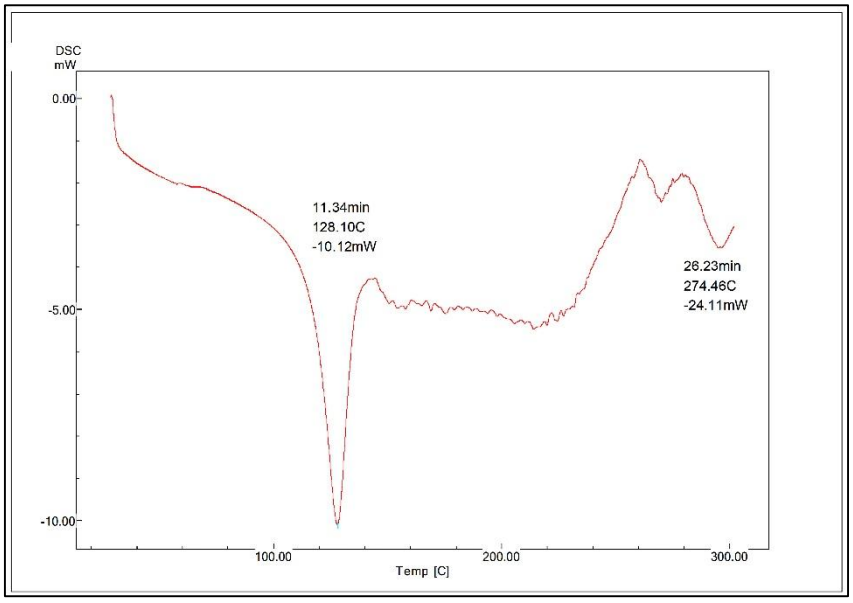

Figure 4.5: DSC thermogram of physical mixture of GCH and excipients.

4.3.2.2 Results of preformulation study of VLB

a) Organoleptic characters: VLB is a yellow amorphous powder, without any odor.

b) Melting point: Melting point of VLB was found to be in the range of 218-223ºC, which is in close proximity to the reported value [7].

c) Solubility: VLB was found to be freely soluble in water, where 1 mg drug required less than 1 m1 of water. Also, it was freely soluble in Ethanol, Methanol and DMSO. Reported aqueous solubility of VLB is >1,000 mg/ml [8].

d) FTIR spectra of VLB is shown in Figure 4.6. Table 4.3 shows the principle functional groups and their characteristic peaks. FTIR study confirms the drug was pure as it do not showed any additional peak. The obtained FTIR spectra is in compliance with the reference spectra of VLB [7].

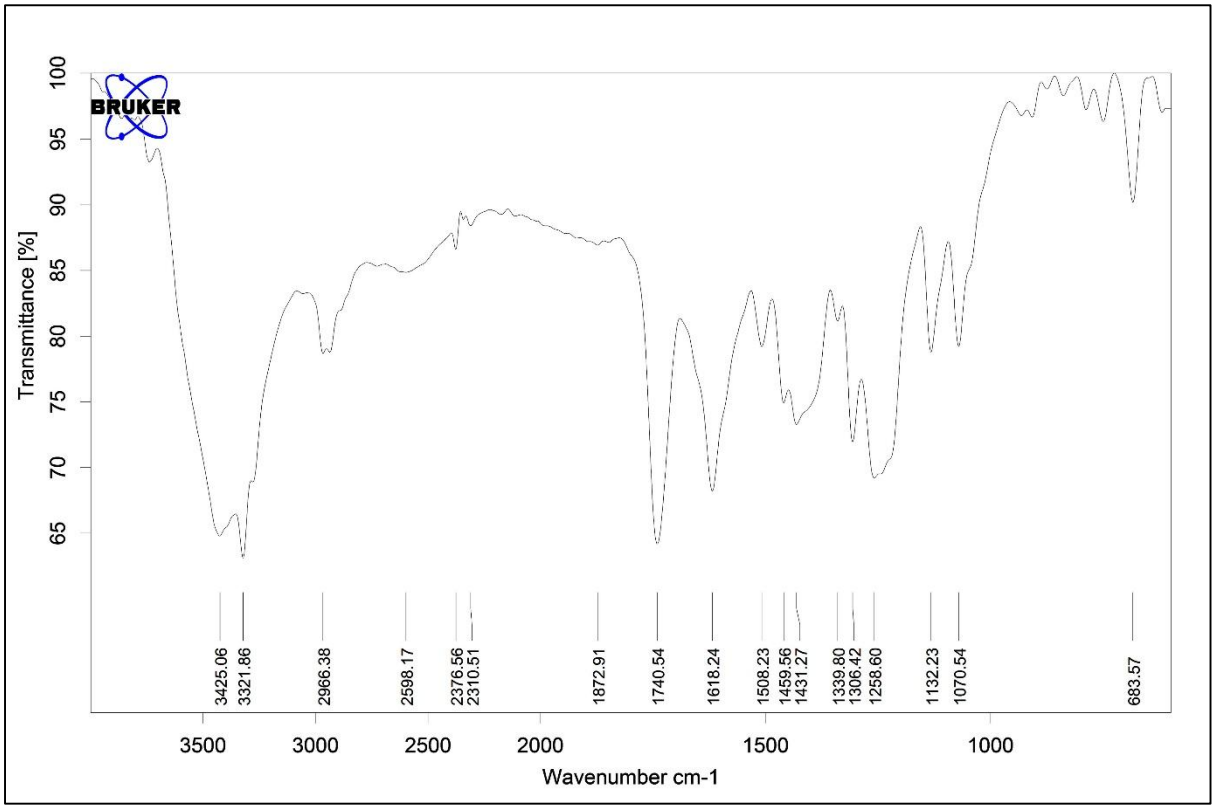

Figure 4.6: FTIR Spectra of VLB.

Table 4.3: Principle functional groups and their characteristic peaks in VLB FTIR spectra.

Functional group	**Characteristic peak (cm^{-1}) (Reference peak [6])**
OH (Stretching)	3425.06 (3200-3600)
2º NH (Stretching)	3321.86 (3300-3500)
C=O (Stretching)	1740.54 (1670-1820)
C=C (Stretching)	1618.24 (1400-1600)

e) DSC: DSC analysis of VLB shown in Figure 4.7 depicts that the drugs thermogram exhibited a single exothermic peak at 221.08ºC. Presence of single exothermic peak at drug's characteristic melting point suggests that the VLB was pure and authentic. The exotherm matches with the melting point of VLB.

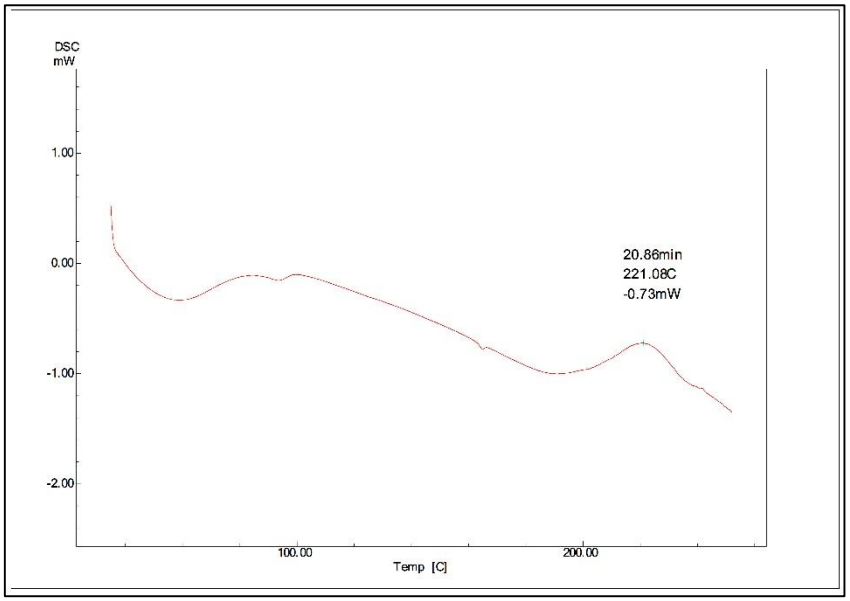

Figure 4.7: DSC thermogram of VLB**.**

f) Drug-Excipient Compatibility: Any possible incompatibility between drug and excipient (Mannitol, Potassium oleate, SPC and Cholesterol) was assessed by performing FTIR and DSC. Physical mixture of VLB and excipients was subjected to FTIR analysis and the resulting spectra shown in Figure 4.8 and Table 4.4 was compared with pure VLB spectra (Figure 4.6 and Table 4.3). It was observed that the characteristic peaks of VLB were retained in the spectra, indicating that there was no incompatibility between the drug and excipients.

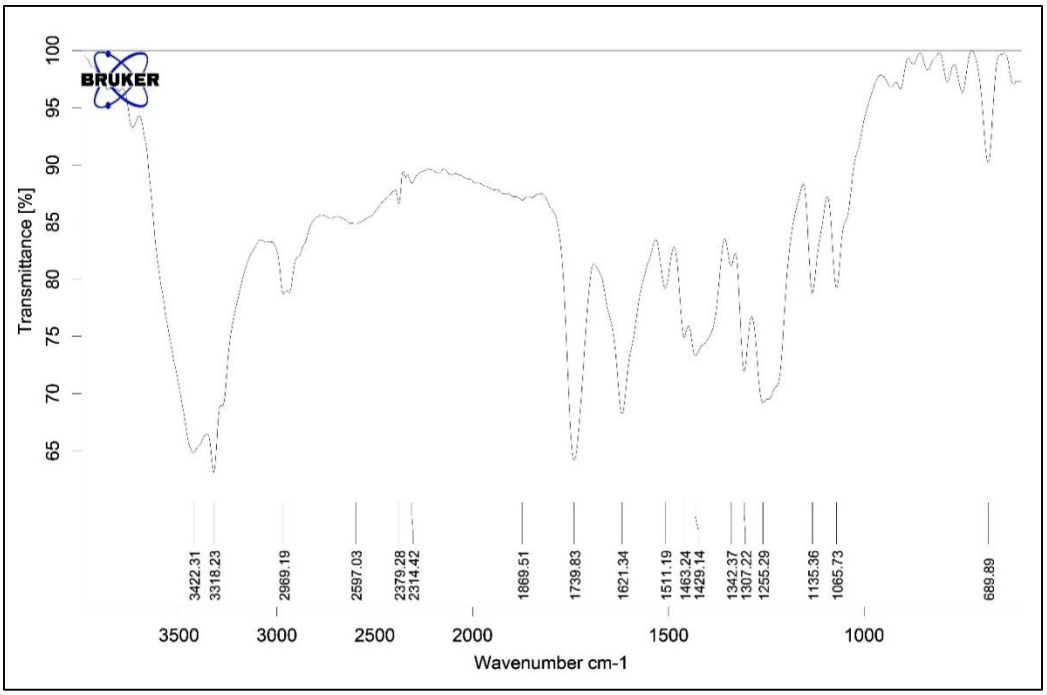

Figure 4.8: FTIR Spectra of physical mixture of VLB and excipients.

Table 4.4: Principle functional groups and their characteristic peaks in physical mixture of VLB and excipients FTIR spectra.

Functional group	Characteristic peak (cm^{-1})
OH (Stretching)	3422.31
2º NH (Stretching)	3318.23
C=O (Stretching)	1739.83
C=C (Stretching)	1621.34

Subsequently, DSC analysis of physical mixture of VLB and excipients was performed, thermogram shown in Figure 4.9. It was found that drug retained its exothermic peak at 220.47ºC. This showed that there was no incompatibility between drug and excipients.

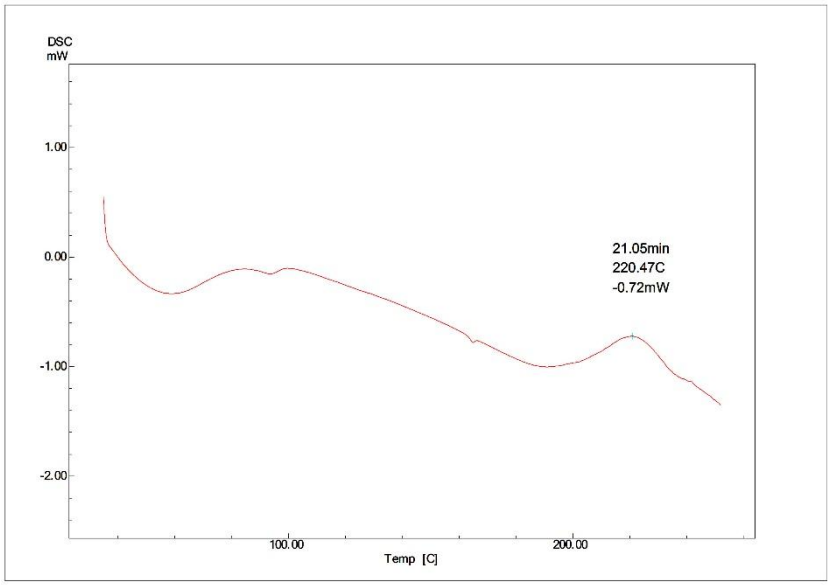

Figure 4.9: DSC thermogram of physical mixture of VLB and excipients.

4.4 Preliminary Optimization

Preliminary optimization was performed in order to identify the formulation and process variables in the course of development of an acceptable drug loaded spherulites formulation. Formulation variables assessed were screening of lipids, SPC concentration, cholesterol concentration which are critical to formulate a good vesicle system. DSPE-PEG 2000 concentration to be added for PEGylation of spherulites was also optimized. Process parameters chosen were type of homogenization assembly, homogenization time and speed, extrusion cycle.

Initially blank (i.e. without drug) spherulites were prepared using a modified method [9]. Briefly, a lipid phase comprising of cholesterol, SPC and potassium oleate was taken in a round

bottom flask (RBF) and 2–3ml of chloroform: methanol mixture (9:1 v/v) (as lipid phase had maximum solubility in this solvent mixture) was added. The solvent was evaporated under negative pressure using a rotary evaporator to obtain a thin lipid film adhered to the walls of RBF. Aqueous phase comprising mannitol was added in the RBF and shaken vigorously to hydrate the lipid film. The obtained lamellar phase was homogenized using a teflon probe and glass cylinder followed by extrusion. PEGylated formulation was prepared by incorporation of a PEGylating agent in the lipid phase and the rest of the method was kept same as described above. The preliminary optimization of formulation and process parameters was carried out step by step.

4.4.1 Preliminary Optimization of formulation variables

4.4.1.1 Screening of Lipids

Spherulites are mainly comprised of a phospholipid and cholesterol. Phospholipids are amphiphillic in nature comprising of a polar head and lipophilic tail. They have the tendency to form vesicular structure when hydrated with aqueous media. For developing spherulites three commonly used phospholipids DMPC, DPPC and SPC were screened to choose a lipid of choice. Maximum % Entrapment Efficiency (%EE) and minimum size of spherulites were the criteria to choose a lipid amongst the screened lipids.

Table 4.5: Screening of lipids (Lipid:Cholesterol ratio kept constant as 1:0.5 M).

Concentration of Cholesterol	**GCH loaded Spherulites**		**VLB loaded Spherulites**	
	% EE	**Size (nm)**	**% EE**	**Size (nm)**
DMPC	53.37±1.54	323±2.23	65.29±1.97	282±1.49
DPPC	54.12±2.07	331±1.74	66.36±2.15	287±2.15
SPC	**59.39±1.92**	**282±1.8**	**68.37±2.49**	**252±2.76**

*Experiment was performed in triplicate (Data represents mean±SD).

Table 4.5 shows the batches prepared using three lipids DMPC, DPPC and SPC. The results indicate that SPC exhibited better %EE with minimum size of spherulites than DMPC and DPPC. DMPC and DPPC both are semi synthetic lipids, whereas, SPC is a natural lipid which is produced at low cost using less solvents and chemicals. Moreover, it is recommended by regulatory authorities to use a natural phospholipid. Furthermore, SPC is cost effective when compared with other two lipids [10], which makes it a choice of lipid for development of spherulites formulation.

4.4.1.2 SPC concentration

Amphiphilic phospholipids have the tendency to form vesicular structure when hydrated with aqueous media. SPC was used to formulate spherulites. Various concentrations of SPC ranging from 30-62% of total formulation was used in preliminary optimization. Results suggested that spherulites were not able to form from concentration 30-46% as evidenced by observing the dispersion under an optical microscope (Figure 4.10A) and by performing size analysis. However, formulation batches containing SPC concentration from 48-62% were observed to have a vesicular structures under an optical microscope (Figure 4.10B) and size analysis confirmed the formation of spherulites.

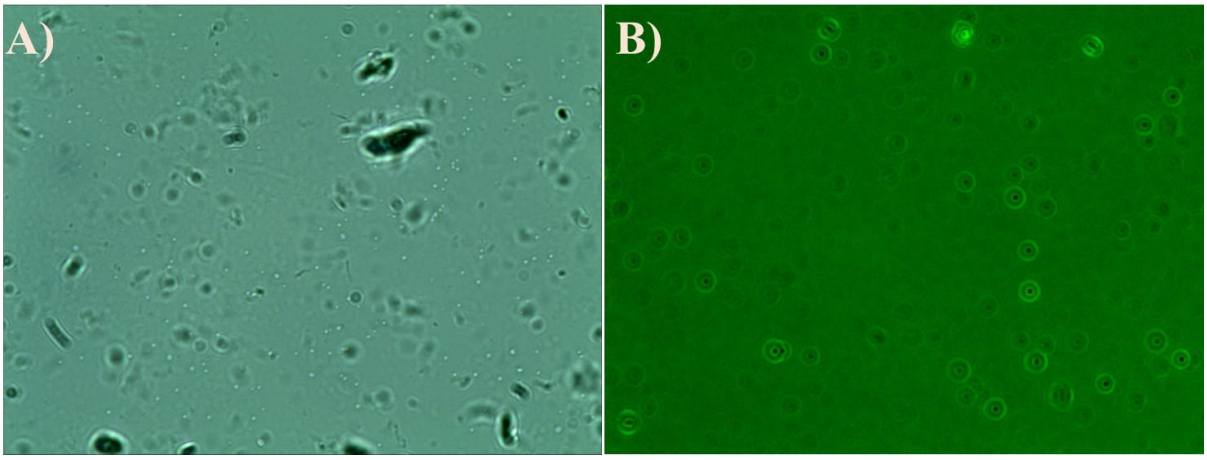

Figure 4.10: Microscopic images of preliminary optimization batches of spherulites prepared by varying concentration of SPC; A) 30-46%, B) 48-62%.

Optical microscopy in confirmation of spherulites formation provides helpful information as the formed vesicles have multilamellar structure which gave them a birefringent property [11] as observed in Figure 4.10B. Figure 4.10A shows no significant structure of spherulites, the visible black particle is phospholipid mass unable to form a vesicular structure due to insufficient concentration of lipid. This preliminary optimization for determining the optimum SPC concentration range suggested that 48-62% was found to be useful for development of drug loaded spherulites.

4.4.1.3 SPC:Cholesterol ratio

Cholesterol is well known for its rigidity imparting function in a vesicular system. It prevents premature leaching of hydrophilic drug molecule from the vesicle. In the present investigation cholesterol was used at 3 different levels i.e. 0.25, 0.5 and 1 M with the lipid i.e. SPC. Results shown in Table 4.6 clearly indicated that use of 0.25 and 0.5 M of cholesterol in the formulation resulted in less encapsulation. Whereas, 1:1 M ratio of cholesterol with SPC gave high % EE. Moreover, it was evident that 1:1 M ratio between lipid and cholesterol is ideal to obtain a

better vesicular system, as it imparts better stability to the formulation. Reason being, cholesterol immobilizes the phospholipids hydrocarbon chains by occupying the half area of a SPC molecule. This helps in stabilization of spherulites by inhibiting the leakage of encapsulated hydrophilic molecule [12, 13].

Table 4.6: Different levels of cholesterol concentration in spherulites (SPC kept constant 1 M).

Concentration of Cholesterol (M)	GCH loaded Spherulites		VLB loaded Spherulites	
	% EE	Size (nm)	% EE	Size (nm)
0.25	37.18±1.68	294±1.2	49.38±1.34	238±1.6
0.5	57.46±2.47	307±3.4	64.27±2.95	257±2.1
1	**70.29±2.19**	**276±2.2**	**89.65±2.23**	**245±1.5**

*Experiment was performed in triplicate (Data represents mean±SD)

4.4.1.4 SPC:DSPE-PEG 2000 ratio (mol %)

PEGylated spherulites were also formulated in order to prolong the circulation time of the vesicles in *in vivo* conditions by avoiding Reticuloendothelial System (RES) uptake. DSPE-PEG 2000 was used as PEGylating agent. Various mol% ratios of SPC:DSPE-PEG 2000 were used to optimize the PEGylating agents concentration in the formulation.

Table 4.7: Different levels of SPC:DSPE-PEG 2000 concentration in spherulites for PEGylation.

Concentration of DSPE-PEG 2000 (mol%)	GCH loaded Spherulites		VLB loaded Spherulites	
	% EE	Size (nm)	% EE	Size (nm)
1	67.32±2.18	269±1.8	84.74±1.59	252±2.1
2	**69.19±1.83**	**281±2.4**	**87.33±2.74**	**243±1.5**
3	64.40±1.57	294±1.9	82.61±2.15	268±2.4
4	62.07±2.64	309±2.7	79.46±2.07	276±1.9
5	59.52±2.37	324±1.9	75.27±1.93	284±2.2

*Experiment was performed in triplicate (Data represents mean±SD).

DSPE-PEG 2000 was incorporated in the spherulites lipid phase from 1-5 mol%. The results shown in Table 4.7 clearly indicate that 2 mol% was found to be appropriate as spherulites obtained exhibited highest entrapment and lower size as compared to other concentrations. Moreover, incorporation of PEG beyond 2 mol% showed changes in morphology of spherulites, where excessive concentration can result into disc shaped spherulites [14].

4.4.2 Preliminary Optimization of process variables

4.4.2.1 Hydration time

Phospholipid hydration is an important step in preparation of Spherulites. The amphilipic lipid used in this case SPC has the tendency to form vesicular structure in presence of water. Due to its polar head and non-polar tail structure the polar head projects outwards i.e. towards water and non-polar tail inwards. The sufficient time provided to the lipid film to hydrate makes the downsizing process easier and improves the homogeneity of the size distribution. However, insufficient hydration of lipid film results into low encapsulation of drug and uneven size distribution of vesicles [15, 16]. Table 4.8 shows the spherulites prepared by different hydration time.

Table 4.8: Effect of hydration time on %EE and size of spherulites.

	Time (Hrs)					
	0		**12**		**24**	
	% EE	**Size (nm)**	**% EE**	**Size (nm)**	**% EE**	**Size (nm)**
GCH loaded Spherulites	34.51±1.43	734.7±1.6	51.27±1.93	594.5±2.3	42.27±1.57	542.1±2.4
VLB loaded Spherulites	39.37±2.18	759.1±2.1	57.51±2.01	605.9±1.6	46.19±1.84	556.3±1.9

*Experiment was performed in triplicate (Data represents mean±SD)

Table 4.8 shows the effect of hydration time of lipid phase on %EE and size of spherulites. It was observed that lipid film with no hydration time exhibited low entrapment and high vesicular size. Moreover, upon standing some settlement was observed in the batch with no hydration time indicating self-aggregation of poorly hydrated lipid. Subsequently, 12 hour and 24 hour hydration time yielded spherulites with less %EE and high size. However, at 24 hours entrapment decreased this could be due to over hydration of lipid film. Over hydrated lipid film leach out the drug encapsulated within the vesicles [17].

4.4.2.2 Type of Homogenization assembly

Homogenization assembly was chosen by observing its effects on % entrapment efficiency and spherulites size. Lipid film upon hydration was subjected to homogenization using a custom fabricated assembly which consisted of a Teflon probe with a glass tube. A homogenization assembly consists of two parts, a rotor and a stator, in this case teflon probe is a rotor and glass tube is a stator. Two different type of glass tubes shown in Figure 4.11 were used one with serrated inner surface and other with smooth inner surface.

Table 4.9: Effect of Homogenization assembly.

Homogenization assembly	**GCH loaded Spherulites**		**VLB loaded Spherulites**	
	% EE	**Size (nm)**	**% EE**	**Size (nm)**
Serrated inner surface	**70.24±2.19**	**234±2.1***	**88.49±1.94**	**229±2.2#**
Smooth inner surface	69.47±1.76	265±3.4	85.25±2.37	250±2.7

Experiment was performed in triplicate (Data represents mean±SD) (Statistically analyzed by t test; $p<0.0002$ (t value = 13.43), # $p<0.0005$ (t value = 10.44)).

Results shown in Table 4.9 indicate that the homogenizer tube with serrated inner surface showed significantly (for GCH loaded Spherulites $p<0.0002$ and for VLB loaded Spherulites $p<0.0002$) lower size values as compared to other homogenizer with smooth inner surface. However, there was not much difference in % entrapment efficiency using both the homogenizers. Hydrated lipid phase is also regarded as lamellar phase. Homogenization in other words means grinding of lamellar phase to control the size of spherulites. It was observed that the serrated inner surface of the glass contributed positively in size reduction of vesicles. Another purpose for using homogenization technique was to obtain vesicles with spherical morphology [18]. Along with the inner surface of the glass tube, the clearance or gap between the teflon probe and glass tube also affects the % entrapment efficiency and size of the spherulites. For preliminary investigation conventional homogenizer tube with teflon probe was used which had a gap of 1 mm. The gap between the glass tube and teflon probe is crucial as it helps in maintaining the constant shear rate while homogenization of the lamellar phase. This assembly grinds the lamellar phase in one direction at constant shear rate without disrupting its structure [19, 20].

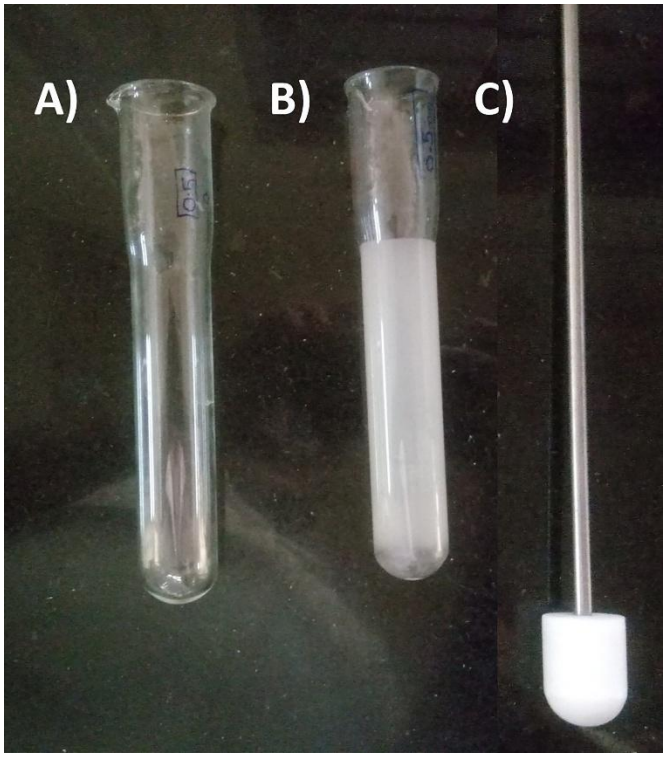

Figure 4.11: Homogenizer tube; A) with smooth inner surface, B) with serrated inner surface and C) Teflon probe.

4.4.2.3 Homogenization time and speed

Homogenization time and speed are two critical parameters to be monitored closely as they directly affect the % entrapment efficiency and size of spherulites. Desirable % entrapment was maximum and size of spherulites were needed in the range of 100-750 nm [21]. Various batches were prepared with varying homogenization time and speed. Homogenization mean shearing, where shearing is defined as the ratio between the velocity of the outer cylinder to the gap size. Shearing of the lamellar phase was done at three levels i.e. 65, 100 and 150 min^{-1} for a period of 15, 30 and 60 minutes (min.) (Table 4.10 and 4.11). Observation from these preliminary trials suggested that 65 min^{-1} of shear rate for 60 min. yielded spherulites with desired size range and % entrapment efficiency.

Table 4.10: Effect of Homogenization time and speed on %EE spherulites.

%EE	Shear rate								
	65 min^{-1}			100 min^{-1}			150 min^{-1}		
	Time (min)			Time (min)			Time (min)		
	15	30	60	15	30	60	15	30	60
GCH loaded Spherulites	64.19 ±1.37	66.54 ±1.41	**69.34 ±1.73**	61.25 ±2.34	56.73 ±2.18	51.82 ±1.57	59.28 ±2.49	52.43 ±2.21	47.34 ±1.84
VLB loaded Spherulites	79.06 ±2.03	82.49 ±1.49	**86.95 ±1.87**	72.19 ±1.96	66.51 ±2.76	61.61 ±2.05	69.32 ±1.57	63.58 ±1.34	57.64 ±1.76

*Experiment was performed in triplicate (Data represents mean±SD).

Table 4.11: Effect of Homogenization time and speed on size (nm) of spherulites.

Size (nm)	Shear rate								
	65 min^{-1}			100 min^{-1}			150 min^{-1}		
	Time (min)			Time (min)			Time (min)		
	15	30	60	15	30	60	15	30	60
GCH loaded Spherulites	284 ±1.7	267 ±2.3	**254 ±1.8**	261 ±1.6	243 ±2.1	227 ±1.9	252 ±1.8	234 ±2.3	213 ±2.2
VLB loaded Spherulites	277 ±2.4	260 ±2.7	**242 ±1.9**	264 ±2.1	249 ±1.6	219 ±2.3	247 ±1.4	229 ±1.4	204 ±2.1

*Experiment was performed in triplicate (Data represents mean±SD).

Results shown in Table 4.10 and 4.11 suggests that effect of homogenization time and speed affected both the parameters i.e. % EE and size of spherulites. Size of spherulites decreased as the speed and time of homogenization increased which is desirable. However, at the same time %EE was observed to be decreasing which is unacceptable. Grinding of lamellar phase for longer period time affected the % EE, this could be because of the rupturing of the vesicles resulting into drug leakage [22].

4.4.2.4 Extrusion

Further size reduction of spherulites was performed by extrusion. A custom made stainless steel syringe filter assembly fitted with 0.22µ membrane filter was used. The dispersion was subjected to extrusion various times viz. 5, 10 and 15 times. %EE and spherulites size were monitored to understand the effect of number of extrusion cycles.

Table 4.12: Effect of number of extrusion cycles on %EE and size of Spherulites.

	Extrusion cycle					
	5 times		**10 times**		**15 times**	
	%EE	**Size (nm)**	**%EE**	**Size (nm)**	**%EE**	**Size (nm)**
GCH loaded Spherulites	**71.19 ±2.13**	**228±1.4**	65.39±1.76	198±2.3	59.47±2.34	173±1.9
VLB loaded Spherulites	**89.67±1.97**	**164±1.9**	80.34±2.15	142±1.8	69.03±2.21	129±1.8

*Experiment was performed in triplicate (Data represents mean±SD).

Results given in Table 4.12 shows that 5 times extrusion of the dispersion effectively reduced the size of the spherulites while the %EE remained unaffected. However, further extrusion of the dispersion exhibited reduced size but compromised the %EE. Consequently, 5 time extrusion was selected for size reduction of the spherulites.

4.5 References

1. Agrawala, P. (1990). Pharmaceutical Preformulation: The Physicochemical Properties of Drug Substances. Journal of Pharmaceutical Sciences, 79(6), 553.
2. Chaurasia, G. (2016). A review on pharmaceutical preformulation studies in formulation and development of new drug molecules. International Journal of Pharmaceutical Sciences and Research, 7(6), 2313.
3. Joshi, G., Kumar, A., & Sawant, K. (2014). Enhanced bioavailability and intestinal uptake of Gemcitabine HCl loaded PLGA nanoparticles after oral delivery. European Journal of Pharmaceutical Sciences, 60, 80-89.
4. Gemcitabine hydrochloride. Available online: https :// www. sigmaaldrich.com/ catalog/ product/ sigma/ g6423?lang=en&region=IN (Accessed on February 07, 2018).
5. Vandana, M., & Sahoo, S. K. (2010). Long circulation and cytotoxicity of PEGylated gemcitabine and its potential for the treatment of pancreatic cancer. Biomaterials, 31(35), 9340-9356.
6. Characteristic IR Absorption Frequencies of Organic Functional Groups. Available online: http://www2.ups.edu/faculty/hanson/Spectroscopy/IR/IRfrequencies.html. (Accessed on February 07, 2018).
7. Yu, X., Wang, W., Ge, Y., Han, X., Zhao, X., Zu, Y., & Li, Y. (2012). A Novel Active Targeting Preparation, Vinorelbine Tartrate (VLBT) Encapsulated by Folate-Conjugated Bovine Serum Albumin (BSA) Nanoparticles&58; Preparation, Characterization and in

Vitro Release Study. Materials, 5(11), 2403-2422.

8. NAVELBINE® (vinorelbine tartrate) Injection, Available online: https://www.fda.gov/ohrms/dockets/ac/04/briefing/4021b1_10_vinorelbine%20label.pdf. (Accessed on February 07, 2018).
9. Simard, P., Hoarau, D., Khalid, M. N., Roux, E., & Leroux, J. C. (2005). Preparation and in vivo evaluation of PEGylated spherulite formulations. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1715(1), 37-48.
10. van Hoogevest, P., & Wendel, A. (2014). The use of natural and synthetic phospholipids as pharmaceutical excipients. European journal of lipid science and technology, 116(9), 1088-1107.
11. Dhanikula, A. B., Lafleur, M., & Leroux, J. C. (2006). Characterization and in vitro evaluation of spherulites as sequestering vesicles with potential application in drug detoxification. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1758(11), 1787-1796.
12. Melzak, K. A., Melzak, S. A., Gizeli, E., & Toca-Herrera, J. L. (2012). Cholesterol organization in phosphatidylcholine liposomes: a surface plasmon resonance study. Materials, 5(11), 2306-2325.
13. Ali, M. H., Moghaddam, B., Kirby, D. J., Mohammed, A. R., & Perrie, Y. (2013). The role of lipid geometry in designing liposomes for the solubilisation of poorly water soluble drugs. International journal of pharmaceutics, 453(1), 225-232.
14. Johnsson, M., & Edwards, K. (2003). Liposomes, disks, and spherical micelles: aggregate structure in mixtures of gel phase phosphatidylcholines and poly (ethylene glycol)-phospholipids. Biophysical Journal, 85(6), 3839-3847.
15. Dua, J. S., Rana, A. C., & Bhandari, A. K. (2012). Liposome: methods of preparation and applications. Int J Pharm Stud Res, 3(2), 14-20.
16. Li, J., Wang, X., Zhang, T., Wang, C., Huang, Z., Luo, X., & Deng, Y. (2015). A review on phospholipids and their main applications in drug delivery systems. Asian journal of pharmaceutical sciences, 10(2), 81-98.
17. Kiafar, F., Shadbad, M. R. S., & Valizadeh, H. (2016). Filgrastim (G-CSF) loaded liposomes: mathematical modeling and optimization of encapsulation efficiency and particle size. BioImpacts: BI, 6(4), 195.
18. Diat, O., & Roux, D. (1993). Preparation of monodisperse multilayer vesicles of controlled size and high encapsulation ratio. Journal de Physique II, 3(1), 9-14.
19. Freund, O., Amédee, J., Roux, D., & Laversanne, R. (2000). In vitro and in vivo stability of

new multilamellar vesicles. Life sciences, 67(4), 411-419.

20. Diat, O., Roux, D., & Nallet, F. (1993). Effect of shear on a lyotropic lamellar phase. Journal de physique II, 3(9), 1427-1452.
21. Dhand, C., Prabhakaran, M. P., Beuerman, R. W., Lakshminarayanan, R., Dwivedi, N., & Ramakrishna, S. (2014). Role of size of drug delivery carriers for pulmonary and intravenous administration with emphasis on cancer therapeutics and lung-targeted drug delivery. RSC Advances, 4(62), 32673-32689.
22. Redkar, M., Hassan, P. A., Aswal, V., & Devarajan, P. (2007). Onion phases of PEG-8 distearate. Journal of pharmaceutical sciences, 96(9), 2436-2445.