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Research Article

Formulation of Solid Lipid Nanoparticles of Cilnidipine for the Treatment of Hypertension

Bhalerao Aparna^{1*}, Choudhari Pankaj²¹ JSPM's Charak College of Pharmacy and Research, Wagholi, Pune, Maharashtra-412207² Micro Labs Limited, Chandivali, Andheri East, Mumbai, Maharashtra-400072

ABSTRACT

Cilnidipine is a fourth generation N and L-type calcium channel antagonists used alone or in combination with another drug to treat hypertension. Cilnidipine is poorly water-soluble, BCS class II drug with 6 to 30 percent oral bioavailability due to first pass metabolism. So to protect the drug from degradation and improve its dissolution, solid lipid nanoparticles were prepared. Glyceryl monostearate was selected as lipid while span 20: tween 20 were selected as surfactant blends. The formulations were evaluated for various parameters, as percent transmittance, drug content, percent encapsulation efficiency; percent drug loading, *In vitro* drug release and particle size. Optimized formulation was lyophilized using lactose as a cryo-protectant. The lyophilized formulation was evaluated for micromeritic properties, particle size and *in vitro* dissolution. It was further evaluated for DSC, XRD, and SEM. Percent encapsulation efficiency and percent drug loading of optimized formulation (F3) were 78.66percent and 9.44percent respectively. The particle size of F3 formulation without drug was 204 nm and with the drug was 214 nm. The particle size of the reconstituted SLN was 219 nm. In DSC study, no obvious peaks for cilnidipine were found in the SLN of cilnidipine indicated that the cilnidipine must be present in a molecularly dissolved state in SLN. In X-ray diffractometry absence of peaks representing crystals of cilnidipine in SLN indicated that the drug was in an amorphous or disordered crystalline phase in the lipid matrix. Thus, solid lipid nanoparticle formulation is a promising way to enhance the dissolution rate of cilnidipine.

Keywords: Cilnidipine, Solid Lipid Nanoparticle, Hypertension**Article Info:** Received 24 March 2019; Review Completed 03 May 2019; Accepted 06 May 2019; Available online 15 May 2019

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*Address for Correspondence:

Dr Aparna V. Bhalerao, Associate Professor, JSPM's Charak College of Pharmacy and Research, Wagholi, Pune, Maharashtra, India-412207

INTRODUCTION

Hypertension also called a silent killer is one of the most important risk factors for cardiovascular disease, a leading cause of death¹. Various classes of a drug as diuretics, α -blockers, β -blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II type 1 receptor blockers (ARB), and organic calcium channel blockers (CCBs) are used alone or in combination for the treatment of hypertension. Short-acting calcium channel antagonists decrease the blood pressure by vasodilation but are unable to decrease the blood pressure associated with sympathetic overactivity which is a secondary to the vasodilation^{1,2}. To lessen or avoid the neurohormonal activation caused by short-acting calcium channel blockers (CCB), fourth generation dihydropyridine (DHP) based N and L-type calcium channel antagonists like cilnidipine have been developed that have potential clinical benefits: gradual onset of action and a long duration of effects along with renal protection by decreasing the activity of the sympathetic nervous system (SNS) and the renin-angiotensin system^{2,3,4}. Cilnidipine is highly lipophilic,

BCS class II drug with very low water solubility, which limits the dissolution rate of cilnidipine in water and in turn oral bioavailability. All calcium channel blocker undergoes extensive hepatic first-pass metabolism⁵, and are also substrates for p-glycoprotein resulting in limited oral bioavailability. Solid lipid nanoparticles (SLN) dissolve the lipophilic drug in lipid which protects the drug from enzymatic degradation and facilitates the transport of drug through lymphatic route thus avoids the hepatic first pass metabolism^{6,7,8}.

MATERIAL AND METHODS

Cilnidipine was obtained as gift sample from JB Chemical, Mumbai, India Labrafil 1944, Compritol, Precirol were obtained as gift samples from Gattefosse Corporation, France, poloxamer 407 was obtained from BASF India, oleic acid, Tween 20, Tween 80, propylene glycol, polyethylene glycol, Span 60, isopropyl myristate, ethyl oleate, isopropyl palmitate, and glyceryl monostearate, were purchased from Research Lab Ltd. All other chemicals used were of analytical grade.

Formulation of solid lipid nanoparticle (SLN) of cilnidipine:

Solubility Study of cilnidipine in solid lipid and surfactant⁶:

The solubility of cilnidipine in various lipids, hydrophilic surfactants, and lipophilic surfactants was checked. Solubility in lipid was checked in molten lipid at 70°C. The solubility of cilnidipine in surfactants was determined by using the shaking method. An excess amount of cilnidipine was added into each 5 ml vial containing 2 ml of selected vehicle i.e. surfactants separately and vortexed for 10 min. to facilitate proper mixing. The mixture vials were then kept at $25 \pm 1.0^\circ\text{C}$ in an orbital shaker (Remi, CIS 24 B2) for 72 h to reach equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 3000 rpm for 15 min, followed by filtration through a 0.45μ membrane filter. The filtrate was suitably diluted with methanol and absorbances were measured using UV visible spectrophotometer (Shimadzu 1700) at 240 nm. The content of cilnidipine in each vehicle was determined using a standard calibration curve of cilnidipine in methanol. From the solubility study lipid, hydrophilic surfactant and lipophilic surfactant showing a maximum solubility were selected for further study.

Selection of Smix⁶:

Each surfactant blend was blended in a concentration giving a required HLB of GMS to form a clear microemulsion.

Lipophilic surfactant was mixed with lipid in a vial and hydrophilic surfactant was added to 1 ml water in a 50 ml beaker. 1 ml of water was then slowly added to the lipid phase in a vial kept on a magnetic stirrer. The temperature of the oil phase and the water phase was kept 70°C .

Selection of surfactant and optimization of surfactant concentration in water⁷:

1ml of clear microemulsion maintained at 70°C was added to 50 ml medium (water) containing hydrophilic surfactant maintained at 2°C under high-speed stirrer rotating at 16,000 rpm. Addition of a drop of microemulsion to cold water results in solidification of the microemulsion. These microemulsified solid drops under high-speed stirring breaks into small particles resulting in the formation of solid lipid nanoparticles. Surfactant concentration in 50 ml cold water was optimized so as to prevent aggregation of solid lipid nanoparticles and imparting stability to a formulation.

Two surfactants i.e. tween 20 and poloxamer 407 were selected and their effect on stability was studied. The concentration of Smix was optimized from the percent transmittance and stability study.

Optimization of lipid concentration:

Lipid concentration had a pronounced effect on particle size, *in vitro* drug release, drug content and drug entrapment efficiency. Lipid: Smix concentration was varied from 1:1 to 1:5. Lipid concentration was optimized from a percent transmittance and stability study.

Table 1: Lipid: Smix ratio

Codes	Drug	Lipid: mix	Lipid(GSM) percent	Smix percent		Water percent
				Tween 20	Span 20	
F1	1.477	0.5:0.5(1:1)	24.63	16.00	8.62	49.2
F2	1.477	0.67:1.33(1:2)	16.42	21.34	11.49	49.2
F3	1.477	0.5:1.5(1:3)	12.31	24.01	12.93	49.2
F4	1.477	0.4:1.6(1:4)	9.85	25.61	13.79	49.2
F5	1.477	0.33:1.66(1:5)	8.20	26.68	14.36	49.2

The formula for suspension:

The suspension formulation of cilnidipine was prepared (Table 2) and used to compare the dissolution efficacy of SLN formulation.

Table 2: Formulation of suspension

Ingredients	Quantity
Cilnidipine	0.5 g
Sodium CMC	0.1556 g
Veegum	0.156 g
Sorbitol solution (70percent)	05.2 ml
Disodium EDTA	0.01 g
Water	50 ml

Evaluation of solid lipid nanoparticles:

Appearance⁹:

The appearance of the SLN formulations was observed for any turbidity or precipitation of drug.

Spectroscopic characterization of optical clarity^{9,10}:

The percent transmittance of SLN formulation was measured using U.V. Spectrophotometer (UV-1700 Shimadzu) at 400 nm.

Drug content^{10,11}:

5 ml of solid lipid nanoparticles formulation was added to 80 ml of methanol. It was heated so that glyceryl monostearate becomes soluble in hot methanol. The drug present inside glyceryl monostearate comes outside in methanol. Methanol was again cooled to room temperature so that glyceryl monostearate precipitates out. It was then filtered out through 0.45μ membrane filter paper. Volume was then adjusted to 100 ml in volumetric flask and absorbance is noted at 240nm.

Percent Encapsulation efficiency and percent drug loading⁶:

A certain volume of cilnidipine solid lipid nanoparticle was accurately taken, dissolved, heated, filtered and diluted with methanol. The resulting solution was determined with UV-Vis spectrophotometer (UV-1700 Shimadzu) at 240 nm and the total amount of cilnidipine was obtained. The same volume of cilnidipine solid lipid nanoparticles was accurately taken in dialysis membrane and it was tied at both the end. It was then suspended in a beaker containing 100 ml methanol. The free drug gets dissolve in methanol and diffuses out through the dialysis membrane. Absorbance was then noted at 240 nm giving the concentration of a free drug.

W_s = Total drug – free drug

$$\text{Entrapment efficiency (percent)} = \frac{W_s}{W_{\text{total}}} \times 100$$

$$\text{DL (percent)} = \frac{W_s}{W_{\text{lipid}}} \times 100$$

W_s : the amount of cilnidipine loaded in SLN,

W_{total} : total cilnidipine in cilnidipine SLN,

W_{lipid} : the weight of the vehicle

In vitro release study^{11,12}:

Solid lipid nanoparticles formulation equivalent to 5 mg of cilnidipine entrapped in dialysis membrane were studied for drug release profiles in 500 ml 0.1N HCl solution using USPXXIII type II dissolution apparatus (DA-3D, Veego) rotating at 50 rpm maintained at $37 \pm 0.5^\circ\text{C}$. Samples were withdrawn at predetermined time intervals and analyzed spectrophotometrically using U.V. spectrophotometer (UV-1700 Shimadzu) at 245nm. Similar drug release profiles in 500 ml phosphate buffer (pH 6.8) were calculated using U.V. spectrophotometer (UV-1700 Shimadzu) at 245nm. A similar procedure was carried out to perform *in vitro* drug release of suspension of cilnidipine.

Freeze-drying of Solid lipid nanoparticles¹³:

To improve the physical and chemical stability of these systems water has to be removed from it. The most commonly used process which allows converting solutions or suspensions into solids of sufficient stability for distribution and storage in the pharmaceutical field is freeze-drying. Some of the cryoprotectants used in literature for the freeze-drying of nanoparticles are glucose, sucrose, trehalose, lactose, mannitol, sorbitol, Aerosil (colloidal silicon dioxide), maltose, poly (vinyl pyrrolidone), fructose, dextran, poly(vinyl alcohol), hydroxypropyl- β -cyclodextrin and gelatin. The cryoprotectant used here was lactose. The SLN suspension in distilled water was cooled and froze stepwise from 25°C to -10°C for 4 h. The primary drying involves drying of frozen product stepwise from -10°C for 40 h to 0°C for 16 h to 10°C for 5 h. secondary drying was performed at 25°C for 10 h.

Evaluation of solid lipid nanoparticles (freeze-dried):

Micromeritic properties¹³:

Freeze dried formulation was analyzed for micromeritics properties like the angle of repose, bulk density and tapped density, Hausner's ratio, and percent compressibility.

Morphological analysis of SLN¹³:

The samples were fixed on a brass disc using the double-sided adhesive tape and were made electrically conductive by coating in a vacuum with platinum using JFC-1600 auto fine coater for 240s at 15mA. The outer macroscopic structure of the SLN was investigated by Scanning Electron Microscopy (SEM) using a Scanning Electron Microscope (JEOL JSM- 6360, Japan), operating at 20 KV.

Thermal analysis^{13,14}:

Thermal analysis of SLN was carried out using differential scanning calorimeter (Mettler STAR[®] SW 9.20 system) with a liquid nitrogen cooling accessory. The analysis was performed under a purge of dry nitrogen gas (40 cc/min). The sample 2mg-4mg was placed in a flat bottom aluminum pan and was firmly crimped with a lid to provide an

adequate seal. The sample was heated from ambient temperature 40°C to 250°C at a pre-programmed heating rate of $10^\circ\text{C}/\text{min}$.

X-ray powder diffraction^{12,13}:

An X-ray diffractometer (Bruker axs, model D8 advance) consisting of 40kV, 40mA generator with a Cu-K α radiation anode tube was used. Diffraction pattern of SLN was scanned over 2θ ranges from 5° to 50° at a rate of 1°C per min. and 0.1° 2θ step size.

Drug content estimation^{10,11}:

SLN formulation equivalent to 5 mg of cilnidipine was dispersed in 80 ml of methanol. The samples were mixed thoroughly to dissolve the drug in methanol. It was heated so as to dissolve GMS. It was again cooled and volume was adjusted to 100 ml with methanol. It was then filtered using 0.45μ membrane filter, suitably diluted with methanol and analyzed using UV visible spectrophotometer (Shimadzu 1700) at 240 nm.

In vitro drug release^{11,12}:

SLN formulation equivalent to 5 mg of cilnidipine in the capsule were studied for drug release profiles in 500 ml 0.1N HCl solution using USPXXIII type II dissolution apparatus (DA-3D, Veego) rotating at 50 rpm maintained at $37 \pm 0.5^\circ\text{C}$. Samples were withdrawn at predetermined time intervals and analyzed spectrophotometrically using U.V.spectrophotometer (UV-1700 Shimadzu) at 245nm. Similar drug release profiles in 500 ml phosphate buffer (pH 6.8) were calculated using U.V spectrophotometer (UV-1700 Shimadzu) at 245nm.

The Droplet size of reconstituted emulsions¹⁵:

The average droplet size, size distribution of resulting emulsions formed from SLN was assessed by photon correlation spectrometer (Mastersizer 2000, Malvern Ltd).

Stability study of reconstituted SLN formulation¹⁴:

The optimized reconstituted SLN formulation was put into empty gelatine capsules and subjected to stability study at $40^\circ\text{C}/75\%$ RH. Samples were charged in stability chambers with humidity and temperature control. The samples were withdrawn at 0, 15th, 30th and 45th days and were analyzed for appearance, *in vitro* drug release and percent transmittance.

RESULT AND DISCUSSION

The solid lipid nanoparticle formulation consisted of lipid and surfactant blend. The prior step before the formation of solid lipid nanoparticle is a formulation of the microemulsion. The microemulsion so formed should be clear. Solubility studies were aimed at identifying suitable lipid phase and surfactant/s for the development of cilnidipine SLN. Identifying the suitable lipid, surfactants/co-solvent having the maximal solubilizing potential for a drug under investigation is very important to achieve optimum drug loading and also to minimize the final volume of SLN. The solubility of cilnidipine in various lipids, surfactants were determined. The data demonstrated that solubility of the lipophilic drug cilnidipine ($\text{Log } P-5.54$)⁴ was found to be higher in glyceryl monostearate. Among the hydrophilic surfactants tested in this study, tween 20, a medium-length alkyl chain with HLB 16.7 was selected as appropriate surfactant because non-ionic surfactants are less toxic than ionic surfactants¹⁴, has good biological acceptance; is powerful permeation enhancer, less affected by pH and ionic strength, and good solubilizer of cilnidipine. Tween 20

inhibits P-glycoprotein efflux of drug and thus increases bioavailability¹³. Among the lipophilic surfactant transcuto P and span 20 showed a good solubility.

Table 3-Solubility of cilnidipine in different lipids, hydrophilic /lipophilic surfactants

Lipid/ surfactants	*Solubility of cilnidipine in mg/ml
Glyceryl monostearate	10
Precirol	3
Compritol	2
Tween 20	126.01 ± 0.89
Tween 80	91.381 ± 0.98
Tween 60	85.31 ± 1.12
Transcutol P	88.93 ± 1.15
Span 20	26.41 ± 0.80
Span 60	28.21 ± 0.92

* Represents mean ± S.D. (n = 3)

The solubility of cilnidipine in surfactants was determined by diluting respective filtrate with methanol and estimated using UV spectrophotometer at λ max 240nm. Upon scanning the λ max of cilnidipine in the presence of various surfactants; it was observed that there was no shift in λ max of cilnidipine. It can be inferred that selected vehicles did not interfere with the analytical method of the drug.

From the solubility study, glyceryl monostearate was selected as a lipid for cilnidipine SLN. Hydrophilic and

lipophilic surfactant blend was selected one which one forms clear microemulsion. Formation of the clear microemulsion is a pre-requisite for the formation of solid lipid nanoparticles.

Formation of microemulsion:

The microemulsion formed by using span 60 and tween 60 was not clear. So it was not selected for further studies. The microemulsions formed by using span 20 and tween 20 and by using transcuto P and tween 20 were clear. So they were used for further studies.

The volume of medium:

The volume of medium given in literature varies from 25ml to 50ml. It is reported that greater the hydrophilicity of a medium, greater is the stability of solid lipid nanoparticles¹². Hence, 50ml medium i.e. water was selected to disperse 1ml of microemulsion maintained at 70°C for uniform dispersion of solid lipid nanoparticles.

Selection of surfactant and optimization of surfactant concentration in water:

Two surfactants were selected for the study. They are poloxamer 407 and tween 20. The surfactant and surfactant concentration which was required to be added in 50ml water so as to dispersed microemulsion drop uniformly under high-speed stirrer was decided from the one showing maximum transmittance and stability. The concentration of both surfactants was varied from 0.2% to 1.0 %.

Table 4: Percent Transmittance and Stability study

Sr no	Percent Surfactant concentration in the polar phase	Percent Transmittance		Stability (at room temperature) in terms of no days	
		Poloxamer 407	Tween 20	Poloxamer407	Tween 20
1	0.2	51	62	Unstable	Unstable
2	0.4	54	66	Unstable	Unstable
3	0.6	58	71	Unstable	Unstable
4	0.8	62	74	Stable (3days)	Stable (5days)
5	1.0	66	78	Stable (5days)	Stable (7days)

From percent transmittance, it was found that the same concentration of poloxamer 407 showed less percent transmittance than tween 20. Percent transmittance increases with an increase in surfactant concentration in case of both surfactants. From stability studies, it was found that stability with poloxamer 407 having the same concentration as that of tween 20 was less. Stability was also found to increase with an increase in the concentration of surfactant in case of poloxamer 407 and tween 20. From percent transmittance and stability studies, tween 20 was

selected as a surfactant, and its concentration selected for further studies was 1.0percent.

Optimizations of surfactant blend in microemulsion:

Two surfactant blend i.e. span 20: tween 20 and transcuto P: tween 20 showed good clarity. Selection of one surfactant blend was further based on stability study. The surfactant blend which does not show aggregation of nanoparticles for a long time was selected as a choice of surfactant blend.

Table 5: Optimization of the surfactant blend

Sr no.	Surfactant blend	Stability study presence of clarity in terms of number of days	Transmittance study (percent)
1	Span20: Tween20	Stable for more than one day	78
2	TranscutolP: Tween20	Stable for one day	86

From the clarity study, it was observed that span 20: tween 20 showed greater stability than transcuto P: tween 20. The percent transmittance obtained with transcuto P: tween 20 was greater than span 20: tween 20. Selected Smix was span 20: tween 20 as it showed greater stability. These Smix selected from the stability point of view of nanoparticles which should be greater.

Effect of lipid on percent transmittance:

Effect of lipid on percent transmittance was checked by changing the lipid: Smix concentration.

Table 6: Change in Percent Transmittance with a change in lipid concentration

Sr no.	Formulation code	Percent transmittance
1.	F1	74
2.	F2	78
3.	F3	81
4.	F4	83
5.	F5	89

The percent transmittance was found to increase from F1 to F5. This increase in percent transmittance was due to an increase in Smix concentration from F1 to F5. Increase in concentration of Smix may be resulting in smaller particle size and hence increase percent transmittance.

Drug content:

Formulation code containing lipid: Smix concentration from F1 to F5 were evaluated for drug content.

Table 7: Drug content of formulated SLN

Sr no.	Formulation code	*Percent drug content
1	F1	98.04 ± 1.02
2	F2	99.12 ± 0.76
3	F3	98.86 ± 0.88
4	F4	98.58 ± 1.21
5	F5	97.45 ± 1.34

* Represents mean ± S.D. (n = 3)

The drug content of all the formulation i.e. from F1 to F5 was within 97% to 100%.

Encapsulation efficiency and Drug loading:

Formulation codes containing lipid: Smix concentration from F1 to F5 were evaluated for encapsulation efficiency and drug loading.

Table 8 Percent encapsulation efficiency & percent Drug loading of formulated SLN

Sr no.	Formulation code	Percent encapsulation efficiency	Percent of drug loading
1	F1	84.00	5.04
2	F2	80.66	7.20
3	F3	78.66	9.44
4	F4	67.33	10.10
5	F5	61.66	11.08

The percent encapsulation efficiency was found to decrease from F1 to F2 and percent drug loading was found to increase from F1 to F2. Formulation F3 showed good percent encapsulation efficiency and percent drug loading.

In vitro release study:

The dissolution study of solid lipid nanoparticles is compared with the suspension of a drug.

Fig 1 In vitro drug release study of formulated SLN in 0.1N HCl

Figure 2: *In vitro* release study of formulated SLN in phosphate buffer pH 6.8

The *in vitro* drug release of all formulation (F1 to F5) was found to be greater in 0.1N HCl than in phosphate buffer pH 6.8. *In vitro* drug release of cilnidipine SLN was showed sustained and improved drug release as compared to cilnidipine suspension. F4 and F5 showed marginally increase in drug release when compared with F3. These may be due to high surfactant concentration which will give finer particle size and greater hydrophilicity. Solid lipid

nanoparticles adhere to the GI membrane or get accumulated in intervillar spaces of GI membrane¹⁶. Hence, they show sustain release of the drug. So, a high concentration of surfactant would cause GI irritation.

So, F3 batch was selected with intermediate lipid: Smix concentration which was showing satisfactory percent encapsulation efficiency, percent drug loading and *in vitro* drug release.

Particle size analysis of solid lipid nanoparticles

Figure 3: Particle size analysis of F3 formulation without drug

Figure 4: Particle size analysis of F3 formulation withh drug

The particle size of F3 without drug was 204 nm, while with the drug it was 214 nm. After the addition of the drug, there was marginal increase in the particle size of solid lipid nanoparticle.

Formulation of Solid lipid nanoparticles by freeze-drying method:

Formulation F3 was subjected to freeze drying. Lactose was used as a carrier as it has good flow properties, surface area, and it is also used as a cryoprotectant in freeze drying.

The Formulation for freeze-drying:

Formulation ready for freeze drying consisted of 2 gm system (i.e. F3) dispersed in 50 ml water to which 1 gm of lactose was added. This formulation was used for freeze drying.

Micromeritics Properties:

Appearance: The freeze-dried SLN were white, non gritty powder

Table 8 Micromeritic properties of optimized formulation

Formulation	Angle of Repose (θ)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Hausner's ratio	Carr's index (percent)
2:1	32.14	0.533	0.59	1.42	30.72

From the results of the angle of repose, Hausner's ratio and Carr's index it indicated that powder possessed satisfactory flow properties.

The Particle size of reconstituted SLN:

The mean globule size of reconstituted solid lipid nanoparticles is presented by Freeze drying method.

There was a slight increase in particle size after reconstitution of solid lipid nanoparticles as compared to liquid-solid lipid nanoparticle. Reconstituted solid lipid nanoparticles showed a particle size of 219 nm while liquid-solid lipid nanoparticle showed particle size of 214 nm.

Figure 5: Particle size analysis of reconstituted formulation F3

Morphological analysis of solid SLN:

Figure 5: Scanning electron micrographs: (A) Cilnidipine; (B) Lactose; (C) SLN by Freeze drying method

Cilnidipine is rectangular plate shaped rough surface crystals. Lactose was detected as aggregates of amorphous particles and with a smooth surface. SLN appeared as rough surfaced lactose particles, indicating that the SLN is adsorbed on lactose.

Differential Scanning Calorimetry (DSC):

The physical state of cilnidipine in the SLN was investigated since it would have an important influence on the *in-vitro*

and *in-vivo* release characteristics. Pure cilnidipine showed a sharp endothermic peak at temperature 108°C-111°C in DSC. No obvious peaks for cilnidipine were found in the SLN of cilnidipine. It might be explained that the crystallization of cilnidipine was inhibited by lactose and surfactants. This also indicates that the cilnidipine must be present in a molecularly dissolved state in solid SLN.

Figure 6: Differential scanning calorimetric thermographs of cilnidipine, lactose, solid SLN prepared by Freeze drying method

DSC of pure lactose showed a first endothermic peak in the range of 145°C to 150°C which was due loss of water molecule and second endothermic peak in the range of 215°C to 220°C which was due to the melting of lactose. DSC of the drug showed melting of the drug started at 108°C. DSC of SLN showed no endothermic peak for drug indicating that crystallization of cilnidipine was inhibited by lactose and

surfactants. Loss of water molecule from lactose due to freeze-drying (DSC of SLN), it did not show a peak in the range of 145°C to 150°C. There was a slight shift in the peak of lactose in formulation from 218°C to 236°C. It might be due to charring of carbohydrate i.e. lactose. From these, we can conclude that the crystallinity of lactose has been suppressed but not totally.

X-ray powder diffractometry:

Figure 7: X-ray powder diffractometry of (A) Cilnidipine; (B) Lactose; (C) SLN prepared by Freeze drying method

The results showed the absence of obvious peaks representing crystals of cilnidipine in SLN indicating that the drug was in an amorphous or disordered crystalline phase in the lipid matrix. The intensity of lactose peak in SLN was also decreased as compared to X-ray of pure lactose, indicating that crystallinity of lactose has been suppressed but not totally.

Drug content of SLN formulations:

Drug content of reconstituted SLN formulations prepared by the freeze-drying method was calculated using a calibration curve of cilnidipine in methanol.

Table 8 Drug content of SLN formulations prepared by Freeze drying method

Formulation	Conc. of Lactose (mg)	SLN formulation (mg)	*Drug content (percent)
F3	1000	2000	98.27± 0.98

* Represents mean ± S.D. (n = 3)

The preparation of Freeze dried SLN from liquid SLN did not significantly affect the drug content of the formulations.

Figure 8: *In vitro* drug release profiles of SLN formulation (F3) prepared by freeze drying method in 0.1N HCl and phosphate buffer pH 6.8

Freeze dried formulation F3 showed 69.24percent drug release and 63.42percent drug release in 0.1N HCl and phosphate buffer pH 6.8 respectively.

Stability study:

The SLN formulation F3 was selected for the stability study. Formulation F3 was stable up to 45days.

Table 9: Stability study of SLN (F3)

Sampling point (days)	percent drug content	*percent drug release in 480 min. in 0.1N HCl	*percent drug release in 60 min. in pH 6.8 Phosphate buffer	percent Transmittance
0	99.20±0.68	67.32±0.12	60.48±0.15	74.54
15	99.43±0.12	66.95±0.23	60.12±0.43	75.12
30	99.03±0.20	68.14±0.45	61.32±0.54	72.86
45	98.07±0.14	67.94±0.65	62.23±0.76	71.09

* Represents mean ± S.D. (n = 3)

CONCLUSION

It can be concluded that solid lipids nanoparticles are promising ways to enhance the dissolution rate of cilnidipine.

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CONFLICT OF INTEREST-None

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