Ind. J. Pharm. Edu. Res., 2023; 57(3):684-694.
https://www.ijper.org

Original Article

Lyophilized NLC of Cinacalcet HCl: Physics of Tablet Compression and Biopharmaceutical Characterization

Sabita Nayak*, Jammula Sruti, Chinam Niranjan Patra, Goutam Kumar Jena

Department of Pharmaceutics, Roland Institute of Pharmaceutical-Sciences-Berhampur, Affiliated to Biju Patnaik University of Technology, Rourkela, Odisha, INDIA.

ABSTRACT

Background: Cinacalcet Hydrochloride (CINH) is a BCS class IV drug. It is mainly used for the treatment of chronic renal disease and parathyroid cancer. It exhibits poor oral bioavailability less than 25%. The main objective is to improve the bioavailability and stability of CINH by formulating the lyophilized Nanostructure Lipid Carrier (NLC) into tablet dosage form. **Materials and Methods:** In this research, Glycerylmonostearate (GMS), labrasol, tween 20 were the main excipients selected for the formulation of NLC. Hot high speed homogenization and ultra-sonication method was used for the NLC formulation of CINH. The selected NLC formulation was lyophilized using three different cryoprotectants at three different concentrations. Physics of tablet compressions study was conducted for the selected lyophilized powders. The pharmacokinetic study was conducted to determine the improvement in bioavailability of the CINH. The cytotoxicity study was performed by using MTT assay method to know the cell viability. **Results:** The lyophilized NLC formulation exhibited high drug entrapment efficiency content with particle size less than 200nm. Physics of tablet compression study showed lyophilized NLC containing 15%w/v mannitol exhibited plastic deformation. Pharmacokinetic study showed 5 folds increase in oral bioavailability for Lyophilized NLC (LNLC3) in comparison to aqueous suspension of CINH. Minimum viability was determined as 94% which indicates the safety of the incubated formulations. **Conclusion:** Lyophilized NLC formulation has the potential to improve the oral bioavailability with high drug loading, stability, and cell viability for CINH with desirable tableting parameters for making tablet.

Keywords: Compactibility, Cryoprotectant, Crystalinity, Bioavailability and Cytotoxicity.

Correspondence:
Mrs. Sabita Nayak
Research Scholar, Roland Institute of Pharmaceutical Sciences-Berhampur, Affiliated to Biju Patnaik University of Technology, Rourkela, Odisha, INDIA.
Email: sabita.rips@gmail.com

Received: 27-09-2022;
Revised: 21-12-2022;
Accepted: 11-04-2023.

INTRODUCTION

Patients with chronic renal disease and parathyroid cancer are treated with cinacalcet HCl (CINH).[1] CINH is poorly water soluble and undergoes first pass metabolism which are primary reasons for its oral bioavailability less than 25%. It is reported in the literature that CINH exhibits improved oral bioavailability in presence of fatty food in healthy male volunteers.[2] This gave an indication that lipid based Drug Delivery System (DDS) can be potentially advantageous in improving delivery of CINH. Literature study on different nanoformulations of CINH revealed that different approaches like solid-SNEDDS,[3] nanocrystals,[4] SMEDDS,[5] Solid Lipid Nanoparticles (SLN)[6] and polymeric nanoparticles[7] have been attempted for improvement of oral delivery. Lyophilized nano structured lipid carriers are attempted. Nanostructured Lipid Carriers (NLC) are produced as emulsions. It is a major challenge for formulator's to maintain physical and chemical stability of the dosage form containing both aqueous and lipid phase. These instabilities are the primary reason for failure of large scale production and long term stability of NLC which can be attributed to the mobility of its constituents. This immobilization can be achieved after lyophilization of liquid formulation of nanoscale.

Literature study reveals that many nanoformulations such as SLN,[8] NLC,[9] PN[10] etc. have been converted into lyophilized free flowing powders for improved long term storage stability. However, no literature is available on the scientific and systematic study on the study of physics of tabeleting of lyophilized powders. Hence purpose of the current research is to determine the physics of tablet compression of lyophilized powders prepared using different cryoprotectants such as mannitol, dextrose and lactose at three different levels. Using the Kawakita, Heckel's, and Leuenberger equation, the flowability and compressibility of lyophilized powders were assessed. The objective is to study the improvements in biopharmaceutical properties of the selected

DOI: 10.5530/ijper.57.3.83

lyophilized formulations by performing pharmacokinetic study in albino rabbits.

MATERIALS AND METHODS

CINH was gifted by RA Chem. Ltd., India. Glyceryl Mono Stearate (GMS) was a gift sample from Loba Chemie, Mumbai, India. Labrasol was procured from Gattefosse, Mumbai, India. Tween 20 was purchased from Merck Life Sciences Pvt. Ltd., Mumbai, India. Tetrabutyl Ammonium Hydrogensulphate (TBHS), chloroform, acetonitrile and methanol were procured from Merck, Mumbai, India. Mannitol, dextrose and lactose were procured from HiMedia Laboratories, India.

Preparation of NLC

Initially trial formulations were attempted to select a suitable NLC formulation for CINH by hot high speed homogenization and ultrasonication method.[11] 1.25 g of GMS and 0.5g of labrasol were used as lipid phase. The aqueous phase was prepared by using stabilizer (2% w/v of tween 20 in water). The drug CINH was introduced to the combination of solid and liquid lipid after the GMS had fully melted. With continuous stirring at 20,000 rpm for 3 hr at the same temperature, 60°C, the organic phase (a combination of solid and liquid lipid) was introduced dropwise to the aqueous phase. The hot O/W emulsion was probe sonicated (amplitude of 60%) for 5 min with on and off of pulse in four seconds and two seconds respectively to obtain the nanoemulsion of CINH followed by cooling to room temperature to obtain NLC of CINH.[12] The obtained product was characterized.

Characterization of NLC

NLC formulation was successfully developed using hot high speed homogenization and ultrasonication method. The NLC formulation showed Entrapment Efficiency (EE) of 74.31%,[13] Particle Size (PS) of 173nm,[14] Zeta Potential (ZP) of-23.5,[15] Poly Dispersity Index (PDI) of 0.346[16] and drug diffusion showed sustained release for 24 hr.[17] This formulation was subjected to lyophilization.

Preparation of lyophilized NLC

The NLC formulation was mixed with three different cryoprotectants such as mannitol, dextrose and lactose in three different concentrations of 5, 10 and 15% w/v to NLC formulation (Table 1) and deep frozen (-20°C) for 24 hr. The substance was then lyophilized for roughly 72 hr at -52°C and 0.002 mbar pressure to produce Lyophilized NLC (LNLC) powder.[9,18-20]

Characterization of lyophilized NLCs

Entrapment efficiency

For the analysis of CINH Reverse Phase Ultrafast Liquid Chromatographic (RP-UFLC) method was followed from literature[21] with the following specifications i.e. C_{18} column (250 mm × 4.6 mm i.d., 5 µm particle), 1:1 Acetonitrile: TBHS-10 mM as mobile phase, flow rate (1 ml/min), PDA detection at 223 nm. Ethyl acetate is used as an extracting solvent. 1 mL of the each NLC formulation was transferred to eppendorf tubes. The tubes were subjected to cooling centrifugation (Remi Instrument Ltd., Mumbai, India) at 10,000 rpm for 30 min at 4°C. Then 0.5 mL of supernatant was collected and mixed with 0.5 mL of ethylacetate followed by vortexing for 10 min, diluted with mobile phase and analyzed using UFLC.[21]

Micromeritics

The bulk density and tap density of LNLC was determined by the tapping smples. The Carr's index and Hausner's ratio was determined.[22]

$$\text{Bulk density} = \text{Mass/Bulk volume} \quad (1)$$

$$\text{Tapped density} = \text{Mass/Tapped volume} \quad (2)$$

$$\text{Carr}'\text{s index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100 \quad (3)$$

$$\text{Hausner}'\text{s ratio} = \text{Tapped density/Bulk density} \quad (4)$$

Kawakita analysis

The Kawakita analysis was used to determine flowability.[23] In this procedure, a glass measuring cylinder (50 mL) was filled with 3 g of each of the pure drugs CINH, LNLC3, LNLC6, and LNLC9. A small metallic spatula was used to level out the piled particles in the cylinder so that the bulk volume 'Vo' could be precisely measured. After that, mechanical tapping was started. After N taps, a change in the powder column volume 'V' was noticed. Each phase of the tapping technique's behaviour was compared using numerical constants that were determined using Kawakita plots.

$$\frac{N}{C} = \frac{N}{a} + \frac{1}{ab} \quad (5)$$

Where a is compactibility, and 1/b is cohesiveness. The C is degree of volume reduction, Vo is original volume and V is tapped volume as follows:

$$C = \frac{Vo - V}{Vo} \quad (6)$$

The slope of the plot of N/C versus number of taps N provides the numerical values for constants a and 1/b. (N =0, 50, 100, 150 and 200).

Heckle analysis

Using plane-face punches with a diameter of 10 mm, tablets (350mg) were compressed on a hydraulic pellet press, M/S Kimaya Engineers Pvt. Ltd., (Type KP) Mumbai, India at various compression pressures. Four different compaction forces (12.5, 25, 37.5 and 50 kg/cm^2) were used for each material (pure drug CINH, LNLC3, LNLC6 and LNLC9). 10 tablets were prepared at

Table 1: Composition/ different concentration of cryoprotectants in lyophilized NLC.

Sl. No. (Formulation code)	Cryoprotectants		
	Mannitol (%)	Dextrose (%)	Lactose (%)
LNLC1	5	-	-
LNLC2	10	-	-
LNLC3	15	-	-
LNLC4	-	5	-
LNLC5	-	10	-
LNLC6	-	15	-
LNLC7	-	-	5
LNLC8	-	-	10
LNLC9	-	-	15

LNLC: Lyophilized Nanostructured Lipid Carriers.

each pressure. A digital slide calliper (Mitutoyo Co., Kawasaki, Japan) was used to measure each compact's dimensions and weigh each one precisely (n = 10). Ten compacts were measured in terms of weight, thickness, and diameter.

The Heckel equation was used to study the powder's compaction characteristics.[24] The Heckel equation (7) is described asfollows.[25]

$$\ln\frac{1}{1-Dr} = kP+A \quad (7)$$

$$Dr = \frac{Da}{Dt} \quad (8)$$

Here Dr, Da and Dt are relative, true and apparent density respectively at applied pressure (P), the slope (K) is equal to the reciprocal of the material's yield Pressure (Py) and A is intercept (function of compact volume).

Leunberger Anaslyis

Tablets were prepared in the same pressure as mentioned in Heckel analysis. Hardness was determined using portable digital tablet hardness tester (EH-1), Electrolab, Mumbai, India to estimate the force necessary to break the compacts' diametral bonds in order to assess compactibility. By applying the following equation (9) tensile strength σ_t of the compacts were determined. Here, F_B is hardness (in kg/cm^2)

D_t= diameter

h_t=thickness of the compacts (in mm).[23]

$$\sigma_t = \frac{2FB}{\pi Dtht} \quad (9)$$

The following equation (10) was used to fit the data for the Leuenberger study. Statistical software (Graph Pad Prism4) was used to produce a non-linear plot of tensile strength with respect to product compaction pressure P and relative density ρ_r. Here, σ_{tmax} is the maximum tensile strength (kg/cm^2) when P will be infinite and ρ_r will be equal to 1, and γ is the compression susceptibility.

$$\sigma_t = \sigma_{tmax}(1- e^{-\rho r \times \gamma \times P}) \quad (10)$$

Particle Size (PS), Polydispersity Index (PDI) and Zeta Potential (ZP)

The mean particle size of LNLC3 was determined using the Nano zetasizer (Malvern, UK). The LNLC3 formulation (100 µL) was analyzed by diluting upto 5000 µL with double distilled water.[26] By using Malvern Zetasizer the PDI and ZP of the LNLC was measured. The sample was analyzed by diluting an aliquot (1mL) of the sample with double distilled water (50 times).[27,28]

In vitro Diffusion

The *in vitro* diffusion study was carried out utilising the dialysis method with 0.1N HCl and phosphate buffer pH 6.8 for CINH and LNLC3.[29] A beaker containing 100mL of medium was used to insert LNLC3 dispersion (equivalent to 30 mg CINH). The samples were diluted and then subjected to RP-UFLC analysis.

Fourier Transform Infra-Red (FT-IR)

FT-IR of CINH, solid lipid GMS, Physical Mixture (PM) of CINH with GMS and NLC3 were conducted on IR Affinity-1 (Shimadzu, Japan). The samples were examined at a scanning speed of 2mm/s with a resolution of 4 cm^{-1} over the range 4000-400 cm^{-1}.[30,31]

Differential Scanning Calorimetry (DSC)

DSC thermal analysis of CINH, GMS, PM of CINH with GMS and LNLC3 wereperformed by using DSC-60 (Shimadzu, Japan). The calibration of instrument was carried out by using Indium as standard. The experiment was conducted at the rate of 10°C rise/ min in temperature range (25 to 225°C).[32]

Powder X-Ray Diffraction Study (P-XRD)

Powder XRD (Multiflex, Japan) studies were carried out for CINH and LNLC3 formulation by scanning (2 to 80°) for 2 hr at a step size of 0.045° and step time of 0.5 sec.[33]

Scanning Electron Microscopy (SEM)

SEM (Hitachi, Tokyo, Japan) was used to examine the morphology of the pure drug CINH and the LNLC3 formulation. The sample was initially attached to a metallic stub that had been coated with carbon before being coated with platinum. The samples were sent through SEM for surface examination.[34]

Stability Study

According to the (ICH) Q1A (R2) guidelines, the LNLC3 was kept in a humidity-controlled oven (TH90 S/G, Thermolab, India) at 25±2°C/60±5% RH for 6 months. Samples were collected at 0, 1, 3 and 6 months and evaluated for EE, PS, ZP and PDI.[35]

Pharmacokinetics studies

The protocol (926/PO/ac/06/CPCSE/100) was approved by IAEC of RIPS. White albino rabbits weighing 2 kg were administered orally with pure drug CINH and LNLC3.[36] Ethyl acetate was used as extracting solvent for the estimation of CINH.[21] Each sample with six number of white albino rabbits was used for the determination of the bioavailability.

Total dose (in humans)× 0.07 (factor for each 1.5 kg weight of rabbit)

$$= (90\times0.07\times2)/1.5 = 8.4 \text{ mg for 2 kg rabbit} = 9 \text{ mg}$$

Aqueous suspension of CINH and LNLC3 formulation (equivalent to 9mg of CINH) were administered to albino rabbits using Ryle's tube. At various times, a blood sample (0.5 mL) was taken from the marginal ear vein.[37] To separate the serum, the blood sample was centrifuged at 3000 rpm for 10 min. CINH was extracted from serum by using ethyl acetate and analysed by reported UFLC method.[21] The pharmacokinetic parameters were calculated.[28]

In vitro cytotoxicity

In vitro cytotoxicity study using MDA-MB (breast cancer cell lines) were performed for pure drug solution and LNLC3 at three different concentration levels *vis-a-vis* 100, 250 and 500µg/mL.[38] At each concentration level, two samples of 10 µL and 100 µL were inoculated on the culture plate.[39] Simultaneously one placebo formulation was also subjected to the above study. A 96-well flat bottomed plate, with each well at a density of 1×10^4 cells was used for the cell plating and incubated for 24hr in the CO_2 incubator at 37°C.[40] Once the cells were attached, three replicate of 10µL and 100µL of the three formulations at above mentioned concentration levels were directly injected to plates followed by incubation of cells in CO_2 a period of 24hr at 37°C.[41] MTT (Sigma, M2128) assay method was used for the evaluation of cytotoxicity by using 3-[4, 5-dimethylthiazole-2-yl]-3,5-diphenyltetrazolium bromide dye.[42] The acetic isopropanol was added in order to dissolve the formzan crystals. After solubilising, the absorbance was measured with EPOCH 2 (Biotek) at a wavelength of 590nm.[43]

Preparation and Quality Control (QC) tests for tablets

The LNLC3 formulation equivalent to 30mg of CINH were compressed into tablets by direct compression method using 10mm flat circular punches(Mini Press II, Karnavati, India).As per standard procedure various QC tests were performed for these tablets.[44]

RESULTS

Characterization of Lyophilized formulations

Entrapment efficiency

All the formulations exhibited higher entrapment of CINH in the range of 48.56 to 75.38% (Table 2).

Micromeritics

The initial micromeritic properties of CINH suggested poor flowability. With increase in the proportion of cryoprotectants (mannitol, dextrose and lactose) from 5 to 15% exhibited significant improvement in flowability as revealed from angle of repose, Carr's index and Hausner's ratio of lyophilized powder. However, cryoprtectants at 15%w/v level showed micromeretic properties in desirable range for further processing into a suitable solid dosage form (Table 2).

Kawakita analysis

Lower value of 'a' i.e.copmactibility for mannitol based lyophilized formulation (LNLC3) showed better flowability than pure drug powder CINH and other lyophilized formulations. Similarly lower value of '1/b' i.e. cohesiveness for LNLC3 showed that it is less cohesive than pure drug CINH and other lyophilized formulations (Table 3).

Heckle analysis

All the lyophilized formulations showed non-linearity at initial stages of compression All the selected lyophilized formulations showed nearly similar values for the intercept 'A' which is significantly higher than the pure drug CINH. Higher K value was observed for LNLC3 indicating good compressibility and plastic deformation (Table 3). Higher value of yield pressure was observed for pure drug CINH.

Leuenberger analysis

The compression susceptibility parameter (γ) for lyophilized formulations was higher in comparison to pure drug CINH

Table 2: % Entrpment Efficiency and Micromeritic properties of Lyophilized NLC.

Formulations	Entrapment Efficiency(%)	Angle of repose (⁰)	Hausner's ratio (HR)	Carr's Index (%)
Pure drug CINH	*	48±1.12	2.17± 0.03	54±1.23
LNLC1	54.24±1.05	41±1.21	1.38± 0.01	27.55±0.91
LNLC2	63.51±1.16	31± 1.32	1.25± 0.04	20±0.46
LNLC3	75.38±2.12	24± 0.85	1.19± 0.04	16±1.13
LNLC4	48.56±0.98	42± 0.91	1.45± 0.01	31±1.05
LNLC5	61.45±1.45	34± 1.23	1.26± 0.03	21±1.08
LNLC6	68.74±2.06	28± 1.12	1.22±0.02	18±1.41
LNLC7	51.63±1.23	44± 0.76	1.40±0.01	28.57±0.95
LNLC8	65.79±1.79	35± 0.43	1.28±0.03	22±0.89
LNLC9	71.27±2.51	29± 1.02	1.20±0.02	17±0.73

*NAMean ± SD, n = 10

Table 3: Parameters of Kawakita, Heckel and Leuenberger analysis.

Type of analysis	Parameters	Formulations			
		Pure drug CINH	LNLC3	LNLC6	LNLC9
Kawakita	Compactibility(a)	0.55	0.17	0.19	0.18
	Cohesiveness(1/b)	23.31	12.85	20.09	19.86
	Coefficient of determination (r^2)	0.989	0.991	0.952	0.978
Heckel	Slope(K)	0.013	0.071	0.026	0.048
	Intercept(A)	0.210	0.743	0.722	0.736
	Yield pressure(P)	76.92	14.08	38.46	20.83
	Coefficient of determination(r^2)	0.922	0.975	0.965	0.958
Leuenberger	Compression susceptibility γ (1/kg/cm^2)	0.01705	0.05280	0.03586	0.08223
	Maximum tensile strength σt_{max} (kg/cm^2)	0.07138	0.2054	0.1487	0.175
	Coefficient of determination (r^2)	0.9967	0.9968	0.9593	0.998

indicates that maximum crushing strength is reached faster at lower compression pressure (29 kg/cm^2) and higher values of γ observed for lactose based lyophilized formulation LNLC9 . A low and high value of σ_{tmax} was observed for pure drug CINH and LNLC3 respectively (Table 3). In case of pure drug CINH, there was an increased deviation of radial crushing strength with higher compression pressures whereas the crushing strength remained nearly similar for all three lyophilized formulation at higher compression pressure. Compression susceptibility for pure dug is at its lowest value. A higher compression pressure could be used to achieve maximal tensile strength, according to CINH.

PS, PDI and ZP

Analysis of the Particle Size (PS) revealed an increase from 173 nm to 193 nm. This increase in particle size may be the result of particle fusion. The LNLC3 exhibited 0.481 and -23.5 values of PDI and zeta potential respectively.

FT-IR

The FT-IR study of pure drug CINH revealed absorption bands at 1517 cm^{-1} assigned to CH_3 group, absorption bands at 1338 cm^{-1} assigned to CH_2 group, absorption bands at 2909 cm^{-1} assigned to NH group, absorption bands at 796 cm^{-1} assigned to CF_3 group and absorption bands at 805cm^{-1} assigned to benzene group. Figure 1 represents the FT-IR spectra.

DSC

The DSC thermograms of CINH, GMS, PM of CINH with GMS and LNLC3 are shown in Figure 2 . The DSC thermogram of CINH showed a prominent endothermic peak at 181.90°C (T_{fus}), with an onset at 178.330°C and latent heat of fusion (H_{fus}) measured at -28.26 mJ, indicating the drug's crystalline form. DSC thermogram of solid lipid GMS showed peak at 60.37°C corresponding to its melting point. Thermogram of PM

showed presence of endothermic peaks of GMS and CINH. The cryoprotectant (mannitol) used for lyophilization of the NLC showed a peak at 167°C which represents the melting point of mannitol (Figure 2).

Powder X-ray Diffractometry (P-XRD)

P-XRD patterns of CINH indicated sharp peaks at 2θ scattered angles of 14.85, 16.73, 19.23, 21.45, 25.31 and 27.69 degrees; these were demonstrating the crystalline nature of drug as shown in Figure 3(a). Figure 3(b) indicates that the LNLC3 has the same crystalline peaks as the CINH, but with much lower intensities.

Scanning Electron Microscopy (SEM)

SEM was used to examine the surface morphology of the pure drug formulations CINH and LNLC3. Figure 4(a) depicts SEM of pure drug which shows large and irregular shaped morphology (crystalline behaviour) of CINH whereas the LNLC3 is found to be polydispersed with a porous, round and smooth surface, as inferred from Figure 4(b).

In vitro Diffusion

As shown in Figure 5(a), the *in vitro* diffusion study for the LNLC3 revealed a comparable diffusion profile to that of the NLC formulation. NLCs and LNLC3 released 96.46±4.72% and 96.53±4.85% of drug respectively, whereas the CINH suspension released 45.31±2.13% of drug for 24hr. To analyze the drug release mechanism from the NLCs and LNLCs system, the release pattern is fitted into several kinetic models such as zero order, first order, Korsmeyer-Peppas, and Higuchi matrix. NLCs and LNLCs system showed higher correlation coefficient for first order equation (0.982 and 0.969) compared zero order equation (0.904 and 0.923) indicating that the drug release/diffusion followed 1st order kinetics.

Stability Study

EE, particle size, zeta potential, and PDI were used to assess the stability of the LNLC3. During the stability study, no significant changes were observed at $p<0.05$ level for 6 months.

Pharmacokinetic Study

Figure 5(b) depicts the serum concentration-time profile. Table 4 provides the pharmacokinetic parameters. The aqueous suspension of the pure drug (CINH) and LNLC3 were both found to have a T_{max} of 6 hr. It was discovered that the C_{max} values for the pure drug and LNLC3 were 627.12 22 ng/mL and 3009.64 185 ng/mL, respectively. In comparision to an aqueous suspension of CINH, LNLC3 demonstrated a 5 times increase in C_{max}. It was found that the AUC values for CINH and LNLC3 were 9556.15 ±124 ng.hr/L and 35050.15 ± 249 ng.hr/L respectively. Similar to this, AUC values of LNLC3 showed an increase in area of about 4 times, indicating greater bioavailability.

In vitro Cytotoxicity

It was found that the concentration of CINH in LNLC3 was not cytotoxic for MDA- MB (breast cancer cell line). The formulations incubated were found to be safe because the minimum viability was assessed to be 94%. Placebo formulation did not exhibit

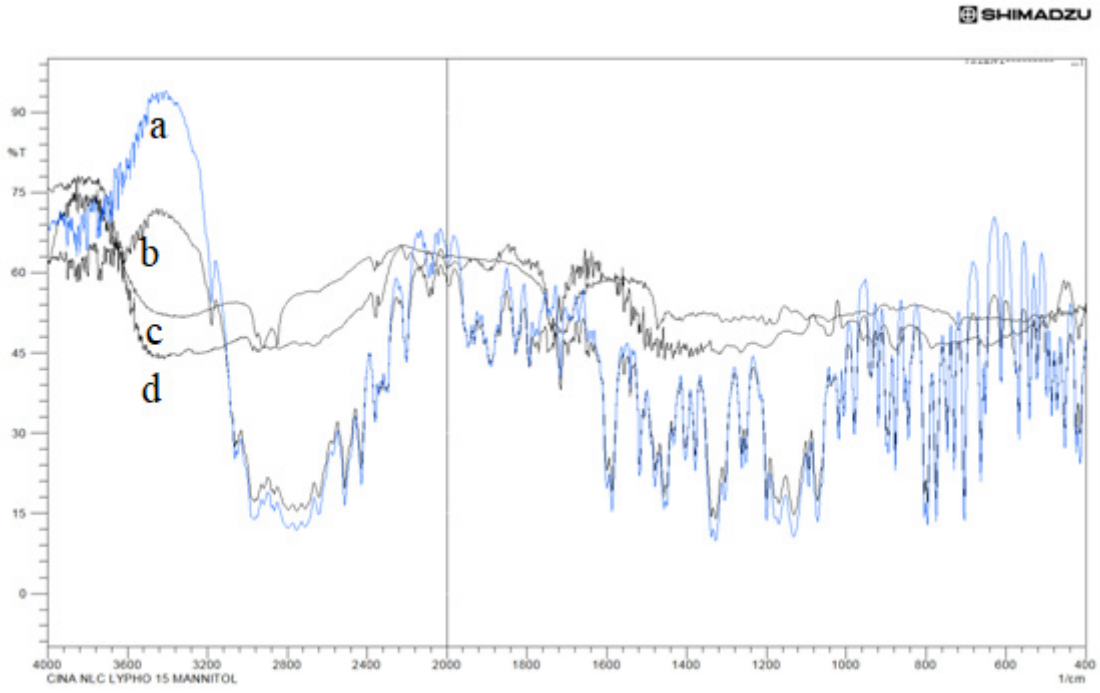

Figure 1: Overlay FT-IR spectra of a) CINH, b) PM of CINH and GMS, c) GMS and d) LNLC3.

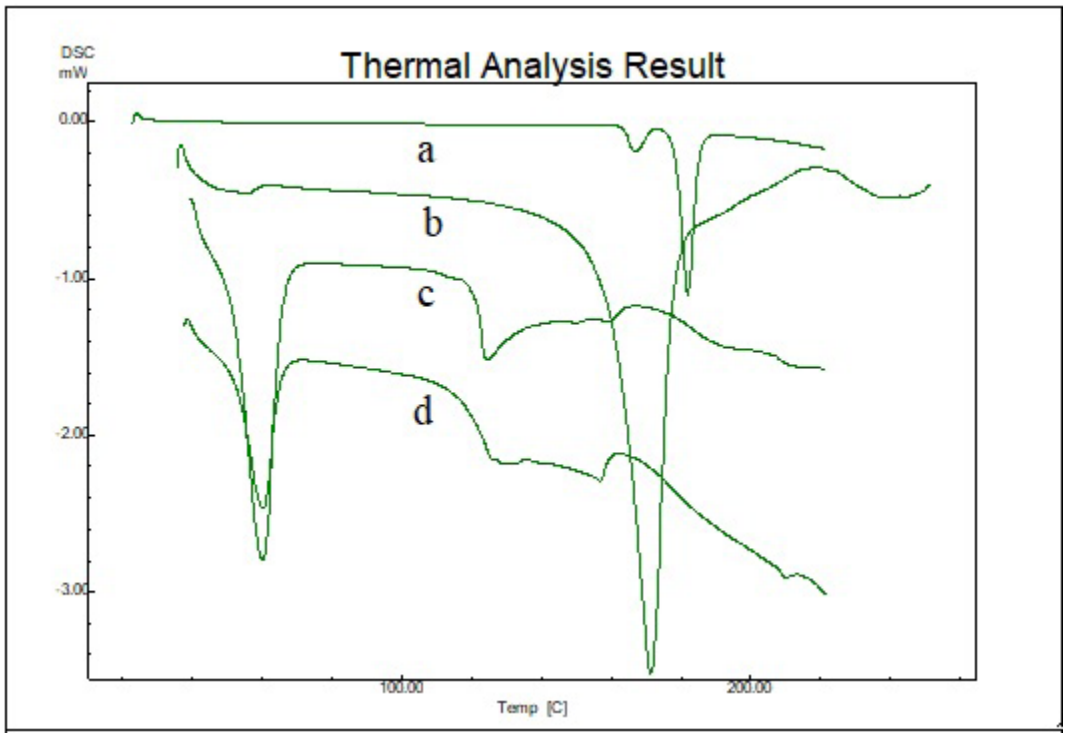

Figure 2: Overlay DSC Thermogram of a) CINH, b) LNLC3, c) GMS and d) PM of CINH and GMS.

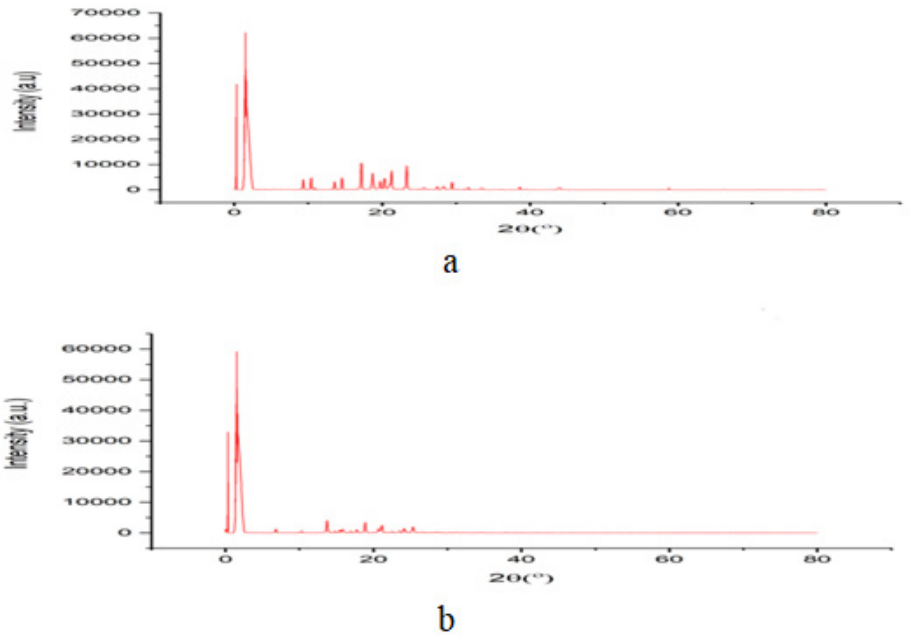

Figure 3: P-XRD of pure drug a) CINH and b) lyophilized NLC.

any cytotoxicity on the cells suggesting the excipients are noncytotoxic.

Preparation and QC tests for tablets

The prepare tablets passed all the QC tests. Drug content was 98.5% which can be attributed to uniform mixing of drug with excipients. The other QC parameters such as weight variation (349±11 mg), hardness (5.1± 1.2kg/cm^2) and friability (0.7±0.2%) were within the official limits. *In vitro* dissolution study showed drug release for 24 hr as observed for LNLC3.

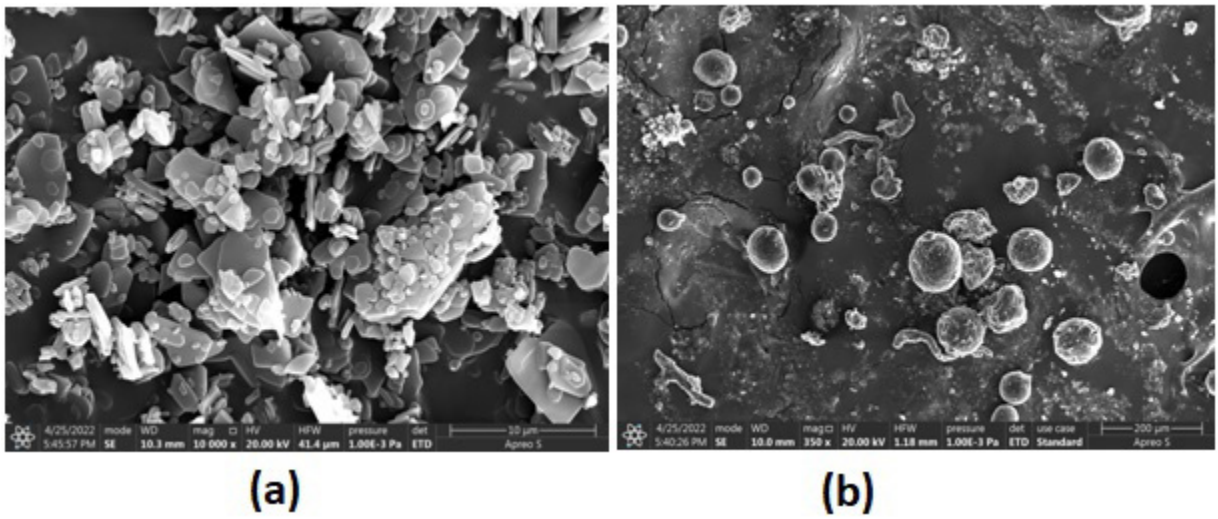

Figure 4: SEM images of pure drug a) CINH and b) lyophilized NLC.

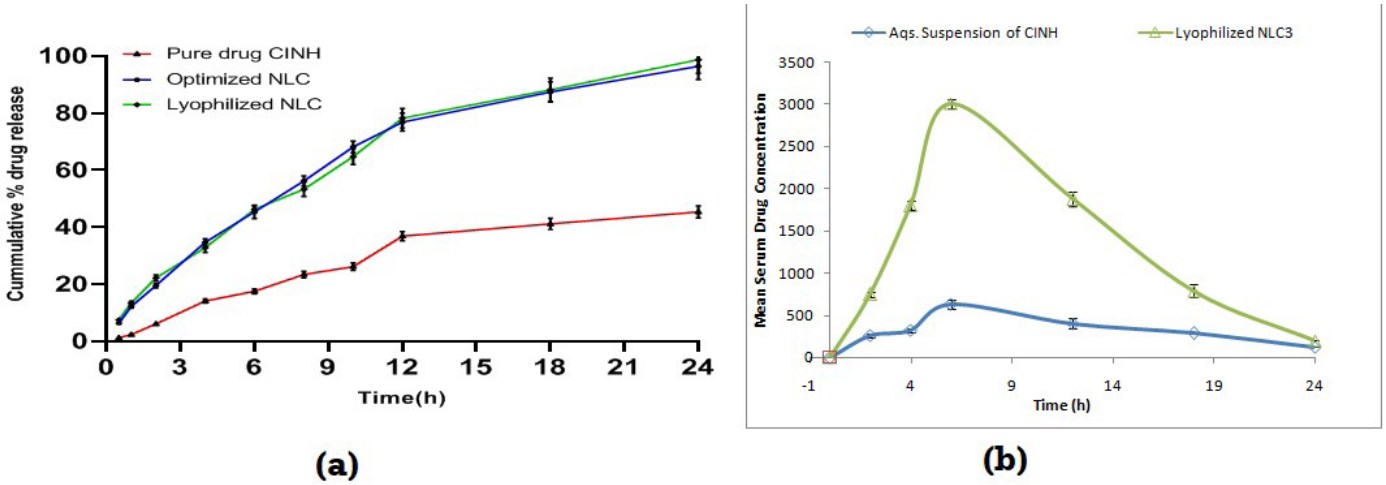

Figure 5: a) *In vitro* diffusion study of pure drug CINH, optimized NLC and lyophilized NLC and b) Pharmacokinetic profile of pure drug CINH and lyophilized NLC in albino rabbit serum following oral administration.

Table 4: Pharmacokinetic data of pure drug CINH and LNLC3.

Pharmacokinetic parameters	Aqueous suspension of CINH	LNLC3
K_E	0.0872± 0.002	0.1493 ± 0.03
C_{max}(ng/mL)	627.12± 22	3009.64 ± 185
t_{max}(h)	6± 0.41	6 ± 0.22
AUC(ng.hr/L)	9556.15 ± 124	35050.15 ± 249

Mean ± SD, $n = 6$.

DISCUSSION

The increase in entrapment efficiency can be attributed to the good solubility of CINH in GMS and labrasol. This also suggests that the critical parameters like amount of solvent, temperature, mixing time etc. were in the optimum range. The micromereticsascribed to the increase in bulk density and reduction of cohesiveness of lyophilized powders. Similarly, increased tap density for lyophilized powder suggest higher degree of compatibility.[45] Hence formulations with 15% of cryoprotectants (LNLC3, LNC6 and LNLC9) were selected for physics of tablet compression study. The lyophilized formulations prepared with 5 and 10 % of cryoprotectant did not exhibit desirable micromeritic properties for processing into tablet dosage form because of their sticky and non-free flowing nature. Kawakita analysis suggest that Lyophilized powder (LNLC3) densified the least (small compressible value) but attained the final packing state slowly because of its less cohesive nature.[46] Non-linearity for lyophilized powder at initial stages of compression can be ascribed to particle rearrangement and initial fragmentation. For lyophilized formulations, a larger value of 'A' denotes greater fragmentation, better packing, and quicker rearrangement. Better compressibility is indicated by more plasticity, or higher slope, or K value. At low pressure, the lyophilized powders were fractured into small size which facilitated further reorganization and close packing. Higher value of yield pressure for pure drug CINH exhibited

high resistance to compaction pressure because of the resistance to movement once the initial die filling occurred.[47] Compression susceptibility parameter (γ) of Leuenberger analysis was high and low for lyophilized powder and pure drug CINH suggests good and poor bonding properties respectively. Similarly higher value of σ_{tmax} was observed for LNLC3 which suggests that it has the capacity to construct stronger compacts. As most of the evaluation tests such as micromeritics, Kawakita, Heckel and Leuenberger suggest that LNLC3 formulation has the desirable flowability and compressibility for processing into tablet dosage form hence LNLC3 was selected for further characterization.

The increase in particle size may be the result of particle fusion. The increase in particle size after lyophilization of nisoldipine-loaded nanostructured lipid carriers was similarly reported by Dudhipala *et al*. in 2018. The negative zeta potential value indicates that the LNLC formulation has sufficient repulsive forces to prevent agglomeration of globules. PDI value (<0.5) is an indication of narrow and uniform size distribution of globules in the selected LNLC. FTIR study revealed that the PM of CINH with GMS and LNLC3 exhibited absorption bands in a similar region, the CINH and carriers are compatible. In case of DSC study it was found that since the drug's crystal nature was lost during molecular level dispersion so the LNLC3 failed to exhibit the peak that would have been considered indicative of the compound. The P-XRD study can be used to confirm this further. The P-XRD study demonstrated partial amorphization of CINH with a lower degree of crystallinity. However, characteristic peaks of CINH were disappeared in LNLC3, which confirmed the absence of crystallinity of drug. SEM study also suggested no significant enlargement of particle size after lyophilization with mannitol as cryorotectant.[48] The *in vitro* diffusion showed a higher correlation coefficient for the Higuchi equation shows that diffusion-controlled release was the primary mechanism of drug release. Korsmeyer release exponent values of both NLC and LNLC3 recommend non-fickian diffusion controlled release mechanism. This first-order release kinetics unveiled the dissolution of the drug from the porous medium. The enhanced drug release from the NLCs and LNLCs might be attributed to the significant decrease in PS and thus increasing the specific surface area and subsequently, the release rate of the drug. During the stability study, there were no appreciable changes to the aforementioned parameters. The improvement in bioavailability can be attributed to the material's excellent redispersibility, substantially faster dissolving, and increased dissolution rate. LNLC3 particles may have a large surface area and result in a quicker rate of drug breakdown because of their small size (less than 200 nm). LNLC3 was found to have a better pharmacokinetic profile than a pure drug aqueous suspension. The *in vitro* cytotoxicity study revealed that the lyophilized formulation was safe (viability 94%).The prepared tablets passed all the QC tests as per official pharmacopoeia.

CONCLUSION

The Lyophilized NLC (LNLC3) exhibited particle size in nano range (< 200 nm) with stable zeta potential value with similar drug release profile as that of liquid NLC. Lyophilization of NLC formulation prepared with mannitol as cryoprotectant showed significant improvement in flowability and compressibility. Physics of tablet compression study by Heckel equation revealed plastic deformation for LNLC3 which is a desirable property for tabletting. Pharmacokinetic study showed 5 folds increase in oral bioavailability for LNLC3 in comparison to aqueous suspension of CINH. The formulations that were incubated were found to be safe because the minimum viability was evaluated to be 94%. Lyophilization of NLC formulation has the potential to improve the oral bioavailability with high drug loading, stability, cell viability for CINH and large scale manufacturing feasibility as tablet dosage form.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Sukant Tripathy, Professor, Department of Physics, Berhampur University for P-XRD and Dr. R. Selvi, Assistant Professor, IISER, Berhampur, India for *in vitro* cytotoxicity study.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest

ABBREVIATIONS

CINH: Cinacalcet hydrochloride; **NLC:** Nanostructure Lipid Carrier; **BCS:** Biopharmaceutics Classification System; **GMS:** Glycerylmonostearate; **LNLC:** Lyophilized Nanostructure Lipid Carrier; **MTT assay:** (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay; **DDS:** Drug Delivery System; **SNEDDS:** Self-Nanoemulsifying Drug Delivery System; **SMEDDS:** Self-Microemulsifying Drug Delivery System; **PN:** Polymeric nanoparticles; **SLN:** Solid lipid nanoparticles; **EE:** Entrapment efficiency; **PS:** Particle Size; **ZP:** Zeta Potential; **PDI:** Poly Dispersity Index; **DSC:** Differential scanning calorimeter; **FT-IR:** Fourier Transform infrared; **hr:** Hour; **min:** Minute; **sec:** Second; **mL:** Milliliter; **AUC:** Area Under Curve; **P-XRD:** Powder X-ray diffraction; **SEM:** Scanning Electron Microscopy; **ICH:** International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; **RH:** Relative Humidity; **IAEC:** Institute Animal Ethics Committee; **UFLC:** Ultra-Fast liquid chromatography; **QC:** Quality Control.

SUMMARY

CINH is a BCS class IV drug. It is mainly used for the treatment of chronic renal disease and parathyroid cancer. It exhibits poor oral bioavailability less than 25%. In this research,

Glycerylmonostearate (GMS), labrasol, tween 20 were the main excipients selected for the formulation of NLC as solid lipid, liquid lipid and surfactant respectively. Hot high speed homogenization and ultra-sonication method was used for the NLC formulation of CINH. The selected NLC formulation was lyophilized using three different cryoprotectants such as dextrose, lactose and mannitol at three different concentrations. The Lyophilized NLC (LNLC3) exhibited particle size in nano range (< 200nm) with stable zeta potential value with similar drug release profile as that of liquid NLC. Lyophilization of NLC formulation prepared with mannitol as cryoprotectant showed significant improvement in flowability and compressibility. Physics of tablet compression study by Heckel equation revealed plastic deformation for LNLC3 which is a desirable property for tabletting. Pharmacokinetic study showed 5 folds increase in oral bioavailability for LNLC3 in comparison to aqueous suspension of CINH. The formulations that were incubated were found to be safe because the minimum viability was evaluated to be 94%. Lyophilization of NLC formulation has the potential to improve the oral bioavailability with high drug loading, stability, cell viability for CINH and large scale manufacturing feasibility as tablet dosage form.

REFERENCES

1. Palmer SC, Nistor I, Craig JC, Pellegrini F, Messa P, Tonelli M, *et al*. Cinacalcet in patients with chronic kidney disease: A cumulative meta-analysis of randomized controlled trials. PLOS Med. 2013;10(4):e1001436. doi: 10.1371/journal.pmed.1001436, PMID 23637579.
2. Padhi D, Harris R. Clinical pharmacokinetic and pharmacodynamic profile of cinacalcet hydrochloride. ClinPharmacokinet. 2009;48(5):303-11. doi: 10.2165/00003088-200948050-00002, PMID 19566113.
3. Panigrahi KC, Patra CN, Rao MEB. Quality by design enabled development of oral self-nanoemulsifying drug delivery system of a novel calcimimetic Cinacalcet HCl using a porous carrier: *in vitro* and *in vivo* characterisation. AAPS PharmSciTech. 2019;20(5):216. doi: 10.1208/s12249-019-1411-2, PMID 31172322.
4. Xu X, Chen G, Li Y, Wang J, Yin J, Ren L. Enhanced dissolution and oral bioavailbility of Cinacalcet hydrochlorde nanocrystals with no food effect. Nanotechnology. 2019;30(5):055102. doi: 10.1088/1361-6528/aaef46, PMID 30511665.
5. Cao M, Xue X, Pei X, Qian Y, Liu L, Ren L, *et al*. Formulation optimization and pharmacokinetics evaluation of oral self-microemulsifying drug delivery system for poorly water soluble drug Cinacalcet and no food effect. Drug DevInd Pharm. 2018;44(6):969-81. doi: 10.1080/03639045.2018.1425428, PMID 29313395.
6. Routray SB, Patra CN, Raju R, Panigrahi KC, Jena GK. Lyophilized SLN of Cinnacalcet HCl: BBD enabled optimization, characterization and pharmacokinetic study. Drug DevInd Pharm. 2020;46(7):1080-91. doi: 10.1080/03639045.2020.1775632, PMID 32486863.
7. Ghose D, Patra CN, Ravi Kumar BVV, Swain S, Jena BR, Choudhury P, *et al*. QbD-based formulation optimization and characterization of polymeric nanoparticles of cinacalcet hydrochloride with improved biopharmaceutical attributes. Turk J Pharm Sci. 2021;18(4):452-64. doi: 10.4274/tjps.galenos.2020.08522, PMID 34496552.
8. Amekyeh H, Billa N. Lyophilized drug-loaded solid lipid nanoparticles formulated with beeswax and theobroma oil. Molecules. 2021;26(4):1-13. doi: 10.3390/molecules26040908, PMID 33572168.
9. Karakash I, Vasileska J, Shalabalija D, Mihailova L, Dodov MG, Raicki RS, *et al*. Freeze-drying of nanostructured lipid carriers loaded with Salvia off. extract for Alzheimer's disease treatment. 2020;66(Suppl 1):219-20.
10. Bohrey S, Chourasiya V, Pandey A. Polymeric nanoparticles containing diazepam: Preparation, optimization, characterization, *in vitro* drug release and release kinetic study. Nano Converg. 2016;3(1):3. doi: 10.1186/s40580-016-0061-2, PMID 28191413.
11. Dolatabadi S, Karimi M, Nasirizadeh S, Hatamipour M, Golmohammadzadeh S, Jaafari MR. Preparation, characterization and *in vivo* pharmacokinetic evaluation of curcuminoids-loaded solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs). J Drug DelivSci Technol. 2021;62:102352. doi: 10.1016/j.jddst.2021.102352.
12. Sun J, Liu J, Zhang J, Xia H. Meclizine-loaded nanostructured lipid carriers to manage nausea and vomiting: Oral bioavailability improvement. J Drug DelivSci Technol. 2021;63:102432. doi: 10.1016/j.jddst.2021.102432.
13. Zhang T, Chen J, Zhang Y, Shen Q, Pan W. Characterization and evaluation of nanostructured lipid carrier as a vehicle for oral delivery of etoposide. Eur J Pharm Sci. 2011;43(3):174-9. doi: 10.1016/j.ejps.2011.04.005, PMID 21530654.
14. Das S, Ghosh S, De AK, Bera T. Oral delivery of ursolic acid-loaded nanostructured lipid carrier coated with chitosan oligosaccharides: development, characterization, *in vitro* and *in vivo* assessment for the therapy of leishmaniasis. Int J BiolMacromol. 2017;102:996-1008. doi: 10.1016/j.ijbiomac.2017.04.098, PMID 28465178.
15. Fathi HA, Allam A, Elsabahy M, Fetih G, El-Badry M. Nanostructured lipid carriers for improved oral delivery and prolonged antihyperlipidemic effect of simvastatin. Colloids Surf B Biointerfaces. 2018;162:236-45. doi: 10.1016/j.colsurfb.2017.11.064, PMID 29197789.
16. Mendes AI, Silva AC, Catita JAM, Cerqueira F, Gabriel C, Lopes CM. Miconazole-loaded nanostructured lipid carriers (NLC) for local delivery to the oral mucosa: Improving antifungal activity. Colloids Surf B Biointerfaces. 2013;111:755-63. doi: 10.1016/j.colsurfb.2013.05.041, PMID 23954816.
17. Piazzini V, Micheli L, Luceri C, D'Ambrosio M, Cinci L, Ghelardini C, *et al*. Nanostructured lipid carriers for oral delivery of silymarin: Improving its absorption and *in vivo* efficacy in type 2 diabetes and metabolic syndrome model. Int J Pharm. 2019;572:118838. doi: 10.1016/j.ijpharm.2019.118838, PMID 31715362.
18. Khan AA, Mudassir J, Akhtar S, Murugaiyah V, Darwis Y. Freeze-dried lopinavir-loaded nanostructured lipid carriers for enhanced cellular uptake and bioavailability: Statistical optimization, *in vitro* and *in vivo*evaluations. Pharmaceutics. 2019;11(2):1-19. doi: 10.3390/pharmaceutics11020097, PMID 30823545.
19. Zhuang CY, Li N, Wang M, Zhang XN, Pan WS, Peng JJ, *et al*. Preparation and characterization of vinpocetine loaded nanostructured lipid carriers (NLC) for improved oral bioavailability. Int J Pharm. 2010;394(1-2):179-85. doi: 10.1016/j.ijpharm.2010.05.005, PMID 20471464.
20. Khan AA, Abdulbaqi IM, AbouAssi RA, Murugaiyah V, Darwis Y. Lyophilized hybrid nanostructured lipid carriers to enhance the cellular uptake of verapamil: Statistical optimization and *in vitro* evaluation. Nanoscale Res Lett. 2018;13(1):323. doi: 10.1186/s11671-018-2744-6, PMID 30324291.
21. Routray SB, Patra CN, Swain S, Jena BR. Analytical quality by design based systematic development and optimization of a sensitive bioanalytical method for estimation CinacalcetHCl in rabbit serum. J Pharm Bioallied Sci. 2021;13(4):360-6. doi: 10.4103/jpbs.jpbs_604_21, PMID 35399796.
22. hyun YJ, Jeong SH, Choi DH, Nam Ah K, Chu KR, Jung YJ,*et al*. 2010;40(4):237-44.
23. Prakash SS, Patra CN, Santanu C, Kumar PH. Studies on flowability, compressibility and *in-vitro* release of *Terminalia chebula* fruit powder tablets. 2011;10(3):393-401.
24. Wünsch I, Finke JH, John E, Juhnke M, Kwade A. The influence of particle size on the application of compression and compaction models for tableting. Int J Pharm. 2021;599:120424. doi: 10.1016/j.ijpharm.2021.120424, PMID 33647406.
25. Powders D, Zhang J. Wu C yu.On identification of critical material attributes for compression behaviour of pharmaceutical. 2017.
26. Schäfer-Korting M, Mehnert W, Korting HC. Lipid nanoparticles for improved topical application of drugs for skin diseases.Adv Drug Deliv Rev. 2007;59(6):427-43. doi: 10.1016/j.addr.2007.04.006, PMID 17544165.
27. Granja A, Vieira AC, Chaves LL, Nunes C, Neves AR, Pinheiro M, *et al*. Folate-targeted nanostructured lipid carriers for enhanced oral delivery of epigallocatechin-3-gallate. Food Chem. 2017;237:803-10. doi: 10.1016/j.foodchem.2017.06.019, PMID 28764070.
28. Dudhipala N, Janga KY, Gorre T. Comparative study of nisoldipine-loaded nanostructured lipid carriers and solid lipid nanoparticles for oral delivery: Preparation, characterization, permeation and pharmacokinetic evaluation. Artif Cells Nanomed Biotechnol. 2018;46(sup 2):616-25. doi: 10.1080/21691401.2018.1465068, PMID 29688077.
29. Jawahar N, Hingarh PK, Arun R, Selvaraj J, Anbarasan A, S S, *et al*. Enhanced oral bioavailability of an antipsychotic drug through nanostructured lipid carriers. Int J BiolMacromol. 2018;110:269-75. doi: 10.1016/j.ijbiomac.2018.01.121, PMID 29402457.
30. Patil-Gadhe A, Pokharkar V. Montelukast-loaded nanostructured lipid carriers: Part i oral bioavailability improvement. Eur J Pharm Biopharm. 2014;88(1):160-8. doi: 10.1016/j.ejpb.2014.05.019, PMID 24878424.
31. Girotra P, Singh SK, Kumar G. Development of zolmitriptan loaded PLGA/poloxamer nanoparticles for migraine using quality by design approach. Int J BiolMacromol. 2016;85:92-101. doi: 10.1016/j.ijbiomac.2015.12.069, PMID 26724690.
32. Zhang Q, Yang H, Sahito B, Li X, Peng L, Gao X, *et al*. Nanostructured lipid carriers with exceptional gastrointestinal stability and inhibition of P-gp efflux for improved oral delivery of tilmicosin. Colloids Surf B Biointerfaces. 2020;187:110649. doi: 10.1016/j.colsurfb.2019.110649, PMID 31767412.
33. Chaudhari VS, Murty US, Banerjee S. Nanostructured lipid carriers as a strategy for encapsulation of active plant constituents: Formulation and *in vitro* physicochemical characterizations. ChemPhys Lipids. 2021;235:105037. doi: 10.1016/j.chemphyslip.2020.105037, PMID 33400968.
34. Khan S, Shaharyar M, Fazil M, Baboota S, Ali J. optimization of production and characterization Department of Medicinal Chemistry. Eur J Pharm Biopharm, Hamdard. 2016;108:277-88. doi: 10.1016/j.ejpb.2016.07.017, PMID 27449630.
35. Sharma T, Katare OP, Jain A, Jain S, Chaudhari D, Borges B, *et al*. QbD-steered development of biotin-conjugated Nanostructured Lipid Carriers for Oral Delivery of chrysin: Role of Surface Modification for Improving Biopharmaceutical Performance.

Colloids Surf B Biointerfaces. 2021;197:111429. doi: 10.1016/j.colsurfb.2020.111429, PMID 33130524.

36. Patel P, Patel M. European Journal of Pharmaceutical Sciences Enhanced oral bioavailability of nintedanibesylate with nanostructured lipid carriers by lymphatic targeting: *in vitro*, cell line and *in vivo* evaluation.2021;159.
37. Dong Z, Iqbal S, Zhao Z. Preparation of ergosterol-loaded nanostructured lipid carriers for enhancing oral bioavailability and antidiabeticnephropathyeffects. AAPS PharmSciTech. 2020;21(2):64. doi: 10.1208/s12249-019-1597-3, PMID 31932990.
38. Du Q, Chen J, Yan G, Lyu F, Huang J, Ren J, *et al*. Comparison of different aliphatic acid grafted N-trimethyl chitosan surface-modified nanostructured lipid carriers for improved oral kaempferol delivery. Int J Pharm. 2019;568:118506. doi: 10.1016/j.ijpharm.2019.118506, PMID 31302169.
39. Moraes S, Marinho A, Lima S, Granja A, Araújo JP, Reis S, *et al*. Targeted nanostructured lipid carriers for doxorubicin oral delivery. Int J Pharm. 2021;592:120029. doi: 10.1016/j.ijpharm.2020.120029, PMID 33130218.
40. Granja A, Neves AR, Sousa CT, Pinheiro M, Reis S. EGCG intestinal absorption and oral bioavailability enhancement using folic acid-functionalized nanostructured lipid carriers. Heliyon. 2019;5(7):e02020. doi: 10.1016/j.heliyon.2019.e02020, PMID 31317081.
41. Vieira R, Severino P, Nalone LA, Souto SB, Silva AM, Lucarini M, *et al*. Sucupira oil-loaded nanostructured lipid carriers (NLC): Lipid screening, factorial design, release profile, and cytotoxicity. Molecules. 2020;25(3):1-22. doi: 10.3390/molecules25030685, PMID 32041134.
42. Chen G, Wang K, Zhou Y, Ding L, Ullah A, Hu Q, *et al*. Oral nanostructured lipid carriers loaded with near-infrared dye for image-guided photothermaltherapy. ACS Appl Mater Interfaces. 2016;8(38):25087-95. doi: 10.1021/acsami.6b07425, PMID 27626389.
43. Üstünda ı-Okur N, Yurdasiper A, Gündo ıdu E, Homan Gökçe E. Modification of solid lipid nanoparticles loaded with nebivolol hydrochloride for improvement of oral bioavailability in treatment of hypertension: Polyethylene glycol versus chitosan oligosaccharide lactate. J Microencapsul. 2016;33(1):30–42.
44. Giri TK, Sahu R, Kumar K, Alexander A, AjazuddinBH, *et al*. *In-vitro* quality control measurement of some commercially available sustained release tablet containing diclofenac sodium. Res J Pharm Technol. 2012;5(5):687-90.
45. Carson J, Pittenger BH, Marinelli J. Characterize bulk solids to ensure smooth flow. ChemEng (United States). 2016;123(4):78-90.
46. Pesonen T, Paronen P. Evaluation of a new cellulose material as binding agent for direct compression of tablets. Drug DevInd Pharm. 1986;12(11-13):2091-111. doi: 10.3109/03639048609042625.
47. Ilkka J, Paronen P. Prediction of the compression behaviour of powder mixtures by the Heckel equation. Int J Pharm. 1993;94(1-3):181-7. doi: 10.1016/0378-5173(93)90022-8.
48. Alihosseini F, Ghaffari S, Dabirsiaghi AR, Haghighat S. Freeze-drying of ampicillin solid lipid nanoparticles using mannitol as cryoprotectant. Braz J Pharm Sci. 2015;51(4):797-802. doi: 10.1590/S1984-82502015000400005.

Cite this article: Nayak S, Sruti J, Patra CN, Jena GK. Lyophilized NLC of CinacalcetHCl: Physics of Tablet Compression and Biopharmaceutical Characterization. Indian J of Pharmaceutical Education and Research. 2023;57(3):684-94.