Development and Characterization of Optimized Cilnidipine Proniosomes

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Abstract

In the contemporary medical model world, the proniosomal system has been serving as a new drug delivery system that is considered to significantly enhance the bioavailability of drugs with low water solubility. In this work, Cilnidipine, a promising 4th generation Ca^{2+} channel blocker with a rational pharmacological profile i.e. dual L/N-type Ca^{2+}channel-blocking action is used in the treatment of hypertension. Cilnidipine loaded Proniosomes (CIL-PRO) were developed to improve the oral bioavailability. Cilnidipine-Proniosomes were prepared by film hydration followed by rotary flask evaporator applying the concepts of Design of Experiments. Box-behnken designwas applied to optimize the formulation variables;Cholesterol (A), Span-60 (B), Sorbitol (C). The particle sizes were in the nanometer range and spherical shaped for all prepared formulations and the zeta potential(-11.2mV) absolute values were high, predicting good long-term stability. Prepared Cilnidipine proniosomes characterized by Differential scanning calorimetry (DSC) analysis and Attenuated total reflection (ATR) analysis, revealed the compatibility of the drug chosen with the ingredient added, Powder X-ray diffractometry (XRD) confirmed the amorphous phase of the prepared proniosomes, and finally, the surfactant layer was observed by Scanning electron microscopy (SEM). In vitro study of Cil-Pro exhibited controlled release profile for at least 24 h. The obtained results revealed that Cilnidipine proniosomes can be successfully prepared by using different carriers. Hence, these proniosomes

could represent as a great potential for a possible alternative to conventional oral formulation in the treatment of hypertension.

Key words: Proniosomes, Cilnidipine, Hypertension, Cholesterol

1. Introduction

Hypertension is one of the most important risk factors for cardiovascular diseases, including ischemic and haemorrhagic stroke, dementia, ischemic heart disease, heart failure, vision loss, and kidney failure. Hypertension is a multifactorial and multifaceted disease in which elevated blood pressure is only one sign of multiple underlying physiological abnormalities, Hypertension or high blood pressure is a leading cause of death.[1-3] The condition is often called a "silent killer" because its symptoms can go undetected until damage to the body has occurred. Because of this, it is one of the most significantly under-diagnosed and under-treated medical conditions all over the world. High blood pressure is usually a lifelong condition. High blood pressure can occur at any age but is particularly prevalent in people with a family history of high blood pressure, people who are overweight or obese, people with diabetes, and heavy drinkers.[4-5]

Cilnidipine is a promising 4th generation Ca^{2+} channel blocker with a rational pharmacological profile; i.e. dual L/N-type Ca^{2+} channel-blocking action. The blockade of N-type Ca^{2+} channels effectively suppresses neurohumoral regulation in the cardiovascular system, including sympathetic nervous system and renin – angiotensin-aldosterone system. Thus, Cilnidipine is expected to be favorable for various types of complications of hypertension. [6-9]

Proniosomes are dry, free-flowing formulations of a surfactant-coated carrier that is suitable for different routes of administration. Proniosomes are rehydrated by brief agitation in hot water to form a multi-lamellar niosomal suspension. Niosomes derived from proniosomes have the ability to enhance the bioavailability of either hydrophilic or lipophilic drugs.[10] A study was carried out in which the Vinpocetine proniosomes were prepared to analyze the effect of proniosomes on the bioavailability of poorly soluble drugs. The study concluded that proniosomes could improve the gastrointestinal absorption of Vinpocetine and can provide an effective mean of delivering poorly water-soluble drugs through the oral route.[11] Proniosomal system also exhibited an improvement in the oral bioavailability of Isradipine[12] and Aceclofenac.[13]Preparation of

proniosomes loaded with various bioactive compounds has been reported. Canthaxanthin was converted into proniosomes using maltodextrin, mannitol, lactose and pullulan as wall materials with entrapment efficiency of 74.1%.[14] Maltodextrin-based proniosomes loaded with Valsartan was reported to have entrapment efficiency of 92%.[15]Similarly, Vinpocetine was converted into proniosomes using Span 60, cholesterol and sorbitol.[16-17]

The main objective of the present investigation was to incorporate Cilnidipine into Cholesterol, to get proniosome to improve the oral bioavailability by passing the first pass metabolism. Accordingly, Cilnidipine-Proniosomes were prepared by film hydration followed by rotary flask evaporator. Prepared proniosomes were characterized and optimal formulation was evaluated.[18-20]

2. Materials

Cilnidipine was obtained as gift sample by J. B. Chemical and Pharmaceuticals Ltd., Mumbai, India. Tablet of Ranolaz of 500mgwas purchased from local pharmacy. Cholesterol (CHO), dicetyl phosphate, and surfactant (SUF) gift sample from SD fine chemicals India. All other chemicals used were of analytical grade.

2.1.Methods

2.1.1. Preparation of Cilnidipine loaded Proniosomes

Proniosomes loaded with Cilnidipine prepared by film hydration (hand shaking). As shown in the table, seventeen formulations were created in total. Initial quantity of cholesterol (CHO), dicetyl phosphate (DCP; charge inducer) and surfactant (SUF) dissolved in the smallest amount of ethanol. The solution was transferred to a round bottom flask (RBF) and subsequently processed in a rotary flask evaporator (Rotary evaporator, RE-2010, Biobase, Mumbai, India). The mixture was then completely dried at 40 °C, 100 rpm, and 16 mm Hg under vacuum to obtain a dried RBF film. A suitable quantity of Cilnidipine was dissolved in phosphate buffer saline (pH 6.8) sorbitol (carrier), which was then added slowly to the RBF containing a thin film of surfactant and cholesterol and vigorously agitated for 40 minutes at room temperature until a good dispersion was obtained. The dispersion was freeze-dried for 24 hours at -80°C in a lyophilizer (BK FD10, Biobase, China) to obtain proniosomes, which were then, stored at 4°C for further evaluation and processing.[19]

2.1.2. Characterization of Cilnidipine proniosomal powder;

Entrapment Efficiency (EE)

In a 100 mL volumetric flask, an accurately weighed quantity of proniosomes (Equivalent amount of 10 mg of drug) was placed, and the minimum amount of ethanol was added and thoroughly mixed. Approximately 10 minutes were spent sonicating (Ultrasonicator, CPX3800-E, Branson) the dispersion. Phosphate buffer with a pH of 6.8 was added to the resultant mixture and the volume was adjusted to the desired level. The dispersion was bath sonicated for an additional 10 minutes, until it became transparent.[21] The resulting mixture was subsequently filtered using a 0.45 m pore size Whatman membrane filter. To quantify drug content, the filtrate was analyzed with a UV-Visible spectrophotometer (Shimadzu UV-1800, Japan) at 241 nm. The entrapment efficiency of drug was calculated by the following equation:

Entrapment efficiency: $\text{EE}\ (\%) = \frac{\text{Mass of drug in proniosomes}}{\text{Initial mass of drug used in proniosomes}} \times 100$ …....(Equation 1)

In-vitro **drug release:**

In vitro drug release of Cilnidipine from the proniosomes was performed by diffusion technique using Franz-diffusion cell. The dialysis membrane; cellophane membrane was cut into equal pieces (6 cm×2.5 cm) and soaked into distilled water for 12 h before use. The drug release studies of the Cilnidipine solution is carried out in 10 ml of phosphate buffer pH 6.8 saline maintained at 37±0.5° with a magnetic stirrer with constant heating equipment (IKA Auto Temp Regulator, Germany). A sample of 2 ml of niosomes suspension was placed in receptor compartment. Aliquot samples of 1 ml were withdrawn at the regular interval and replaced with same volume of fresh buffer. The aliquots were diluted with fresh media, if necessary. Amount of drug diffused through the membrane was measured by using U.V. spectrophotometer at the wavelength 241 nm against phosphate buffer (pH 6.8) as the blank.[22]

2.1.3. Characterization of optimized Cilnidipine

1. ATR study of Optimized formulation

Attenuated total reflection (ATR) is a sampling technique used in conjunction with infrared spectroscopy which enables samples to be examined directly in the solid or liquid state

without further preparation. ATR spectroscopy is particularly useful for online monitoring of polymer composition. Its ability to fingerprint chemical components allows IR to determine the constituents of a chemical process. The study was conducted for optimized formulation by ATR Bruker Opus 7.0, Germany.[23-28]

2. Surface morphology, Particle size and zeta potential of optimized formulation

Morphology of the prepared optimized Cilnidipne proniosome powder was observed under scanning electron microscope. The sample was attached to the slab surface with double sided adhesive tape and the scanning electron microscope (S3700N-Hitachi, Japan) photomicrographs were taken at different magnifications. Similarly, proniosomesevaluated for particle size and polydispersity index value using the scattering light intensity technique (Malvern zetasizer, ATA scientific, USA). [24,29]

3. Differential Scanning Calorimetry

Thermal characteristics of the Cilnidipine proniosomes after hydration with PBS pH 7.4 were evaluated using differential scanning calorimetry (Perkin Elmer 4000, USA) instrument. The analysis was performed on 1 mg proniosomal powder samples sealed in standard aluminum pans. Thermogram of Cilnidipine proniosomes and bulk Cilnidipine was obtained at a scanning rate of 10 °C/min in a temperature range of 30 to 300 °C. [25,30]

4. X-ray diffraction

Cilnidipine proniosomes after hydration with PBS were evaluated for solid-state characteristics by Xray diffraction technique. Bulk Cilnidipine and drug-loaded proniosomal dispersion was scanned at a scanning speed of 2°/min using a Phillips X-ray diffractometer equipped with an X-ray generator operating at a 40 kV voltage and 20 mA current.[28,31]

3. Results and Discussion

3.1.Preparation of Cilnidipine loaded Proniosomes

A three-level three-factor Box-Behnken experimental design was used in the present study to evaluate the effects of selected independent variables on the responses. Three independent factors such as sodium Cholesterol (A), Span-60 (B), Sorbitol (C) considered. The responses

recorded in the experiment are EE, Drug release in 12th hour and particle size. Mathematical fitting and analysis were performed by the *polynomial equation*. The optimized formula was solved by graphical optimization technique along with a numerical method using the confidence interval value of alpha 0.05. For the three-level three-factor Box-Behnken experimental design, a total of 17 experimental runs as provided in table 1.

Table 1 Formulation of Cilnidipine proniosome

	Factor 1	**Factor 2**	**Factor 3**	**Response 1**	**Response 2**	**Response 3**
Run	**A:Cholesterol (%)**	**B:Span-60 (%)**	**C:Sorbitol (%)**	**EE (%)**	**Drug release at 12 h (%)**	**Particle size (**nm)
F1	27.5	1.25	20	54.38	78.19	632
F2	5	0.5	20	53.61	79.82	511
F3	50	1.25	30	71.83	85.28	812
F4	27.5	1.25	20	55.72	74.27	627
F5	27.5	0.5	30	62.28	81.29	738
F6	50	2	20	61.36	88.15	696
F7	50	0.5	20	65.52	65.74	719
F8	27.5	1.25	20	54.28	73.92	612
F9	27.5	2	10	55.49	93.68	658
F10	27.5	1.25	20	54.29	75.51	629
F11	27.5	1.25	20	53.28	71.93	635
F12	50	1.25	10	63.35	67.52	683
F13	27.5	0.5	10	56.18	80.59	701
F14	5	2	20	45.58	99.98	598
F15	27.5	2	30	59.38	91.77	759
F16	5	1.25	10	48.73	83.73	486
F17	5	1.25	30	51.82	70.58	541

The EE data can be found in Table 1. It observed that, formulations loaded with high amount of cholesterol (50%) found high EE. Formulation "F3" have maximum 71.83%, F6 and F7 entrapped 61.36% and 65.52% respectively. Similarly the percentage amount of sorbitol signified the EE as it can be seen that 30% of sorbitol in F3 possessed highest EE whereas formulation (F12) with less amount of sorbitol i.e. 10% exhibited comparatively lesser EE of 63.35%. The least EE (45.58%) of drug found from F14. This could be the least 5% of cholesterol availability in niosome formulation. Finally it can be concluded that, a suitable combination of cholesterol and carrier such as sorbitol with preferable high quantity can develop a proniosome with good EE.

3.2.In vitro dissolution study

In vitro dissolution study of Niosomes suggested by BBD was evaluated. In all the formulations it noted that, maximum 20% drug released in initial 30minutes. It found that, F14 released maximum 99.98% of drug in 12h of dissolution study. Whereas, F7 exhibited the minimum 65.74 % of drug in 12h. It found that, there is direct relationship with the amount of cholesterol and span-60 in dissolution study. The lesser amount of cholesterol and more amount of Span-60 contributed faster release as seen in F14. Similarly more amount of cholesterol and less amount of span-60 contribute lesser drug release as seen in F14. While developing the Niosomes; sorbitol used as carrier and have profound effect of drug release. The more the quantity of sorbitol forms a barrier surrounding drug crystal and retards drug release. A comparison was made between F16 and F17 in relation to percentage of sorbitol involved; it found lesser amount of drug release i.e. 70.58% at 12h in F17 whereas F16 exhibited higher amount of release of 83.73% at 12h. This ascertained the quantity influence of sorbitol in drug release. In one more instance it observed F2 exhibited only 79.82% of drug, whereas F14 with 2% span-60 exhibited 99.98% of drug. This confers the wetting property of span-60 which can emulsify drug and promoted faster drug release (Figure 1 and Figure 2).

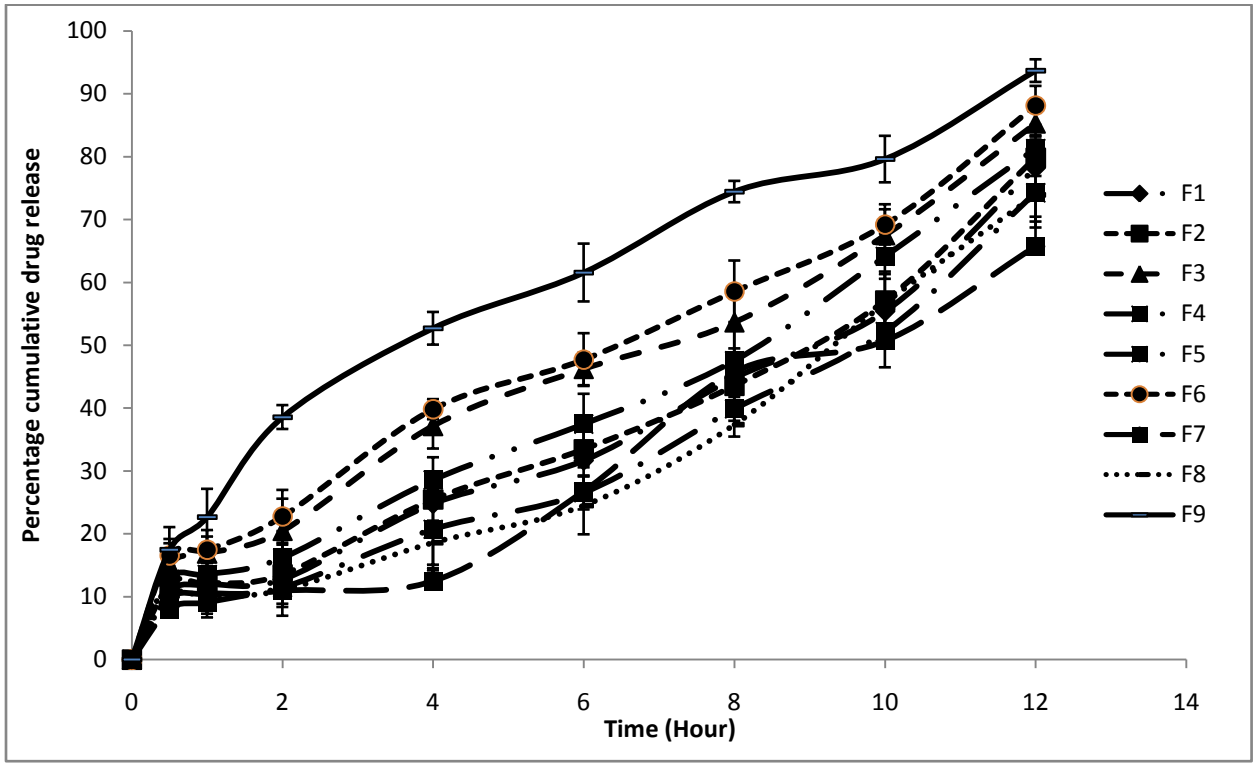


Figure 1: In vitro evaluation study of niosomess F1-F19

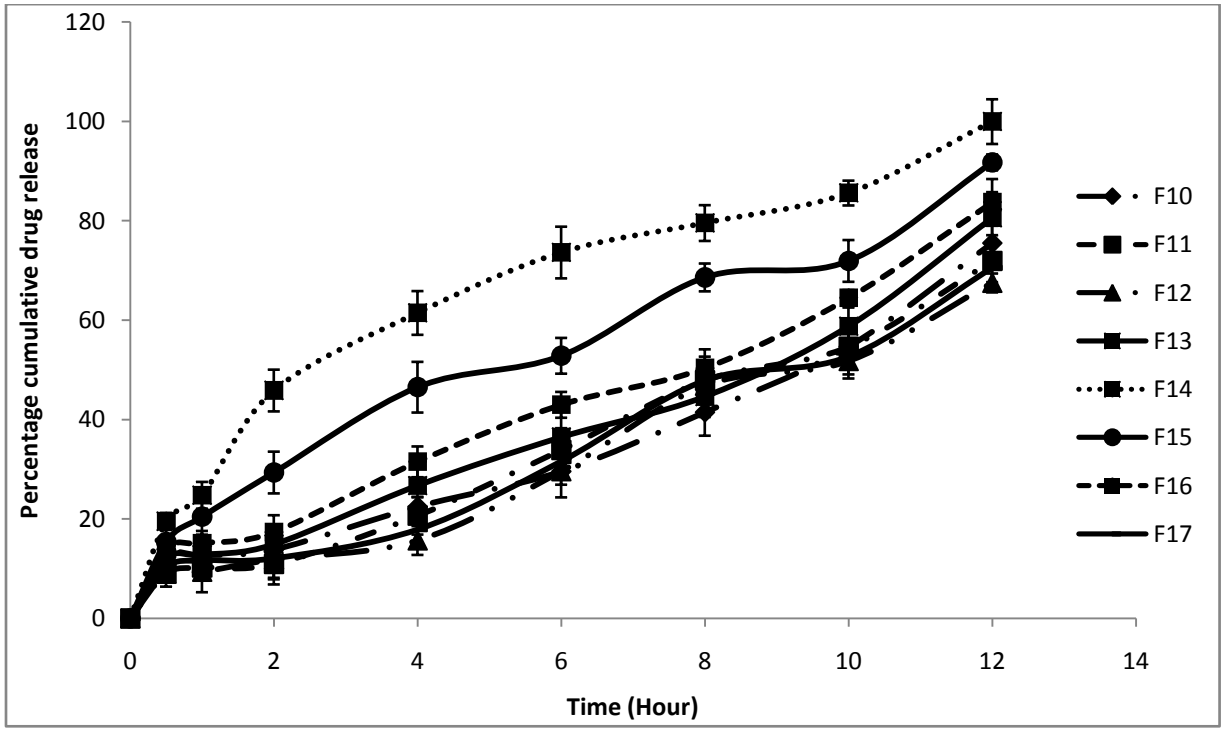
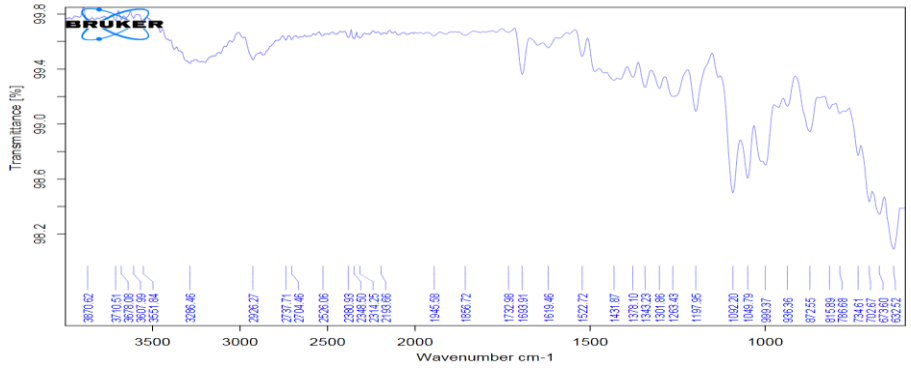


Figure 2: In vitro evaluation study of niosomess F10-F17

3.3.Optimised formulation evaluation

It observed in the ATR study that there was no such major interaction took place with excipients. Peak at 1522.72 cm^{-1} contributed by N-O stretching in nitro group, C-H stretching (dimethyl group) exhibited a medium band at 2926.27 cm^{-1}, as well as a strong C=O stretching at 1693.91 cm^{-1} appeared. A medium C-H bending exhibited at 1343.23 cm^{-1} for methyl group as well as the medium peak by aromatic amine (N-H) displayed peak at 3286.46 cm^{-1}. A sharp peak at 1092.20 cm^{-1} appeared because of aromatic amine (C-N stretching) in pyridine ring. Aromatic C-H in plane bending observed at 1049.79 cm^{-1}. Similarly esterified C-O stretching developed strong peak at 1263.43 cm^{-1}.



Fig; 3 ATR spectra of optimized formulation

The scanning electron micrograph (SEM) of proniosomal dispersion of optimized cilnidipine proniosome (Fig. 3) shows spherical morphology and size in the nano dimensions.

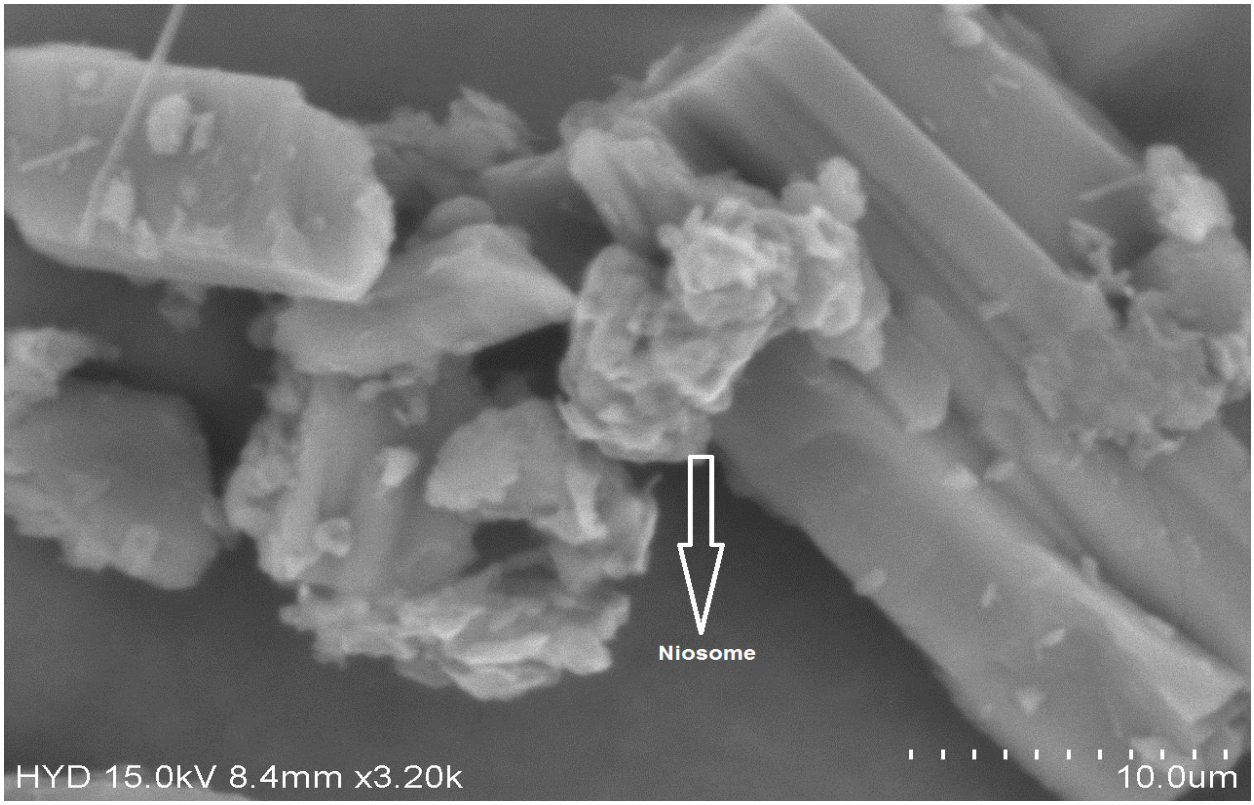


Fig 4 **SEM study of Optimized formulation (20.0 µm)**

The particle size study revealed average size of 829.7 nm of optimized formulation. Similarly polydispersity index (PI) revealed the value of 0.529. As per the literature stated the PI value of less than 0.5 indicates homogenous dispersion. However the data stated 0.529 which indicated heterogenous dispersion.

Zeta potential analysis The value of zeta potential was found to be −11.2 mV mV for optimized formulation , It indicates prepared proniosomes have sufficient surface charge to prevent aggregation of the vesicles.

Table 2 Optimised Formulation characteristics

Factor	**Name**	**Level**	**Response**				
			EE (%)	Drug release at 12h(%)	**Particle size(nm)**	**PI**	**Zeta potential**

							(mV)
1	Cholesterol (%)	22.06	57.28	81.34	829.7	0.529	-11.2
2	Span-60 (%)	0.9316					
3	Sorbitol (%)	20.48					

DSC study (Figure 5) highlighted a sharp endothermal peak at 99.97 °C; which is far deviated from the pure Cilnidipine as recorded earlier value of 112.35°C. This ascertained a significant change in endothermal peak and indicated interaction with excipients considered in this study. It also inferred decreased in thermal stability of pure drug.

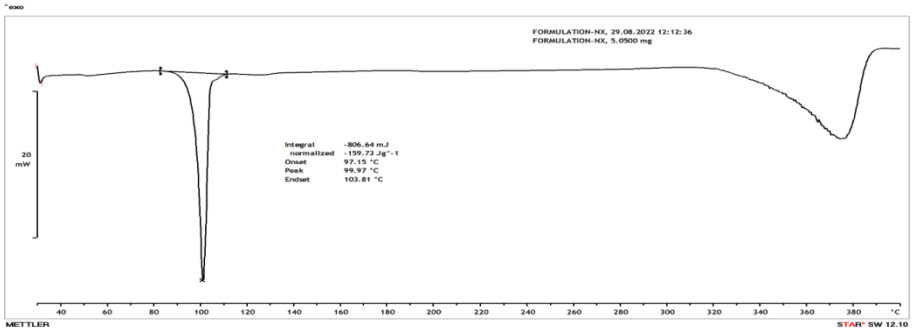


Fig5. DSC thermogram of Optimized formulation

XRD study (Figure 6) highlighted significant characteristic peak at position 11.61, 13.886, 18.68, 22.62, 25.49,29.02 and 33.749 (2Theta). Those peaks signified such changes as observed in pure Cilnidipine. However the intensity reduced to below than 2000; which could be due to presence of solvent and reduced crystallinity during formulation development.

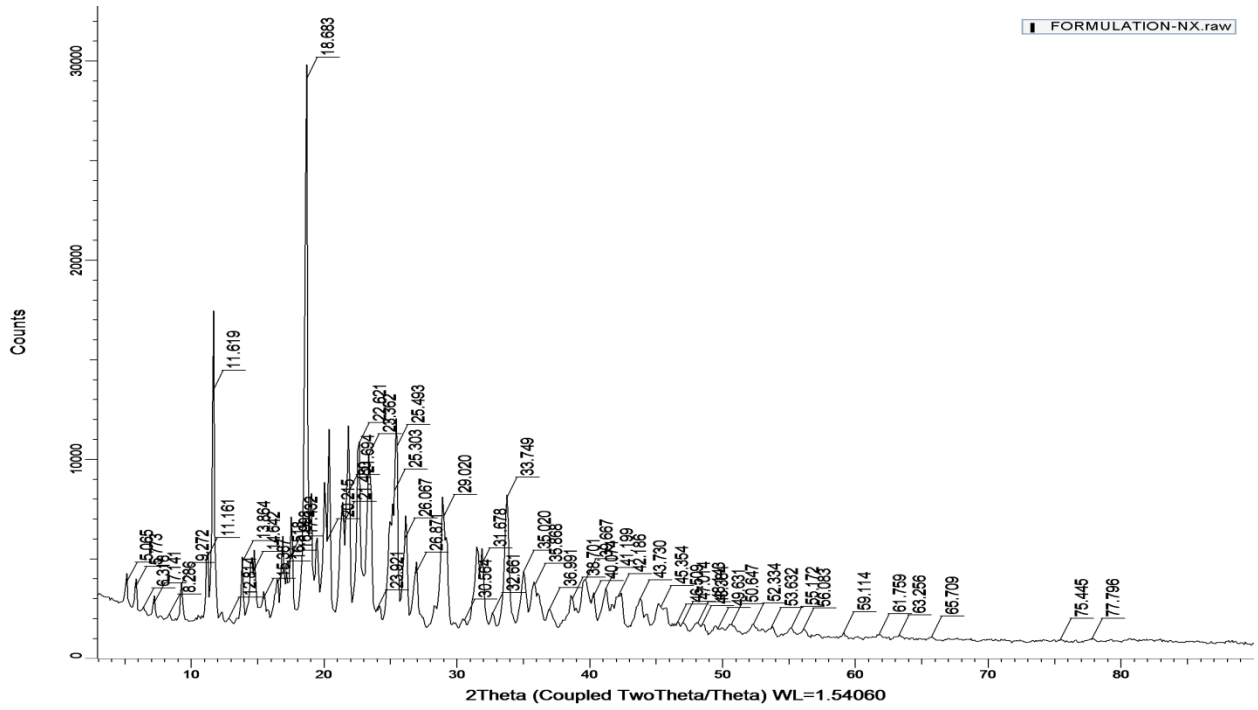


Fig 6 XRD spectra of Optimized formulation

4. Conclusion

Cilnidipine proniosomes were prepared successfully by using different carriers such as Cholesterol, span 80 and sorbitol. Physicochemical characterization concluded the possibility of preparing proniosomes by the use of different carriers, However, niosomal characterization concluded non-significant differences in term of entrapment efficiency, vesicle size, and content uniformity. Providing different alternatives for such formulation gives a good opportunity for the market to offer different products not only for Cilnidipine, but also for any other drug with low bioavailability. Proniosomes can be prepared easily by the slurry method and niosomes derived from proniosomes prepared exhibited a niosomal suspension with good stability, high entrapment efficiency (57.28%), and drug content. Proniosomes can be hydrated with a minimum amount of water, which is acceptable for adult administer.

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