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Advances in Bioresearch

ORIGINAL ARTICLE

Development and Characterization of Optimized Cilnidipine and Manidipine Proniosomes

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ABSTRACT
In this work, Cilnidipine and Manidipine loaded proniosomes are used in the treatment of hypertension to improve oral bioavailability. Cilnidipine and Manidipine proniosomes were prepared by film hydration followed by a rotary flask evaporator applying the concepts of Design of Experiments. The box-behnken design was applied to optimize the formulation variables. The particle sizes were in the nanometer range and spherical shaped for all prepared formulations predicting good long-term stability. Prepared proniosomes were characterized by differential scanning calorimetry (DSC) analysis and attenuated total reflection (ATR) analysis, revealed the compatibility of the drug chosen with the ingredient added, powdered 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 studies of Cilnidipine and Manidipine proniosomes exhibited a controlled release profile for at least 24 h. The obtained results revealed that Cilnidipine and Manidipine proniosomes can be successfully prepared by using different carriers. Hence, these proniosomes could represent as great potential for a possible alternative to conventional oral formulation in the treatment of hypertension.
Keywords*: Proniosomes, Cilnidipine, Manidipine, Hypertension, Cholesterol, in-vitro study.*

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INTRODUCTION

Hypertension is one of the most important risk factors for cardiovascular diseases, including ischemic and hemorrhagic 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 as "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 underdiagnosed 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 Ca2þ channel blocker with a rational pharmacological profile; i.e. dual L/N-type Ca2þ channel-blocking action. The blockade of N-type Ca2þ channels effectively suppresses neurohumoral regulation in the cardiovascular system, including the sympathetic nervous system and renin-angiotensin-aldosterone system. Thus, Cilnidipine is expected to be favorable for various types of complications of hypertension [6-9]. Manidipine binds to voltage-dependent calcium channels on smooth muscle cells and dissociates them, thus blocking the entrance of extracellular calcium into the cell, and preventing this contraction [10-12]. This produces vasodilation which decreases blood pressure. 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 can enhance the bioavailability of either hydrophilic or lipophilic drugs [13]. 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 main objective of the present investigation was to incorporate Cilnidipine and Manidipine into Cholesterol, to get proniosome to improve oral bioavailability by bypassing the first-pass metabolism. Accordingly, Cilnidipine & Manidipine proniosomes were prepared by film hydration followed by a rotary flask evaporator. Prepared proniosomes were characterized and optimized formulation was evaluated [14,15].

MATERIAL AND METHODS

Manidipine and Cilnidipine were purchased from Dr. Reddy's Laboratories, India. Dicetyl phosphate, surfactant, and cholesterol were purchased from SD Fine Chemicals, India.

Methods

Method of preparation of Proniosomes

Proniosomes loaded with Cilnidipine and Manidipine were prepared by film hydration (slurry method**)**. As shown in the table, seventeen formulations were created in total. The initial quantity of cholesterol (CHO), dicetyl phosphate (DCP; charge inducer), and surfactant (SUF) dissolved in the smallest amount of ethanol[16]. 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 and Manidipine 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 niosomes, which were then stored at 4°C for further evaluation and processing [17].

Optimization Formulations by Box-Behnken design (BBD)

In the experiment, a Box-Behnken experimental design with three levels and three factors. The current study aims to measure the impact of selected independent factors on the responses [18,19]. The polynomial equation was used for fitting and analysis in mathematics. The optimized formula was solved by graphical optimization technique along with a numerical method using the confidence interval value of alpha 0.05.

Table 1: Formulation Table of Manidipine proniosome

	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Run	A: Cholesterol	B: Poloxamer-407	C: Sorbitol	EE	Drug release at 12 h	Particle size
	%	%	%	%	%	nm
1	27.5	1.5	20	56.96	80.75	276
2	5	0.5	20	55.32	81.97	186
3	50	1.5	30	73.82	87.58	495
4	27.5	1.5	20	57.47	76.24	253
5	27.5	0.5	30	68.73	83.36	342
6	50	2.5	20	63.96	90.17	284
7	50	0.5	20	67.3	67.89	397
8	27.5	1.5	20	56.67	75.06	209
9	27.5	2.5	10	57.05	95.14	214
10	27.5	1.5	20	56.16	77.63	207
11	27.5	1.5	20	55.45	73.07	249
12	50	1.5	10	65.28	69.24	237
13	27.5	0.5	10	58.33	82.99	396
14	5	2.5	20	47.09	99.97	129
15	27.5	2.5	30	61.29	92.42	347
16	5	1.5	10	50.47	85.39	195
17	5	1.5	30	53.23	74.61	185

Table 2: 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

Characterization of niosomes

Entrapment efficiency

A precisely weighed quantity of niosomes (comparable to 10 mg of drug) was put into a 100 mL volumetric flask, and the smallest amount of ethanol was then added and properly mixed [20]. The dispersion was sonicated (Ultrasonicator, CPX3800-E, Branson) for around ten minutes. The resulting combination was mixed with a phosphate buffer with a pH of 6.8, and the volume was then set at the required amount. The dispersion underwent a further ten minutes of bath sonication to make it translucent. A Whatman membrane filter with a resulting mixture was then filtered using a device with a 0.45 m pore size. Using a UV-visible spectrophotometer (Shimadzu UV-1800, Japan) at respective wavelengths, the filtrate was analyzed for drug content.

$$EE\ (\%) = \frac{\text{Mass of drug in niosomes}}{\text{Initial mass of drug used in niosomes}} \times 100 \quad \text{.....(Equation 2)}$$

In-vitro drug release:

In vitro drug release of Cilnidipine and Manidipine from the niosomes was performed by diffusion technique using Franz-diffusion cell. The dialysis membrane; and cellophane membrane were cut into equal pieces (6 cm×2.5 cm) and soaked in distilled water for 12 h before use. The drug release studies of the solutions were 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 the receptor compartment. Aliquot samples of 1 ml were withdrawn at regular intervals and replaced with the 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 a U.V. spectrophotometer at the wavelength of 241 nm and 230 nm for Cilnidipine and Manidipine respectively against phosphate buffer (pH 6.8) as the blank [21].

Characterization of optimized Cilnidipine and Manidipine Proniosomes

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 [22].

Differential Scanning Calorimetry

Thermal characteristics of the Cilnidipine and Manidipine 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 and Manidipine proniosomes and bulk Cilnidipine and Manidipine was obtained at a scanning rate of 10 °C/min in a temperature range of 30 to 300 °C [23].

X-ray diffraction

Cilnidipine and Manidipine proniosomes after hydration with PBS were evaluated for solid-state characteristics by X-ray diffraction technique. Bulk Cilnidipine and Manidipine, and drug-loaded proniosomal dispersion were 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 [24].

Surface morphology, Particle size and zeta potential of optimized formulation

Morphology of the prepared optimized Cilnidipine and Manidipine proniosome powder was observed under a 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, proniosomes were evaluated for particle size and polydispersity index value using the scattering light intensity technique (Malvern zetasizer, ATA scientific, USA)[25].

RESULTS AND DISCUSSION

Optimization

Effect of the independent variable on EE %

Response 1 [EE]=56.54+8.03A-2.53B+3.24C+1.22AB+1.44AC -1.54BC +0.61A^2+ 1.26B^2+ 3.54 C^2 ……..(1)

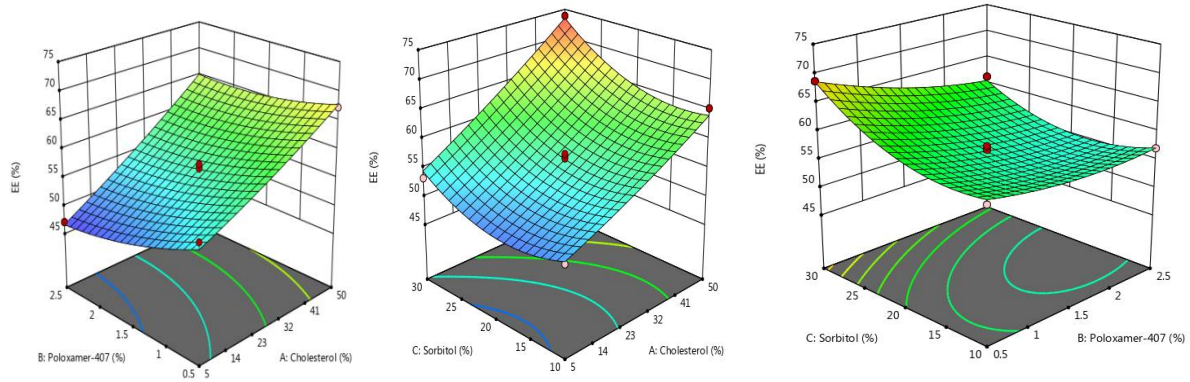

Figure 1: 3D simulation curve of Response 1(EE); Cholesterol Vs Poloxamer-407, Cholesterol Vs Sorbitol, Poloxamer-407 Vs Sorbitol

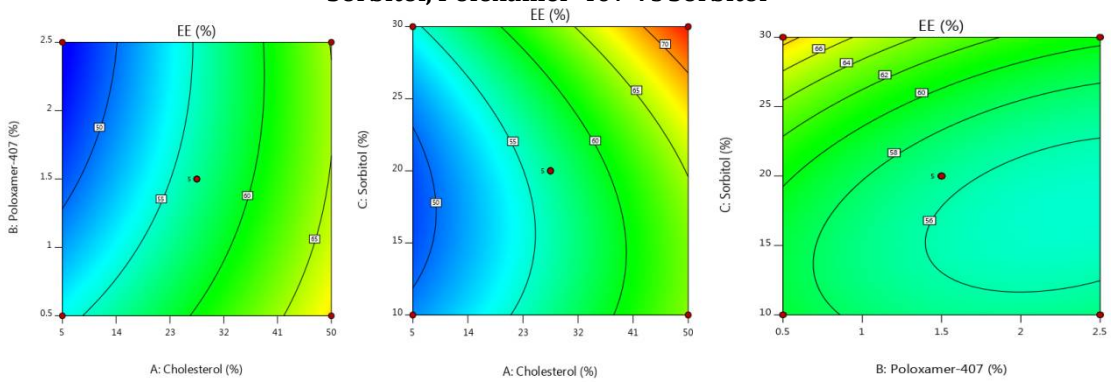

Figure 2: 2D Contour plot of Response 1(EE); Cholesterol Vs Poloxamer-407, Cholesterol Vs Sorbitol, Poloxamer-407 Vs Sorbitol

Effect of the independent variable on Drug released at 12th Hour

Response 2 [Drug released at 12th Hour] = 76.55-3.38A**+7.**68B+0.65C+1.07AB**+7.**28AC -0.77BC -0.41A^2**+**8.86B^2+3.06C^2…….. (2)

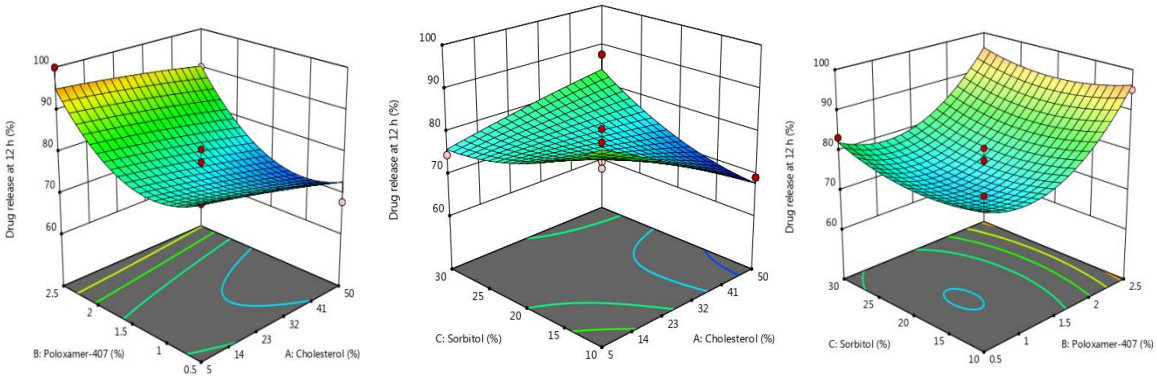

Figure 3: 3D simulation curve of Response 2(Drug released at 12th Hour); Cholesterol Vs Poloxamer-407, Cholesterol Vs Sorbitol, Poloxamer-407 Vs Sorbitol

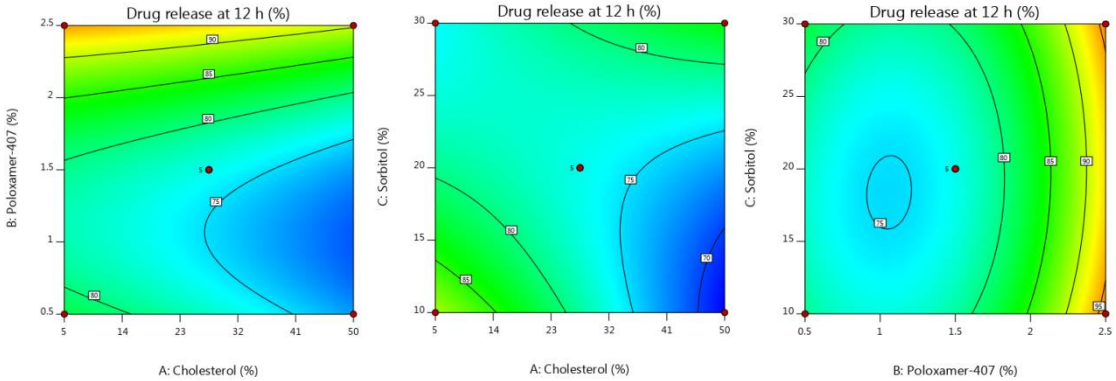

Figure 4: 2D Contour plot of Response 2(Drug released at 12th Hour); Cholesterol Vs Poloxamer-407, Cholesterol Vs Sorbitol, Poloxamer-407 Vs Sorbitol

Effect of the independent variable on Particle size

Response 3 [Particle size] = 238.8**+89.75A+43.37B+40.87C**-14.0AB**+67.0AC +46.75BC**-18.27A^2**+**28.47B^2**+57.47C²**…….. (3)

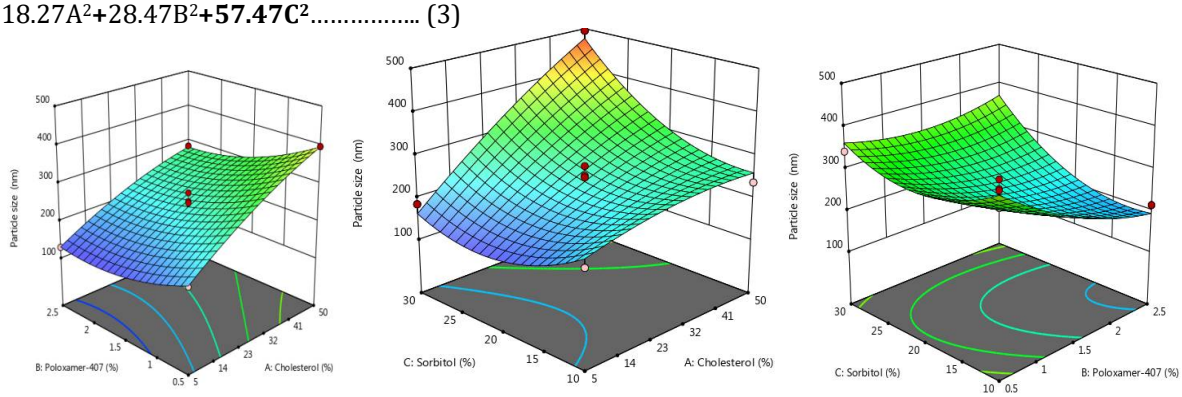

Figure 5: 3D simulation curve of Response 3(Particle size); Cholesterol Vs Poloxamer-407, Cholesterol Vs Sorbitol, Poloxamer-407 Vs Sorbitol

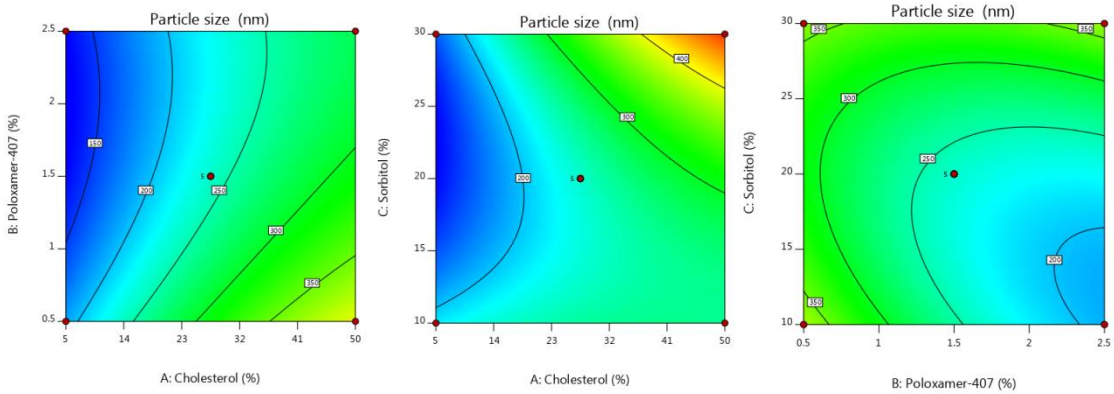

Figure 6: 2D Contour plot of Response 3(Particle size); Cholesterol Vs Poloxamer-407, Cholesterol Vs Sorbitol, Poloxamer-407 Vs Sorbitol

Optimization of study

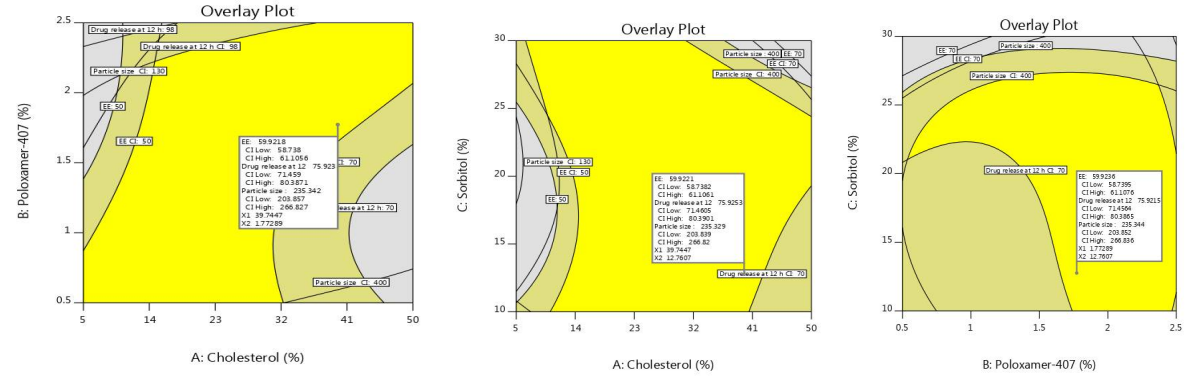

Figure 7: Overlay plot of region highlighting the optimized space and values

Table 3: Point Prediction table of optimized formulation

Response	Predicted Mean	Predicted Median	Observed
EE	59.9232	59.9232	63.41
Drug release at 12 h	75.9237	75.9237	81.22
Particle size	235.334	235.334	156.5

EE

The EE data of the Manidipine proniosome can be found in Table 1. It was noted that formulations with high cholesterol content (50%) had significant EE. The maximum percentage for "F3" is 73.82%, while F5 and F7 are locked at 68.93% and 67.3%, respectively. The formulation (F3) with 30% of sorbitol had the greatest EE while the formulation (F12) with less sorbitol, or 10%, had a relatively lower EE of 65.28%. The smallest amount of substance from F14 was discovered (47.09%).

The EE of Cilnidipine proniosome data can be found in Table 2. The formulation “F3” has a maximum of 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 the 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 drugs 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 a preferable high quantity can develop a proniosome with good EE.

In-vitro drug release

In all Manidipine formulations, it was stated that the majority of the drug was delivered in the first 30 minutes. The most drug released by F14 over the 12-hour dissolving period trial was 99.97%. Poloxamer-407 was used more and less, which resulted in a speedier release as witnessed in F14. Similar to F14, greater cholesterol, and less Poloxamer-407 contribute to lessening medication release. The more sorbitol there is, the more of a barrier it creates around the drug crystal, delaying the release of the medicine. Comparing F16 and F17 in terms of the amount of sorbitol used, it was found that F17 released less medication at 12 hours—74.12%—while F16 released more medication at that time—85.39%.

Another instance showed that F2 only displayed 81.97% of the drug, but F14, which had 2.5% Poloxamer-407, displayed 99.97% of the drug.

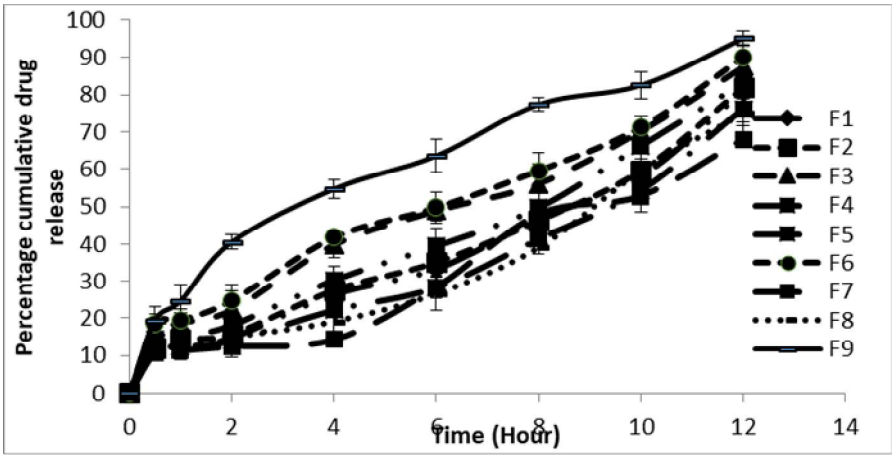

Figure 8: In vitro evaluation study of Manidipine niosomes F1-F9

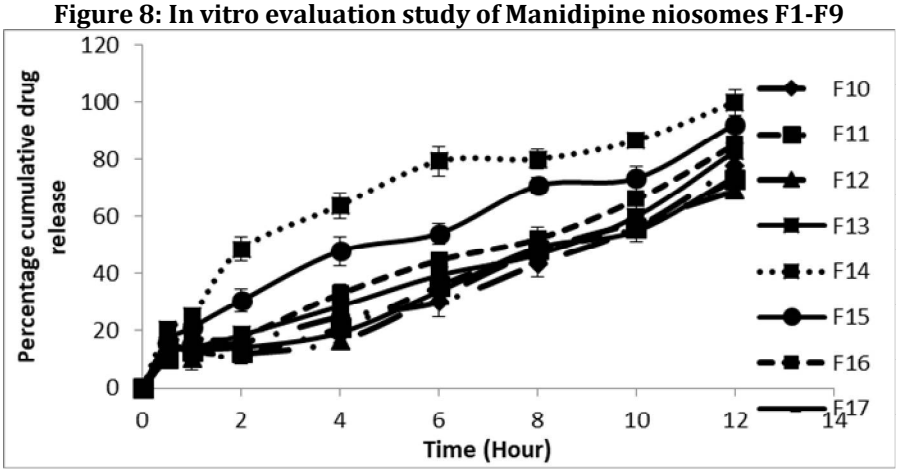

Figure 9: In vitro evaluation study of Manidipine niosomes F10-F17

In all the formulations of Cilnidipine, it was noted that a maximum of 20% drug was released in initial 30 minutes. It found that F14 released a maximum of 99.98% of the drug in 12h of dissolution study. Whereas, F7 exhibited the minimum 65.74 % of the drug in 12h. It found that there is a direct relationship between the amount of cholesterol and Span-60 in the dissolution study. The lesser amount of cholesterol and higher amount of Span-60 contributed to faster release as seen in F14. Similarly, more amount of cholesterol and less amount of span-60 contribute to lesser drug release as seen in F14. While developing the Niosomes; sorbitol is used as a carrier and has a profound effect on 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 about the percentage of sorbitol involved; it was found, a lesser amount of drug release i.e. 70.58% at 12h in F17 whereas F16 exhibited a higher amount of release of 83.73% at 12h. This ascertained the quantity influence of sorbitol in drug release. In one more instance, it was observed F2 exhibited only 79.82% of the drug, whereas F14 with 2% span-60 exhibited 99.98% of the drug. This confers the wetting property of span-60 which can emulsify the drug and promote faster drug release.

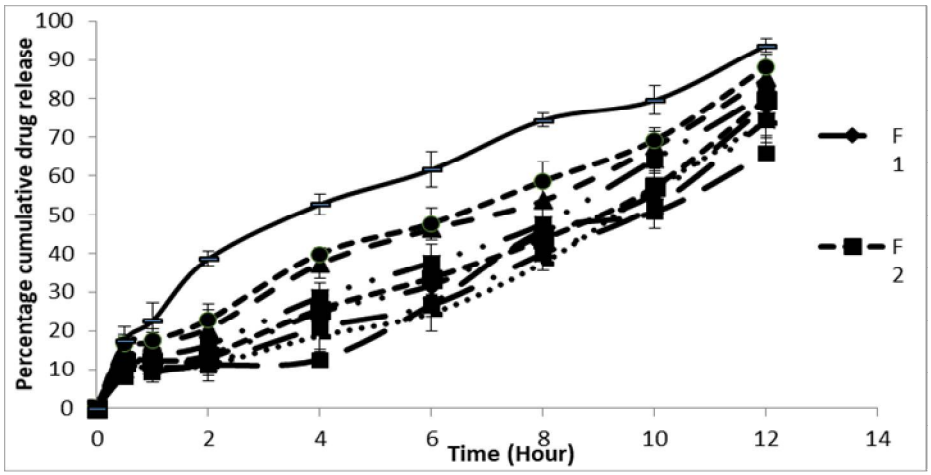

Figure 10: In vitro evaluation study of Cilnidipine niosomess F1-F9

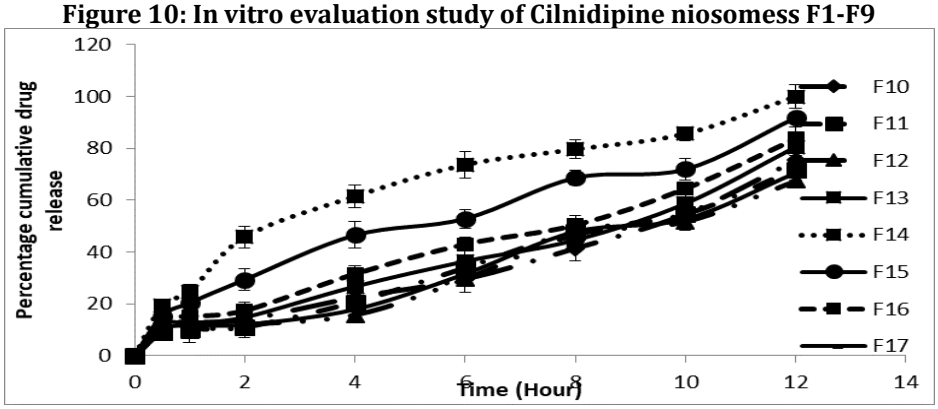

Figure 11: In vitro evaluation study of Cilnidipine niosomess F10-F17

ATR study of Drug and Excipients

Manidipine showed distinctive peaks at 1265.07 cm^{-1} owing to aromatic amine group C-N stretching, at 3201.61 cm^{-1} due to N-H stretch, and at 1530.19 cm^{-1} due to C=O stretch. Furthermore, the spectra showed bands at 1363.50 cm^{-1} caused by C-N bending, supporting the purity of Manidipine. It was noted that there was no significant interaction with excipients. Significant distinctive peaks in the formulation were seen at 3167.42 cm^{-1} for the N-H stretch, 1525.06 cm^{-1} for the C=O stretch, and 1275.64 cm^{-1} for the aromatic amine group C-N stretch. As a result of C-N bending, the spectra also revealed bands at 1365.92 cm^{-1}, indicating the existence of Manidipine.

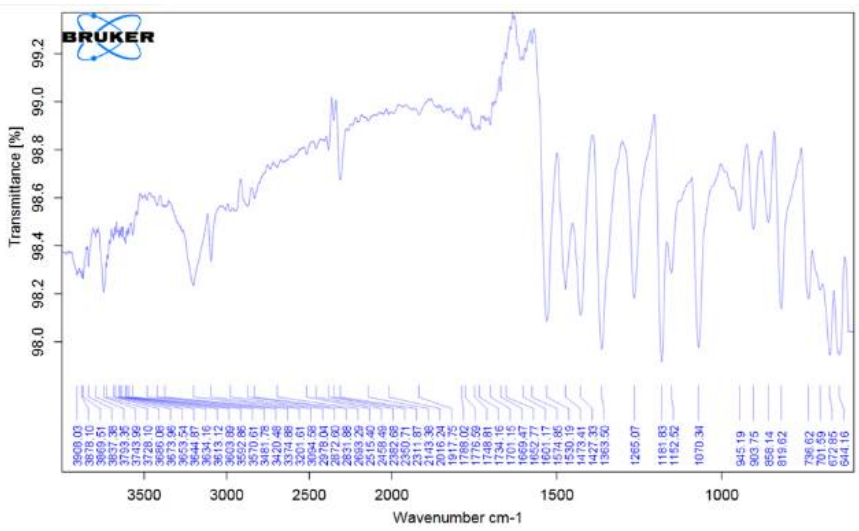

Figure 12: ATR spectra of Manidipine

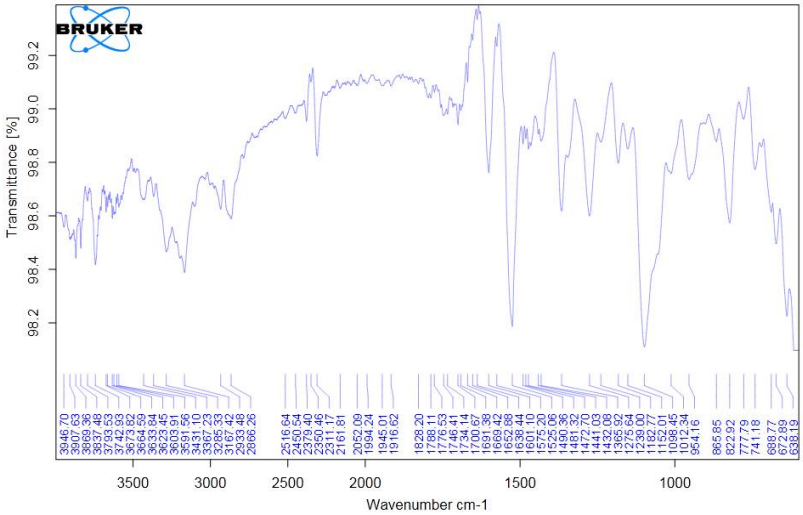

Figure 13: ATR spectra of optimized formulation

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 was observed at 1049.79 cm^{-1}. Similarly esterified C-O stretching developed strong peak at 1263.43 cm^{-1}.

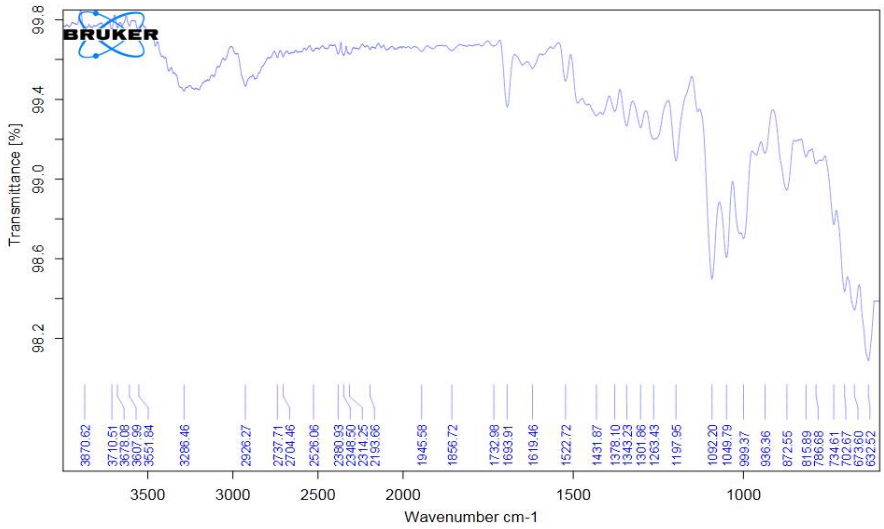

Figure 14: ATR spectra of Cilnidipine optimized formulation

Differential Scanning Calorimetry (DSC) study

DSC analysis revealed a pronounced endothermal peak at 300.89 °C, which is significantly different from the prior result of 439.1 °C for pure Manidipine. This confirmed a considerable shift in the endothermal peak and showed interaction with the study's excipients. Additionally, it suggested that the pure drug's thermal stability had deteriorated.

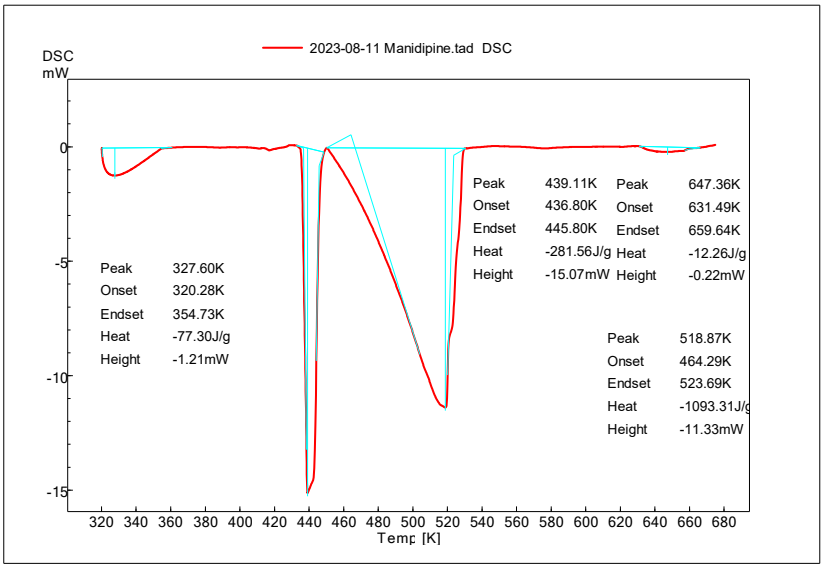

Figure 15: DSC of Manidipine

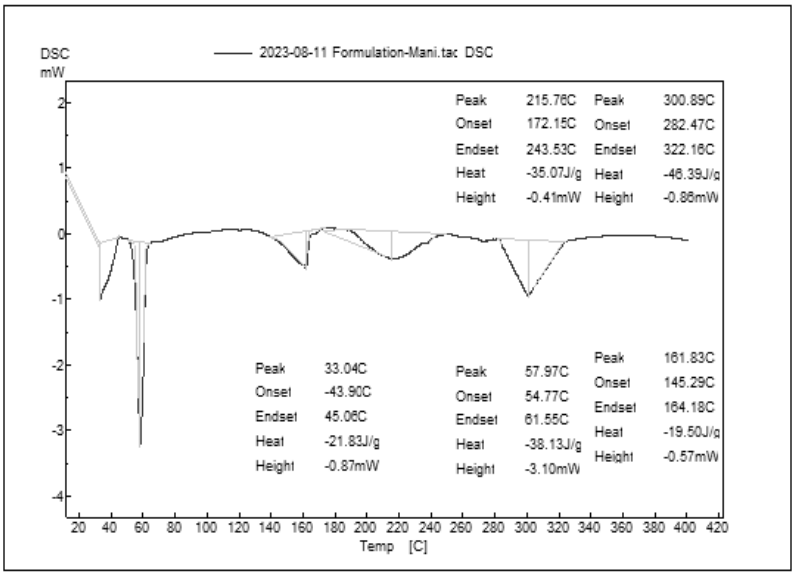

Figure 16: DSC thermogram of Manidipine Optimized formulation

DSC study 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 a decrease in the thermal stability of pure drugs.

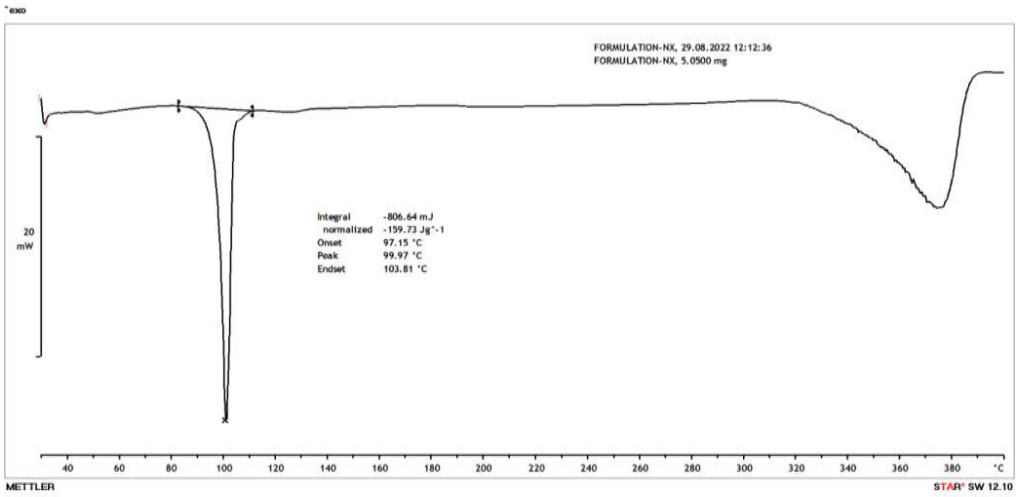

Figure 17: DSC thermogram of Cilnidipine optimized formulation

X-ray diffraction (XRD) study

It noted peaks at position 12.22 (2Theta) with intensity of 23000, 25.307 (2Theta) with intensity of 9000, 27.38 (2Theta) with intensity of 3000, 33.12 (2Theta) with intensity of 2500. Few more characteristic peaks appeared at 13 (2Theta), 16 (2Theta) and 25 (2Theta) with intensity below 2000. The XRD examination found significant unique peaks at locations 12.13, 18.98, 27.238, 27.818, and 33.222 (2Theta). These peaks suggested similar alterations to those seen in pure Manidipine. Nevertheless, the intensity decreased to below 3500 throughout formulation development, which was presumably caused by the presence of a solvent and a fall in crystallinity.

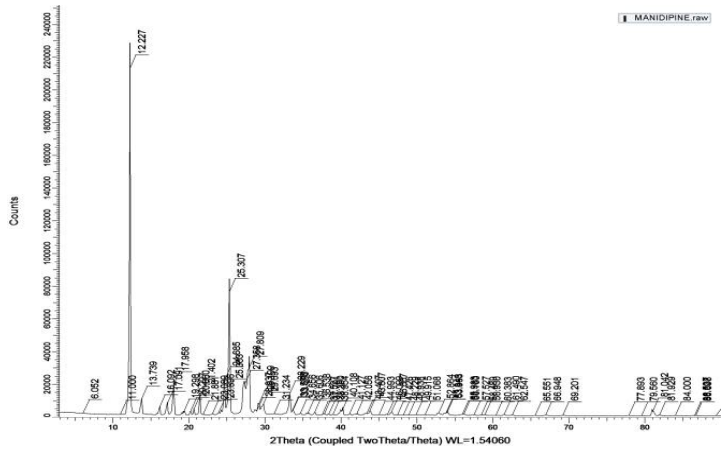

Figure 18: XRD of Manidipine

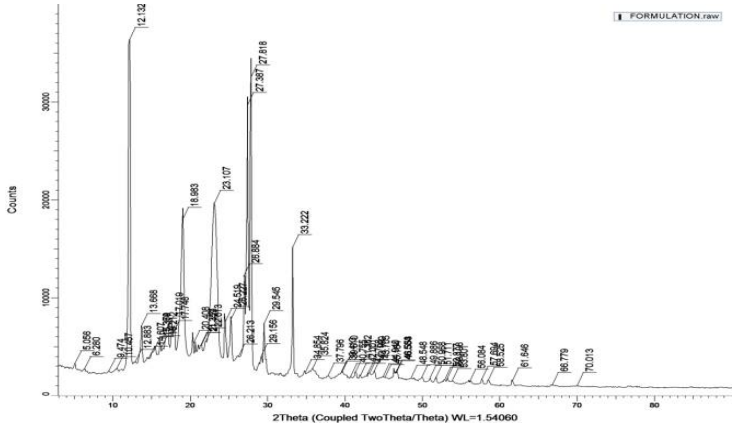

Figure 19: XRD spectra of Optimized formulation

XRD study highlighted significant characteristic peaks 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 2000; which could be due to the presence of solvent and reduced crystallinity during formulation development.

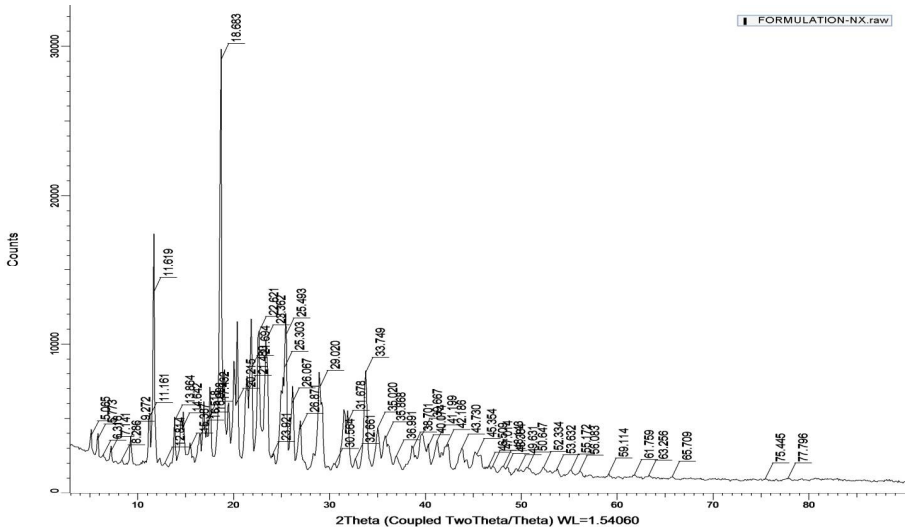

Figure 20: XRD spectra of Cilnidipine optimized formulation

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

Niosomes and drug crystals were seen to be dispersed in small areas during the SEM analysis. The research also uncovered the surface and look of optimized proniosomes. The structure of niosomes is asymmetrical. Although the drug crystals weren't connected to niosome production, they did emerge as crystals.

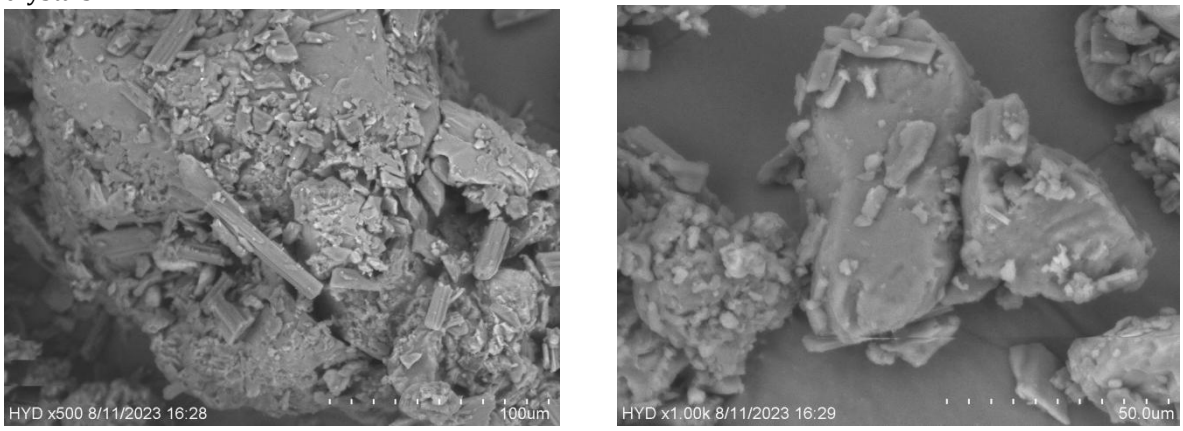

Figure 21: SEM study of Manidipine Optimized formulation

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

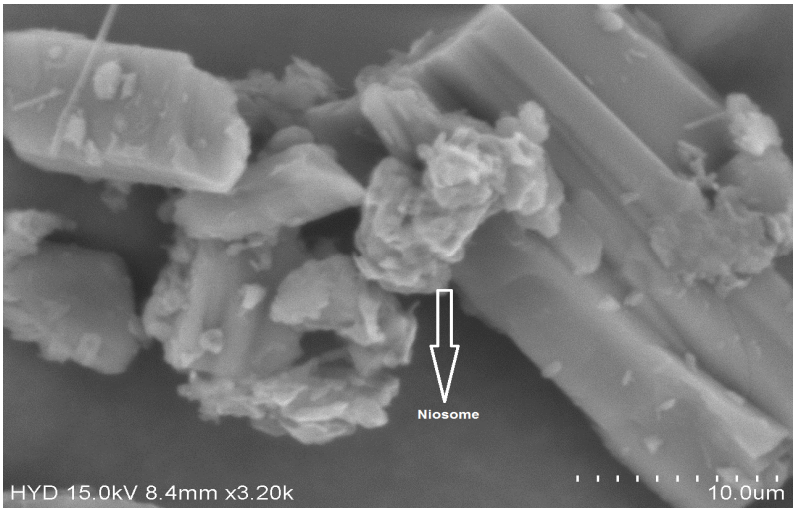

Figure 22: SEM study of Cilnidipine optimized formulation (20.0 µm)

The research on particle size showed the average size of 156.5nm Manidipine optimized formulation. Likewise, the polydispersity index (PI) demonstrated the importance of 0.449. According to the literature, homogeneous dispersion is indicated by a PI value of less than 0.5. As data stated 0.449 which indicated homogenous dispersion. Niosomes dispersed in acetate buffer pH 4.0 zeta potential were estimated. The study showed a zeta value of -6.7 mV; indicating stability as per the literature available.

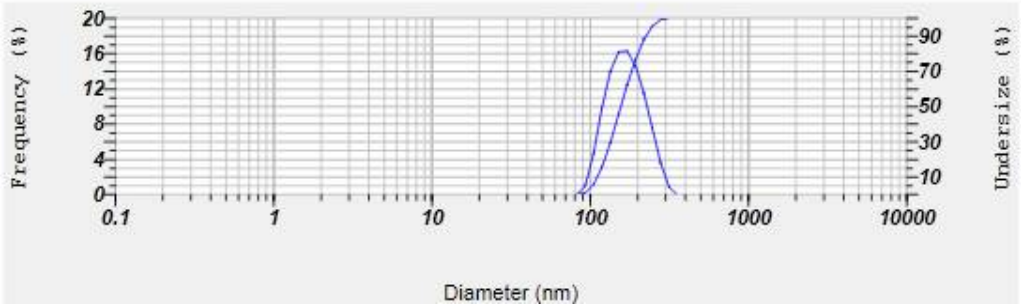

Figure 23: Particle size distribution

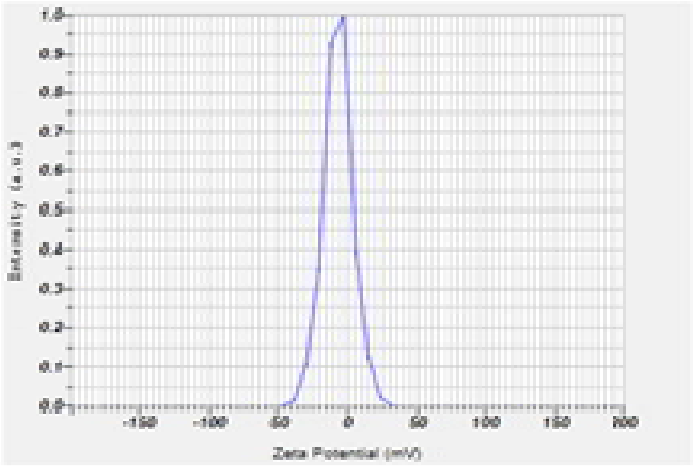

Figure 24: Zeta potential of optimized formulation

The particle size study revealed an average size of 829.7 nm of the optimized formulation. Similarly, the polydispersity index (PI) revealed a 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. The value of zeta potential was found to be −11.2 mV for the optimized formulation, it indicates prepared proniosomes have a sufficient surface charge to prevent aggregation of the vesicles.

Table 4: Cilnidipine 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					

CONCLUSION

Proniosomes, which have a structure like that of liposomes, offer a viable medication delivery technique. They may thus be a different vesicular system. By using the film hydration (slurry process), Manidipine and Cilnidipine-loaded proniosomal formulations were effectively formed. The generated formulations were tested for particle size, entrapment effectiveness, and Drug Release at 12 hr. Box-Behnken design statistical optimization was used. The improved niosomes were further assessed for permeation depth using surface morphology, FTIR, ATR, and DSC. The improved niosomes were also assessed for an in-vitro. According to the optimized niosomes formulation, F14 released 99.97% of the drug at its highest concentration in under 12 hours.

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