RESEARCH ARTICLE

Novel Proniosomes of Manidipine: Optimization and *In-vivo* Evaluation

J Ashwini, Vasudha Bakshi*

Department of Pharmaceutics, School of Pharmacy, Anurag University, Hyderabad, Telangana, India.

Received: 27th November, 2023; Revised: 19th March, 2024; Accepted: 23nd April, 2024; Available Online: 25th June, 2024

ABSTRACT

The objective of the current study is to produce proniosomes of manidipine using the thin film hydration method for the management of hypertension. Statistical optimization was performed using the Box-Behnken design. The generated formulations were tested for particle size, entrapment effectiveness, and drug release at 12 hours. The improved proniosomes were further assessed for surface morphology, ATR, and differential scanning calorimetry (DSC). The improved proniosomes were also assessed for an *in-vitro* and *in-vivo* investigation on dexamethasone-induced hypertension. It was noted that there was no significant interaction with excipients. DSC indicated a considerable shift in the endothermal peak and showed interaction with the study's excipients. Niosomes and drug crystals were seen to be dispersed in small areas during the scanning electron microscope (SEM) analysis. The study also showed that the optimized proniosomes have asymmetrical surfaces and appearances. The zeta value in the research was -6.7 mV, indicating stability. According to the optimized proniosomes formulation, F14 released 99.97% of the drug at its highest concentration in 12 hours. ANOVA results from an *in-vivo* investigation confirmed a satisfactory outcome in decreasing raised blood pressure, as shown by the F and *p-values*.

Keywords: Manidipine, Proniosomes, Zeta potential, Niosomes dexamethasone.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.2.03

How to cite this article: Ashwini J, Bakshi V. Novel Proniosomes of Manidipine: Optimization and *In-vivo* Evaluation. International Journal of Drug Delivery Technology. 2024;14(2):630-638.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

There is currently no one medication delivery method that satisfies all the requirements, however, efforts are made using cutting-edge strategies. Targeted and regulated medication distribution is one of the goals of innovative drug delivery systems.[1,2] The most attention has been focused on colloidal delivery and nanotechnology because they are promising systems with a localized effect. Dry, free-flowing preparations covered with a surfactant are referred to as proniosomes. By briefly agitating proniosomes, they quickly rehydrate within minutes, giving rise to the production of multi-lamellar niosomes. Niosome suspension is suitable for administering medicine by many methods.[3]

An antihypertensive drug is manidipine. Its low water solubility and low bioavailability (50%) place it in the Biopharmaceutical Classification System (BCS) class II of drugs. Proniosomes boost the therapeutic benefits of medication, reduce or eliminate negative side effects, and increase its efficacy. They are used to avoid oral delivery-related undesirable side effects such as gastrointestinal tract (GIT) incompatibility and pre-systemic metabolism.[4,5]

MATERIALS AND METHODS

In Hyderabad, India's Dr. Reddy's Laboratories, one may get manidipine. Purchased from SD Fine chemicals: Dicetyl phosphate, surfactant, and cholesterol.

Method of Preparation of Proniosomes (Slurry Method)

Proniosomes loaded with manidipine are made using the slurry technique of film hydration. Diacetyl phosphate (DCP; charge inducer), surfactant (SUF), and cholesterol (CHO) were initially dissolved in the least amount of ethanol. The rotating flask evaporator, RE-2010, Biobase, Mumbai, India used to treat the solution after it had been transferred to a round bottom flask (RBF). The mixture was subjected to thorough vacuum drying at a temperature of 40°C, a speed of 100 rpm, and a pressure of 16 mmHg in order to produce a dry RBF film.[6] A certain amount of manidipine, which is known to promote dispersion, was dissolved in phosphate-buffered saline (pH 6.8) with sorbitol serving as the carrier. The RBF was progressively supplemented with a thin layer of surfactant and cholesterol. The niosomes were extracted by freeze-drying the dispersion in lyophilizers for a duration of 24 hours at a temperature

**Author for Correspondence: vasudhapharmacy@anurag.edu.in*

of -80°C. The niosomes were then stored at 4°C for further processing and analysis.

Optimization Formulations by Box-Behnken Design

Three components and three levels make up the Box-Behnken experimental design used in the study. The goal of the current study is to quantify how certain independent variables affect the responses.[7,8] The following three variables were taken into account: Cholesterol (A), poloxamer-407 (B), and sorbitol (C). The experiment's recorded responses include EE, drug release after 12 hours, particle size, and so on. In mathematics, the polynomial equation was utilized for fitting and analysis. Using a graphical optimization methodology and a numerical approach, an alpha-valued confidence interval of 0.05 was used to develop the improved model. The equation was worked out shown in a Table 1.

Characterization of Niosomes

A 100 mL volumetric flask was filled with a precisely weighed quantity of niosomes (equivalent to 10 mg of medicine), to which the least amount of ethanol was added and well mixed.[9] Approximately five minutes were spent sonicating the dispersion. After the mixture was combined with a pH 6.8 phosphate buffer, the volume was adjusted to the necessary value. To achieve translucency, the dispersion was subjected to 10 further minutes of bath sonication. A membrane filter from Whatman with a After filtering the combination at 230 nm using a Shimadzu UV-1800 (Japan), the filtrate's drug content was examined.

$$DL\ (\%) = \frac{\text{Drug content in niosomes}}{\text{Total weight of niosomes}} \times 100 \quad \text{..... (Equation 1)}$$

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

Entrapment efficiency

Utilise the removal of manidipine from niosomes was successfully achieved *in-vitro* by the utilization of the Franz-diffusion cell and diffusion technology. In order to facilitate the utilization of the cellophane dialysis membrane, it was partitioned into uniform segments measuring 6 by 2.5 cm and subjected to immersion in distilled water for a duration of 12 hours. The drug release was evaluated in a 10 mL solution of pH 6.8 saline in phosphate buffer at a temperature of 37° ± 0.5° utilizing a magnetic stirrer and a German company's IKA Auto Temp Regulator for continuous heating. Experiments are performed on the manidipine solution. A 2 mL sample of niosome solution was introduced into the receptor compartment.[10] About 10 mL of phosphate buffer was prepared at a temperature of 37° ± 0.5°, with 6.8 pH saline being substituted with a new buffer of the same volume. As required, the aliquots were diluted using new media. The quantity of medication that spread over the membrane was measured using a 230 nm UV spectrophotometer, with phosphate buffer (pH 6.8) serving as the reference solution.

ATR study of drug and excipients

For online polymer composition monitoring, ATR spectroscopy is very helpful.[11] IR can identify the elements of a chemical process by using its capacity to fingerprint chemical components. Germany's ATR Bruker Opus 7.0 conducted the study.

Table 1: Formulation table of manidipine

	F-1	*F- 2*	*F- 3*	*R- 1*	*R- 2*	*R- 3*
Run	*A:Cholesterol*	*B:Poloxamer-407*	*C:Sorbitol*	*EE*	*Drug release at 12 hours*	*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

DSC study

A predetermined number of samples was introduced into aluminum crucibles, while a reference aluminum crucible was employed as a blank. The crucibles were subjected to a constant nitrogen flow rate of 20 mL/min. Nitrogen was employed as a purge gas in a Mettler Toledo (Ohio, USA) differential scanning calorimeter, namely model DSC. The scanning range encompassed temperatures ranging from 100 to 450°C, with a heating rate of 10°C per minute.[12,13]

Analysis of the improved formulation's surface shape, particle size, and zeta potential.

Double-sided adhesive tape was used to secure the sample to the slab surface, and photomicrographs were acquired under the S3700N-Hitachi, Japan, scanning electron microscope at various magnifications. Niosomes were examined utilizing the light-scattering output method using the Malvern Zetasizer from ATA Scientific in the United States.[14]

In-vivo study in dexamethasone induced hypertension

The research was done in compliance with CPCSEA recommendations. The research was done in compliance with CPCSEA recommendations. The study project received approval from the institutional animal ethical committee before conducting the *in-vivo* investigation with approval number CPSCEA/IAEC/JLS/19/02/2023/163.

Wistar rats, weighing 200 to 250 g, were separated into six groups before the investigation. About 5 days for additional acclimatization, and throughout this time they were being watched for any symptoms of stress. Two times was the acclamation cycle set. A 20-second interval was specified between each set. The maximal occlusion pressure that the tail cuff would cause was set at 250 mmHg, and the 20-second deflation period was used to reduce the pressure. The rats were fasted for the whole night before the experiment, and an IR thermometer was also used to measure body temperature. The rat was gently placed in a restrainer holder by the tail. The back screw was tightened as soon as the rat's face turned towards the front to keep it in place. On a plate, the restraint holder was kept. That keeps food at a constant 350°C. The rat was given 20 minutes to become used to the environment. Carefully, while the sensor cuff was safely positioned 2 mm away from the occluser, the occlusion cuff was attached, without being irritated, to the base of the tail. Finally, the system was prepared to automatically gather information, such as heart rate, and two blood pressure readings: systolic and diastolic. Because there was a risk of mistake during the acclamation, the first five readings were eliminated.[15]

In-vivo blood pressure estimation

The heart rate average was recorded from The minimal tail-cuff method. Dexamethasone was administered for 10 days to induce hypertension. Rat blood pressure was measured and noted as the mean value. Rat grouping and treatment with both the reference medication and the test sample "Optimized formulation" were administered. After systolic blood pressure, the particular acclamation and diastolic blood pressure was recorded.

RESULTS AND DISCUSSION

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+ 3.54C^2 …….. (1) shown in Figures 1 and 2

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) shown in a Figures 3 and 4

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 + 57.47C^2…….. (3) shown in a Figures 5 and 6. The

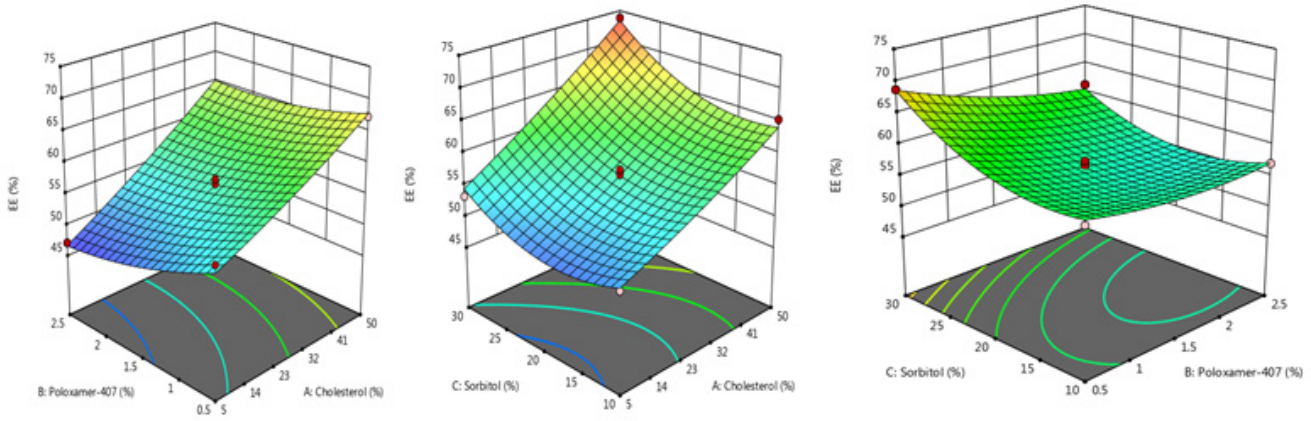


Figure 1: 3D simulation curve of response 1(EE); Cholesterol *vs* poloxamer-407, cholesterol *vs* sorbitol, poloxamer-407 *vs* sorbitol

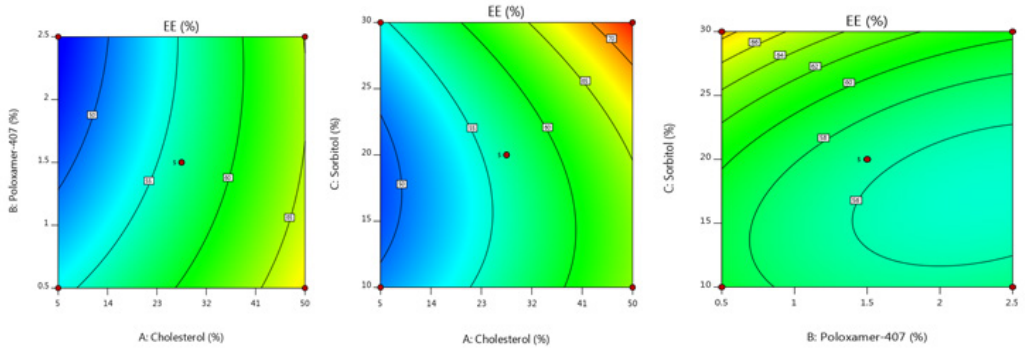


Figure 2: 2D Contour plot of response 1 (EE); Cholesterol *vs* poloxamer-407, cholesterol *vs* sorbitol, poloxamer-407 *vs* sorbitol

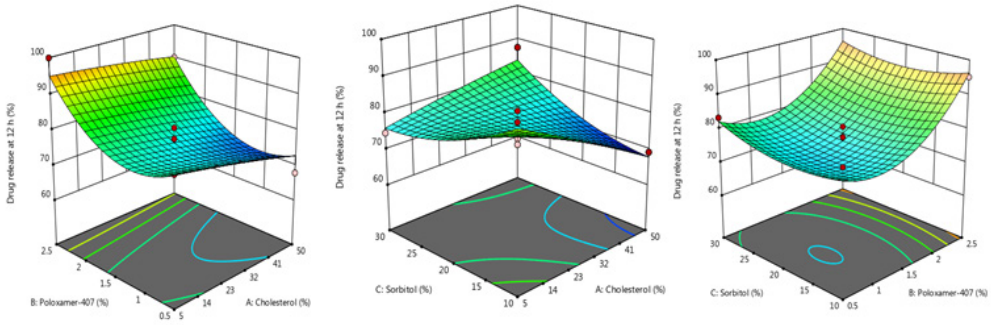


Figure 3: 3D simulation curve of response 2 (drug released at 12th hour); cholesterol *vs* poloxamer-407, cholesterol *vs* sorbitol, poloxamer-407 *vs* sorbitol

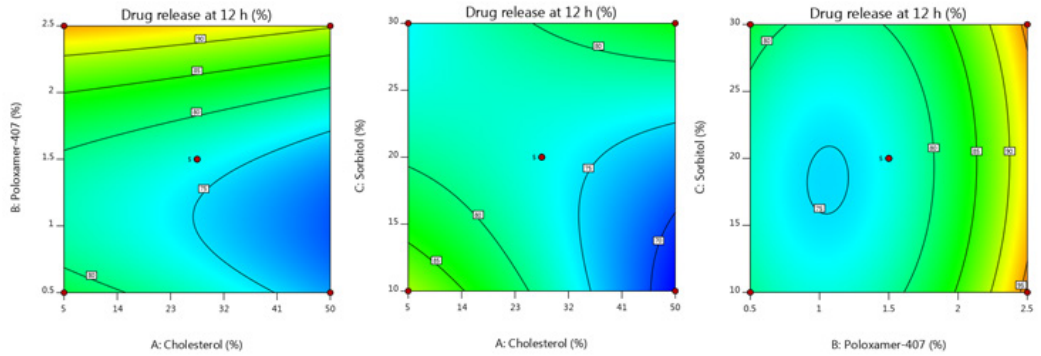


Figure 4: 2D contour plot of response 2 (drug released at 12th hour); cholesterol *vs* poloxamer-407, cholesterol *vs* sorbitol, poloxamer-407 *vs* sorbitol

Overlay Plot Illustrates the region has been optimized in terms of space and values are shown in Figure 7.

Optimization of Study

Drug Loading and EE

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. In a similar vein, it can be observed that the percentage quantity of sorbitol indicated the EE. 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%). Finally, it can be said that a niosome with excellent EE may be created by mixing sorbitol, a carrier, and cholesterol in an appropriate amount shown in Tables 2 and 3.

In-vitro Drug Release

Maximum 10% In all formulations, it was stated that the majority of the medicine 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

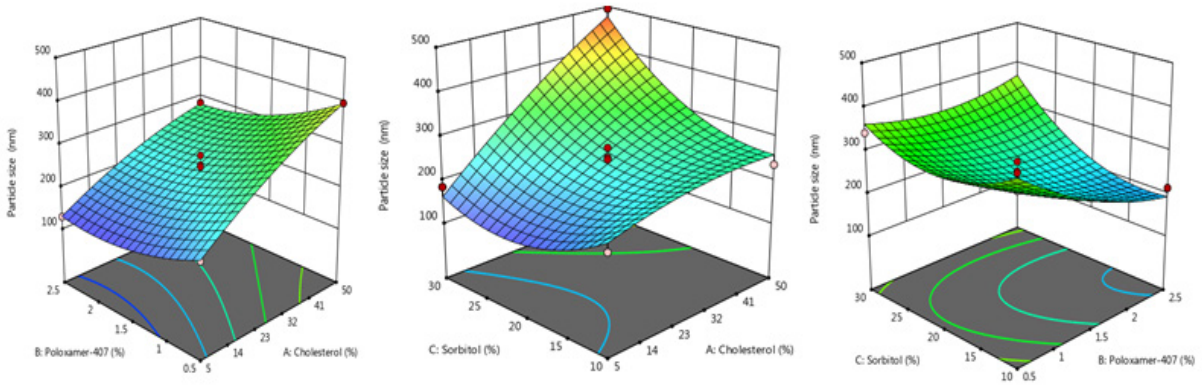


Figure 5: 3D simulation curve of response 3 (particle size); cholesterol *vs* poloxamer-407, cholesterol *vs* sorbitol, poloxamer-407 *vs* sorbitol

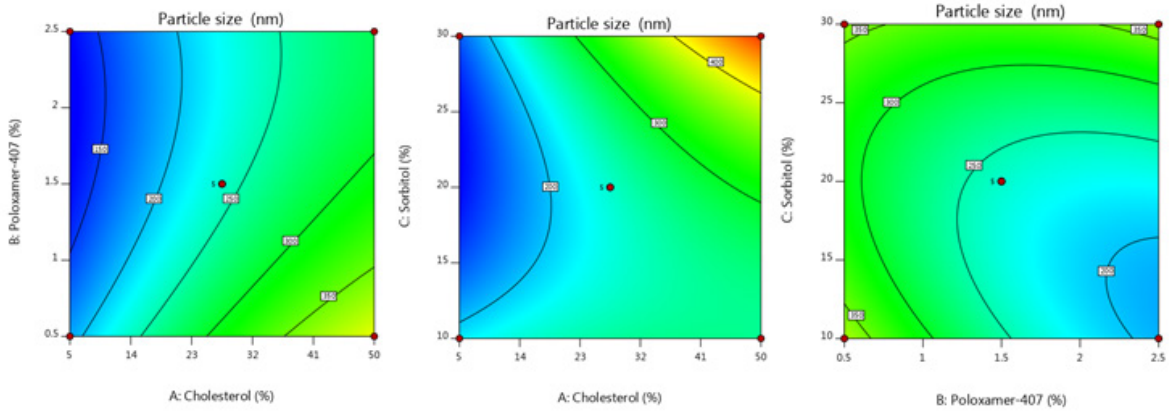


Figure 6: 2D Contour plot of response 3(particle size); cholesterol *vs* poloxamer-407, cholesterol *vs* sorbitol, poloxamer-407 *vs* sorbitol

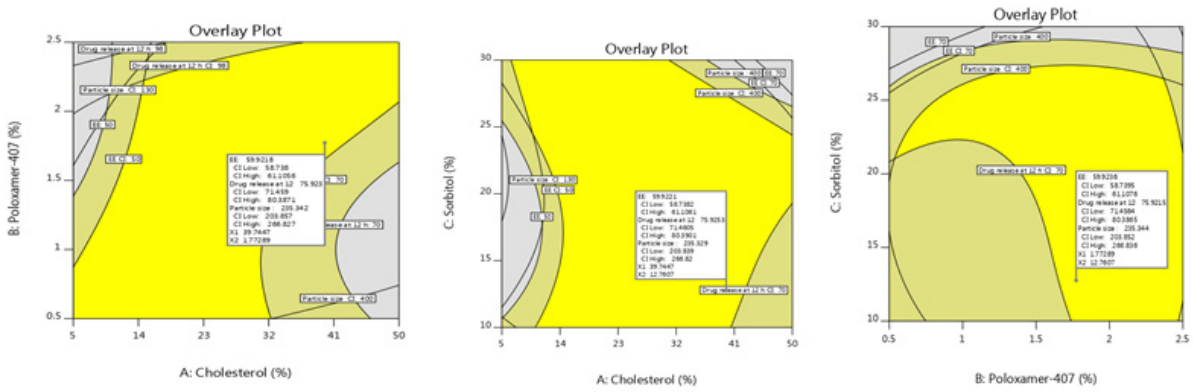


Figure 7: The overlay plot illustrates the region that has been optimized in terms of space and values

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. As a result of Poloxamer -407, its wetting characteristic accelerates the release of medicines by emulsifying them shown in Figures 8 and 9.

ATR Study of Drug and Excipients

Manidipine (Figure 10) showed distinct 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.

Table 2: Table displaying the predicted points for the optimized formulation

Response	*Predicted mean*	*Predicted median*	*Observed*
EE	59.9232	59.9232	63.41
Drug release at 12 hours	75.9237	75.9237	81.22
Particle size	235.334	235.334	156.5

Table 3: Drug loading of formulations F1-F17

Run	*Drug loading*
F1	68.97
F2	67.96
F3	85.19
F4	70.05
F5	76.13
F6	77.65
F7	80.32
F8	68.42
F9	69.10
F10	68.77
F11	63.18
F12	78.95
F13	71.27
F14	60.95
F15	73.82
F16	62.19
F17	65.76

No significant interaction with excipients was observed. The formulation exhibited notable characteristics (Figure 11) with distinct peaks seen at 3167.42 cm^{-1} for the N-H bond, 1525.06 cm^{-1} for the C=O bond, and 1275.64 cm^{-1} for the aromatic amine group C-N bond. The presence of manidipine was indicated by the presence of bands at 1365.92 cm^{-1} in the spectra, which were seen as a consequence of C-N bending.

Differential Scanning Calorimetry Study

The DSC analysis showed a clear endothermal peak at 300.89°C, which is notably lower than the previous result of 439.1°C for pure manidipine (Figure 12). 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 13).

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 (Figure 14). The research also uncovered the surface and look of the optimized. The structure of niosomes is asymmetrical. Although the drug crystals weren't connected to niosome production, they did emerge as crystals.

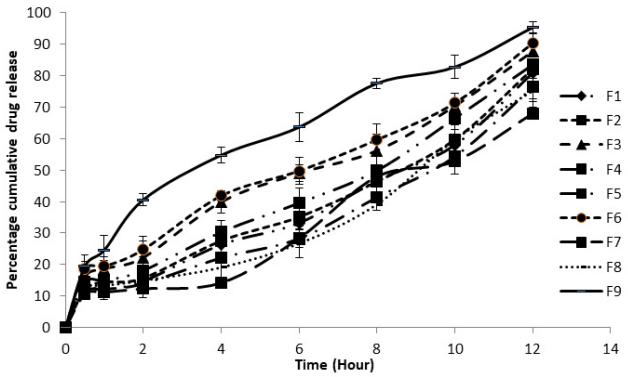


Figure 8: *In-vitro* evaluation study of niosomes F1-F9

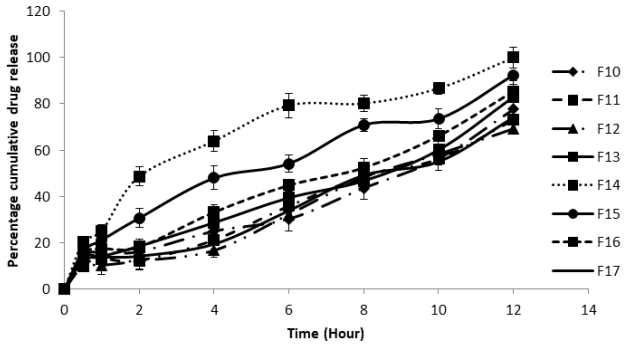


Figure 9: *In-vitro* evaluation study of niosomes F10-F17

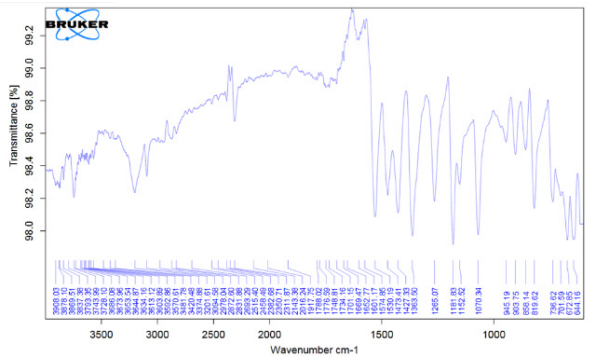


Figure 10: ATR spectra of manidipine

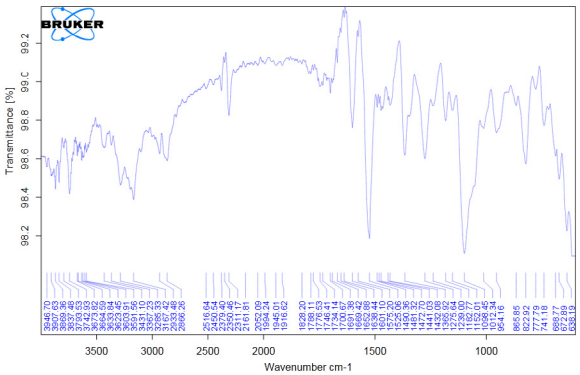


Figure 11: ATR spectra of optimized formulation

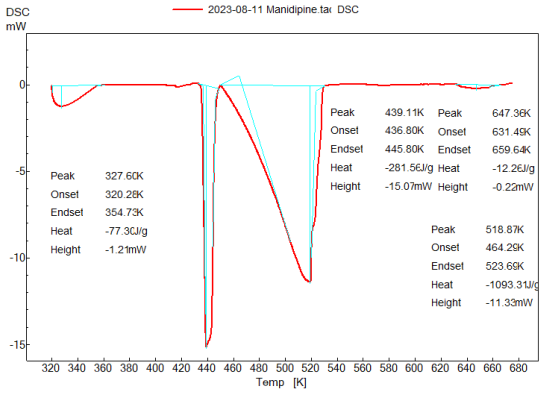


Figure 12: DSC of manidipine

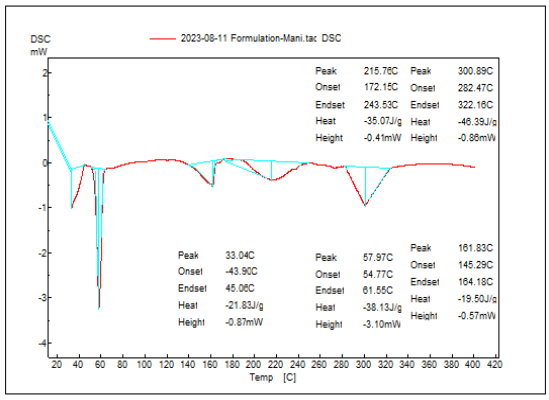


Figure 13: DSC thermogram of optimized formulation

The research on particle size revealed that the average size of the optimized formulation was 156.5 nm. (Figure 15). The polydispersity index (PI) highlighted the significance of 0.449 (Figure 16). The literature suggests that a PI value of less than 0.5 is an indication of homogeneous dispersion. The data showed a value of 0.449, indicating a homogenous dispersion.

The zeta potential of niosomes dispersed in acetate buffer at pH 4.0 was determined. The study revealed a zeta value of -6.7 mV, which suggests stability based on the existing literature.

One-way ANOVA was carried out to assess the significant difference in systolic blood pressure as a result of groupings are shown in Table 4. The "*p-value*" and "F-value" were also

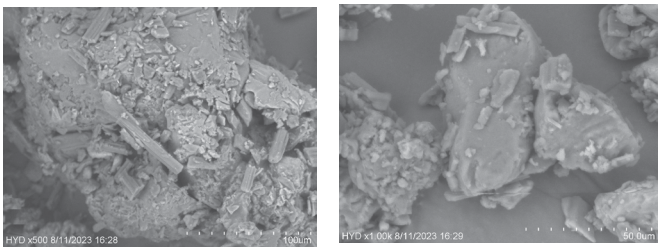
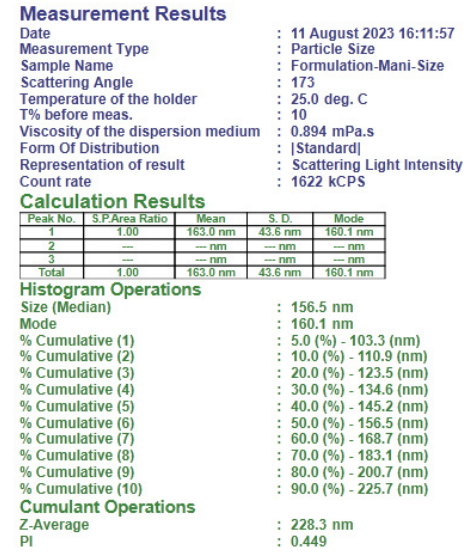


Figure 14: Study conducted on the optimized formulation using SEM



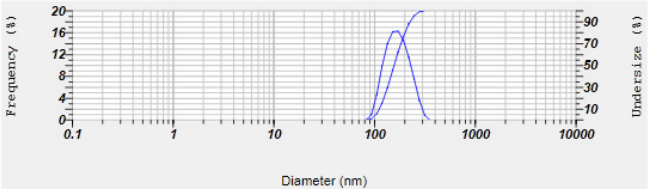


Figure 15: Particle size distribution

determined. To determine the significant difference between the sections of the ANOVA Table 5 an analysis of Tukey's HSD was used.

The ANOVA findings' F-value and *p-value* indicate that the therapy was effective in lowering the elevated blood pressure values shown in Table 6.

Table 4: Mean systemic blood pressure recorded from groups

Groups	*Systolic blood pressure (mm/Hg)*							
	Day 1	*Day 2*	*Day 3*	*Day 4*	*Day 5*	*Day 6*	*Day 7*	*Day 8*
I	125.6 ± 1.2	125.3 ± 2.6	125.8 ± 1.5	125.3 ± 2.3	126.9 ± 1.1	125.1 ± 1.3	125.7 ± 1.4	126.2 ± 1.4
II	147.4 ± 1.3	147.8 ± 3.4	149.2 ± 2.4	149.8 ± 1.8	149.2 ± 2.5	150.6 ± 2.2	150.6 ± 3.2	151.3 ± 2.2
III	126.1 ± 2.1	125.9 ± 1.9	125.2 ± 1.7	126.4 ± 1.3	126.1 ± 3.1	127.3 ± 3.8	126.4 ± 1.5	126.7 ± 3.5
IV	129.8 ± 1.7	131.3 ± 2.4	129.9 ± 1.6	130.5 ± 3.1	131.7 ± 1.9	130.5 ± 1.2	131.8 ± 3.5	132.2 ± 2.3
V	128.2 ± 2.2	127.8 ± 3.2	128.1 ± 2.2	126.8 ± 2.5	125.2 ± 1.7	125.7 ± 3.1	123.4 ± 2.2	124.9 ± 1.9
VI	125.3 ± 3.1	125.9 ± 1.8	124.1 ± 1.1	125.9 ± 1.9	124.3 ± 3.4	124.1 ± 1.9	122.6 ± 1.9	122.3 ± 1.5

The values are expressed as Mean ± Standard error of the mean (n = 3)

Table 5: Mean diastolic blood pressure recorded from groups

Groups	*Diastolic blood pressure (mm/Hg)*							
	Day 1	*Day 2*	*Day 3*	*Day 4*	*Day 5*	*Day 6*	*Day 7*	*Day 8*
I	85.8 ± 2.1	85.1 ± 2.8	84.8 ± 3.1	85.3 ± 2.4	86.9 ± 2.7	87.5 ± 1.9	85.6 ± 1.4	85.8 ± 2.5
II	95.5 ± 2.2	94.1 ± 3.1	99.7 ± 1.4	96.9 ± 1.5	96.3 ± 1.2	95.5 ± 2.7	94.7 ± 2.1	98.9 ± 1.6
III	86.7 ± 1.1	87.3 ± 2.6	86.4 ± 1.8	85.8 ± 1.2	85.4 ± 2.2	84.3 ± 1.5	84.5 ± 1.2	85.8 ± 2.1
IV	89.6 ± 1.7	88.7 ± 3.2	90.9 ± 1.1	87.2 ± 2.4	85.5 ± 2.2	86.4 ± 2.7	84.9 ± 1.3	84.3 ± 1.9
V	88.5 ± 2.1	89.2 ± 1.6	86.6 ± 2.4	87.9 ± 1.4	84.8 ± 2.1	86.1 ± 3.6	83.6 ± 1.8	84.5 ± 2.2
VI	87.4 ± 1.1	88.2 ± 3.1	86.4 ± 2.5	85.8 ± 3.7	82.8 ± 2.4	83.5 ± 2.2	81.8 ± 1.8	82.3 ± 1.3

The values are expressed as Mean ± Standard error of the mean (n = 3)

Table 6: One-way ANOVA

	Sample size	*Mean*	*Standard deviation*	*SE of mean*
Group I	8	125.7375	0.58294	0.2061
Group II	8	149.4875	1.37471	0.48603
Group III	8	126.2625	0.61164	0.21625
Group IV	8	130.9625	0.91016	0.32179
Group V	8	126.2625	1.74351	0.61642
Group VI	8	124.3125	1.36741	0.48345
DF	Sum of squares	Mean square	F value	Prob > F
Model 5	3660.94667	732.18933	526.64215	0
Error 42	58.3925	1.3903		
Total 47	3719.33917			

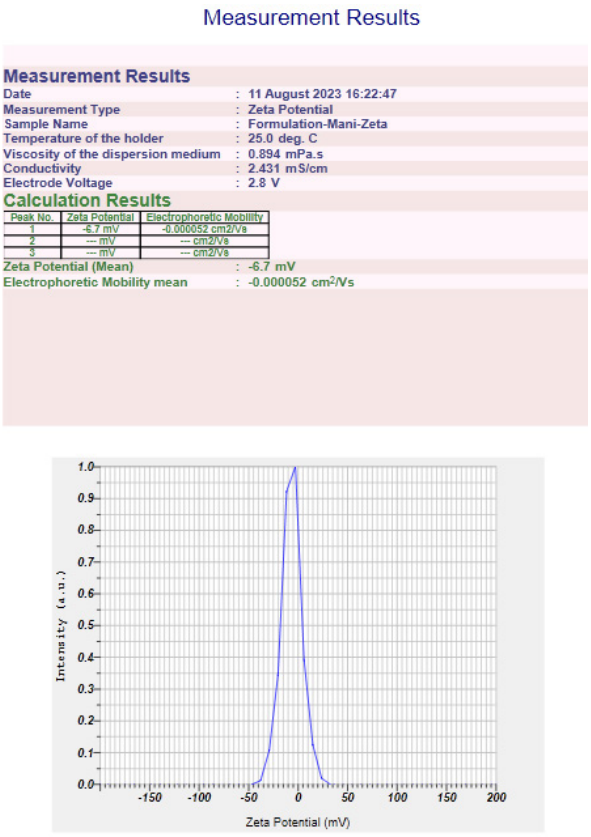


Figure 16: Zeta potential of optimized formulation

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-loaded proniosomal formulations were effectively created. The generated formulations were tested for particle size, entrapment effectiveness, and drug release at 12 hours. 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* and *in-vivo* investigation on dexamethasone-induced hypertension. According to the optimized niosomes formulation, F14 released 99.97% of the medication at its highest concentration in under 12 hours. ANOVA results from an *in-vivo* investigation confirmed a satisfactory outcome in decreasing raised blood pressure, as shown by the F and *p-values*.

REFERENCES

1. Pham TT, Jaafar-Maalej C, Charcosset C, Fessi H. Liposome and niosome preparation using a membrane contactor for scale-up. Colloids and Surfaces B: Biointerfaces. 2012 Jun; 94:15–21.
2. Janga KY, Jukanti R, Velpula A, Sunkavalli S, Bandari S, Kandadi P, et al. Bioavailability enhancement of zaleplon via proliposomes: Role of surface charge. European Journal of Pharmaceutics and Biopharmaceutics. 2012 Feb;80(2):347–57.
3. Payne NI, Browning I, Hynes CA. Characterization of

Proliposomes. Journal of Pharmaceutical Sciences. 1986 Apr;75(4):330–3.
4. Om Prakash Katare, Vyas SP, Dixit VK. Effervescent granule based proliposomes of ibuprofen. 1990 Jan 1;7(4):455–62.
5. Baillie Aj, Florence At, Hume Lr, Muirhead Gt, Rogerson A. The preparation and properties of niosomes-non-ionic surfactant vesicles. Journal of Pharmacy and Pharmacology. 1985 Dec;37(12):863–8.
6. Schreier, H., & Bouwstra, J. (1994). Liposomes and niosomes as topical drug carriers: Dermal and transdermal drug delivery. Journal of Controlled Release, 30(1), 1-15. https://doi.org/10.1016/0168-3659(94)90039
7. Solanki AB, Parikh JR, Parikh RH. Formulation and optimization of piroxicam proniosomes by 3-factor, 3-level box-behnken design. AAPS Pharm SciTech. 2007 Oct;8(4).
8. Ge X, Wei M, He S, Yuan WE. Advances of Non-Ionic Surfactant Vesicles (Niosomes) and Their Application in Drug Delivery. Pharmaceutics. 2019 Jan 29;11(2):55.
9. Uchegbu, I. F., & Vyas, S. P. (1998). Non-ionic surfactant based vesicles (niosomes) in drug delivery. International Journal of Pharmaceutics, 172(1-2), 33-70.
10. Carafa M, Santucci E, Lucania G. Lidocaine-loaded non-ionic surfactant vesicles: characterization and *in-vitro* permeation studies. International Journal of Pharmaceutics. 2002 Jan;231(1):21–32.
11. Alsarra IA, Bosela AA, Ahmed SM, Mahrous GM. Proniosomes as a drug carrier for transdermal delivery of ketorolac. European Journal of Pharmaceutics and Biopharmaceutics. 2005 Apr;59(3):485–90.
12. Proniosomes: A Novel Drug Carrier Preparation. International Journal of Pharmaceutics 1999 ;185(1):23–35.
13. Radha GV, Rani TS, Sarvani B. A review on proniosomal drug delivery system for targeted drug action. Journal of Basic and Clinical Pharmacy [Internet]. 2013 Mar 1;4(2):42–8.
14. Patel NN, Roopchandani KV, Gupta A, Gupta A. Proniosomes for better transdermal drug delivery: A review, J Pharmacy Research,2013; 62–80.
15. Mokhtar M, Sammour OA, Hammad MA, Megrab NA. Effect of some formulation parameters on flurbiprofen encapsulation and release rates of niosomes prepared from proniosomes. International Journal of Pharmaceutics. 2008 Sep;361(1-2):104–11.