RESEARCH ARTICLE

Enhancement of Solubility by Hydrotropic Techniques of Lercanidipine HCl: *In-situ* Nasal Gel Development

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ABSTRACT

Lercanidipine HCl is a novel, third-generation, potent, vasoselective 1, 4- dihydropyridine calcium channel antagonist and a Biopharmaceutical Classification System (BCS) class II drug that aids in preventing hypertension. The final aim of the present work was to promote the solubility of lercanidipine HCl *via* hydrotrophy and then include it in the in-situ gel formulation. This enhancement of drug solubility was carried out by using citric acid as a hydrotropic agent. Simultaneously, using a mixture of two polymers, namely Poloxamer 407, a thermosensitive polymer with reversible thermal characteristics. Carbopol 940P is a high-viscosity builder exploited for developing *in-situ* gel. The optimization of preliminary batches of mixtures of both polymers was highly accepted by using 32 factorial designs. F4 was found to be highly optimized, according to all the evaluation parameters and Poloxamer 407 (18% w/v) and Carbopol 940P (0.2% w/v), with drug release of 93.23%. Goat mucosa *ex-vivo* investigation yielded a value of 79.14% flux at 6 hours. According to ICH guidance, the *in-situ* Lercanidipine HCl gel has turned up to be stable following a 6-month stability investigation. Thus, the subsequent thermoreversible *in-situ* gel was scouted to act as a potent nasal distributor with greater bioavailability and patient compliance for the nasal medication.

Keywords: Lercanidipine HCl, Hypertension, Poloxamer407, Carbopol 940P, Thermoreversible, 3^2 Factorial design, Franz diffusion cell.

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INTRODUCTION

The absorption and dispersion of drugs through mucous membranes are the primary goals of nasal medication administration. So, several mechanisms have been proposed based on drug absorption into systemic circulation *via* nasal drug delivery that mainly includes paracellular, transcellular carrier-mediated transport and endocytic process. Lercanidipine HCl is a novel, potent, vasoselective, third-generation 1, 4- dihydropyridine Ca^{2+} channel antagonist and a Biopharmaceutical Classification System (BCS) class II drug possessing soaring lipid solubility and weak aqueous solubility with slower onset of action which is intended to treat hypertension. It works by preventing calcium ion inflow through L-type calcium channels in cell membranes. In order to select lercanidipine HCl, an oral dose of 10 to 20 mg must be completely absorbed from the GIT. Furthermore, after humans receive a single dose of 0.01 and 0.02 gm of lercanidipine HCl, with a pKa value of 6.83, the production of inactive metabolites during hepatic first-pass metabolism can reduce the drug's absolute bioavailability to roughly 10%, lowering the half-lives by 2.8 and 4.4 hours. The present exploratory work aims to design, create, and evaluate a thermoreversible *in-situ* nasal gel of hydrophobic lercanidipine HCl using Poloxamer 407 as a gelling agent, Carbopol 940P as a mucoadhesive agent, and sodium citrate as a hydrotropic agent. This will allow for the development of *in-situ* gel with reversible thermal characteristics. We analyzed several aspects such as medication content, gelation temperature (Tsol–Tgel), pH, mucoadhesive strength, viscosity, statistical analysis, histopathology research, accelerated stability study, *in-vitro* diffusion study, and *ex-vivo* permeation study in order to enhance our formulation.[1]

MATERIAL AND METHOD

The sample of lercanidipine HCl of USP grade was used in this experiment and was purchased from Glenmark Pharmaceuticals, Gujarat. Poloxamer, Carpool 940P, Sodium citrate, sodium benzoate, urea, PEG AR (Polyethylene glycol) were acquired from Analab Fine Chemicals, Mumbai and

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benzalkonium chloride AR was acquired from Molychem, Mumbai.[1]

Characteristics of Lercanidipine HCl

The drug component was analyzed for properties of life, physical appearance, and nature and the melting point was identified by the melting point apparatus with the help of capillary.[2]

UV Spectroscopy

The drug was dissolved in phosphate buffer 6.8, and its dissolution was monitored in the 200 to 400 nm range. The λ_{max} was found to be 337 nm using a UV spectrophotometer (Make: Varian, Model: Carry 100, Japan).[2]

Fourier Transform Infrared Spectroscopy

With the help of KBr pellet technique, our parent molecule is blended with IR-graded potassium bromide (KBr) and the pellets are subjected to an fourier-transform infrared spectroscopy (FTIR) spectrophotometer (Make: Varian, Model: 640 IR, Japan) in the range of 400 to 4000 cm^{-1} for FTIR spectral analysis.[3]

Study of Drug-Excipient Compatibility

FTIR study

The sample preparation was done by employing different combinations of our drug and other excipients (i.e., hydrotropic agents, Polymers) in a ratio of 1:1 covered in aluminum foil and stored at a relative humidity of 25°C and the IR spectra were obtained at 400 to 4000 cm^{-1}.[2]

Differential scanning calorimetry

The differential scanning calorimetry (DSC) apparatus (Model: DSC 7020, Make: Hitachi, Germany) was used to obtain the thermogram of the parent molecule and other excipients by appropriately weighing and placing our drug molecule in a closed aluminum pan along with an empty aluminum pan serving as the reference standard. The DSC apparatus was designed to scan at a speed of 10°C/min between 40 and 220°C and to flow 40 mL of nitrogen gas per minute.[2]

Pre-formulation Study

Pre-formulation batches

The preparation of introductory batches was done by using different compositions of Poloxamer 407 and Carbopol 940P based on trial and error in screw cap vials and the blend of selected hydrotropic agent (Sodium citrate) was well incorporated along with the calculated distilled water volume and stored at 4°C. At the final stage, properties like gelation temperature help us to finalize our preliminary batches, as illustrated in Table 1.[4,5]

Formulation of in-situ nasal gel

Using the cold procedure, which entails adding a determined amount of Poloxamer 407 to cold water containing 1% PEG 400 as a permeation enhancer while constantly stirring the mixture, the *in-situ* gel was prepared, which is housed at 4°C for an overnight period. On the next day, carbopol 940P was incorporated in the above solution with constant stirring with the blend of selected aqueous hydrotropic agent (Sodium citrate) containing lercanidipine HCl. Finally, the incorporation of benzalkonium chloride takes place. The prepared mixture was properly mixed by the help of a magnetic stirrer with a magnetic bar in a beaker to form the homogeneous gel and later, it was stored at 4°C.[5,6]

Design of experiment (DoE)

Using the data and considering the range of formulation variables, a preliminary batch of lercanidipine HCl *in-situ* gel, comprising Poloxamer 407 as well as Carbopol 940P polymer, was selected utilizing a 3^2 factorial design, where in Poloxamer 407 (X2) and Carbopol 940P (X1) serve as independent variables, it was optimized. Also, %cumulative drug release (Y_1), gelation temperature (Y_2), and mucoadhesive strength (Y_3) act as a dependent variable illustrated in Table 2 and the prepared formulation batch is depicted in Table 3 to which drug (%), sodium citrate, PEG 400, benzalkonium chloride and distilled water (mL) were added in 0.035, 8% w/v, 1%, 0.002% w/v and 100 mL (q.s), respectively [6,7]

Evaluation of in-situ nasal gel

The following parameters were assessed for *in-situ* nasal gel:

Visual inspection

It was performed to check the color, clarity and presence of particles by the naked eye.[8]

pH

A pH meter that had been calibrated was used to determine the produced gel's pH.[7]

Drug content

A pH 6.8 phosphate buffer was used in conjunction with a UV-visible spectrophotometer to measure the medication concentration at 337 nm.[7]

Gelation temperature ($T_{sol\text{-}gel}$)

Gelation Temperature ($T_{sol\text{-}gel}$) was determined by incorporating an aliquot solution of 2 mL of our prepared formulation in the test tube by immersing it in a water bath. Thus, by slowly raising the temperature of a water bath, the visual inspection of the sample for gelation takes place, which can be observed by checking the tilt of the meniscus at 90°, which no longer exists in a test tube.[9]

Mucoadhesive strength

The mucoadhesive potential was evaluated by applying nasal gel formulation to the bottom of an inverted glass vial by using the support of double-sided adhesive tape and the mucosal membrane was used in the experiment. For adhesion of gel formulation to the mucosal membrane, the intermediate spaces between the two vials were adjusted by applying pressure on both sides for 10 seconds. A consistent weight was applied to induce pressure for detaching both vials from the adapted balance utilized for mucoadhesion. Mucoadhesive strength (dynes/cm^2) is determined by determining the minimum weight

Table 1: Variations in the composition across different trial batches

Batch No.	*P1*	*P2*	*P3*	*P4*	*P5*	*P6*	*P7*
Poloxamer 407(%)	16	17	18	18	20	22	24
Carbopol 940P	0.1	0.2	0.1	0.3	0.2	0.1	0.2

Table 2: Levels for variable of different batches (F_1-F_9)

Independent variables	*Coded levels*		
	-1	*0*	*+1*
Carbopol 940P (w/v %)	0.1	0.2	0.3
Poloxamer 407 (w/v %)	18	20	22

Table 3: Different batches of formulation of *in-situ* nasal gel

S No	*Components*	*Formulation batches*								
		F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
1	Poloxamer 407 (% w/v)	18	20	22	18	20	22	18	20	22
2	Carbopol 940P (% w/v)	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.3

necessary to detach the mucosal membrane from the surface of each formulation.

This is calculated using the formula: Mucoadhesive strength (dynes/cm^2) = (mg) / A,

where A = the area of exposed mucosa, g = the acceleration caused by gravity (980 cm/s^2), and m = the weight necessary for separation (in grams).

Viscosity

Brookfield viscometer (Brookfield Engineering Labs.) was used to measure the viscosity with approaches like steady shear sweep test at steady temperatures of 4 and 37°C.[5,6]

Drug Release Study

In-vitro diffusion study

This *in-vitro* diffusion investigation was conducted with a Franz diffusion cell. A pH 6.8 phosphate buffer-dipped cellophane membrane acted as the substrate, onto which a gel containing 2 mg of medicine was deposited. The gel-containing membrane was then inserted into the donor compartment of the diffusion cell. To replicate physiological conditions, 37 ± 0.5°C was maintained outdoors with water, and a magnetic stirrer ensured constant stirring to prevent any influence from the receptor component's boundary layer. This experiment was run at 37 ± 0.5°C for 6 hours while being constantly stirred. Every 15 minutes for an hour, 2 mL samples were extracted from the receptor compartment, and at the same time, an equivalent volume of pH 6.8 phosphate buffer was added. Prior to analysis, the extracted materials were passed through Whatman filter paper for filtering. A UV spectrophotometer (Varian, carry 100) was used to measure the amount of medicine that had diffused at a wavelength of 337 nm after the diffusion research.[6,10]

Ex-vivo permeation study

Fresh nasal mucosa from a goat that had been slaughtered nearby was obtained for this investigation. The nasal membrane was carefully removed, cleansed with Ringer's solution, and allowed to equilibrate in receptor buffer for about an hour. After that, the mucosal membrane was placed between the donor and recipient chambers and *ex-vivo* permeation research was carried out utilizing a technique similar to the *in-vitro* diffusion study.[5,10]

Histopathological study

The histopathological examination of the nasal *in-situ* gel involved obtaining goat nasal mucosa from a local slaughterhouse. The nasal mucosal membrane was carefully isolated, and the underlying tissue was extracted. A comparison of histopathological findings was made between tissues treated with phosphate buffer of pH 6.8 and those placed in Franz diffusion cell chambers containing our formulated *in-situ* gel. Tissues were fixed using 10% buffered formalin (pH 6.2) and embedded in paraffin before being sectioned and stained with hematoxylin and eosin. Optical microscopy (Motic, Japan) was utilized to observe morphological changes in the tissue and detailed images were captured to assess histological components.[11]

Accelerated stability study

Applying the ICH's recommendations, an expedited stability evaluation of the improved batch of *in-situ* gel was successfully completed. The estimated values for the physical and chemical stability research were 40 ± 2°C and 75 ± 5% RH. A sufficient amount of thermoreversible *in-situ* gel was transferred into screw-cap vials and kept in various stability states.[11]

RESULT AND DISCUSSION

Characteristics of Lercanidipine HCl

The melting point of lercanidipine HCl was noted at 161°C and it was found to be amorphous in nature. The color was found to be slightly yellowish.[1]

UV spectroscopy

From the spectra obtained, λ_{max} of 337 nm was observed when a phosphate buffer of 6.8 was used as a solvent.[1]

FTIR spectroscopy

Every chemical structure possesses a unique FTIR spectrum, distinguishing it from others with precision. The generated FTIR spectrum gives the characteristics of the whole molecule along with the information related to the structure by referring to the peaks of each functional group. Figure 1 displays the FTIR spectra of lecarnidipine HCl, while Table 4 represents the corresponding FTIR scan value.[1]

Drug- Excipient Compatibility Study

FTIR study

This study looked into the relationship between medication and excipient incompatibility. There was no major modification in the drug's peak when measured against reference values and

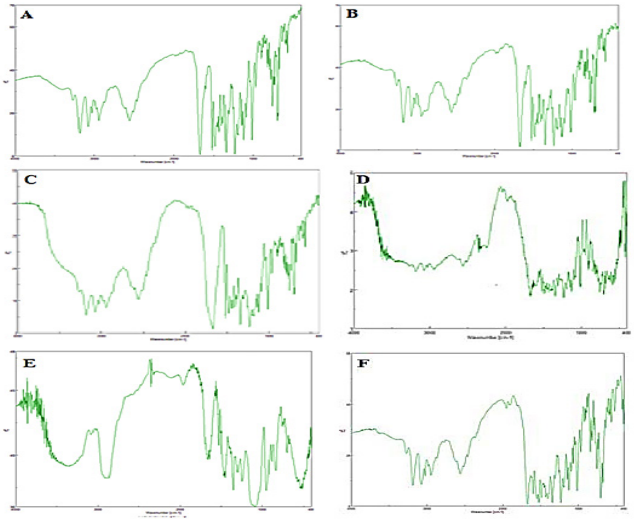


Figure 1: FTIR spectra of (A) pure lecandipine HCL, (B) lercanidipine HCl + Poloxamer 407, (C) Lercanidipine HCl + Carbopol 940P, (D) Lercanidipine HCl + Sodium citrate, (E) Lercanidipine HCl + PEG 400, (F) Lercanidipine HCl *in-situ* gel formulation

Table 4: FTIR spectrum of lercanidipine HCl

Functional group	*Range (cm^{-1})*
NH stretching vibration	3185
Stretching of alkyl and phenyl groups	3078–2800
N-H stretching	2743
C=O stretching	1671
Asymmetric and symmetric stretching of NO_2 group	1524
Bending of geminal methyl groups	1347
Out of plane bending of 5 and 3 adjacent hydrogens on aromatic rings	697

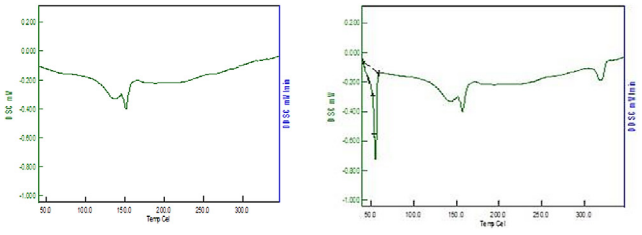
no chemical interaction can be predicted between both drug-polymer and drug-hydrotropic agents. Hence, drug-excipient compatibility can be seen from the FTIR spectra, which has been illustrated in Figure 1.[1]

Differential scanning calorimetry

The combination and pure thermographs of the medication exhibit no appreciable variation in melting point. Therefore, as seen in Figure 2, the drug's peak does not change when combined with the polymer, indicating that there is no evidence of a chemical interaction between the two. The pure drug's DSC showed a high endothermic peak at 156.4°C. Poloxamer 407 indicates a strong peak at 56.5°C, whereas its physical mixture displays a distinctive peak at 157.2°C.[1]

Pre-formulation batches

As shown in Table 5, the pH and gelation temperature of each manufactured preliminary batch were assessed. The initial attempts to meet the gelation criterion using batches of 0.1% Carbopol 940P and 16% Poloxamer 407, 0.2% Carbopol 940P and 17% Poloxamer 407, and 0.2% Carbopol 940P and 24% Poloxamer 407 were a complete failure. Thus, the 0.1 and



(I) (II)

Figure 2: DSC thermogram of (I) Pure drug, (II) Drug – Polymer mixture

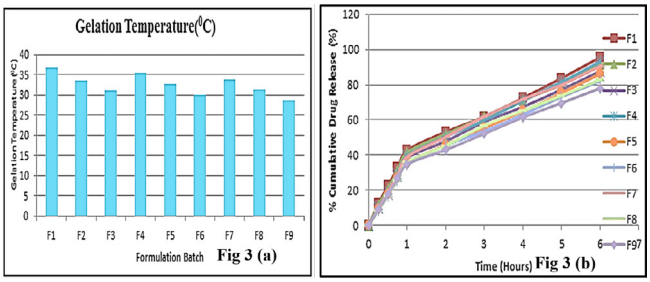


Figure 3(a): Gelation temperature of all batches of *in-situ* gel lercanidipine HCl, Figure 3(b): Study of *in-vitro* drug release of *in-situ* gel lercanidipine HCl

18% Poloxamer 407 and Carbopol 940P batches were created. Based on the aforementioned results, a factorial design was created and followed by evidence of an increase in Carbopol 940P concentration and Poloxamer 407 resulted in excellent gelation and pH that was also maintained.[1]

Design of Experiment (DoE)

Visual appearance

It was discovered that every batch of nasal gel formulations that had been made had good clarity and a translucent look.[1]

pH

The values of the pH of is well are displayed in Table 5 for all prepared formulations. The nasal mucosa's standard physiological range is 4.5 to 6.5. The prepared nasal formulation was found under the pH range of 5.23 to 5.78 by means of a pH meter.[1]

Drug content

Table 5 gives an obvious illustration of the drug content values for each formulation. On the basis of the findings, it was found that the drug content %ranged between 97.47 and 99.60, which was considered satisfactory and nearly consistent. Thus, uniformity found in drug content indicates that the procedure utilized for the preparation of gel has the capability of formulation of gel with uniform drug content and minimum variability.[1]

Gelation temperature ($T_{sol-gel}$)

The ideal range of 28.66 to 36.61°C was discovered to be the gelation temperature of all developed formulations, shown in Figure 3 (a) and Table 5; the appropriate range for the nasal gel is 32 to 35°C.[1]

Table 5: Evaluation of different batches of formulation of *in-situ* gel

Batch	*pH*	*Gelation temperature (in °C)*	*Gel strength (in sec.)*	*Drug content (%)*	*Mucoadhesive strength (dyne/cm^2)*	*%Drug release at 6 hours*
F_1	5.23 ± 0.050	36.61 ± 0.22	24.24 ± 0.781	98.40 ± 0.074	1623 ± 28.47	95.49
F_2	5.56 ± 0.063	33.7 ± 0.081	32.44 ± 0.191	98.61 ± 0.076	2707 ± 48.21	91.67
F_3	5.78 ± 0.080	31.46 ± 0.12	39.45 ± 0.109	97.56 ± 0.157	3441 ± 28.61	87.59
F_4	5.45 ± 0.041	35.63 ± 0.20	27.37 ± 0.220	99.60 ± 0.053	1795 ± 25.45	93.23
F_5	5.41 ± 0.052	32.66 ± 0.20	34.56 ± 0.275	98.48 ± 0.057	2863 ± 29.00	86.19
F_6	5.60 ± 0.037	30.26 ± 0.17	44.57 ± 0.343	97.47 ± 0.051	3736 ± 27.74	82.02
F_7	5.69 ± 0.052	33.86 ± 0.20	30.56 ± 0.374	97.78 ± 0.099	2253 ± 28.89	90.52
F_8	5.46 ± 0.030	31.33 ± 0.20	35.37 ± 0.375	97.63 ± 0.041	3140 ± 39.37	82.88
F_9	5.51 ± 0.033	28.66 ± 0.17	47.45 ± 0.191	98.35 ± 0.024	4462 ± 37.96	77.97

Table 6: Model terms for (%) drug release, gelation temperature and mucoadhesive strength.

Parameter	*%Drug release values*	*Gelation temperature values*	*Mucoadhesive strength values*	*Interpretation*
R^2 Value	0.9867	0.9952	0.9798	Model indicates high linearity
Prob > F	< 0.05	< 0.05	< 0.050	Model terms are statistically significant
Pred R-squared	0.9469	0.9895	0.9482	Predictive capability aligns well with model
Adj R-squared	0.9787	0.9936	0.9731	Adjusted for model complexity
Adequate precision	> 4	> 4	> 4	Indicates adequate signal-to-noise ratio
Signal-to-noise ratio	32.616	32.616	30.734	Strong signal quality
Influential factors	Carbopol 940P and Poloxamer 407	Carbopol 940P and Poloxamer 407	Carbopol 940P and Poloxamer 407	Factors significantly impacting the response

Mucoadhesive strength

Based on the findings, it was determined that the concentrations of Carbopol 940P and Poloxamer 407 directly correlate with mucoadhesive strength; that is, as polymer concentration rises, so does mucoadhesive strength. Table 5 illustrates this relationship and shows that mucoadhesive strength falls between 1623 and 4462 dynes/cm^2. The mucoadhesive strength is highest in batch F9 and lowest in batch F1.[1,12]

Viscosity

Measurement of viscosity (cP) of all formulations at 4 and 32°C at the shear rate of 2, 4, 10 and 20, respectively. At 4°C: All formulations (F1 to F9) exhibit higher viscosities at lower shear rates (2 and 4 rpm) compared to higher shear rates (10 and 20 rpm). Among these formulations, F5 and F9 consistently show the highest viscosities at all shear rates, suggesting a more resistant or slower drug release pattern at 4°C. At 32°C: Again, higher viscosities are generally observed at lower shear rates for all formulations. At 32°C, F9 continues to display the highest viscosities across all shear rates, indicating a slower drug release pattern even at a higher temperature. F6 also exhibits relatively high viscosities, indicating a less rapid drug release at 32°C, particularly at lower shear rates. The viscosity measurements suggest that F5 and F9 tend to have slower drug release patterns, both at 4 and 32°C, while other formulations exhibit varying degrees of viscosity and, by extension, drug release behavior under different temperature and shear rate conditions.

Drug Release Study

In-vitro diffusion study

All batches of formulation exhibited drug release in the range of 77.97 to 95.49% over a period of 6 hours as shown in Figure 3(b). Hence, it was confirmed that the release of lercanidipine HCl was affected by the concentration of Poloxamer 407 as well as Carbopol 940P.

Experimental Design

Effect of formulation variable on drug release at 360 minutes

The regression equation for the release of drugs at 360 minutes (%) for all batches given in Figure 3 (b) was established as follows

Drug release (at 360 minutes) = +92.78 – 2.74A – 5.28B

Where, Carbopol 940P and Poloxamer 407 are A and B, respectively.

The model terms given in Table 6 were found to be significant. Consequently, one may utilize the model to traverse the design space. Hence, the significance can be achieved by using model terms which indicates the adequacy of our model to possess linearity.

The interaction between Carbopol 940P and Poloxamer 407 (X1X2) was shown by a two-dimensional contour plot

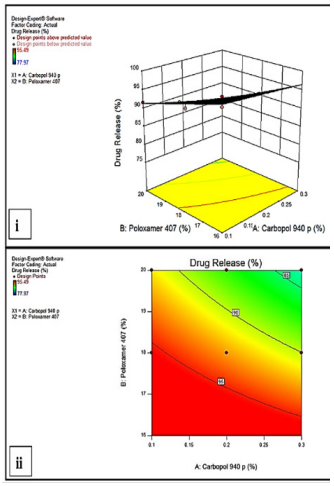


Figure 4: (I) The two-dimensional contour plot, (II) Three-dimensional surface response plot illustrating the effect on drug release of Poloxamer 407 and Carbopol 940P. [1]

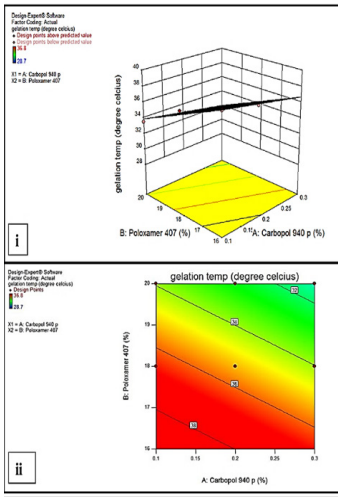


Figure 5: (I) The two-dimensional contour plot, (II) Three-dimensional surface response plot illustrating the effect on drug release of Poloxamer 407 and Carbopol 940P.

that was nonlinear and represented the interaction between two variables Figure 4(i). The inverse link between polymer concentration and drug release percentage was demonstrated by a three-dimensional response surface plot Figure 4(ii).

Effect of formulation variables on gelation temperature

This is the regression equation for gelation temperature, which was identified:

Gelation temperature = +35.32 - 1.28A- 2.67B

Where A is Carbopol 940P and B is Poloxamer 407.

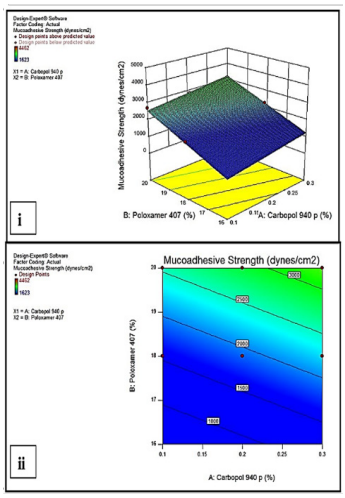


Figure 6: (I) The two-dimensional contour plot, (II) Three-dimensional surface response plot illustrating the effect on drug release of Poloxamer 407 and Carbopol 940P.

Table 6's model terms were determined to be significant. Consequently, one may utilize the model to traverse the design space.[1]

The relationship between Carbopol 940P and Poloxamer 407 (X1X2) was displayed by a two-dimensional contour plot that was nonlinear, indicating an interaction between two variables Figure 5(i). The inverse connection between polymer concentration and drug release% appears in a three-dimensional response surface plot Figure 5(ii).[1]

Formulation variables on mucoadhesive strength and its impact

The following is the regression equation for mucoadhesive strength that was found:

Mucoadhesive strength = +1896.44+347.33A-994.67B

Where A is Carbopol 940P and B is Poloxamer 407.

Table 6 model terms were determined to be significant. Consequently, one may utilize the model to traverse the design space.[1]

The relationship between Carbopol 940P and Poloxamer 407 (X1X2) was displayed by a two-dimensional contour plot that was non-linear, indicating an interaction between two variables Figure 6(i). The inverse connection between polymer concentration and drug release % appears in a three-dimensional response surface plot Figure 6(ii).[1]

Validation of statistical model

Several gel formulations were created and adjusted based on mucoadhesive strength (Y3), gelation temperature (Y2), and

Table 7: Compression of predicted and actual values of various parameter

Polymers	*Coded level*	*Actual level*	*Response*	*%Drug Release*	*Gelation temperature*	*Mucoadhesive strength*
			Predicted value	*92.78*	*35.32*	*1896.44*
			Observed value	*93.23*	*35.4*	*1795*
Carbopol 940P	0	0.2	Standard deviation	0.844	0.2057	151.26
Poloxamer 407	0	18	Standard error of the mean	0.4448	0.1084	79.721

Table 8: Accelerated stability assessment of the optimized formulation

Sr no	*Observation*	*Before stability testing*	*After stability testing*			
			1 month	*2 month*	*3 month*	*6 month*
1	pH	5.35	5.33	5.32	5.25	5.29
2	Drug content (%)	98.73	98.29	97.5	98.18	98.35
3	Gelation temperature (°C)	34.51	34.56	34.63	34.69	34.78

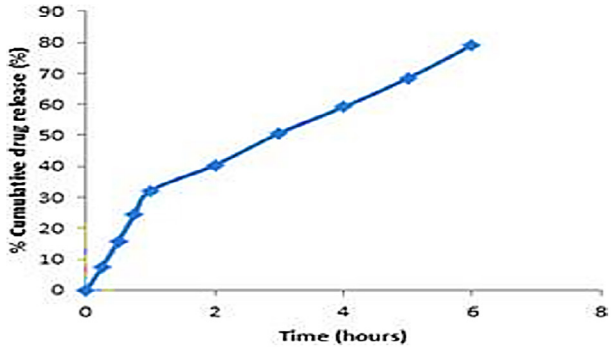


Figure 7: *Ex-vivo* permeation study of optimized batch (F_4).

in-vitro drug release (Y1) after model equations derived from the effects and responses were formed. The ideal values of these answers were ascertained by numerical analysis and desirability criteria; design-expert software was then utilized to choose the optimized batch. The estimates of the percentage drug release at 360 minutes, the gelation temperature, and the mucoadhesive strength were all within a very small range of the anticipated values as shown in Table 7, indicated by the minimal standard deviation for the gelation temperature and the design-expert software for the mucoadhesive strength. The F4 batch demonstrated a highly optimized batch, hence demonstrating the effective validation of our model.[1]

Ex-vivo permeation study

From the study of the validated statistical model, an *ex-vivo* permeation study was performed, given in Figure 7, only for batch F_4, which was found to be highly optimized and the criteria of selecting batch F_4 was done from the evaluation parameters like gelation temperature as well as *in-vitro* diffusion study. The value of *ex-vivo* drug permeation study of this optimized batch was 79.14% at 6 hours. From Figure 7, we can depict the drug permeation exhibits a synergistic mechanism with *ex-vivo* drug release.[1]

Histopathological study

From the study of a validated statistical model, batch F_4 was selected for the purpose of histopathology study of control and treated nasal mucosa. It was well observed from Figure 8. From the observations of microscopic data, formulation F_4 doesn't show any changes in the microscopic structure of goat nasal mucosa and, thus, exhibits the safety and nil irritation effect of *in-situ* gel formulation on goat nasal mucosa.[13-15]

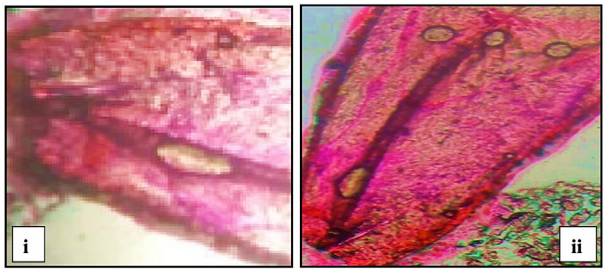


Figure 8: Goat nasal mucosa histopathology (I) following phosphate buffer treatment (pH 6.8) and (II) following *in-situ* nasal gel formulation therapy.

Accelerated stability study

Based on the results of stability tests carried out on the refined formulation, it is reasonable to believe that the qualities of formulations stay the same (F4), it showed clarity & transparency before, during and after the testing period. Table 8 illustrates that the drug content of the optimized formulation that was generated was found to be consistent prior to the stability research.

CONCLUSION

Utilizing a 3^2-factorial design method, nasal *in-situ* gel was efficiently created utilizing Poloxamer 407 and Carbopol 940P as independent variables for thermoreversible *in-situ* gel optimization for nasal release of lercanidipine HCl. F4 was discovered to be noteworthy and significant in connection to mucoadhesive strength, gelation temperature, and drug release percentage. It was discovered that the highly optimized batch F4 had a medication release percentage of 99.60 ± 0.053% in relation to the cellophane membrane. The microscopic examinations show that the goat nasal mucosa's microscopic structure is not significantly affected, indicating that the mucosa's structure is well-maintained. Based on these findings, it may be inferred that lercanidipine HCl can avoid the first pass metabolism impact. Additionally, the nasal dosage form exhibits enhanced bioavailability, allowing for a potential reduction in dosing frequency.

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REFERENCES

1. Fatouh AM, Elshafeey AH, Abdelbary A. Intranasal agomelatine solid lipid nanoparticles to enhance brain delivery: formulation, optimization and in vivo pharmacokinetics. Drug Design, Development and Therapy. 2017;11:1815-1825. Available from: doi.org/10.2147/DDDT.S102500
2. Ahmed EM. Hydrogel: Preparation, characterization, and applications: A review. Journal of Advanced Research. 2015;6:105-121. Available from: doi.org/10.1016/j.jare.2013.07.006.
3. Reddy MS, Navya SK, Ul Haq S. Method development and

validation of UV spectroscopic method for the determination of lercanidipine. World Journal of Pharmaceutical Research. 2015;4(10):1341-1348.
4. Pande V. Exploration of mixed hydrotropy strategy in formulation and development of ketorolac injection. Journal of Nanomedicine Research. 2016;3(4):1-7. Available from: doi.org/10.15406/jnmr.2016.03.00063.
5. Godbole MD, There PW, Dangre PV. Formulation and optimization of prolonged release nasal in situ gel treatment of migraine. Indo American Journal of Pharmaceutical Research. 2014;4(2):1320-1332. Available from: doi.org/10.1016/j.ejps.2015.01.
6. Ahiwale R, Mahaparale P, Chakor R. Formulation and evaluation of nasal in situ gel of bupropion hydrochloride. World Journal of Pharmacy and Pharmaceutical Sciences. 2015;4(1):595-614.
7. Agrawal S, Maheshwari RK. Formulation development of in-situ nasal drug delivery system of poorly water soluble drug (Indomethacin) using mixed solvency concept and their evaluation. Bulletin of Pharmaceutical and Medical Sciences. 2014;2(1):2128-2138. Available from: doi.org/10.4103/0973-8398.91988.
8. Mahakalkar NG, Upadhye KP. Zolmitriptan nasal in-situ gel using sterculia foetida Linn gum as natural mucoadhesive polymer. International Journal of Pharmaceutical Sciences Review and Research. 2013;22(3):206-213.
9. Nerella A, Dontha P, Uppuluru A, Konda SK. Formulation and evaluation of in situ mucoadhesive nasal gel of montelukast sodium. Der Pharmacia Sinica. 2014;5(2):1-8.
10. Sathe S. Formulation and evaluation of thermo reversible in-situ nasal gel of terbutaline sulphate. Indo American Journal of Pharmaceutical Research. 2015;5(9):3680-3687. Available from: doi.org/10.1208/s12249-017-0747-8.
11. Parmar VJ, Lumbhani AN. Formulation and development of thermo-reversible mucoadhesive intranasal in situ hydrogel by using a combination of polymers. Bulletin of Pharmaceutical Research. 2012;2(3):167-174.
12. Mythri G, Kavitha K, Kumar MR, Singh SJ. Novel mucoadhesive polymers – a review. Journal of Applied Pharmaceutical Science. 2011;1(8):37-42.
13. Yadav DJ, Kunjwani HK, Suryawanshi SS. Formulation and evaluation of thermosensitive in situ gel of salbutamol sulphate for nasal drug delivery system.
14. Devasani SR, Dev A, Rathod S, Deshmukh G. An overview of in situ gelling systems. Pharmaceutical and Biological Evaluations. 2016;3(1):60-69.
15. Dargude S, Dhake P, Hole A, Pinjari RR, Hingne S. A Review on Curcumin Loaded Nano Drug Delivery Systems: Nano Formulations and Recent Advances. European Chemical Bulletin. 2023;12(7):5986-6002. Available from: doi.org/10.48047/ecb/2023.12.si7.524.