https://doi.org/ 10.33472/AFJBS.6.5.2024. 6884-6911

African Journal of Biological Sciences

Formulation, characterization and evaluation of *in-situ* nasal gel of Amitriptyline Hydrochloride

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Article History
Volume 6, Issue 5, 2024
Received: 15 May 2024
Accepted: 22 May 2024
doi: *10.33472/AFJBS.6.5.2024. 6884-6911*

ABSTRACT

***Introduction*:** Amitriptyline Hydrochloride faces challenges related to extensive hepatic first-pass metabolism, limiting its bioavailability.

Objectives: The study aimed to formulate and evaluate thermoreversible in-situ nasal gel formulations of Amitriptyline Hydrochloride.

Methodology: Thermally triggered in-situ nasal gels were prepared using Poloxamer 407 as the gelling polymer and HPMC K4M as the mucoadhesive polymer. Formulations underwent comprehensive characterization, including clarity assessment, pH determination, gelation temperature measurement, viscosity analysis, and determination of drug content. Mucoadhesive strength, gel strength, *In-vitro* and *Ex-vivo* drug release studies, and histopathological examination were conducted.

Results: Formulations exhibited clear appearances with gelling temperatures ranging from 39°C to 32°C. Drug content exceeded 78.33% across all formulations. Viscosity increased with temperature and polymer concentration. pH values fell within the nasal physiological range (4.7-5.9). The optimized formulation (F4) demonstrated 96.64% *In- vitro* drug release in 8 hours and 87.52% *Ex-vivo* drug release. Histopathological examination revealed no adverse effects.

Conclusion: Thermoreversible in-situ nasal gels offer a promising drug delivery strategy for Amitriptyline Hydrochloride, circumventing hepatic first-pass metabolism and enhancing drug bioavailability. The developed formulations exhibit favorable properties and show potential as a therapeutic option for depressive disorders.

Key words: *In-situ* nasal gel, Amitriptyline Hydrochloride, Thermoreversible nasal gels, Poloxamer 407, HPMC K4M

INTRODUCTION

Depression stands as a pervasive mental health challenge globally, affecting a substantial portion of the population. With an estimated 3.8% of the populace, including 5.0% of adults, grappling with this condition, its impact reverberates across communities, families, and individuals. Alarmingly, depression's severity can culminate in dire outcomes such as suicide, underscoring the urgency of effective intervention strategies. Central to understanding and addressing depression is recognizing its neurobiological underpinnings, chiefly characterized by the dysregulation of neurotransmitters like serotonin, norepinephrine, and dopamine.

In clinical practice, various antidepressants offer avenues for mitigating depressive symptoms and restoring individuals' wellbeing. Among these, tricyclic antidepressants (TCAs) constitute a notable therapeutic option, with amitriptyline emerging as a cornerstone in managing conditions like anxiety or agitated depression. Renowned for its potent sedative effects and efficacy, amitriptyline holds promise in ameliorating mood disturbances, alleviating anxiety, and improving sleep quality [1-3]

However, conventional oral formulations of amitriptyline encounter hurdles such as hepatic first-pass metabolism and limited gastrointestinal permeability, impeding optimal drug delivery and bioavailability. Moreover, traversing the blood-brain barrier presents an additional challenge, necessitating innovative strategies to enhance therapeutic efficacy.

Nasal Route:

The nasal cavity, long revered for its therapeutic potential in ancient healing practices like Ayurveda's 'Nasyakarma,' emerges as a promising conduit for drug delivery. With its intricate anatomy and physiological characteristics, the nasal cavity facilitates efficient systemic drug absorption, including access to the central nervous system (CNS). Notably, the respiratory region, rich in permeable mucosa and vascular networks, serves as a primary site for drug absorption [4].

In-Situ Nasal Gel:

Aiming to surmount limitations associated with conventional oral formulations, in-situ nasal gels offer a compelling solution for enhancing drug bioavailability and efficacy. These temperature-responsive formulations undergo phase transition upon contact with nasal mucosa, transitioning from a liquid to gel state. Leveraging stimuli-responsive polymers like Poloxamer 407, these gels exhibit superior mucoadhesive properties, ensuring prolonged drug retention and sustained release [4-5].

Objectives of the study

1. Formulate thermoreversible in-situ nasal gel formulations of Amitriptyline Hydrochloride.
2. Optimize polymer concentrations in the nasal gel formulations.
3. Characterize the properties of the nasal gel formulations and evaluate their drug release profiles and mucosal compatibility.

MATERIALS AND METHOD:

The pure drug Amitriptyline Hydrochloride was purchased from Dhamtech pharma consultant (Navi Mumbai), Poloxamer 407 was purchased from Yarrow Chem (Ghatkopar, West Mumbai,India) and HPMC K4M was obtained as a gift sample from Colorcon Asia Pvt. Ltd. (Verna, Goa, India). All other reagents used were of analytical grade. Distilled water was used for the study.

➢ *Pre-formulation Studies:*

UV Estimation:

A standard stock solution of Amitriptyline Hydrochloride was prepared by dissolving 10mg of the drug in a 100ml volumetric flask using a mixture of methanol and distilled water (9:1) as the solvent. The volume was adjusted to 100ml with diluent. From this stock solution, 25ml was pipetted into a separate 100ml volumetric flask, and the volume was made up to the mark with diluent (methanol: distilled water, 9:1 v/v). Further aliquots were prepared from this solution within the Beer's range (5-25µg/ml). Absorbance of each solution was measured at selected wavelengths (λmax) ranging from 200-400nm against a blank containing only the diluent [6].

Fourier transforms infrared spectral studies (FTIR):

FTIR spectra were conducted on pure Amitriptyline Hydrochloride and the polymers Poloxamer 407 and HPMC K4M, both individually and in 1:1 combinations, to investigate potential chemical interactions. Spectra were analyzed from 4000 to 350 cm-1 using an FTIR spectrophotometer (IRSPIRIT-L2 21) to assess any interference of polymers with the drug [7-8].

Differential scanning colorimeter (DSC) method:

DSC measurements were conducted using a Shimadzu DSC 60 plus. Samples (4-6 mg) of pure drug and physical mixture were sealed in aluminum pans and heated under nitrogen gas flow (20 mL/min) at 10°C/min from 50 to 300°C [1].

X-Ray Diffraction (XRD):

X-ray diffraction patterns were obtained for pure drug and physical mixture using a Rigaku SmartLAB SE X-ray diffractometer. Measurements were conducted over a 2θ range of 5-90° at a scan rate of 10.00°/min [3].

Experimental Design: In the present study randomized full factorial design (3^2) was used for the preparation of in-situ nasal gel using SYSTAT software 13.2 version. Two factors were evaluated for three levels and obtained number of batches was prepared. Two independent variables chosen were concentration of Poloxamer 407 (22, 23 and 24%) and HPMC K4M (1, 1.25 and 1.5%). Dependent variables were the gelation temperature, mucoadhesive strength (dyne/cm^2) and percent cumulative drug release after 8 hours.

- **Preparation of in-situ nasal gel**

The cold method was adopted for preparation of in-situ nasal gel. The weighed quantity of Poloxamer 407 is slowly dissolved in cold water with continuous stirring with the help of magnetic stirrer at 500rpm for 15min. This solution is then refrigerated overnight to form a clear solution. The mucoadhesive polymer, permeation enhancer, preservative and drug were slowly added to the above mixture with continuous stirring. This solution was kept in the refrigerator until it forms a clear liquid [9].

- **Preparation of simulated nasal electrolyte solution (SNES):**

8.77g of sodium chloride (NaCl), 2.98g of potassium chloride (KCl) and 0.59g of calcium chloride ($CaCl_2$) were weighed and dissolved in 1000ml of distilled water. The pH was adjusted to 5.5 and kept at 37± 5℃ temperature [10].

Evaluation of Amitriptyline Hydrochloride in-situ nasal gel

1) Clarity:

To check the clarity of the formulation we have used the technique of visual inspection in front of black and white background.

2) Gelation temperature:

2 mL of the prepared gel was added to test tubes and heated gradually in a water bath at a rate of 1°C/minute. Gelation was confirmed when the meniscus ceased to move upon tilting the test tube at a 90° angle [11,12].

3) Gelation time:

A glass slide was equilibrated in a water bath at approximately 37°C for 15-20 minutes. A single drop of the formulation was placed on the slide, maintained at a 120° angle, and the time for gel formation was recorded [13].

4) Determination of gelling capacity:

Gelling capacity was determined based on the formulation behaviors like gelling time and erosion time of formed gel due to the environmental changes [14].

\+ - Gelled after few minutes and dissolves rapidly (within minutes).

++ - Gelled after few minutes and remains intact for few hours.

+++ - Gelled immediately and remains intact for extended period of time.

5) Measurement of Gel Strength:

An accurately weighed quantity (10g) of gel was placed in a 25ml measuring cylinder and was allowed to form a gel.A weight of 5gm was placed on the gel. The time taken by the weight to sink 5cm down the gel was measured [14].

6) Drug content:

1ml of gel was taken in 10ml volumetric flask then it was diluted with 10ml of methanol. Aliquot 1ml from this solution was diluted up to 10ml methanol again to get the final concentration. The absorbance of prepared solution was measured at 240nm by using UV visible Spectrophotometer [15].

$$\%\ \textbf{Drug content} = \frac{\textbf{concentration of drug in sample solution}}{\textbf{Equivalent conc.of drug taken}} \times \mathbf{100} \text{--- (2)}$$

7) pH:

pH of all the formulations were determined by using Digital pH meter. This was previously calibrated by pH 4 and pH 7. The pH values were recorded immediately after preparation and after 15 days.

8) Rheology Study:

The rheological properties of gels were determined by the Brookfield viscometer. Viscosity of the formulations at solution states i.e. at 25ºC and in gel state at 37ºCwith Spindle No. TL6 at 10, 20,30,40,50 and 60rpm was measured in cps [15].

9) Spreadability :-

Spreadability was assessed by placing approximately 1 g of the formulation at the center of a glass slide post-gelation. Another slide was gently positioned atop the formulation with a weight of approximately 1 kg. After 1 minute, the spread circle diameter was measured in cm [16].

10) Mucoadhesive Strength:

Mucoadhesive strength was assessed using a modified weighing balance setup. The mucosal membrane was fixed between two glass slides moistened with SNES pH 5.5. Gel was applied, and a contact time of two minutes was allowed. Gradual weight addition in the right pan detached the mucosa. Mucoadhesive force (detachment stress) was calculated in dyne/cm2 using the equation [17].

Detachment stress(dyne/cm^2)=(m×g)/A

Where,

m=Weight required for detachment in grams,

g = Acceleration due to gravity [980cm/s^2],

A = Area of tissue exposed ($A = \pi r^2$)

11) *In- vitro* drug release through diffusion studies:

The drug permeation study involved using a Franz diffusion cell with a dialysis membrane of 12,000-14,000 KDa molecular weight cutoff and 70µ pore size. Prior to use, the dialysis membrane was pre-treated with SNES pH 5.5. The membrane was positioned between the donor and receptor compartments, with the gel containing 10mg of the drug applied onto its surface. The receptor compartment held 12ml of SNES pH 5.5. The cell was stirred at 50rpm and kept at 37℃. At hourly intervals over 8 hours, 1ml samples were withdrawn and replaced with equal volumes of SNES. Absorbance was measured at 240nm to assess drug permeation [18,19].

12) *Ex-vivo* release through diffusion studies:

Fresh goat nasal mucosa was washed and used in a Franz diffusion cell with SNES (pH 5.5) in the acceptor chamber. The donor chamber contained 10mg Amitriptyline hydrochloride. Samples were withdrawn at 1-hour intervals over 8 hours, replaced with SNES. Analysis was done spectrophotometrically at 240nm to determine drug permeation [20,21].

13) Histopathological Evaluation of Mucosa:

Histopathological evaluation was conducted on tissues incubated in simulated nasal electrolyte solution (SNES) at pH 5.5 and compared with tissues exposed to the gel formulation in the diffusion chamber. Tissue sections of 4µm thickness were prepared and stained with hematoxylin and eosin. Examination under a light microscope by a blinded pathologist aimed to detect any tissue damage during the *In- vitro* permeation process [22].

14) Statistical Analysis:

The data of pharmacodynamic study was analyzed by SYSTAT software 13.2 by using Full factorial design. Statistical comparison of results was performed using ANOVA. Data were considered statistically significant when $p<0.05$ [7,8].

RESULTS AND DISCUSSION

- ***Pre-formulation Studies:***

a) **UV- Visible spectrophotometric studies:**

The standard calibration curve for Amitriptyline hydrochloride was developed using a solvent mixture of methanol and distilled water (9:1). Dilutions were prepared to achieve final concentrations of 5, 10, 15, 20, and 25µg/ml. Absorbance of each concentration was measured at 240nm using a UV spectrophotometer. The resulting calibration curve confirmed that the drug follows Beer's law within the range of 5-25µg/ml in the specified solvent. The linear regression equation generated was Y=0.0073x + 0.0223, with a correlation coefficient (R^2) of 0.993. UV spectrum of Amitriptyline Hydrochloride is shown in Figure 1.

b) **Fourier transform infrared spectroscopy (FT-IR):**

The drug was mixed with polymers in a 1:1 ratio to produce a physical mixture, which was stored for 1 month. At the end of the month, samples were analyzed for physical and chemical changes using FTIR spectroscopy. The spectra showed that the characteristic peaks of the pure drug were retained in both the drug and physical mixture samples. This indicates that no incompatibilities were observed between the drug and the excipients. FTIR Spectrum

of pure drug and physical mixture is shown in Figure 2 and Figure 3.

c) Differential scanning calorimetry (DSC):

The drug and polymers were mixed in a 1:1 ratio to produce a physical mixture. Differential scanning calorimetric (DSC) analysis revealed that the endothermic peak of the pure drug ranged from 195.60℃ to 205.60℃. In contrast, the endothermic peak of the physical mixture began at 190.94℃ and ended at 200.30℃. Amitriptyline hydrochloride displayed a sharp endothermic peak at 198.47℃ with an enthalpy of 65.18J/g, consistent with literature. However, in the physical mixture, it exhibited an endothermic peak around 196.08℃ with an enthalpy of 30.98J/g. DSC thermogram of pure drug and physical mixture was shown in Figure 4 and Figure 5.

d) X-Ray Diffractometry (XRD):

The drug and polymers were mixed in a 1:1 ratio to form a physical mixture, which was analyzed using powder X-ray diffractometry. Results showed sharper and more intense peaks in the physical mixture compared to the pure drug, indicating an enhancement in its crystalline nature. No interactions between the components were observed. XRD pattern of pure drug Amitriptyline Hydrochloride and physical mixture.was shoen in Figure 6 and Figure 7.

➢ Evaluation of Formulated Amitriptyline

1) Clarity:

All the formulations were found to be clear and transparent without any foreign particles.

2) Gelation temperature:

The gelation temperature, crucial for transitioning liquid to gel, ranged from 29-39°C, aligning with the 32-35°C range of nasal mucosa. Gelation below 25°C poses manufacturing and handling challenges, while higher temperatures lead to nasal dripping. Increasing poloxamer 407 and HPMC K4M concentrations lowered gelation temperatures by enhancing

intermolecular bonding. Combining HPMC K4M with poloxamer 407 adjusted gelation to match nasal physiological temperature effectively shown in table 2.

3) Gelation time:

The time to transition from solution to gel, known as gelling time, for all formulations was within seconds. This rapid gelation occurred well before muco ciliary clearance (MCC) time, eliminating the risk of expulsion from the nasal cavity due to MCC

4) Determination of gelling capacity:

Formulations F1 and F2 gelled within minutes but dissolved quickly, whereas F3 and F4 gelled within seconds and maintained integrity for a few minutes. In contrast, formulations F5 to F9 underwent immediate gelation and sustained the gel state for an extended duration. The values are depicted in table 2

5) Measurement of gel strength:

A 10ml sample of each formulation was poured into a 25ml measuring cylinder and converted into gel. Then, a 5g weight was added, and the time taken for it to sink 5cm was recorded. Gel strength below 25 seconds may lead to washout from the nasal cavity, while over 50 seconds may cause discomfort due to stiffness. Increasing polymer concentration resulted in a denser lattice pattern and increased gel strength. Higher percentages of mucoadhesive agent correlated with greater gel strength. All formulations exhibited suitable gel strength. The values are depicted in table 2

6) Drug content:

Drug content of all the formulations was found in the range of 91.22 to 96.23% which indicates that drug is uniformly distributed in polymer. Hence, the gel was capable of giving uniform drug content with minimum variability. The values are depicted in table 2

7) pH:

The nasal mucosa's pH range (4.5-6.5) supports lysozyme's antimicrobial action, while an alkaline environment renders it inactive, increasing infection risk. Formulations should maintain a pH within this range, as shown in the table, ensuring non-irritancy and stability even after 15 days. The values are depicted in table 2

8) Viscosity study:

Viscosity measurements were taken pre and post gelation for all formulations at different RPMs. Optimal viscosity is crucial for in-situ gel, ensuring easy administration and conversion to gel upon contact with nasal conditions. Viscosity increased with temperature and polymer concentration but decreased with higher shear rates (RPM). The values are depicted in table 3

9) Spreadability:

Spreadability test for the nasal formulation was done and it was observed that as the concentration of polymers increased the spreadability decreased. The results indicated that the *in-situ* nasal gel has excellent spreadability according to acceptable range (2.5 – 7.5cm) which is desired for the application of the nasal *in-situ* gel. The values are depicted in table 3

10) Mucoadhesive strength:

All formulations underwent mucoadhesion testing, revealing increased mucoadhesive strength with higher concentrations of polymers, specifically poloxamer 407 and HPMC K4M. This enhancement is attributed to HPMC's wetting and swelling, enabling close contact with mucin molecules and forming weak chemical bonds between entangled chains. Strong mucoadhesive force prevents the gelled solution from exiting the nasal cavity. However, excessive mucoadhesive strength can result from higher levels of HPMC. The values are depicted in table 3

11) In- vitro drug release through diffusion study:

In the *In- vitro* drug release studies conducted over 8 hours, formulations F1, F2, F4, and F5 exhibited maximum cumulative drug release percentages, ranging from 92.20% to 96.64%. Conversely, formulations F3, F6, F7, F8, and F9 demonstrated controlled drug release. Increased polymer concentrations led to a significant decrease in cumulative drug release percentages, attributed to higher formulation viscosities hindering drug diffusion by reducing water channels. The addition of PEG 400 aimed to enhance drug permeation by opening tight junctions between cells and increasing vascularity at the basal membrane, facilitating absorption of hydrophilic drugs across the mucus membrane. Graphical representation of *In-vitro* drug release of F1-F9 formulations was shown in Figure 8.

12) *Ex-vivo* drug permeability study:

Ex-vivo diffusion study of the optimized formulation (F4) was carried out for 8hours with goat nasal membrane which showed 87.52% drug release. Compared to dialysis membrane the drug release from the goat nasal membrane was found to be less due to the thickness of nasal membrane. *Ex-vivo* drug permeation study of optimized formulation (F4) was shown in Table 4

13) Histopathology:

Histopathology studies were carried out for goat nasal membrane incubated with optimized formulation (F4) and goat nasal membrane incubated in SNES at pH 5.5. In histological sections of normal nasal mucosa and the mucosa treated with *in-situ* nasal gel. They showed pseudo stratified ciliated columnar epithelium with sub epithelial seromucinous glands. There was no such evidence of hemorrhage or necrosis found in *in-situ* gel treated nasal mucosa. Histopathological study of goat nasal mucosa Incubated in pH 5.5 -Control and Histopathological study of goat nasal membrane for optimized formulation (F4) was shown in Figure 9 and Figure 10.

14) Statistical analysis of 3^2 full factorial design:

All 9 formulation batches were developed using two variables as Poloxamer 407 (X_1) and HPMC K4M (X_2) and three responses, Gelation studies (Y_1), mucoadhesive strength (Y_2) and % cumulative drug release (Y_3) using 3^2 full factorial design. The results of the regression analysis for response Y_1, Y_2 and Y_3 were obtained with 3D response surface plots of fitted model and residual v/s predicted were obtained. Positive coefficient indicated that response is favorable, while a negative value indicates that the response is unfavorable. P value less than 0.05 indicated it to be significant. All the variables with their results are shown in Table No.6

➢ Effect of formulation variable on Gelation temperature (Y1):

- In an OLS regression analysis of gelation temperature, the model demonstrated a multiple R value of 0.921 and an R^2 value of 0.848. These values indicate a strong linear relationship and suggest that the model explains 84.8% of the variance in gelation temperature. The efficiency of the model is 92.1% shown in Table No. 7.
- Regression analysis revealed that both Poloxamer 407 ($p = 0.003$) and HPMC K4M ($p = 0.015$) significantly affect gelation temperature, with p-values less than 0.05 indicating statistical significance(Table No.8). A 3D response surface graph showed that increasing concentrations of these agents reduce gelation temperature(Figure 11,12).

- ANOVA results supported the model's validity with a regression source p-value of 0.004(Table No.9). The polynomial equation derived from the regression coefficients is:
- Gelation temperature (Y1)=37.889−3.500X1−2.500X2\text{Gelation temperature (Y1)} = 37.889 - 3.500X1 - 2.500X2 Gelation temperature (Y1)=37.889−3.500X1−2.500X2
- This equation reflects the significant impact of both factors on gelation temperature.

➢ Effect of formulation variable on in-vitro drug release (Y2):

- In an OLS regression analysis of in-vitro drug release, the model showed a multiple R value of 0.936 and an R^2 value of 0.876, indicating a strong linear relationship. The model is 93.6% efficient, explaining 87.6% of the variance in drug release (Table No. 10).
- Regression analysis identified that both Poloxamer 407 (p = 0.004) and HPMC K4M (p = 0.004) significantly impact in-vitro drug release, with p-values less than 0.05(Table No. 11). A 3D response surface graph indicated that increasing concentrations of these agents reduce drug release (Figure 13,14).
- ANOVA results confirmed the model's validity with a regression source p-value of 0.002(Table No. 12). The derived polynomial equation for drug release is:
- In-vitro drug release (Y2)=96.732−2.615X1−2.632X2\text{In-vitro drug release (Y2)} = 96.732 - 2.615X1 - 2.632X2 In-vitro drug release (Y2)=96.732−2.615X1−2.632X2
- This equation reflects the significant effects of both factors on in-vitro drug release.

➢ Effect of formulation variable on Mucoadhesive strength of formulation (Y3):

- In an OLS regression analysis of mucoadhesive strength, the model showed a multiple R value of 0.950 and an R^2 value of 0.903, indicating a strong linear relationship. The model is 95.0% efficient, explaining 90.3% of the variance in mucoadhesive strength (Table No. 13).
- Regression analysis identified that both Poloxamer 407 (p = 0.001) and HPMC K4M (p = 0.006) significantly impact mucoadhesive strength, with p-values less than 0.05(Table No. 14). A 3D response surface graph indicated that increasing concentrations of these agents enhance mucoadhesive strength (Figure 15,16).
- ANOVA results confirmed the model's validity with a regression source p-value of 0.001(Table No. 15). The derived polynomial equation for mucoadhesive strength is:
- Mucoadhesive Strength (Y3)=923.297+195.063X1+130.042X2\text{Mucoadhesive Strength(Y3)}=923.297+195.063X1+130.042X2Mucoadhesive Strength (Y3)=923.297+195.063X1+130.042X2

- This equation reflects the significant effects of both factors on mucoadhesive strength.

FIGURES

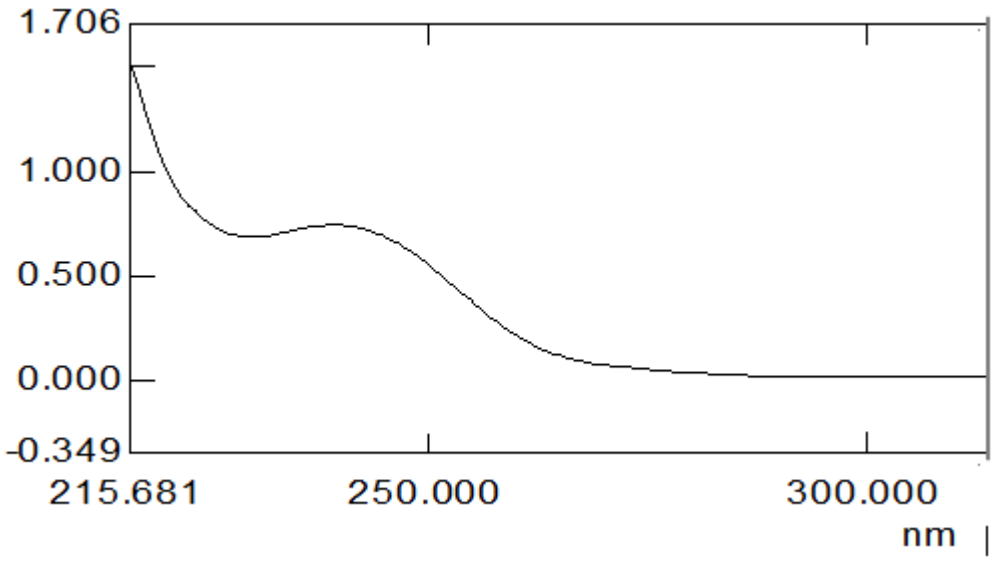

Figure 1: UV spectrum of Amitriptyline Hydrochloride

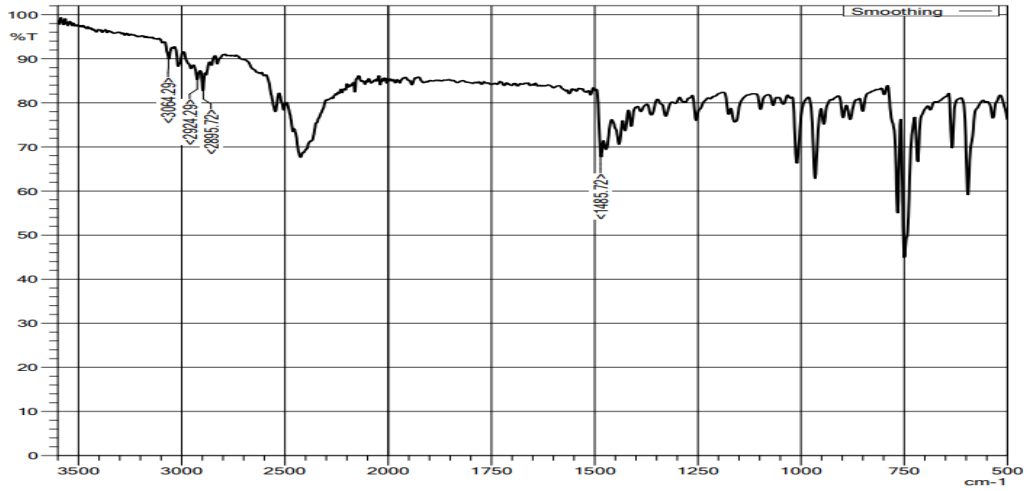

Figure 2: FTIR spectrum of pure drug

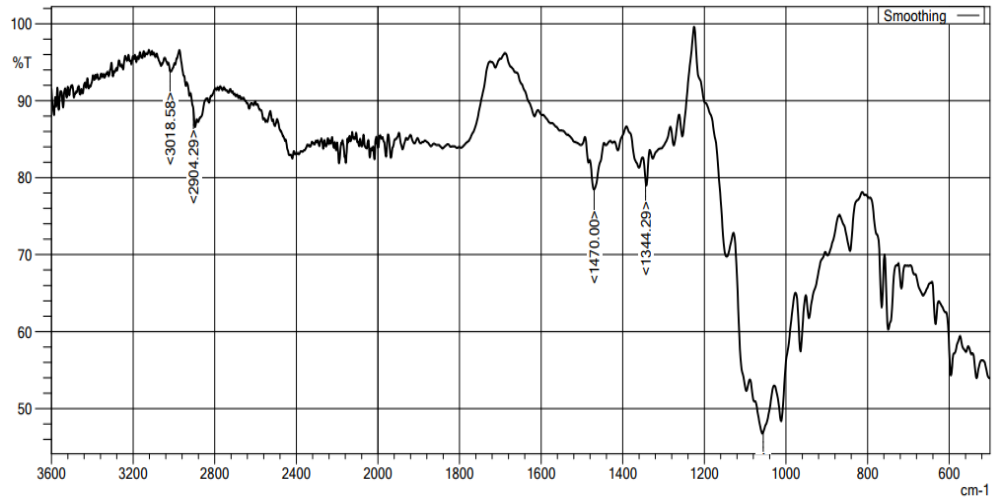

Figure 3: FTIR spectrum of Physical mixture

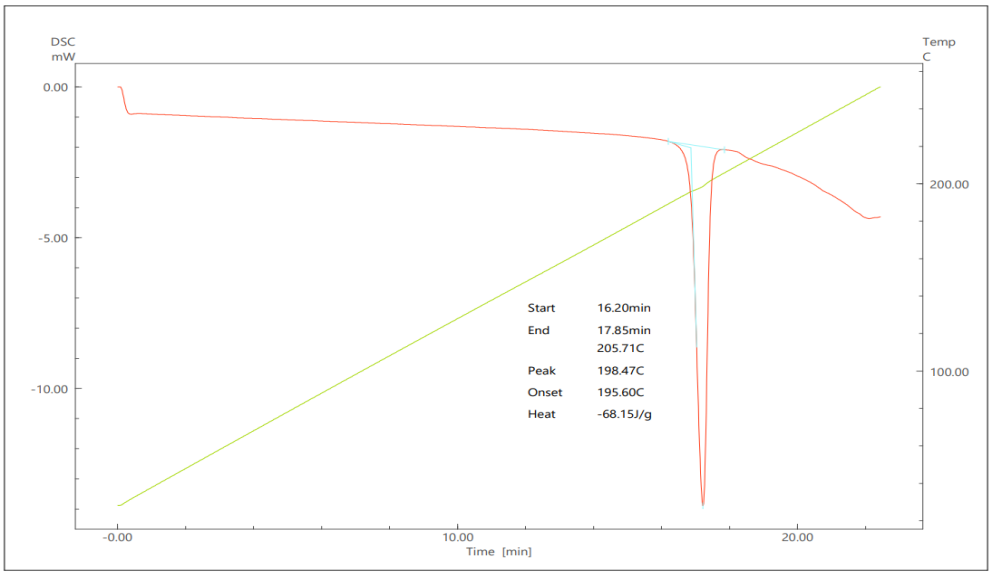

Figure 4: DSC thermogram of pure drug

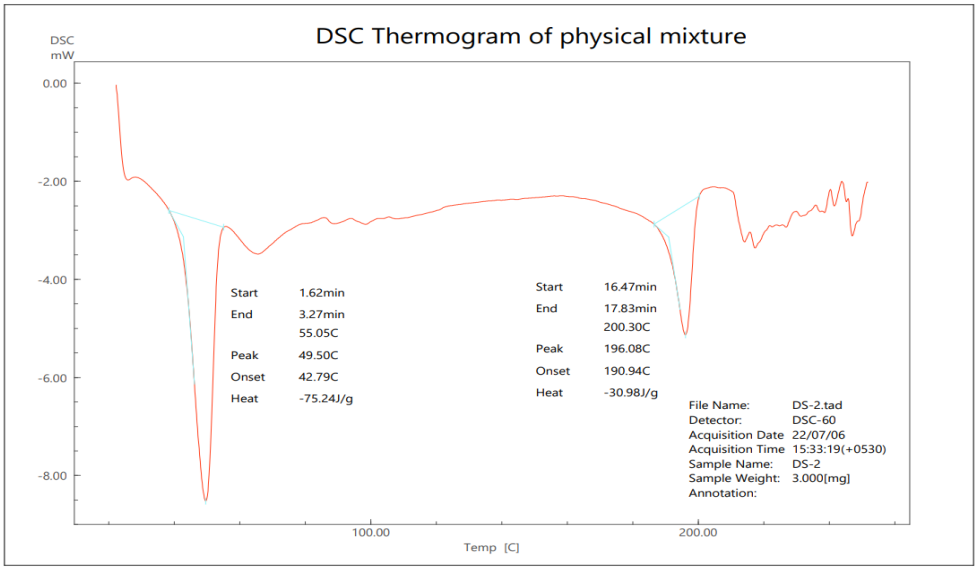

Figure 5: DSC thermogram of physical mixture

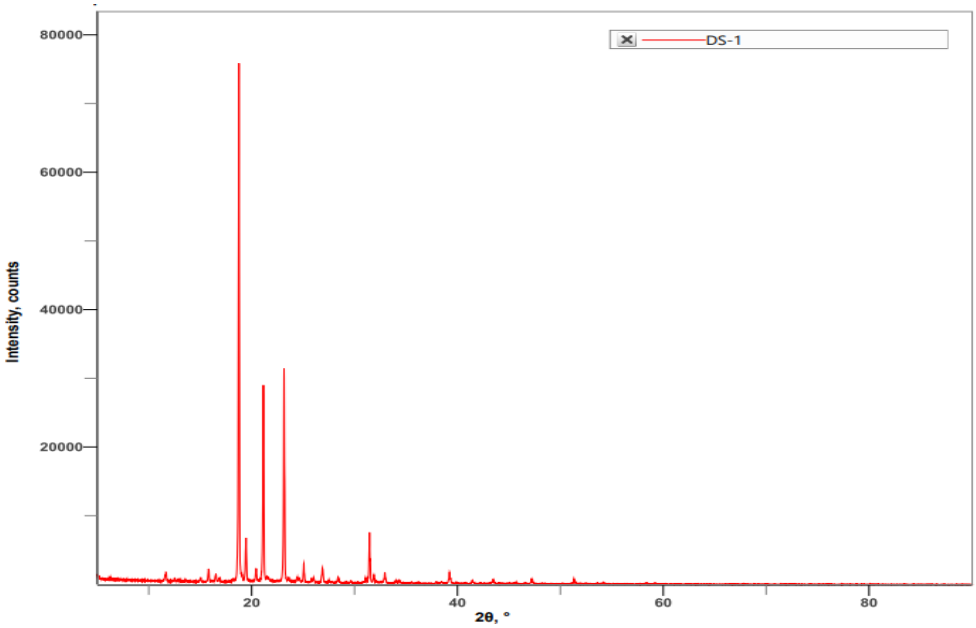

Figure 6: XRD pattern of pure drug Amitriptyline Hydrochloride

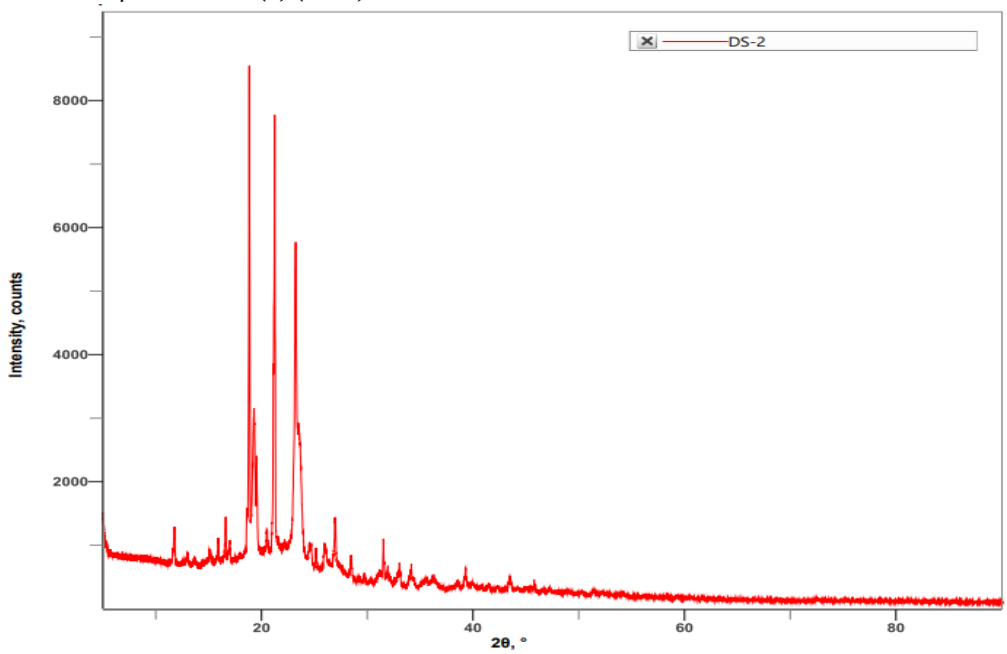

Figure No. 7: XRD pattern of physical mixture.

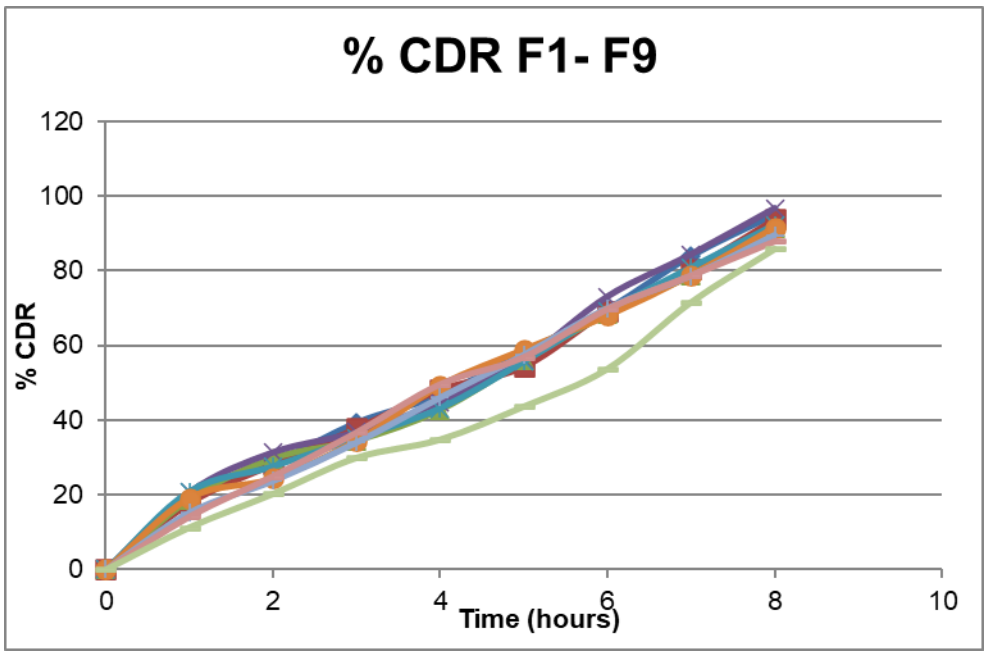

Figure 8: Graphical representation of In- vitro drug release of F1-F9 formulations

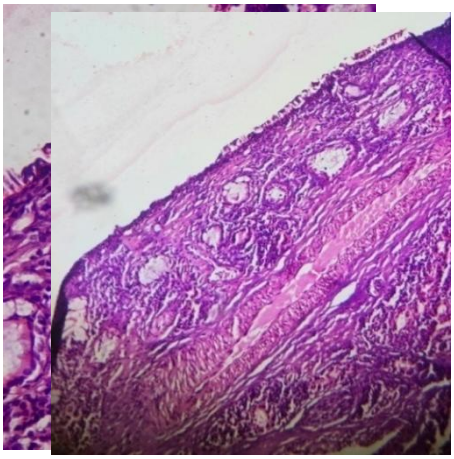

Figure 9 (a): Histopathological study of goat nasal mucosa Incubated in pH 5.5 -Control

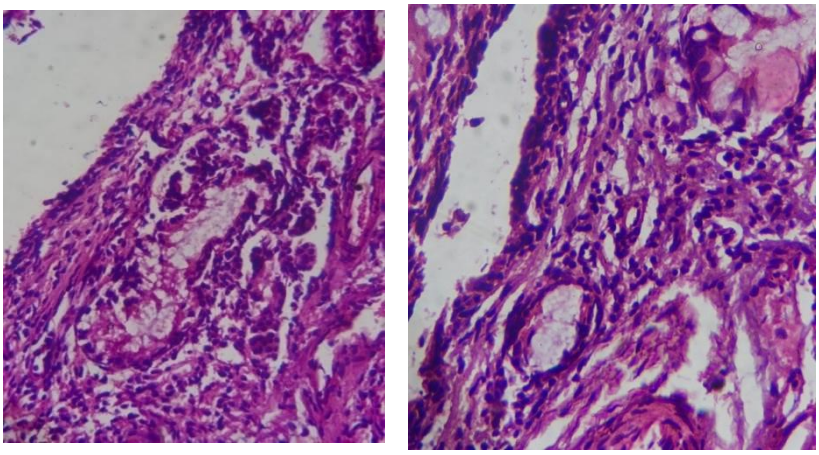

Figure 10 (b): Histopathological study of goat nasal membrane for optimized formulation (F4)

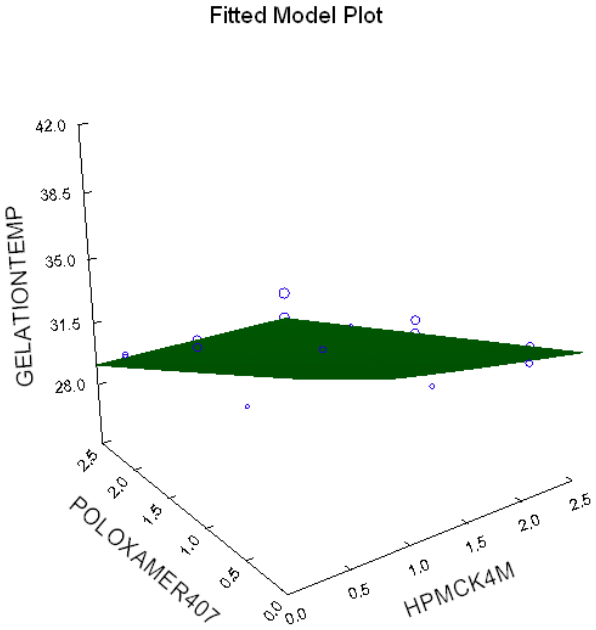

Figure No. 11: 3D Response surface plot – Gelation Temperature v/s Factor-1, Factor-2

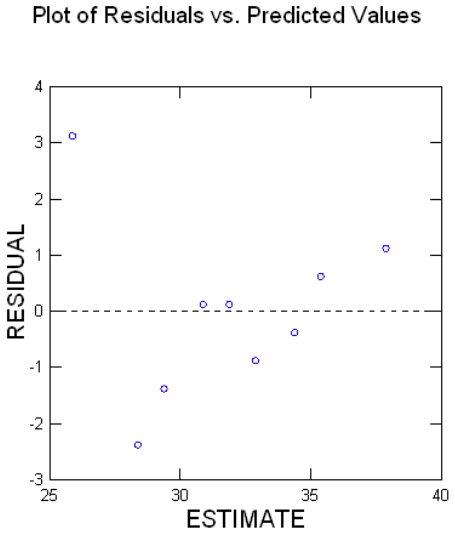

Figure No. 12: Residual v/s Predicted plot of Gelation Temperature

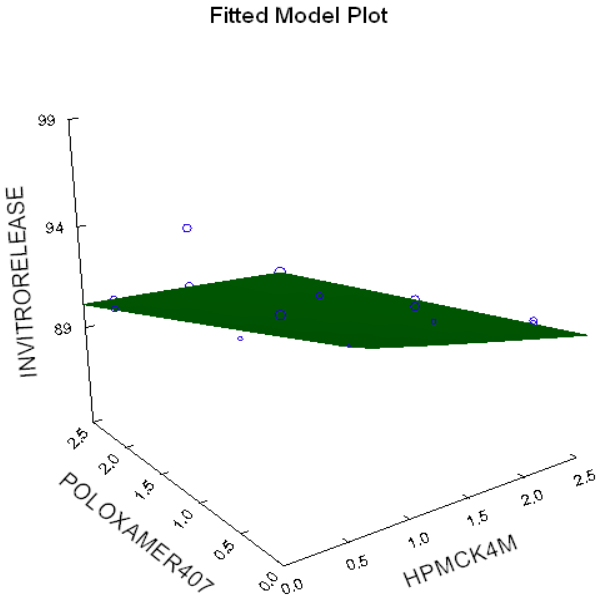

Figure No.13: 3D Response surface plot – in-vitro drug release v/s Factor-1, Factor-2

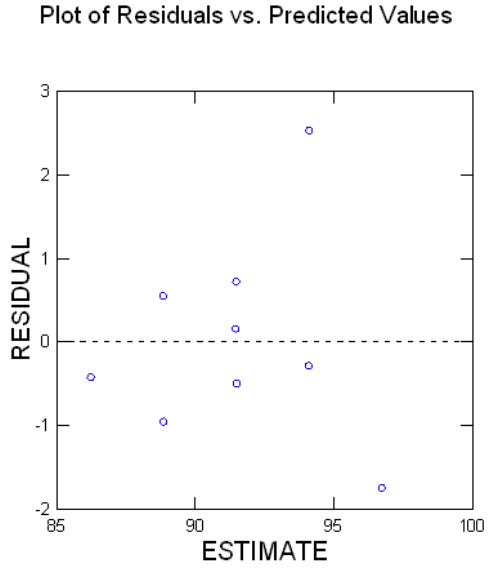

Figure No. 14: Residual v/s Predicted plot of in-vitro drug release study

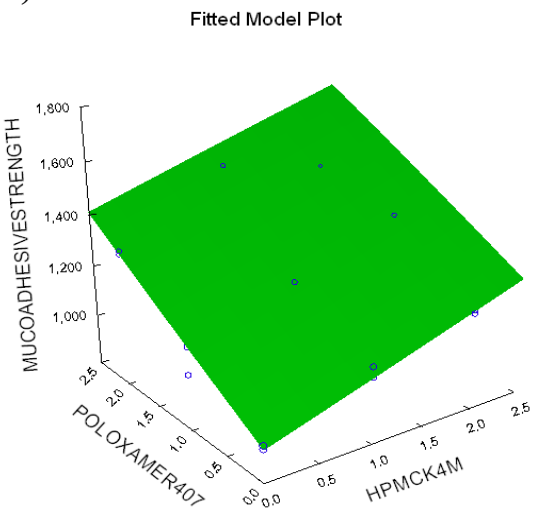

Figure No. 15: 3D Response surface plot – Mucoadhesive strength v/s Factor-1, Factor-2

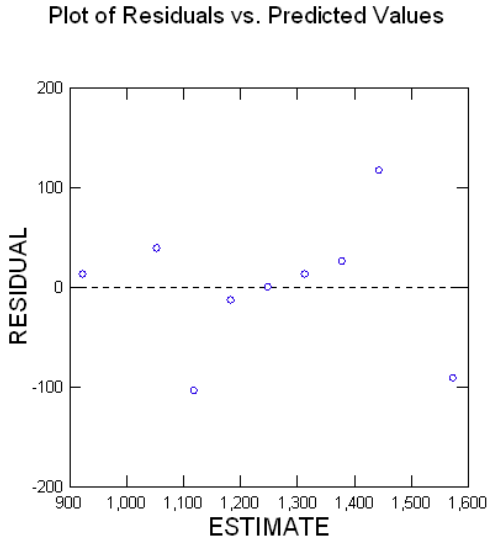

Figure No. 16: Residual v/s Predicted plot of Mucoadhesive strength

TABLES

Table 1: Formulation Table Amitriptyline Hydrochloride *in-situ* nasal gel

INGREDIENTS (W/V)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Amitriptyline hcl (%)	10	10	10	10	10	10	10	10	10
Poloxamer 407 (%)	22	22	22	23	23	23	24	24	24
Hpmc k4m (%)	1	1.25	1.5	1	1.25	1.5	1	1.25	1.5
Peg 400 (%)	1	1	1	1	1	1	1	1	1
Benzalkonium chloride (%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Distilled water (ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Table 2: Evaluation of Amitriptyline Hydrochloride for various parameters

Code	**Gelation Temperature (℃)**	**Gelling time (sec)**	**Gelling Capacity**	**Gel strength (sec)**	**Drug content %**	**pH**
F1	39	10.4	+	27.20	95.80	5.6±0.02
F2	36	8.53	+	29.45	93.55	5.1±0.06
F3	32	6.32	++	33.86	93.67	5.9±0.03
F4	34	5.01	++	35.44	92.25	5.5±0.01
F5	32	5.38	+++	38.67	96.23	5.7±0.02
F6	28	4.12	+++	40.87	92.70	5.5± 0.01
F7	31	5.66	+++	41.17	91.22	5.3± 0.00
F8	29	3.95	+++	47.11	92.89	4.8± 0.01
F9	26	4.06	+++	52.29	93.33	4.7± 0.01

Table 3: Evaluation of Amitriptyline Hydrochloride for Spreadability, Mucoadhesive strength and Viscosity

Code	**Spreadability (cm)**	**Mucoadhesive strength (dyne/cm2) (±S.D)**	**Viscosity of sol at 40 RPM (cps)**	**Viscosity of gel 40 RPM (cps)**
F1	5.0 ± 0.08	936.30 ± 0.12	31.19	55.14
F2	4.8 ± 0.41	1092.35± 0.2	101.21	178.36
F3	4.5 ± 0.11	1170.38 ± 0.3	141.92	268.84
F4	4.9 ± 0.32	1014.33 ± 0.2	206.45	319.05
F5	4.2 ± 0.15	1248.40 ± 0.1	304.18	621.15
F6	3.5 ± 0.21	1404.45 ± 0.4	602.50	863.12
F7	3.8 ± 0.12	1326.43 ± 0.3	874.26	1005.72
F8	3.2 ± 0.49	1560.50 ± 0.2	854.69	1154.32
F9	2.9 ± 0.17	1482.48 ± 0.3	965.21	1205.32

Table No. 5: *Ex-vivo* drug permeation study of optimized formulation (F4)

Code	**Time(hr)**	**%CDR**
F1	0	0
F2	1	7.71
F3	2	11.27
F4	3	27.75
F5	4	43.31
F6	5	53.12
F7	6	58.85
F8	7	72.28
F9	8	87.52

Table No. 6: Results for dependent and independent variables

Formulation Code	Runs	Independent Variables		Dependent variables		
		X1	X2	Gelation temperature (°C) Y1	In-vitro drug release (hours) Y2	Mucoadhesive strength (dynes/cm$^{2)}$ Y3
F1	1	0	0	39	94.98	936.30 ± 0.12
F2	2	0	1	36	93.81	1092.35± 0.23
F3	3	0	2	32	91.62	1170.38 ± 0.31
F4	4	1	0	34	96.64	1014.33 ± 0.20
F5	5	1	1	32	92.20	1248.40 ± 0.15
F6	6	1	2	28	89.40	1404.45 ± 0.40
F7	7	2	0	31	91.00	1326.43 ± 0.32
F8	8	2	1	26	87.91	1560.50 ± 0.22
F9	9	2	2	29	85.81	1482.48 ± 0.35

Table No. 7: OLS Regression Analysis of Gelation Temperature

Dependent Variable	Gelation Temperature
N	9
Multiple R	0.921
Squared Multiple R	0.848
Adjusted Squared Multiple R	0.797
Standard Error of Estimate	1.821

Table No. 8: Regression coefficient report of Gelation Temperature

Regression Coefficients B = $(X'X)^{-1}X'Y$ (Gelation Temperature)						
Effect	Coefficients	Standard error	Standard coefficient	Tolerance	T	p-value
CONSTANT	37.889	1.214	0.000	---	31.216	0.000
POLOXAMER4	-3.500	0.743	-0.749	1.000	-4.709	0.003

07						
HPMCK 4M	-2.500	0.743	-0.535	1.000	-3.363	0.015

Table No. 9: ANOVA study reports of Gelation Temperature

Analysis of Variance (Gelation Temperature)					
Source	SS	Df	Mean Squares	F-Ratio	p-Value
Regression	111.000	2	55.500	16.743	0.004
Residual	19.889	6	3.315	---	---

Table No. 10: OLS Regression Analysis of *in-vitro* drug release

Dependent Variable	***In-vitro* drug release**
N	9
Multiple R	0.936
Squared Multiple R	0.876
Adjusted Squared Multiple R	0.834
Standard Error of Estimate	1.397

Table No. 11: Regression coefficient report of *in-vitro* drug release

Regression Coefficients B = $(X'X)^{-1}X'Y$ (*In-vitro* drug release)						
Effect	Coefficients	Standard error	Standard coefficient	Tolerance	T	p-value
CONSTANT	96.732	0.931	0.000	---	103.865	0.000
POLOXAMER 407	-2.615	0.570	-0.660	1.000	-4.585	0.004
HPMCK4M	-2.632	0.570	-0.664	1.000	-4.614	0.004

Table No. 12: ANOVA study reports of *In-vitro* drug release

Analysis of Variance (*In-vitro* drug release)					
Source	SS	Df	Mean Squares	F-Ratio	p-Value
Regression	82.583	2	41.292	21.158	0.002
Residual	11.709	6	1.952	---	---

Table No. 13: OLS Regression Analysis of Mucoadhesive strength

Dependent Variable	**Mucoadhesive strength**
N	9
Multiple R	0.950
Squared Multiple R	0.903
Adjusted Squared Multiple R	0.870
Standard Error of Estimate	76.932

Table No. 14: Regression coefficient report of Mucoadhesive Strength

Regression Coefficients B = $(X'X)^{-1}X'Y$ (Mucoadhesive strength)						
Effect	Coefficients	Standard error	Standard coefficient	Tolerance	T	p-value
CONSTANT	923.297	51.288	0.000	---	18.002	0.000
POLOXAMER 407	195.063	31.407	0.791	1.000	6.211	0.001
HPMCK4M	130.042	31.407	0.527	1.000	4.141	0.006

Table No. 15: ANOVA study reports of Mucoadhesive Strength

Analysis of Variance (Mucoadhesive strength)					
Source	SS	Df	Mean Squares	F-Ratio	p-Value
Regression	329,763.234	2	164,881.617	27.859	0.001
Residual	35,510.803	6	5,918.467	---	---

CONCLUSION:

The in-situ nasal gel of Amitriptyline hydrochloride was successfully designed, formulated, and evaluated. This study aimed to create a thermoreversible nasal gel using Poloxamer 407 as a gelling agent and HPMC K4M as a mucoadhesive agent, utilizing the cold method.

Pre-compatibility studies, including UV, FTIR, DSC, and XRD, were conducted for the pure drug and its physical mixture, revealing no incompatibilities. A 2^3 full factorial design was employed to examine the effects of two factors on three variables. The formulations were clear, transparent, and had a pH that matched nasal physiology, thus avoiding nasal irritation.

Results indicated that increasing the concentrations of Poloxamer 407 and HPMC K4M led to higher viscosity and mucoadhesive strength, lower gelation temperature, and reduced percent drug release. Gelation time and gelling capacity were found to be optimal, and drug content ranged between 78.33% and 94.23%. The viscosity of the formulation increased with both temperature and gelling agent concentration, while the spreadability of the gel was within the acceptable range of 2.5-7 cm.

In-vitro drug release studies demonstrated that higher polymer concentrations resulted in decreased cumulative drug release. The optimized formulation F4, containing 23% Poloxamer 407 and 1% HPMC K4M, achieved the highest drug release, reaching 96.64% over 8 hours. Histopathological studies confirmed that the optimized formulation (F4) was safe, causing no tissue damage or cell necrosis.

Statistical analysis showed significant values, and polynomial equations and 3D response surface plots were generated. The 3D response surface plots revealed that as the concentration of the gelling and mucoadhesive agents increased, gelation temperature decreased. Similarly, in-vitro drug release decreased, and mucoadhesive strength increased with higher agent concentrations.

ANOVA results for gelation temperature, mucoadhesive strength, and in-vitro drug release

showed regression source p-values of less than 0.05, indicating significant effects of the studied factors.

Ethical Statement :

Not applicable

Competing Interest :

Authors declare no conflict of interests

REFERENCES

1. Manashi P. Valavi*, Sandip A. Tadavi AS and SPP. Review on in Situ Gel a Novel Nasal Drug Delivery System. Int J Pharm Rev Res. 2018;50(1):175–81.
2. Karavasili C, Fatouros DG. Smart materials: In situ gel-forming systems for nasal delivery.Drug Discov Today [Internet]. 2016; 21(1):157–66.
3. Chonkar A, Nayak U, Udupa N. Smart polymers in nasal drug delivery. Indian J Pharm Sci. 2015; 77(4):367–75.
4. Rokade M. In situ gel-sustained nasal drug delivery Manisha Rokade *, Bhavna Tambe and Manjula Ruparel Department of pharmacy, S.M.B.T. Institute of Diploma of Pharmacy Dhamangaon, Nashik, MS, India. 2015;6(12):4958–66.
5. Dr P, Ul P, Bv B, Dj D, Gv P. Nasal In-Situ Gel: A Novel Drug Delivery System. Int J Pharm Res Sch. 2012; 457–73.
6. Kaur G, Sehrawat S. Development and evaluation of a method for qualitative and quantitative analysis of Amitriptyline using UV visible. World Journal of Pharmaceutical Research. 202;(June).
7. Naresh WR, Dilip DV, Sunil KP. Xyloglucan based nasal in situ gel formulation of mirtazapine for treatment of depression. Indian J. Pharm. Educ. Res. 2020 Apr 1; 54:s210-9.
8. A, S. S. S., Asha, A. N., & Devi, V. K. Formulation and Evaluation of Niosomes of Mirtazapine for Nasal. Indo American Journal of Pharmaceutical Research, 2019: 9(05).
9. Sharwaree H, Priyanka B, Ashok B, Swapnil M. Development of mucoadhesive nasal in situ gel to sustain the release of amitriptyline hydrochloride. Int J Biol Pharm Res. 2012; 3:980-9.
10. De PK, Ghatak S. Formulation Optimization, Permeation Kinetic and Release Mechanism Study of In-Situ Nasal Gel Containing Ondansetron. 2020;4929.
11. Padalkar R, Madgulkar A. International Journal of Pharmaceutical Sciences and

Drug Research In situ Nasal Gel of Granisetron for Enhancement of Bioavailability over Oral Delivery : Formulation , Optimization , and In vivo Evaluation. 2020;12(5):544–53.

12. Patil SK, Dhage AN, Patil SV, Patil SS. Formulation and evaluation of nasal in situ gel for Alzheimer disease. Int. Res. J. Pharma. Bio. Sci. 2015; 2(2):41-58.
13. Jagadeesh R, Kumar PA, Manjunath K, Mancy SP. Development of sustained release in situ nasal gel of ondansetran HCl using mucoadhesive polymers. 2019;5(2):23–31.
14. Lakshmi PK, Harini K. Design and Optimization of Thermo-reversible Nasal in situ Gel of Atomoxetine Hydrochloride Using Taguchi Orthogonal Array Design. 2019; 18(2)
15. Saudagar RB, Kulkarni MM. Formulation Development and Evaluation of In-Situ Nasal Gel of Ziprasidone Hydrochloride. 2017; 10(9):195–211.
16. Kassab HJ. Formulation and evaluation of sustained release sumatriptan mucoadhesive intranasal in-situ gel. Iraqi Journal of Pharmaceutical Sciences (IJPS). 2019 Dec 22;28(2):95-104.
17. Shete Prathmesh M. Formulation and optimization of thermoreversible in-situ gel of venlafaxine hcl. Evaluation.2014;2(10):11.
18. Patil PR, Salve VK, Thorat RU, Shahi SR. Formulation and evaluation of ion-sensitive in- situ nasal gel of zolmitriptan. Int J Pharm Pharm Sci. 2015 Jan 1; 7:47886.
19. Kempwade A, Taranalli A. Formulation and evaluation of thermoreversible, mucoadhesive in situ intranasal gel of rizatriptan benzoate. Journal of sol-gel science and technology. 2014 Oct; 72(1):43-8.
20. Singh RM, Kumar A, Pathak K. Thermally triggered mucoadhesive in situ gel of loratadine: β-cyclodextrin complex for nasal delivery. AAPS PharmSciTech. 2013 Mar; 14(1):412-24.
21. Jagdale S, Shewale N, Kuchekar BS. Optimization of Thermoreversible In Situ Nasal Gel of Timolol Maleate. Scientifica (Cairo) 2016; 2016: 6401267

22. Abouhussein DM, Khattab A, Bayoumi NA, Mahmoud AF, Sakr TM. Brain targeted rivastigmine mucoadhesive thermosensitive In situ gel: Optimization, in vitro evaluation, radiolabeling, in vivo pharmacokinetics and biodistribution. Journal of Drug Delivery Science and Technology. 2018 Feb 1; 43:129-40.