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Original Article

# Design and Characterization of pH Dependent Phase Transition System of Almotriptan Malate for Nasal Drug Delivery System by Employing Factorial Design

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## ABSTRACT

**Introduction:** Nasal medication delivery uses nasal mucosa for faster and higher drug absorption. Transmucosal nasal administration is promising. Many medicines obtain superior systemic absorption via nasal route than orally. **Objectives:** This research aimed to construct and characterize a pH-dependent almotriptan malate nasal *in situ* gel for acute migraine treatment. It eases discomfort, headaches, and migraine symptoms. **Materials and Methods:** Gels were delivered by changing the grouping of pH-sensitive and mucoadhesive polymers, explicitly HPMC-E15 and chitosan hydrochloride, in a cycle that was done by applying cool method Results. The range of drug content was between 95.87±0.45 to 99.58±0.56%. The pH value was measured to fall between 6.10±0.92 and 6.41±0.11. The obtained results notably agreed with the change in phase+transition, and the formulations portraits the rheology in a pseudoplastic behavior. This was a very exciting discovery. The mucoadhesiveness of all of the formulations rose with the HPMC-E15: Chitosan HCl concentration which was found to be in the range of 2133±1.58 to 3111.47±1.32 dynes/cm$^2$ for the most effective formulations. The *in vitro* release of drug results of the optimized formulation, which consisted of 5 g HPMC-E15, 1 g Chitosan hydrochloride, and 1.5 g PEG400, indicated that 94.24±0.96% release occurred at the end of 12 hr. Studies using histopathology indicated that the AMF4 formulation had a non-toxic effect on the microscopic structure of the nasal mucosa when it was subjected to *ex vivo* permeation tests which showed the drug release of 78.85±0.87 %. Based on the findings of the stability research, a temperature range of 5°C±3°C is recommended for storing the formulations. **Conclusion:** As per these discoveries, nasal *in situ* gel containing almotriptan malate is probably going to turn into a selection of arrangements for treating migraines and other symptoms associated with migraines in the near future and in a nutshell, an *in situ* gel formulation of almotriptan malate that is pH dependent has the potential to function as an efficient method of treating migraines.

**Keywords:** *In situ* Gel, Migraine, Triptan, pH Sensitive, Nasal Gel, Almotriptan Malate.

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## INTRODUCTION

Nasal medicine delivery has gained popularity as a convenient and reliable method. The nasal route has gained popularity, attracting this attention.[1] Lower membrane permeability, fast clearance, and enzymatic breakdown in the nasal antrum impede polar drug and macromolecule absorption. Intranasal medication delivery to the brain is non-invasive, feasible, and an alternate approach. Large nasal surface area and high blood flow allow for fast absorption by nasal medication delivery.[2,3] This approach eliminates first-pass liver elimination. It's straightforward to administrate, low-cost, and handy for the user.[4]

Women (15%) are more often than males (6%) to get migraines. Migraines can induce Cognitive and motor impairment if left untreated. Symptoms include headaches, anorexia, Nausea, Vomiting, and Photophobia. Migraines aren't just headaches. It's a multi-symptom Neurological condition that occasionally doesn't cause headaches. Misdiagnosed or misunderstood, it affects people in many ways.[5,6]

Almotriptan malate is $C_{21}H_{31}N_3O_7S$ which is selective serotonin receptor agonists. It binds to the 5-HT1D, the 5-HT1B, and the 5-HT1F receptors with a high degree of affinity. There is a mean unbound fraction of more than sixty percent for almotriptan, indicating that it does not have a strong affinity for plasma proteins. Therefore, the extent to which plasma proteins are bound to almotriptan is not a significant factor in the drug's pharmacokinetics.[7,8]

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The purpose of this study is to plan and evaluate the characteristics of Intra Nasal delivery of pH-dependent gel containing almotriptan malate as a treatment for migraines. By employing a design known as a factorial design, the researcher of the current study attempted to create an *in situ* nasal gel formulation of Almotriptan Malate.

## MATERIALS AND METHODS

### Materials

Almotriptan Malate was obtained as a generous gift sample from AZAKEM Labs Private Limited, Hyderabad, India. Chitosan Hydrochloride was procured from Sulab Laboratories, Gujarat. HPMC E15 was obtained as a gift sample from Colorcon, Goa. Benzalkonium chloride and PEG 400 was procured from S.D. Fine Labs, Mumbai, India. Other than those mentioned, all chemicals and reagents utilized were of analytical grade.

### Drug Excipients Interaction Study

Analysis of FTIR and DSC data was used to further evaluate probable interactions between excipients and the medication.

### Fourier Transform Infrared Spectroscopy

Shimadzu affinity-l (Shimadzu Affinity) spectrophotometer, conventional KBr plate technique was used. Using a mortar and pestle, we combined the medication in a ratio of 1:1 with powdered potassium bromide. The IR spectrum of the medication was generated, and the major matched to standard FTIR informational indexes.[9,10]

### Differential Scanning Calorimeter

In order to obtain Thermograms of Almotriptan Malate and polymers, aluminium pans were used in a Differential Scanning Calorimeter (DSC). For each sample, a fresh set of temperature parameters were programmed. The cooling unit was used to remove the nitrogen. The DSC temperature was traditionally calibrated using an indium standard. In aluminium pans, the samples were hermetically sealed and heated to temperatures ranging from 0 to 550°C at a steady rate of 20°C/min. Purging nitrogen at a flow rate of 100 mL/min was used to maintain the inert environment.[11,12]

### Standard Calibration Curve

Out of a solution that contained 100 micrograms of active ingredient per mL, pipette aliquots of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 mL. The final concentration was 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 mg/mL after the addition of phosphate buffer 6.8. Further the absorbance of each dilution was determined by measuring the absorbance at 230 nm.[13]

### Methods

Nine formulations were created using $3^2$ full factorial designs, and the effects of independent variables such as PEG 400 ($X_1$) and HPMC E15 ($X_2$) on dependent variables such as Viscosity ($Y_1$), Mucoadhesion ($Y_2$), and Drug release ($Y_3$) were studied. The statistical experimental design was generated and evaluated using Design Expert 11.0. Table 1 and Table 2 show the experimental design, which includes the various independent factors. Both extreme high and low values were assigned the values of +1 and -1, which are the mean and median values, respectively. In order to accurately measure the effects on the response variables of a factor, the range of the factor must be selected. Each factor's range was determined based on preliminary study. Since it has enough room for both factor interactions and principal effects of the experiment, this design was chosen. In order to determine which of the control factors have a meaningful influence on the response variables, it was essential to carry out a regression analysis in a stepwise fashion.[14]

### *In situ* Gelation Procedure

After first dissolving the mixture of HPMC and Chitosan HCl in distilled water and then stirring it for thirty min at a temperature of ninety degrees Celsius in a water bath, the mixture was allowed to cool to room temperature before being dissolved in a PEG 400 solution that had been prepared in advance. Following the

**Table 1: Experimental design.**

| Formulation Code | Coded Value | |
|---|---|---|
| | X1 | X2 |
| AMF1 | -1 | -1 |
| AMF 2 | 0 | 0 |
| AMF 3 | 0 | -1 |
| AMF 4 | 0 | 1 |
| AMF 5 | -1 | 0 |
| AMF 6 | 1 | 0 |
| AMF 7 | -1 | 1 |
| AMF 8 | 1 | -1 |
| AMF 9 | 1 | 1 |

addition of 6 mg of almotriptan HCl to the HPMC Chitosan HCl solution, this solution was very carefully added to the PEG 400 solution while it was continuously mixed. As an osmotic agent, mannitol had to be added in the appropriate quantity, and benzalkonium chloride was essential for the formulation's preservation. The pH sensitive gel formulations that contain almotriptan that have been created are outlined in Table 3.[14,15]

## Preparation of Simulated Nasal Fluid

An artificial nasal fluid or a synthetic nasal fluid matrix is a type of solution that is designed to imitate the content and pH of the fluid that is found in the nasal cavities. It was required that 250 mL of double-distilled water be produced, into which 2.1925 g of sodium chloride, 0.145 g of calcium chloride, and 0.745 g of potassium chloride were respectively dissolved. The pH of the artificial nasal fluid was kept at 6.4±0.3.[16]

## Evaluation Parameters

### *Measurement of pH*

First, the pH meter had to be calibrated, which involved using a number of different solutions ranging in pH from 7 to 2.3. After transferring 1 mL of each formulation to a beaker, the volume of the beaker was brought up to 25 mL by further addition of distilled water. The volume of the beaker was then measured and recorded. In order to obtain a precise reading of the pH level of the solution, a digital pH meter was utilized.[17]

## Measurement of Gelation Time

To summarizes, 5 mL of the formulation was swirled magnetically at 80 rpm in a glass beaker containing 10 mL of the simulated nasal fluid while the temperature was maintained at 37.5±0.5°C. The time of gelation was determined by noting when the magnetic bead stopped revolving and writing it down. The measurements were taken three times to ensure accuracy.[18]

## Rheological Studies

Utilizing a thermostatically controlled Brookfield programmable digital rheometer, each formulation's rheological parameters were analysed in order to make a determination about flow property (Model DV III, USA). The samples' viscosities were tested in triplicate for the sol phase and for the gel phase. Shearing was performed on samples using spindle number 21 at 5, 10, 20, 30, 40, and 50 revolutions per min. It was observed that the shear rate denoted by γ, shear stress denoted by τ, and viscosity denoted by η all differed. Plots of RPM vs Viscosity were used in order to arrive at an understanding of the rheological behavior.[19]

## Analysis of the Gel Strength

The test used a lab-modified gel testing device. Simulated nasal fluid gelled 50 g of *in situ* gel in a 100 mL measuring cylinder. Up to 35 g of pressure was applied at various places on the gel testing device. The gel's strength was evaluated by the minimum weight needed to sink an instrument five centimeters.[20]

## Determination of Mucoadhesive Force

The mucoadhesive strength of each nasal *in situ* gel formulation was determined using goat nasal mucosal tissue. This evaluation

**Table 2: Amount of variable in $3^2$ factorial designs batches.**

| Formulation Code | Coded Value | |
|---|---|---|
| | X1 | X2 |
| -1 | 1% w/v | 1:5 % w/v |
| 0 | 1.5% w/v | 2:2% w/v |
| +1 | 2% w/v | 5:1 % w/v |

**Table 3: Formulation chart of almotriptan *in situ* nasal gel.**

| Ingredients | AMF1 | AMF2 | AMF3 | AMF4 | AMF5 | AMF6 | AMF7 | AMF8 | AMF9 |
|---|---|---|---|---|---|---|---|---|---|
| Almotriptan HCl | 6 mg | 6 mg | 6 mg | 6 mg | 6 mg | 6 mg | 6 mg | 6 mg | 6 mg |
| PEG 400 | 1 g | 1.5 g | 1.5 g | 1.5 g | 1 g | 2 g | 1 g | 2 g | 2 g |
| HPMC-E15 | 1 g | 2 g | 1 g | 5 g | 2 g | 2 g | 5 g | 1 g | 5 g |
| Chitosan HCl | 5 g | 2 g | 5 g | 1 g | 2 g | 2 g | 1 g | 5 g | 1 g |
| Mannitol | 1 g | 1 g | 1 g | 1 g | 1 g | 1 g | 1 g | 1 g | 1 g |
| Benzalkonium Chloride | 0.02 g | 0.02 g | 0.02 g | 0.02 g | 0.02 g | 0.02 g | 0.02 g | 0.02 g | 0.02 g |
| Distilled Water (q.s) | 100 mL | 100 mL | 100 mL | 100 mL | 100 mL | 100 mL | 100 mL | 100 mL | 100 mL |

was carried out using goat nasal mucosal tissue obtained from a local butcher and a customized mucoadhesive measurement instrument. A piece of nasal mucosa was cut and secured with a rubber band to the top of each glass vial, leaving the mucosal side exposed. Each mucosal membrane was 2 cm in length. At 37°C, nasal mucosa vials were used for 5 min. On the equilibrium, one vial containing mucosa was set upper side, and one more was put on a level customizable container. Each formulated gel was applied to the nasal mucosa of the principal vial. The level of the subsequent vial was then changed so their mucosal surfaces contacted. To guarantee private tissue test contact, two min of power were applied. While associated with the equilibrium, the upper vial was lifted. The offset's dish was loaded up with steady loads. Subsequent to adding loads, two vials isolated. The mucoadhesive power, communicated as separation stress in dyne/cm$^2$, was determined for every definition utilizing the littlest loads those isolated tissues from the surface.[21]

$$\text{Detachment Stress (dyne/cm}^2) = m \times g/A$$

Where m=Weight required for detachment of two vials in g.

*g*=Acceleration due to gravity (980 cm/s$^2$).

*A*=Area of tissue exposed, which is equal to $\pi r^2$.

## Drug Content

To check for consistency in the drug concentration, a modified UV Visible spectroscopic method was applied. The gel was made in a volumetric flask, and after filtering, 83.33 mL of the gel formulation were added to 100 mL of phosphate buffer with a pH of 6.4. That yielded 5 mL of almotriptan malate.[22] Absorbance was measured spectrophotometrically (with a UV-spectrophotometer) at 230 nm after diluting 1 mL of the filtered solution to a volume of 10 mL with phosphate buffer with a pH of 6.4.

## *In vitro* Drug Release Study

*In vitro* diffusion investigations were performed on the gel utilising a US-made Franz diffusion cell and a dialysis membrane soaked in receptor media the night before usage. The donor compartment had 5 mg of almotriptan malate, whereas the receiver contained 15 mL of phosphate buffer 6.4. Both compartments were swirled at 100 rpm using a magnetic stirrer (Remi, INDIA) and kept at 34±1°C. The experiment was stopped after collecting 1 mL aliquots from the receptor compartment and replacing them with new dialysis media at predefined intervals for 12 hr. Each sample was spectrophotometrically assessed at 230 nm against phosphate buffer (pH 6.4). The samples were all taken in triplicate before the analysis. A calibration curve was utilized in order to ascertain the amount of medication that was dispersed.[23-25]

## *Ex vivo* Permeation Study

In this evaluation parameter, the nasal mucosa of goat was utilized as a model. The saturation examinations were led involving newly extracted goat nasal mucosa from a slaughterhouse nearby. A short time later, the mucosal side of the nasal mucosa was mounted in the Franz diffusion cell (0.785 cm$^2$ pervasion region) with the giver compartment confronting the mucosal side. A magnetic stirrer was utilized to foment Phosphate Buffer Saline (PBS) pH 6.4 in the beneficiary compartment while an *in situ* gel containing 5 mg almotriptan malate was placed on the mucosal surface in the giver chamber. 1 mL test Aliquots were taken from the recipient compartment at determined intervals and recharged with replaced with fresh dissolution media to maintain the sink condition for 12 hr. The samples were examined spectrophotometrically at 230 nm after filtering and appropriate dilution.[26,27] The procedure performed for the dialysis membrane was same as of nasal mucosa.

## Histopathological Evaluation of Nasal Mucosa

After 12 hr of permeation investigations, the vitality of the mucosa was examined using histological examinations of goat nasal tissue, and the results were compared to mucosa that had not been treated. After the tissue had been treated and embedded in paraffin, it was first fixed in a 10% buffered formalin solution. Additional paraffin slices with a thickness of 7 micrometers were stained with Haematoxylin and Eosin, and each slice was scrutinized in great detail using a microscope in order to search for evidence of any tissue damage that may have been produced by the *ex vivo* permeation method.[28,29]

## Release Kinetics

The model with the highest correlation coefficient was found to be the one that could best explain the mechanism by which drugs are released from a gelling system. This system had been prepared by fitting *in vitro* permeation data to the zero-order and first-order Higuchi release model, the Hixson and Crowell method, and the Korsemeyer-Peppas model using the Kinetics DS software.[30]

## Stability Studies

The *in situ* gel that was created was subjected to short term stability tests, which proved both the physical and chemical stability of the gel. We stored the samples in glass vials with screw caps at 5°C±3°C and at ambient temperature 25°C±2°C with a relative humidity of 60%±5% RH for a period of three months. After thirty days, sixty days, and ninety days, the substance was put through a physical stability test in which its appearance, pH, drug content, gelation pH, and drug release rate were measured.[31,32]

## RESULTS AND DISCUSSION

### Drug Interaction Study with Excipients

#### *Fourier Transform Infrared Spectroscopy*

The graphs produced by FTIR will be of assistance in determining the functional groups as well as the potential physical and chemical interactions that may take place during the process of combining the medicine with the excipients. Figure 1 represents the FTIR of pure drug with the characteristic peaks at 2976.59 $cm^{-1}$ representing CH stretching, 1433.82 $cm^{-1}$ representing CH bending, 3429.78 $cm^{-1}$ representing NH stretching, and 1433.82 $cm^{-1}$ representing C=C aromatic groups. While Figure 2 represents the similar characteristic peaks of almotriptan at 2954.41 $cm^{-1}$ representing CH stretching, 1322.98 $cm^{-1}$ representing CH bending, 3499.66 $cm^{-1}$ representing NH stretching, and 1586.67 $cm^{-1}$ representing C=C aromatic groups. By differentiating the IR range of unadulterated almotriptan with the range of the actual blend, it was feasible to presume that there was no impedance between the dynamic fixing in the medication and the excipients that were utilized in the study.

### Differential Scanning Calorimeter

The drug's DSC scan in Figure 3 showed a distinct endothermic peak, as determined by thermal analysis. Almotriptan malate crystallized and melted at 102.08°C, as shown by a sharp

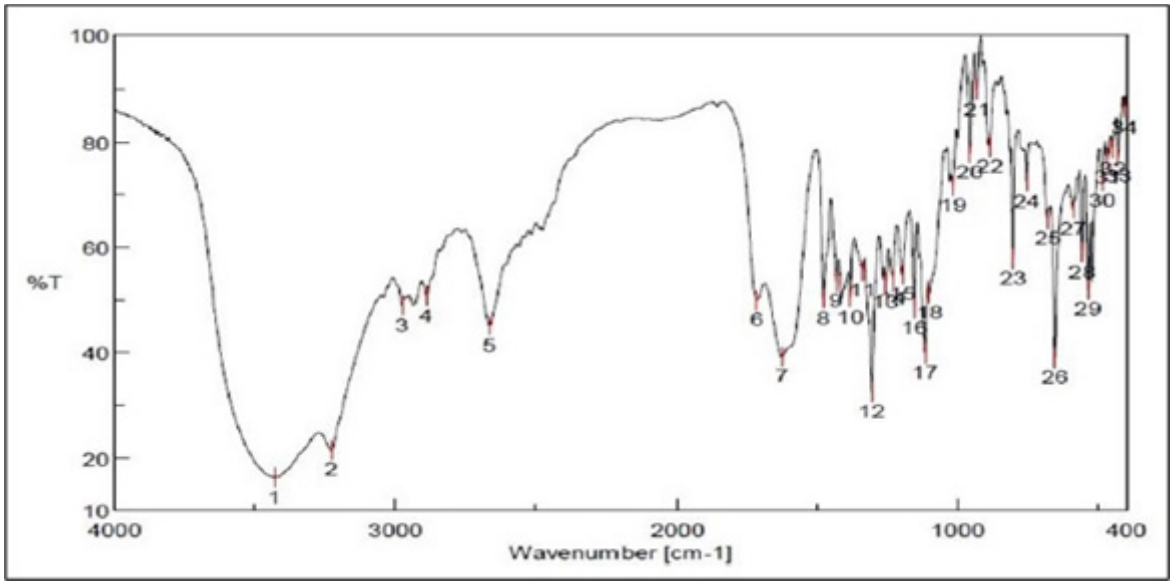


**Figure 1:** FTIR spectrum of pure drug almotriptan malate.

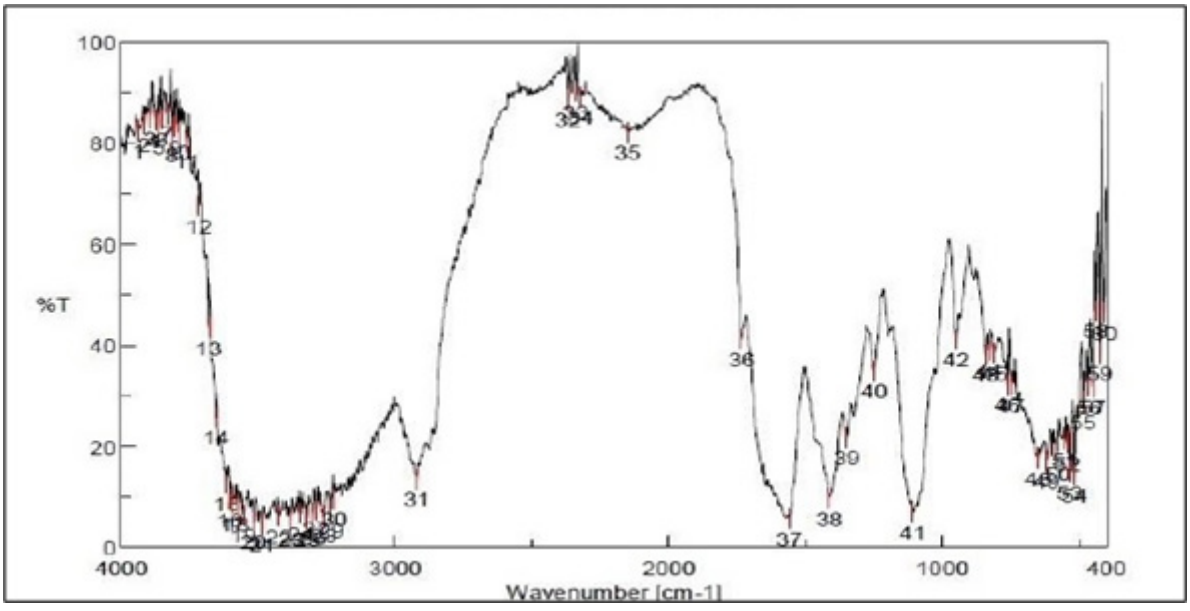


**Figure 2:** FTIR spectrum of pure drug almotriptan malate with excipients.

endothermic peak in the drug's thermogram. The sharp peak of almotriptan malate in a DSC spectrum of a physical mixture containing excipients and the drug does not appear as shown in Figure 4. As a result, we may infer that almotriptan malate was in a partially crystalline state in the physical mixture of excipients and drug and that the drug did not interact with the polymer.

## Standard Calibration Curve

The drug's concentration between 5 and 50 µg/mL shows good linearity in calibration curve studies using the spectrophotometric method (beers lamberts law). $R^2$ for the relationship between concentration and absorbance which can be seen in the Figure 5 was 0.998, and the resulting equation was $y = 0.3975x\text{-}0.4627$.

## Evaluation Parameters

### *Measurement of pH*

Regardless of the way that it's generally expected physiological pH goes from 4.5 to 6.5, the nasal mucosa can endure pH somewhere in the range of 3 and 10. To guarantee definitions are alright

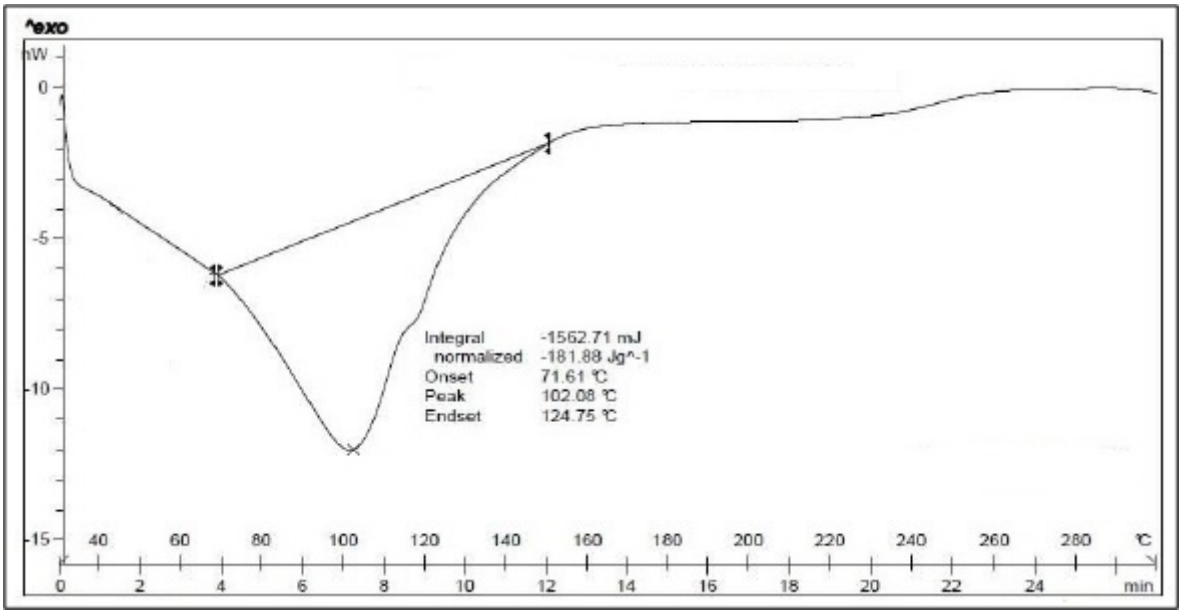


**Figure 3:** DSC thermogram of pure drug almotriptan malate.

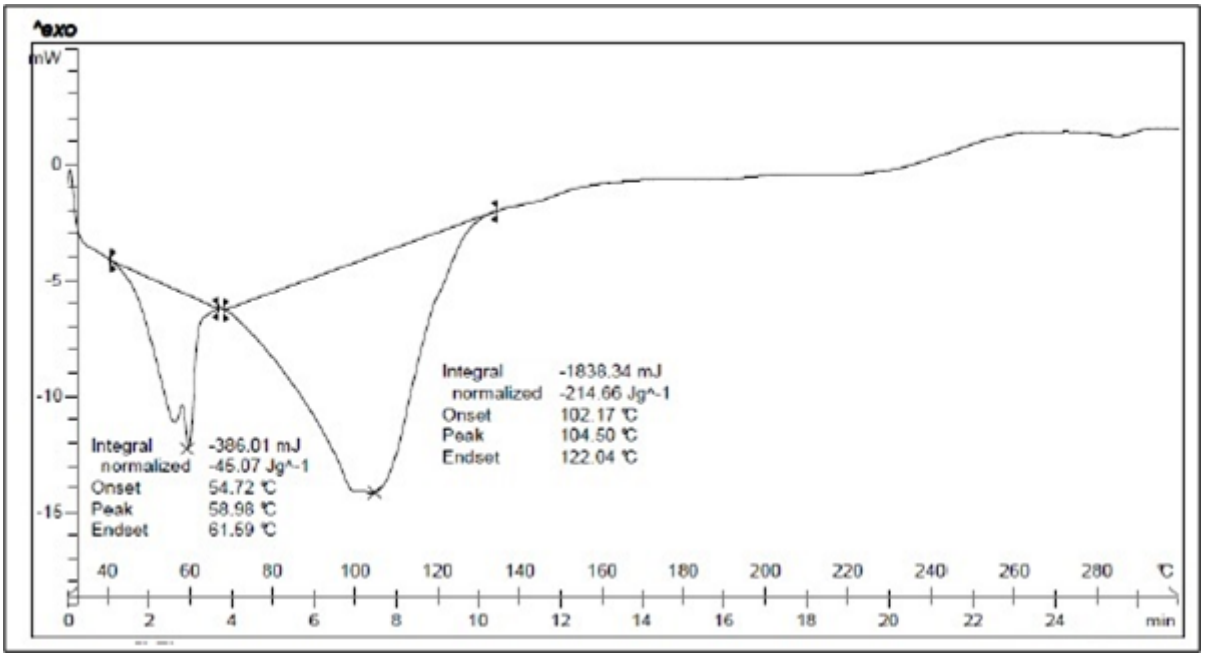


**Figure 4:** DSC thermogram of pure drug almotriptan malate with excipients.

for nasal mucosa, they ought to fall inside the typical pH range (5.5-6.5). All prepared gel formulations were found to have a pH between 6.10±0.92 and 6.41±0.11, which is in the same range as the nasal mucosa (Table 4). Differences in pH were traced back to changes in the ratio of HPMC: Chitosan Hydrochloride.

## Gelation Time

It was calculated that the prepared formulation needed between 43.64±2.31 sec and 80.25±4.72 sec to undergo the sol-to-gel transition. It must be converted to a gel at the optimal pH to prevent it from draining out of the nasal cavity after installation, as the formulation would be lost if it remained in a liquid state. Gelation times rose across the board as polymer compositions were changed (Table 3). A shorter gelation period should result in a quicker gelation. The results are shown in Table 4.

## Gel Strength

The severity of postnasal drip or leaking can be reduced by adjusting this parameter. Preparation of the formulation revealed that HPMC-E15 and chitosan hydrochloride concentrations affected the gel strength as shown in Table 4, which was reported to be in the range of 64±1.58 to 119.4±2.21 sec. The gel strength is significantly improved by both mucoadhesive polymers. There is only a little improvement in gel strength with increasing concentrations of chitosan hydrochloride (Table ).

Some of these polymers and water may form hydrogen bonds, which could make the gel more stable. The molecular weight of the hydrophobic molecule and the Ethylene Oxide/Propylene oxide ratio affect the congealing strength of HPMC and Chitosan Hydrochloride. Even while gel dehydrates at a higher pH, significant structural changes are prevented thanks to the presence of strong Hydrogen bonds and densely connected micelles. However, the structure of the gel is destroyed by significant changes in pH.

## Determination of Mucoadhesive Force

Formulations with a higher concentration of the blend of HPMC and chitosan Hydrochloride had greater Mucoadhesive qualities, as measured by detachment stress. The compositions Mucoadhesive strength improved with increased polymer concentration (Table 4) and was seen in the range of 2133±1.58 dynes/cm$^2$ to 3111.47±1.32 dynes/cm$^2$. Mucoadhesion depends intensely on the speedy admission of liquid from the bodily fluid layer, which permits the polymer chain to enter the mucin organization and make tacky associations. An increase in mucoadhesive strength

**Table 4: Evaluation parameters of almotriptan *in situ* nasal gel.**

| Batch | pH | Gelation time (sec) | Gel strength (sec) | Mucoadhesion force (dynes/cm$^2$) | Drug content (%) |
|---|---|---|---|---|---|
| AMF1 | 6.11±0.084 | 43.64±2.31 | 64±1.58 | 2133±1.58 | 96.77±1.41 |
| AMF2 | 6.23±0.064 | 51.21±1.41 | 79.64±2.06 | 2458.57±1.05 | 96.04±0.25 |
| AMF3 | 6.22±0.098 | 45.58±2.56 | 73.64±1.71 | 2718±1.66 | 97.48±0.59 |
| AMF4 | 6.41±0.11 | 55.31±2.06 | 94±2.78 | 2272±1.45 | 99.58±0.56 |
| AMF5 | 6.17±0.05 | 59.31±5.31 | 86.35±3.08 | 2535.53±1.78 | 98.58±1.21 |
| AMF6 | 6.25±0.09 | 70.25±2.41 | 104.02±1.66 | 2865.57±1.32 | 97.69±1.45 |
| AMF7 | 6.21±0.90 | 63.64±2.21 | 96±2.38 | 229.77±1.11 | 97.36±1.49 |
| AMF8 | 6.10±0.92 | 76.58±2.68 | 112.8±5.31 | 2871.77±1.33 | 96.36±1.41 |
| AMF9 | 6.25±0.095 | 80.25±4.72 | 119.4±2.21 | 3111.47±1.32 | 95.87±0.45 |

**Table 5: Rheological behavior of the prepared nasal *in situ* gel.**

| Batch | Viscosity (cps) at 50 rpm | | Consistency index (*K*) | | Flow index (*n*) | |
|---|---|---|---|---|---|---|
| | Sol Phase | Gel Phase | Sol Phase | Gel Phase | Sol Phase | Gel Phase |
| AMF1 | 36.56±1.52 | 418.3±0.38 | 77.92 | 5061.18 | 0.851 | 0.442 |
| AMF2 | 38.14±0.29 | 679.1±0.42 | 84.85 | 6305.52 | 0.853 | 0.505 |
| AMF3 | 31.53±0.77 | 315.5±0.36 | 115.31 | 9648.89 | 0.725 | 0.211 |
| AMF4 | 47.79±0.43 | 761.28±0.51 | 81.58 | 7971.15 | 0.894 | 0.481 |
| AMF5 | 46.54±0.37 | 757.1±0.38 | 81.85 | 4911.69 | 0.896 | 0.571 |
| AMF6 | 51.83±1.58 | 840.5±0.55 | 93.58 | 12031.47 | 0.887 | 0.372 |
| AMF7 | 52.18±0.47 | 805.3±0.48 | 89.58 | 4471.28 | 0.882 | 0.595 |
| AMF8 | 56.77±0.56 | 899.15±0.59 | 115.28 | 12748.11 | 0.851 | 0.385 |
| AMF9 | 59.25±0.51 | 811.08±0.48 | 119.48 | 12889.85 | 0.852 | 0.387 |

in the delivery method may improve retention and absorption in mucosal tissues for a longer period of time. Hydrophilic cationic polymers like HPMC and Chitosan Hydrochloride has numerous polar functional groups. The Glycoprotein chains of mucin become entangled with the polymeric chains of HPMC upon hydration, leading to Bioadhesion.

## Drug Content

In the current study, the medication was integrated into a gel. Considering probable phase transition with medication due to the pH shift essential for the dosage form during intranasal delivery, drug content was measured. The drug concentration of all formulations was observed in the range of 95.91±1.41%-99.47±0.66% (before gelation) and 96.04±0.25%- 99.58±0.56% (after gelation).

After gelation, there was no discernible change in the concentration of the active ingredient, which is evidence that the medication was distributed evenly throughout the gel, as shown in Table 4.

**Table 6: Data obtained for stability studies of the selected formulation AMF4 at 5°C±3°C.**

| Parameters | 5°C±3°C | | | |
|---|---|---|---|---|
| | 0 day | 30 days | 60 days | 90 days |
| Appearance | Transparent | Transparent | Transparent | Transparent |
| pH | 6.41±0.11 | 6.41±0.58 | 6.41±0.74 | 6.21±0.98 |
| Drug Content (%) | 99.58±1.87 | 99.58±1.56 | 98.58±1.65 | 98.18±1.42 |
| Gelation pH | 6.4±1.25 | 6.4±1.75 | 6.4±1.53 | 6.4±1.23 |
| Drug Release (After 12 hr) | 94.24±0.96 | 94.24±0.96 | 94.15±1.89 | 93.85±1.94 |

**Table 7: Data obtained for stability studies of the selected formulation AMF4 at 25°C±2°C/60% RH±5%.**

| Parameters | 25°C±2°C/60% RH±5% | | | |
|---|---|---|---|---|
| | 0 day | 30 days | 60 days | 90 days |
| Appearance | Transparent | Transparent | Transparent | Cloudy |
| pH | 6.41±0.11 | 6.41±0.18 | 6.31±1.85 | 6.05±1.21 |
| Drug Content (%) | 99.58±0.56 | 96.85±1.95 | 95.87±0.25 | 92.18±0.35 |
| Gelation pH | 6.4±0.35 | 6.6±0.89 | 6.9±0.74 | 6.9±1.87 |
| Drug Release (After 12 hr) | 94.24±0.96 | 94.15±1.85 | 93.75±1.84 | 93.35±1.98 |

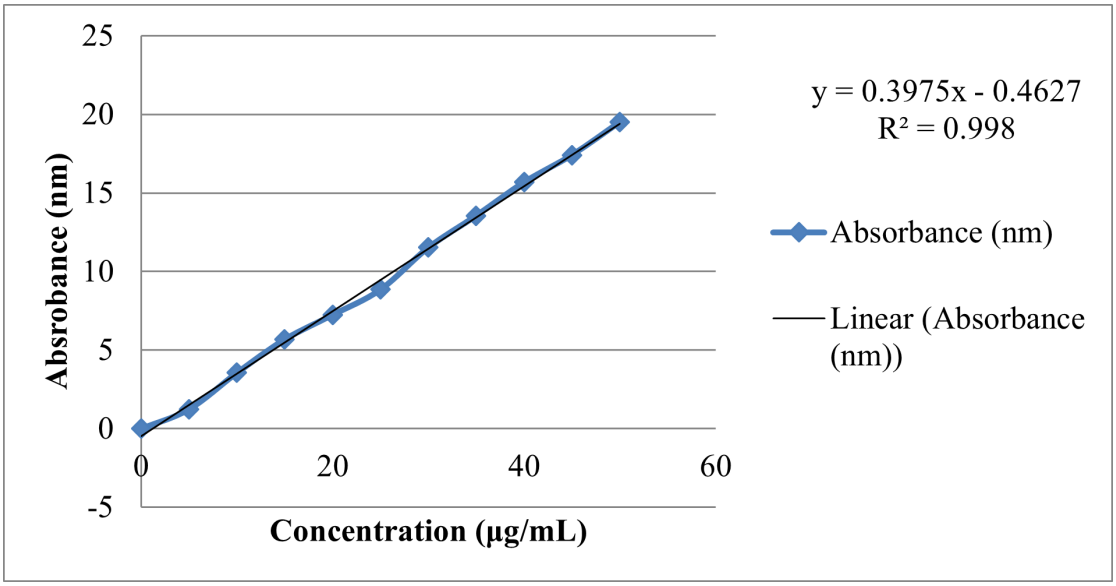


**Figure 5:** Standard calibration curve of pure drug almotriptan malate.

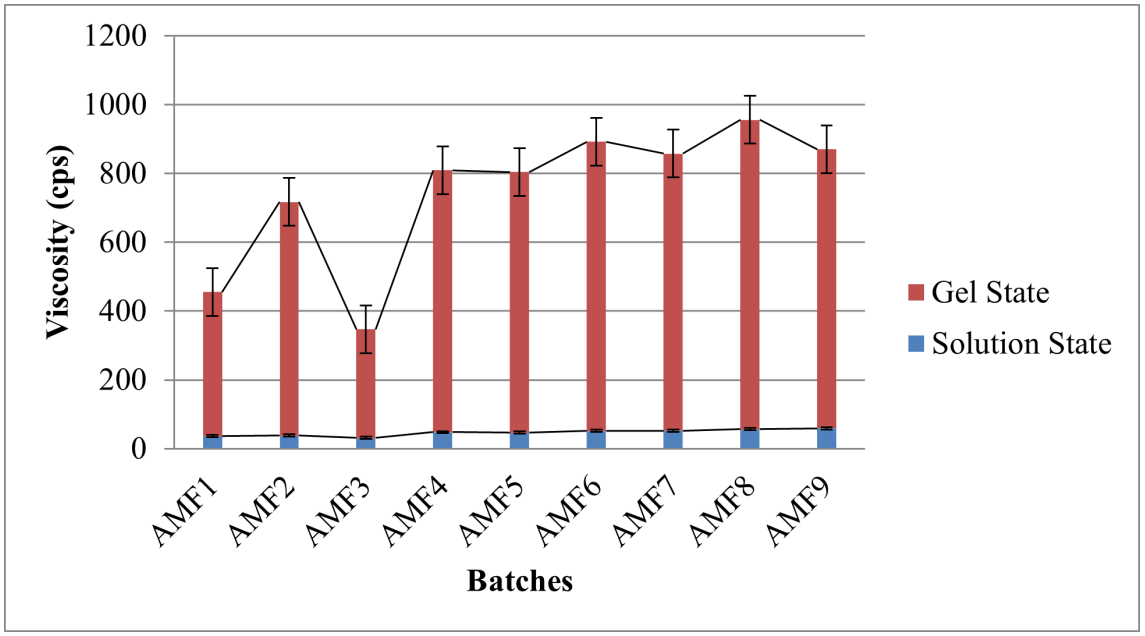


**Figure 6:** Viscosity in cps at 50rpm of formulations AMF1-AMF9.

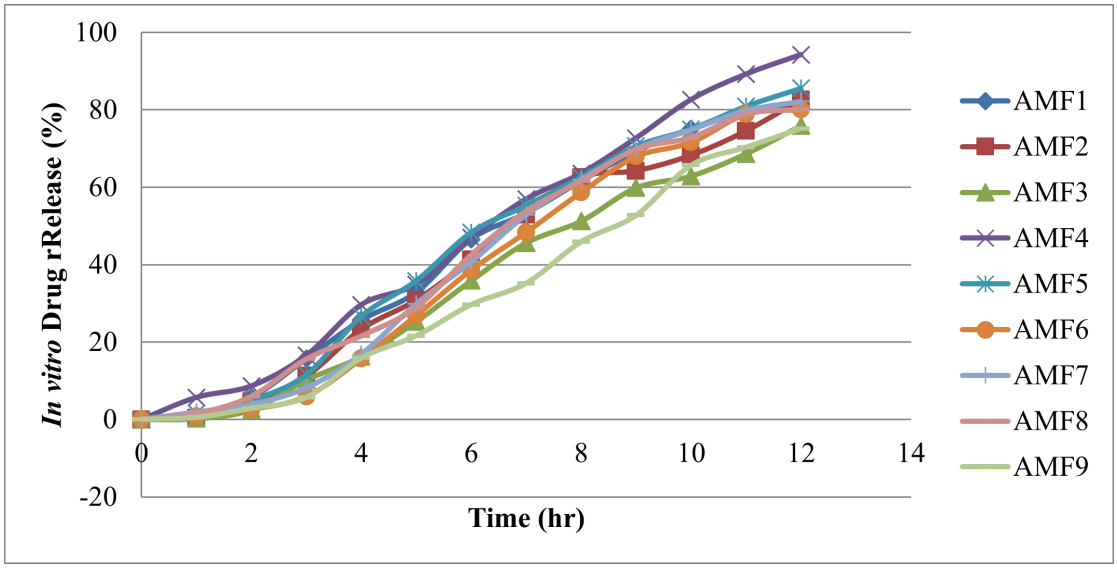


**Figure 7:** Comparative *in vitro* drug release profiles of formulations AMF1-AMF9.

## Rheological Study

The best nasal *in situ* gel detailing would have a low consistency when imparted into the nasal cavity yet would increment in thickness after organization, waiting where it was applied. The blend of the mucoadhesive polymer HPMC and Chitosan Hydrochloride expanded the thickness of the *in situ* gel in a fixation subordinate style. The rheological data for all details is recorded in Table 5. Figure 6 likewise exhibits that the created formulation chart fits the power-regulation rheological condition successfully in both the arrangement and gel states.

Consistency index (*K*) and flow index (*n*) qualities were gained for every detailing, the two of which are dimensionless qualities. If '*n*' is equal to one, then the flow is Newtonian; otherwise, the flow is shear-thinning. Greater shear thinning of the formulation is seen for smaller values of (*n*). All 9 formulations tested had flow indices (*n*) above 0.5 at solution state, making them suitable for nasal administration without the need for refrigeration thanks to their low consistency indices (*K*) and Newtonian flow behavior. The formulations became more viscous after the phase transition. Figure 6 display the viscosity distributions for each formulation.

A notable shear-thinning behavior (*n* less than 0.5) and a significant (*p* less than 0.05) rise in viscosity were noticed while the phase transition was taking place. The increase in viscosity that was measured at the targeted pH provided conclusive evidence that a sol may transform into a gel under those conditions. At the solution state, the gel's viscosity profiles displayed Newtonian

behavior; however, non-Newtonian behavior presented itself as a dramatic increase in viscosity as the gel phase approached. The fact that the viscosities of gels decreased as the shear rate rose is evidence that supports the hypothesis of Pseudoplastic rheology.

## *In vitro* Drug Release Study

*In vitro* dialysis tests were performed in phosphate buffer solution at pH 6.4 on all eight almotriptan-containing *in situ* gel formulations. There was a 50% drug release within 6 hr across all formulations, followed by a steady release over the next 12 hr. In order to have an anti-migraine impact, this release pattern is ideal, followed by a sustained release pattern to keep the loading dose in effect for the treatment of chronic migraine and other symptoms related to migraine. As the concentration of HPMC Chitosan HCl increased, the drug diffusion rate decreased. Drug diffusion percentages for AMF1-AMF9 were calculated to be 80.52±0.48%, 82.65±0.58%, 74.85±0.81%, 94.24±0.96%, 85.52±0.54%, 85.13±0.75%, 80.12±0.92%, 82.18±0.25%, 80.12±0.84%, and 75.1±0.47%, respectively (Figure 7). The fact that formulations AMF6-AMF9 had decreased drug release in comparison to formulations AMF1-AMF5 shows the impact of high polymer fixation on the private relationship of micelles following gelation, which confines drug discharge. This was demonstrated by the fact that formulations AMF6-AMF9 had decreased drug release. One possible explanation for the slowed release of the medication is that it became trapped inside the hydrophobic core. As the mucoadhesive polymer concentration rises, so does the gel's viscosity. In addition, the squeezing effect that the mucoadhesive polymer has on the aqueous channels

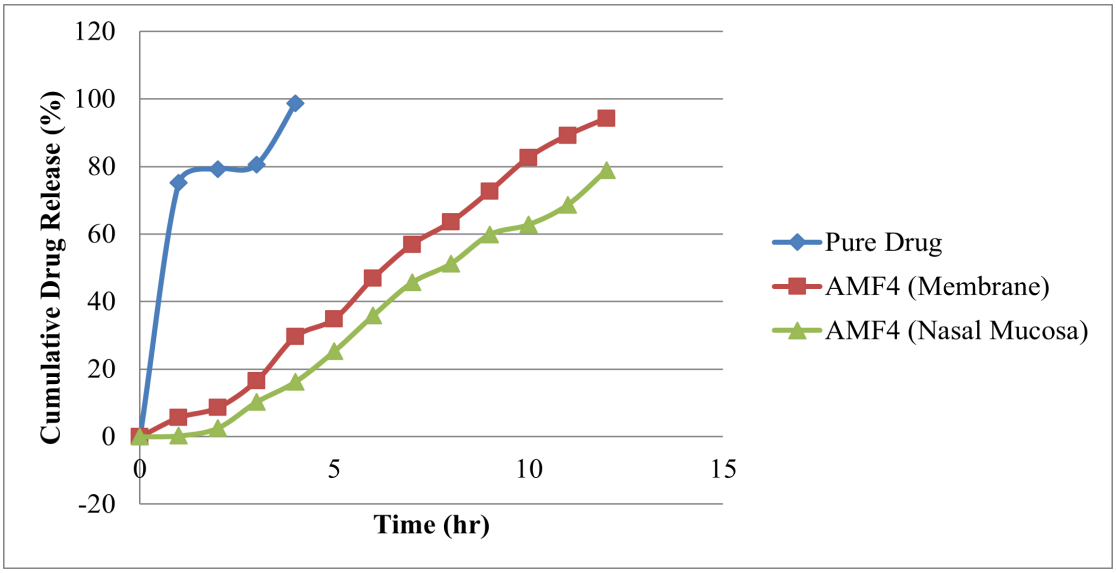


**Figure 8:** Comparative graphical representation of % cumulative drug permeation of formulation by *ex vivo* method.

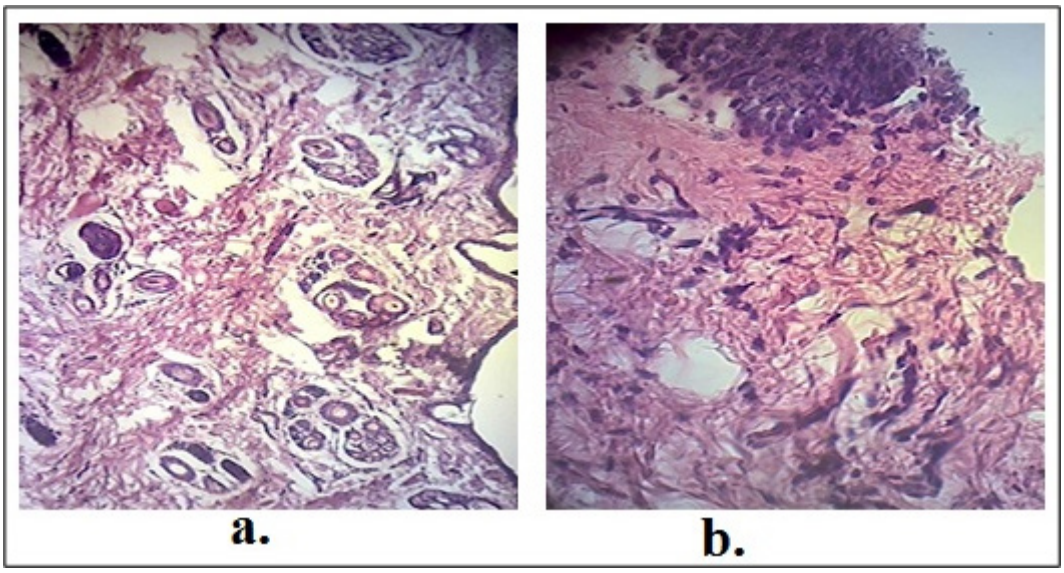


**Figure 9:** Histopathological evaluation of goat nasal mucosa (a. Nasal mucosa treated with PBS (control); b. Nasal mucosa treated with formulation AMF4).

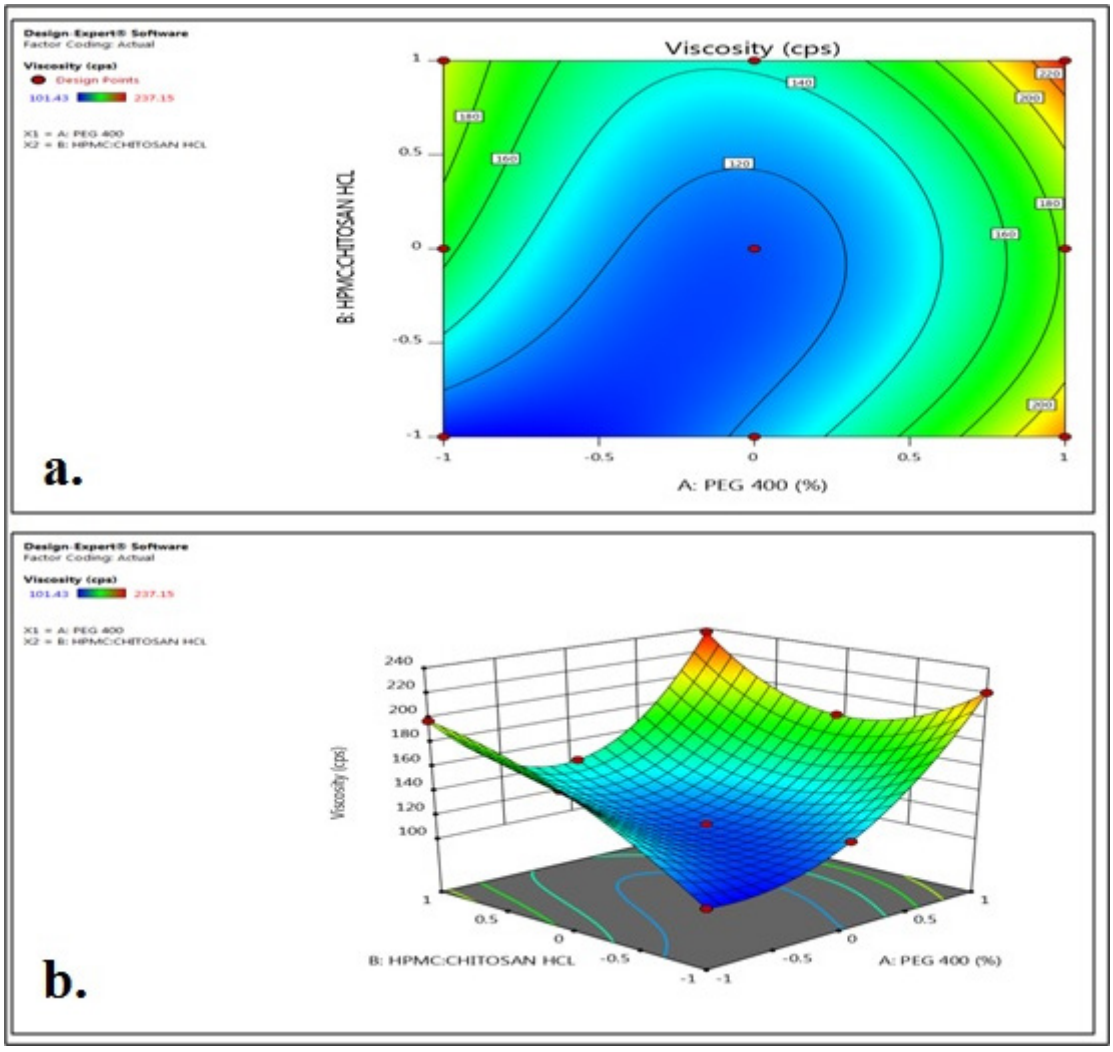


**Figure 10:** Response surface plot (a. contour graphs for viscosity; b. 3D surface plot for viscosity).

which works to prevent the diffusion of almotriptan. After 12 hr, drug diffusion was 94.24±0.95% more efficient with the improved formulation, AMF4.

## *Ex vivo* Permeation Study

As can be seen in Figure 8, the cumulative percentage of medication that passed through the nasal membrane of goat after a period of 12 hr was found to be 78.85±0.87%. Because of the presence of polymers, the medication is released into the environment more slowly than usual. These polymers prevent the drug from being released from the gel matrix and into the surrounding environment. It is desirable over have a slower delivery to accomplish an impact for a more drawn-out time frame period and to lessen the dosing interval.

## Histopathological Evaluation of Nasal Mucosa

The improved formulation (AMF4) had no appreciable impact on the mucosa's microscopic structure, according to Histopathological investigation, as evidenced by the lack of cell necrosis or epithelium loss (Figure 9a and 9b). Evaluation of the nasal mucosa of goat demonstrated a lack of toxicity via histopathology. At the microscopic level, the membranes, both treated with the optimized formulation and untreated, displayed a comparable tissue architecture, consistent with the undamaged columnar organisation of epithelial cells. Compared to PBS-treated mucosa, gel formulation for nasal administration was shown to be safe since the epithelial layer remained unharmed and the basal membrane and superficial area of the sub-mucosa showed no changes.

## Release Kinetics

*In vitro* drug release data from the optimized formulation were utilized to evaluate the mechanism of release and the release rate kinetics of the dosage form. Models representing zero order, first order, Higuchi, and Korsemeyer-Peppas were fitted to the data. There is less of an appearance of linearity when the data are displayed using the first-order equation. Because of this, we are able to draw the conclusion that the major drug-release mechanism demonstrates zero-order kinetics, as indicated by a regression coefficient ($R^2$) value of 0.9702. A mathematical model

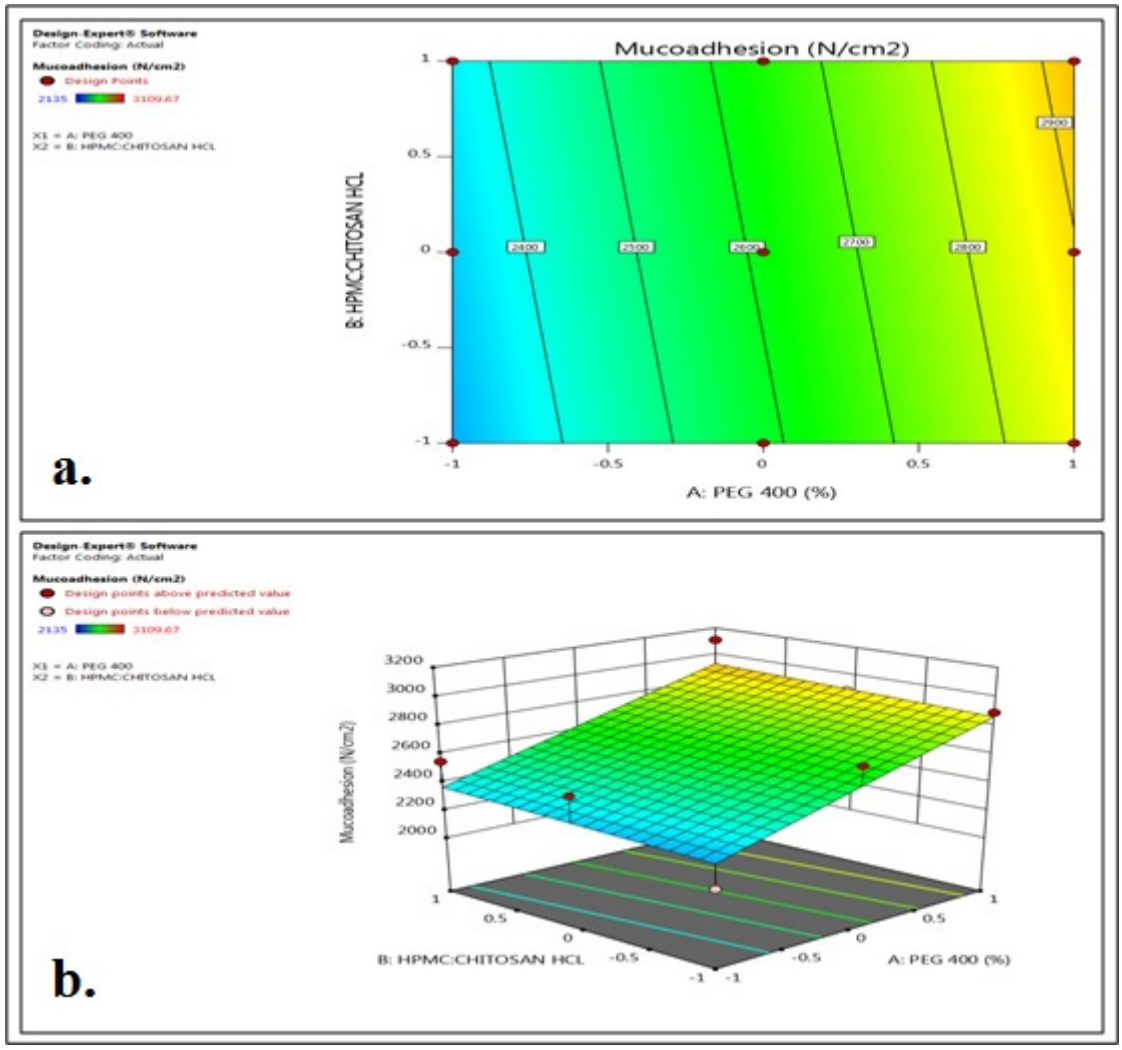


**Figure 11:** Response surface plot (a. contour graphs for mucoadhesion; b. 3D surface plot for mucoadhesion).

known as the Korsemeyer-Peppas plot can be utilized to infer information regarding the drug-release mechanism. The plot of the percentage of release against the square root of the passage of time is linear. In most cases, data based on first-order models will provide evidence for the diffusion process that is involved in the release of drugs from sustained-release delivery systems. Because of this, the overall quantity of pharmaceuticals present in the matrix has a significant impact on drug release. Because the optimized formulation showed an n value greater than 0.5, it is possible that the diffusion rate of the formulation and the relaxation rate of the polymer matrix were responsible for regulating the release of the drug.

## Analysis of Data

To lead the examination of the answers, a measurable model that included both intelligent and polynomial factors was applied.

$$Y=b0+b1X1+b2X2+b12X1X2+b11X12+b22X22$$

Where Y is the reliant variable, b0 is the number-crunching mean reaction of the nine runs, and b1 (b1, b2, b12, b11, and b22) is the assessed coefficient for the comparing factor X1 (X1, X2, X12, X11, and X22), this addresses the typical results got by continuously expanding the worth of one element until all values have been expanded. Changes in the reaction because of concurrent adjustments to two elements are addressed by the cooperation term (X1, X2).

$$Y\ (Viscosity)=65.47+9.47X1+28.83X2$$

The positive coefficients of X1 and X2 in the above condition show that the thickness increments with the convergence of gellan gum and HPMC.

$$Y\ (Mucoadhesive\ strength)=+2468.26+208.89\ X1+290.78\ X2-5.25\ X1\ X2+80.45\ X12\ 4.89\ X22.$$

From above condition the positive coefficient of variable X1 and X2 demonstrate that, as convergence of HPMC Chitosan HCl and PEG 400 expanded the mucoadhesive strength was additionally increased.

$$Y\ (\%\ Drug\ discharge)=+88.89-2.52\ X1-6.85\ X2$$

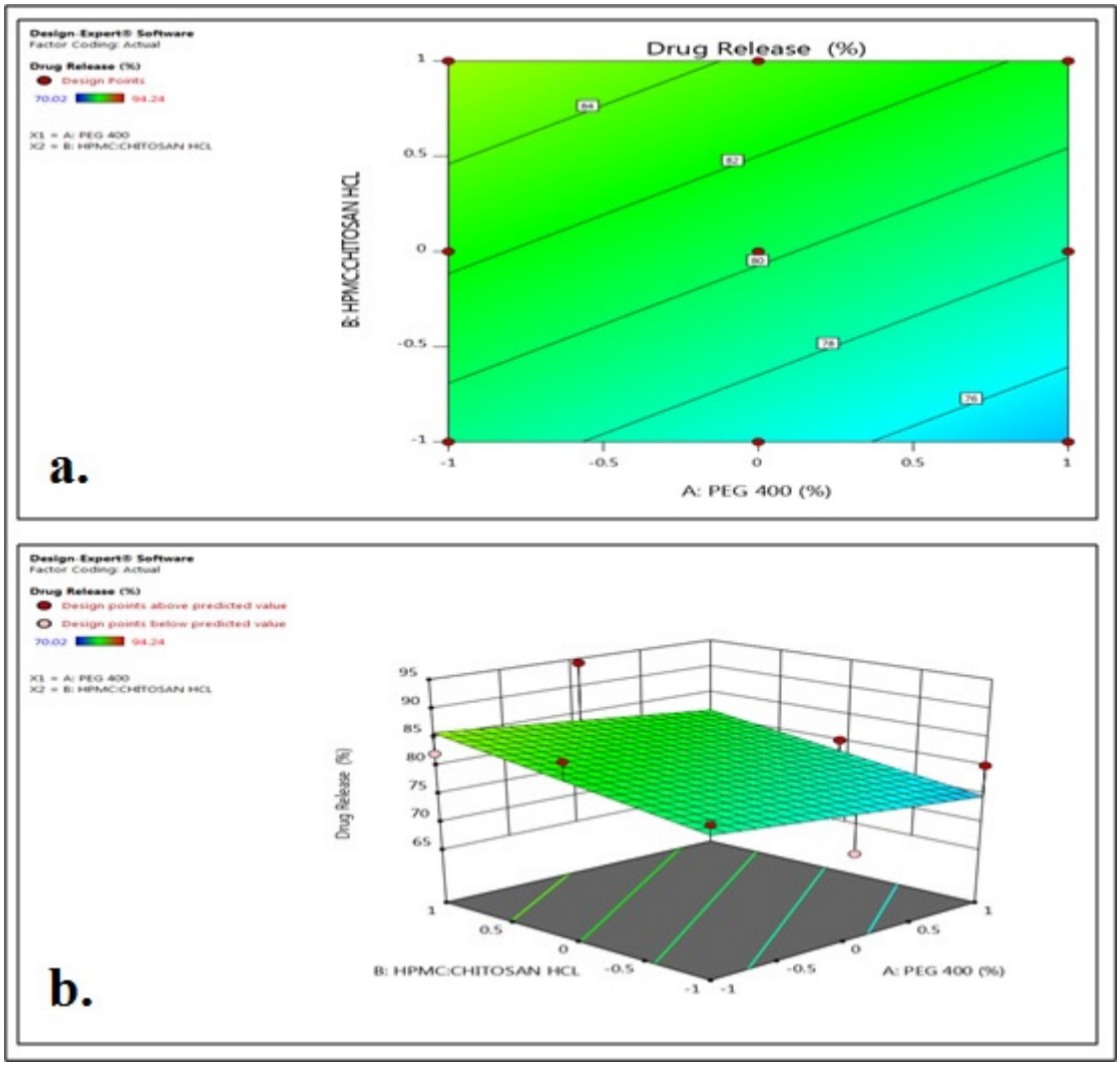


**Figure 12:** Response surface plot (a. contour graphs for drug release; b. 3D surface plot for drug release).

The way that the level of medication discharge dropped as the grouping of HPMC: Chitosan HCl rose uncovers that the negative coefficient of the consolidated variable X1 and X2 recommends that the level of medication discharge diminishes as the proportion of polymer increments.

## Response Surface Plot (RSP)

Based on the results of the RSP examination, it was discovered that the content of HPMC Chitosan HCl rose, along with an increase in mucoadhesive strength and viscosity. While the opposite is true in the case of drug release, a rise in polymer concentration results in a decrease in permeability release, as seen in Figure 10, Figure 11, and Figure 12. The results of the RSP were comparable to the data from the mathematics.

## Stability Studies

Table 6 and Table 7 show the results of stability tests conducted on the optimised formulation AMF4. AMF4 formulation held at room temperature showed a minor change in actual appearance. Transparency of the formulation didn't change at 5°C±3°C. Changes in pH levels were recognized. With time, the definition's pH dropped, arriving at 6.21±0.98 following 90 days, all through both temperature settings. The almotriptan malate content of the AMF4 put away at 5°C±3°C fluctuated barely marginally. In any case, following 90 days at room temperature, the medication content diminished from 99.58±0.56% to 92.18±0.35% because of dryness of the gel. The total level of prescription delivery didn't modify observably between capacity settings. So too with the gelation pH. These discoveries recommend that in-situ gels kept up with at 5°C±3°C are ideal.

## CONCLUSION

Good therapeutic action and prolonged nasal residence time can be attained with intranasal administration of a pH-dependent in-situ gel formulated from almotriptan malate. The mucoadhesive properties of the gel prolonged the drug's stay in the nasal cavity, and the gel's pH-dependent nature made it easy to administer and handle. It has been determined that the optimised formulation of

HPMC-E15 (5gm), Chitosan HCL (1gm), and PEG 400 (1.5gm) is suitable to provide adequate mucoadhesive strength and the desired pH (6.4±1.25), and it is stable at 5°C±3°C and acceptable for routine nasal administration. Therefore, the pH-dependent gel system appears to be a promising formulation for the intranasal delivery of almotriptan malate for the safe and effective treatment of migraine and its associated symptoms.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**RSP:** Response Surface Plot; **HPMC:** Hydroxyl Propyl Methyl Cellulose; **FTIR:** Fourier Transform Infra-Red Spectroscopy; **DSC:** Differential Scanning Calorimetry; **UVS:** Ultra Violet Spectroscopy.

## SUMMARY

The planning and evaluation of the characteristics of intra nasal delivery of a pH-dependent gel containing almotriptan malate as a treatment for migraines is the purpose of this study. The researcher of the current study attempted to create an *in situ* nasal gel formulation of almotriptan malate by using a design that is known as a factorial design. 95.87±0.45 to 99.58±0.56% drug content. pH was 6.10±0.92 to 6.41±0.11. The results corresponded with the phase transition, and the formulations showed pseudoplastic rheology. This revelation was exciting. Mucoadhesiveness rose with HPMC-E15 Chitosan HCl concentration, which was 2133±1.58 to 3111.47±1.32 dynes/cm$^2$ for the most successful formulations. The improved formulation of 5 g HPMC-E15, 1 g Chitosan hydrochloride, and 1.5 g PEG400 released 94.24±0.96% medication after 12 hr. Histopathology studies demonstrated that the AMF4 formulation was non-toxic to the nasal mucosa when submitted to *ex vivo* penetration tests, which showed 78.850.87% drug release. The stability research recommends storing formulations at 5°C±3°C. According to these observations, nasal *in situ* gel containing Almotriptan Malate may soon be used to treat migraines and related symptoms. In a nutshell, a pH-dependent *in situ* gel formulation of Almotriptan Malate has the potential to cure migraines effectively.

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