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Formulation design and evaluation of nasal *in situ* gel as a novel vehicle for Azelastine hydrochloride.

Inayat Bashir Pathan[1]*, Vijay Chudiwal[1], Irfan.farooqui[1], Prakash Shingare[1]

***Corresponding author:**

Inayat Bashir Pathan

[1]Department of Pharmaceutics. Government College of Pharmacy, Aurangabad, Vedant road, Osmanpura -431003 Maharashtra, India.

Abstract

For locally acting intranasal drugs, an extended residence time in the nasal cavity is desirable and related to a prolonged effect. The aim of the present work was to design a nasal delivery system with improved mucoadhesive properties that could provide prolonged retention time for the treatment of allergic rhinitis. A 3^2 factorial design was used to investigate effect of amount of gellan gum and mucoadhesive polymer namely HPMC E4M as independent variable. Viscosity and mucoadhesive strength were taken as dependent variables. The formulations were tested for gelation study, viscosity study, gel strength, mucoadhesion study, shot weight study, drug content, histopathological evaluation, and stability study. Gelation was determined by physical appearance. Viscosity study of sol and gel formulations indicated that increase in polymer concentration increases the viscosity. Shot weight of the formulations was found to proportionally vary with the viscosity of formulations. Gel strength was found in the range of 22-55 sec. The mucoadhesive force in terms of detachment stress increased with increase in the concentration of HPMCE4M. Histopathological examination of sheep nasal mucosa with control and optimized formulation did not show any change in the nasal tissue. A stability study for optimized AZ5 formulation as per ICH guideline for 90 days showed no change in pH, drug content, and viscosity. The developed in situ gelling system for azelastine hydrochloride using gellan gum in combination with HPMC E4M with improved mucoadhesive properties that could provide prolonged retention time for the treatment of allergic rhinitis.

Keywords: 32 factorial design, In situ nasal gel, azelastine hydrochloride, gellan gum

Introduction

The nasal route is an important mode of drug delivery, with a growing number of products available for administration through this route for systemic and local action, such as for allergic rhinitis. For locally acting anti-allergic drugs, an extended residence time in the nasal tissue is related to a prolonged pharmacologic activity. An additional advantage is the slow distribution into systemic circulation resulting in low plasma concentrations and therefore a low risk of systemic toxicity [1]. One of the constraints of delivering drugs via the nose is the capacity of the nasal cavity; the maximum volume administered is typically only about 0.2 ml per nostril. Even at these low volumes, delivery of many nasal products, typically simple aqueous solutions, is sub-optimal, notably when post nasal drip or run-off into the throat gives rise to discomfort/tolerability issues or reduced/variable absorption and therefore efficacy. Gel formulations have the potential to reduce mucociliary clearance, post-nasal drip and anterior leakage. But the effective delivery of a nasal product formulated as a gel is technically challenging, especially ensuring effective deposition and distribution within the nasal cavity [2]. It is well known that the allergic rhinitis is an inflammatory disease of the upper airway, which is accompanied by sneezing, itching, congestion, rhinorrhea and loss of the sense of smell. These symptoms are considered to be caused by antigen–antibody reaction on mast cells that are located on the epithelia of the nasal cavity [3]. Azelastine hydrochloride is an intranasal antihistamine indicated for use in patients with seasonal allergic rhinitis and non-allergic vasomotor rhinitis [4]. In situ gel is a new dosage form which has been applied in nasal drug delivery recently. Compared with liquid nasal formulations, nasal in situ gels are instilled as low viscosity solutions into the nasal cavity. Upon contact with the nasal mucosa, the polymer changes conformation producing a gel, so that it can not only prolong the contact time between the drug and the absorptive sites in the nasal cavity, but also releases drug slowly and continuously. Hence, it is especially useful for those drugs used chronically. Gellan gum is an anionic deacetylated, exocellular polysaccharide secreted by Pseudomonas elodea. The mechanism of gelation involves the formation of double-helical junction zones followed by aggregation of the double-helical segments to form a 3-D network by complexation with cations and hydrogen bonding with water [5]. Since human nasal mucosa is covered with approximately 0.1 ml mucus, which consists of sodium, potassium, and calcium ions, a solution-gel phase transition can be expected. Till date, there is no report on preparation of in situ nasal gel of azelastine

hydrochloride. The objective of the present work was to design a nasal delivery system for azelastine hydrochloride based upon the concept of ion activated in situ gelation with improved mucoadhesive properties that could provide prolonged retention time for the treatment of allergic rhinitis.

Materials

Azelastine Hydrochloride was a gift sample from MSN Laboratories, India. Gellan gum were procured as gift samples from CPKelco Signet Chemical Corporation Ltd. HPMC E4M was a gift sample from Colorcon, Mumbai, India. Nasal device procured as a gift sample from Vinis Products Pvt. Ltd, Mumbai, India. Mannitol and Benzalkonium chloride were obtained from Wockhardt Ltd, Aurangabad. All other chemicals were of analytical reagent grade.

Methods

Preparation of in situ gels

Gellan gum was weighed and dispersed in ultra-pure water. The dispersions were then stirred by mechanical stirrer (Remi motors ltd, Mumbai, India, type RQ-122) for 30 min at 90 C in a water bath and then cooled to room temperature. HPMC E4M was weighed and dissolved in ultra-pure water and heated at 90^0C then cooled to room temperature. 10 ml of chilled water was added to it. Azelastine hydrochloride (AZ HCL) (0.1% w/v) was added in small volume of ultra-pure water and sonicated (Toshcon ultrasonic cleaner, Toshniwal instrument Pvt. Ltd. Ajmer) for 30min. HPMCE4M solution and drug solution were added in gellan gum solution slowly with continuous stirring. Appropriate quantities of mannitol and benzalkonium chloride (BKC) were added simultaneously. The formulations were filled in bottles. The formulation layout for the factorial design batches (AZ1 to AZ9) are shown in Table 1.

Table 1: Composition of all nasal in situ gel formulation

Sr no	Ingredients	AZ1	AZ2	AZ3	AZ4	AZ5	AZ6	AZ7	AZ8	AZ9
1	AZ HCL	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
2	Gellan gum	0.3	0.3	0.3	0.4	0.4	0.4	0.5	0.5	0.5
3	HPMC E4M	0.05	0.1	0.15	0.05	0.1	0.15	0.05	0.1	0.15
4	Mannitol	4	4	4	4	4	4	4	4	4
5	BKC	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
6	Purified water quantity sufficient.	100ml	100ml	100ml	100ml	100ml	100ml	100ml	100ml	100ml

Experimental design

A 3^2 randomized full factorial design was used in the present study (Design Expert 8.0.7.1). Two independent factors were evaluated, each at 3 levels, and experimental trials were performed for all 9 possible combinations. The concentration of gellan gum (X1) and concentration of HPMC E4M (X2) were chosen as independent variables. Viscosity and mucoadhesive strength were taken as dependent variables.

Evaluation of prepared in situ gels

Appearance

The developed formulations were inspected visually for clarity in sol and gel form.

Gelation study

The Simulated Nasal Fluid, SNF (aqueous solution containing 8.77 mg/ml NaCl, 2.98 mg/ml KCl and 0.59 mg/ml CaCl2 per liter), having the cationic composition of nasal secretions, was prepared according to the report. Gellan gum is a polymer which undergoes change from sol to gel in the presence of cations. Gelation is the process by which the liquid phase (sol) makes a transition into gel. Azelastine hydrochloride in situ gel and simulated nasal fluid were mixed in 1:1v/v ratio. The gelation study was done on magnetic stirrer (1MLH magnetic stirrer, Remi). The gelation point was determined when the magnetic bar stopped moving due to gelation. The consistency of formed gel was checked and graded, as indicated in Table 2.

Viscosity and rheological study

The viscosity of nasal in situ gel formulation before and after gelation was determined using Brookfield Rheometer R/S-CPS +1600, Lauda Ecoline Staredition RE-204, having cone-and-plate geometry by using spindle coaxial CP75-1. The shear rate was varied from 1 to 1000/s. Samples were applied to the plate using a spatula(approximately 2 ml) to ensure that formulation shearing did not occur. Each point is the average of at least three readings [6].

pH study

pH of all formulations was determined by using pH meter (Model No. EQ-621, Equip Tronix microcontroller pH meter).

Gel strength study

The gel strength is an indication of the viscosity of the nasal in situ gel at physiological condition. It is expressed in terms of seconds required by a 35 g piston for penetration of 5 cm distance, through the 50 g gel formulation. This test was performed using 'Gel strength apparatus' modified at the laboratory [7]. In situ gel formulation (50 g) was placed in a 100 ml-measuring cylinder and gelation was induced by SNF. The apparatus for measuring gel

strength (weight: 35 g) was then placed on the gel. In case the apparatus took more than 10 min to drop into the gel, various weights were placed on top of the apparatus. The gel strength was measured by the minimal weight that pushed the apparatus 5 cm down through the gel [8].

Mucoadhesive strength study

Mucoadhesive Strengths of gel was determined by the method reported [9,10]. Nasal mucosal tissue, obtained from the local slaughterhouse (Aurangabad), was carefully removed from the nasal cavity of sheep and mounted on glass surface using adhesive tape while another mucosal section was fixed in inverted position to the cylinder. 50mg of gel was placed on mucosal surface. The glass mounted mucosal surface with gel formulation and mucosal surface attached to cylinder were held in contact with each other for 2min to ensure intimate contact between them. In second pan, the weights were increased until the two mucosal tissues got detached from each other. The nasal mucosa was changed for each measurement.

The mucoadhesive force expressed as the detachment stress in dynes/cm^{2}was determined from the minimal weight that detached the mucosal tissue from surface of each formulation.

Mucoadhesive Strength (dynes/cm 2) = mg/A (1)

Where, m = weight required for detachment in gram,

g = Acceleration due to gravity (980cm/s^2),

A = Area of mucosa exposed.

Shot weight study

Shot weight study was performed to assess pump-pump reproducibility and to evaluate the delivery from the pump (137 µl, PP Bottles). Shot weights were assessed by weighing the spray pumps prior to and after each actuation using an analytical balance (A and D Company, Ltd, Japan) having maximum weighing capacity of 210 gm with readability to 0.1 mg. In general, pump spray weight delivery acceptance criteria should control the weight of the individual spray within 15% of the target weight and the mean weight within 10% of the target weight [11].

Drug content

1 ml of formulation was taken in 10 ml volumetric flask, diluted to 10 ml with phosphate buffer pH 6.6 and shaken to dissolve the drug. The content of the drug was estimated on UV-Visible Spectrophotometer (Shimadzu, UV-1700, LabIndia) at λmax 212.20nm.

Histopathological study

Nasal mucosal tissue incubated in phosphate buffer (control) after collection was compared with tissue incubated with in situ gel formulation (AZ5). Tissue was fixed in 10% buffered formalin (pH 7.2), routinely processed and embedded in paraffin. Paraffin sections (7 µm) were cut on glass slides and stained with hematoxylin and eosin (HE). Sections were examined under a light microscope to detect any change in the tissue.

Drug-excipients interaction study

Differential Scanning Calorimeter DSC-60 (Shimadzu, Japan.DSC-60) was used to study the interaction between the drug and excipients. Samples, 5.4mg, were weighed and sealed in standard aluminum pan and then scanned over a temperature range from 150^{0}C to 300^{0}C at a heating rate of 20.00^{0}C / min.

Stability studies

The stability of optimized formulation AZ5 was tested according to ICH guideline, at 40^{0}C±2^{0}C/ 75%RH± 5% condition in stability camber (HMG, India) for three months [12]. Tablets were tested for drug content for 30, 60, and 90 days.

Result and Discussion

Gellan gum, forms gel in presence of cations. HPMC E4M, a mucoadhesive polymer, was added to improve mucoadhesion. Mannitol was used for adjusting tonicity and benzalkonium chloride as preservative.

Appearance

All formulations were found clear in both sol and gel form.

Gelation study

In vitro gel study was carried out by using simulated nasal fluid. Gelation was assessed on a scale ranging between – and +++, as shown in Table 2. The composition of nasal electrolyte was rich in cationic content. After instillation into the nasal cavity the liquid solution should undergo a rapid change from sol-to-gel transition by means of ionic gelation. All gellan gum formulations showed instantaneous gelation, depending upon the polymer concentration. Formulation AZ1 showed less gelation as compared to AZ9. This indicates that with an increase in concentration of gellan gum, the gelation point increases (immediate gelation). From the gelation study, it was observed that the HPMC E4M did not affect the gelation property but the gellan gum plays a critical role in hydrogel formation.

Viscosity and rheological study

The main requirement of in situ gelling system for nasal administration is optimum viscosity that will allow easy spray as a liquid from nasal device, which then undergoes a rapid sol-gel transition due to ionic interaction. In addition, the formed gel should preserve its integrity to facilitate sustained release of drug locally for prolonged period without dissolving or eroding quickly. For satisfactory gel strength the selection of the concentration of gellan gum for use as a delivery vehicle and an acceptable viscosity for ease of spraying from the nasal device. Viscosity of all formulations containing liquid and gel was studied using Brookfield viscometer. The increase in viscosity of all formulations was observed after sol-to-gel transition. A large change in viscosity of formulations was observed with gelling polymer, gellan gum, compared to HPMC E4M. The viscosity of both, solution and gel formulations, was

found to be proportionate to the increase in polymer concentration. All the formulations in solution state showed Newtonian flow whereas gels exhibited non-Newtonian flow as shown in Figure. 1.

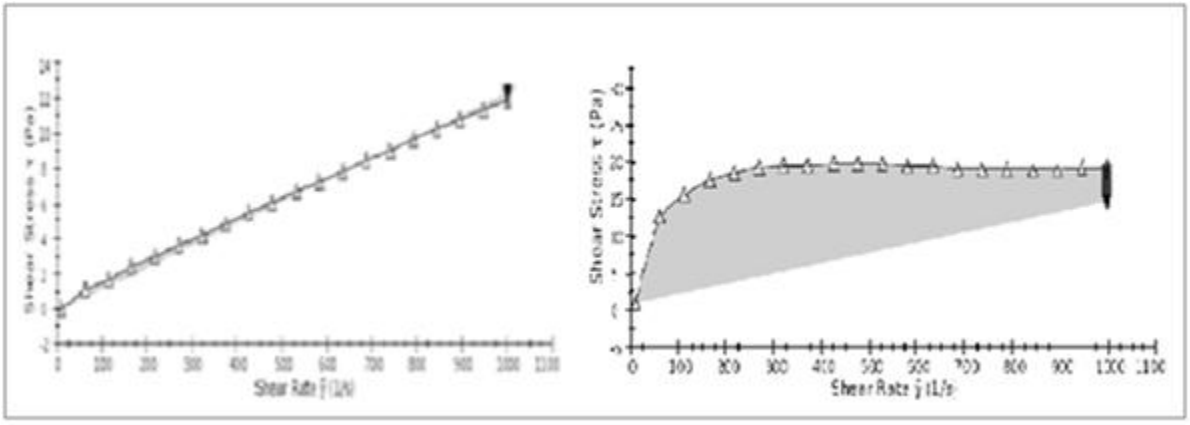

Figure 1 Showing Newtonian flow for sol and non-Newtonian flow for gel.

Table 2: pH, Drug content, Gelation capacity, Viscosity, Gel strength , Shot weight studies and Mucoadhesive strength of all formulation

Batch code	pH*	Drug content*	Gelling capacity	Viscosity (cps)		Gel strength(sec)*	Shot weight (mg)	Mucoadhesive strength(dynes/cm^2)*
				Solution*	Gel			
AZ1	6.53±0.05	98.48±0.3	++	28.16±2.14	98.6	22.33±1.15	136.974	2044.37
AZ2	6.54±0.02	98.92±0.2	++	34.17±2.34	115.12	26.67±1.53	136.960	2395.11
AZ3	6.54±0.08	98.96±0.1	++	43.41±1.04	130.19	31.00±1.00	136.951	2709.97
AZ4	6.56±0.06	98.62±0.0	+++	50.15±3.06	150.11	32.33±0.58	136.945	2159.65
AZ5	6.58±0.05	98.18±0.3	+++	55.55±0.99	166.25	35.67±0.58	136.938	2504.66
AZ6	6.60±0.05	98.04±0.8	+++	67.85±2.83	180.94	37.33±0.58	136.934	2858.80
AZ7	6.62±0.01	97.82±0.1	+++	79.46±1.36	205.94	42.33±1.15	136.925	2390.22
AZ8	6.64±0.06	98.15±0.1	+++	96.31±2.04	222.14	46.33±1.53	136.914	2711.45
AZ9	6.65±0.03	98.29±0.1	+++	103.03±2.1	243.84	52.33±1.53	136.911	3030.14

(+ +) Immediate gelation remains for few hours (less stiff gel) (+ + +) Immediate gelation remains for extended period (stiff gel).*denotes all values with standard deviation (S.D), n=3

pH study

The normal physiological pH of nasal mucosa is 4.5 – 6.5. However, the nasal mucosa can tolerate solutions within pH range of 3-10. The pH of all formulations was found to be in the range of 6.53-6.65 as shown in Table 2.

Gel strength

In the development of a nasal in situ gelling system, the gel strength is an important criteria, which allows easy administration as droplets and extends the post-nasal drip of the nasal formulation. The gel strength values between 25–50 sec are considered adequate. The gel strength less than 25 sec may not retain its integrity and may erode rapidly while gel having strength greater than 50 sec is too stiff and may cause discomfort to the mucosal surfaces. All the formulations had gel strength between 22.33 sec to 52.33 sec in triplicate as shown in Table. 2 and were considered suitable for nasal administration.

Mucoadhesive strength study

Mucoadhesive strength was determined in terms of detachment stress. All formulations were subjected to in vitro mucoadhesion strength test. Our study indicates that the variation in concentration of HPMC E4M and gellan gum changes mucoadhesive strength. Figure 2 shows the effect of gellan gum and HPMC E4M on mucoadhesion strength. The significant effect was observed with HPMC E4M as compared to gellan gum. This was due to wetting and swelling of HPMC, which permits intimate contact with nasal tissue, interpenetration of mucoadhesive HPMC chains with mucin molecules leading to entanglement and formation of weak chemical bonds between entangled chains. Due to stronger mucoadhesive force, it can prevent the gelled solution coming out of the nose and increases its residence time in nasal cavity. But higher ratio of

HPMC, responsible for excessive mucoadhesive force, and gellan gum can damage the nasal mucosal membrane. Mucoadhesion strength of all formulations is shown in Table 2.

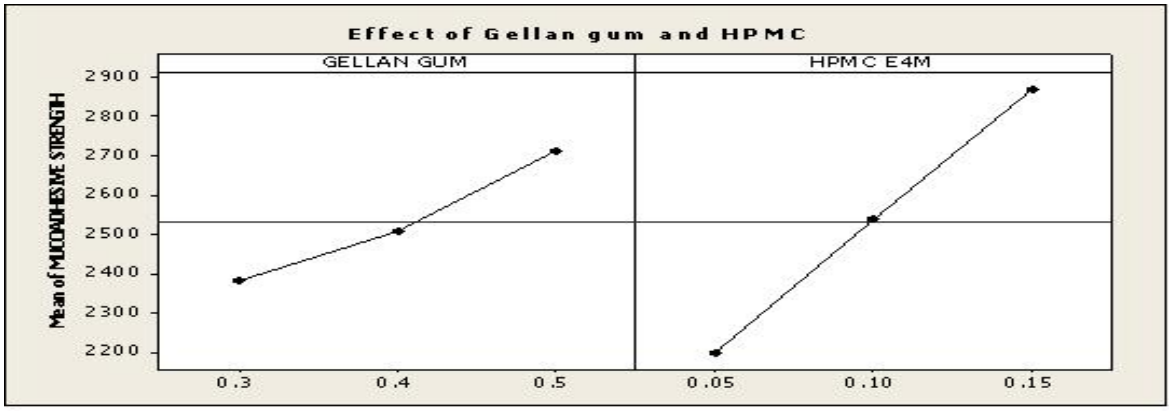

Figure 2 Displays the effects of gellan gum and HPMC E4M on mucoadhesion force.

Shot weight study

The delivery of formulation from nasal device mostly depends on the device chemistry and viscosity of the formulation. To assess device-device reproducibility, it is essential to carry out shot weight study. The study was done on all formulations, and it was found that as the viscosity of the formulation increases, the weight of formulation after the actuation decreases. Table 2 shows the shot weight of all nasal in situ gel formulation.

Drug content

The percent drug content of all formulations was found to be in the range, 97.82-98.92%, which is within the acceptable limit. The results are shown in Table.2.

Histopathological study

The histopathological study showed no significant effect of the optimized formulation on the microscopic structure of the mucosa. As shown in Figure 3. The epithelium layer was intact and there were no alterations in basal membrane and superficial part of submucosa as compared with phosphate buffer treated mucosa (control). Thus, the formulation seems to be safe for nasal administration.

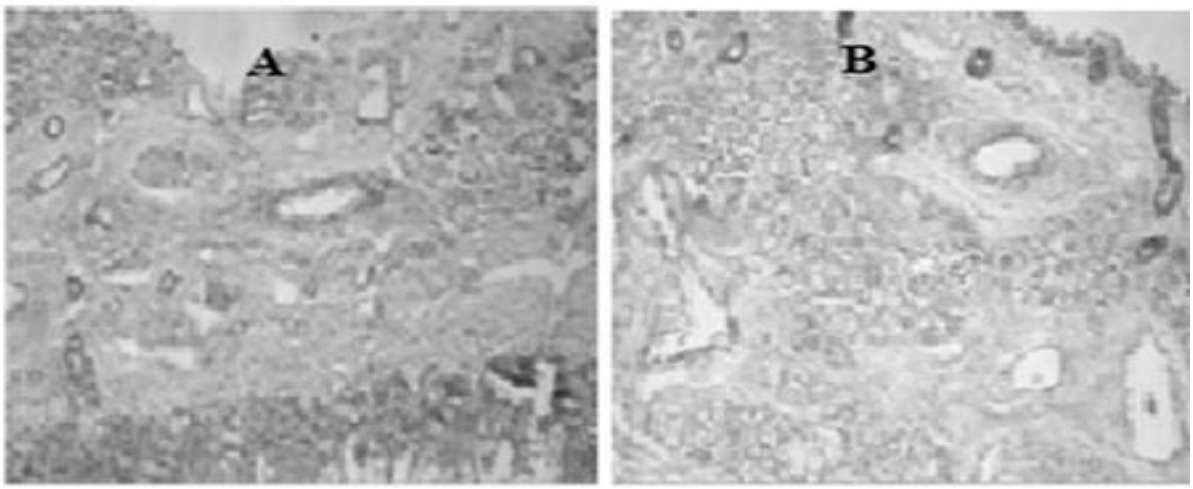

Figure 3 Histopathological examination of nasal mucosa.

Drug-excipients interaction study

Differential scanning calorimetric determination revealed that, the endothermic peak of pure drug starts from 218.75^{0}C and ends at 236.47^{0}C, where as endothermic peak of the formulation starts from 214.43^{0}C and ends at 231.25^{0}C. Azelastine hydrochloride exhibited a sharp endothermic peak at 228 C while formulation showed an endothermic peak at around 225 C. This indicates no interaction between drug and excipients. The findings are shown in Figure 4.

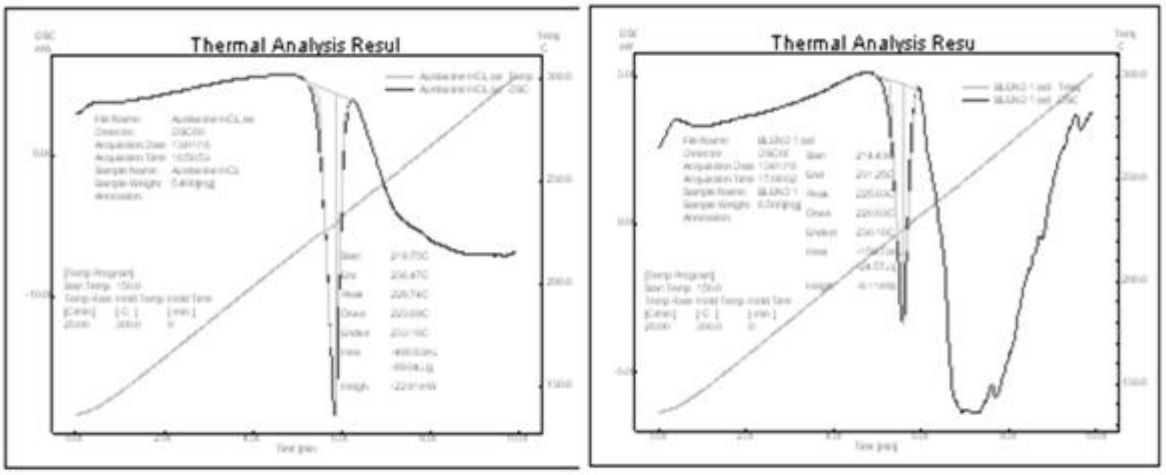

Figure 4 Showing DSC of drug and formulation.

Validation of the Experimental Design

In order to validate the experimental design using a polynomial equation, three parameters namely viscosity and mucoadhesive strength were selected. The following second order polynomial equation was applied as a tool of mathematical modeling:

$$Y= b_0+b_1 X_1+b_2 X_2+b_{12} X_1X_2+b_{11}X_1^2+b_{22}X_2^2$$

Where, Y is the dependent variable, b_0 is the arithmetic mean response of the nine runs and b_1 (b_1,b_2,,b_{12},b_{11} and b_{22}) is the estimated coefficient for corresponding factor X_1 (X_1,X_2,X_{12},X_{11},and X_{22}), which represents the average results of changing one factor at a time from its low to high value. The term X_1^2 and X_2^2 indicate curvilinear relationship. The interaction term (X_1X_2) depicts the changes in the response when two factors are simultaneously changed.

The parameter viscosity can be described by the model equation,

Y (viscosity) =+62.01+28.84X1+9.42X2

The positive sign for coefficient X1 and X2 indicates that as concentration of gellan gum and HPMC E4M increases, viscosity increases. R^2 value 0.978 for viscosity indicating good correlation between independent and dependent variable. The term with (P<0.0001) were considered significant.

The parameter mucoadhesive strength can be described by model equation,

Y(mucoadhesivestrength) =+2533.81+163.729X1+334.110X2

The positive sign for coefficient X1 and X2 indicates that as concentration of gellan gum and HPMC E4M increases mucoadhesive strength increases.R^2 value 0.995 for mucoadhesive strength indicating good correlation between independent and dependent variable. The term with (P<0.0001) were considered significant.

The computer generated response surface for dependent variables are shown in Figure 5.

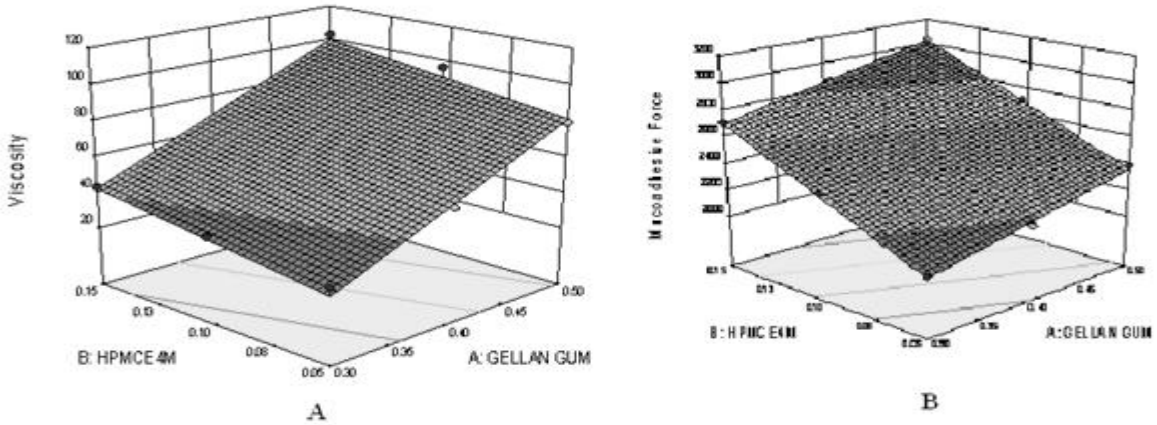

Figure 5 Surface response plot for A) viscosity, B) mucoadhesive strength.

Stability study

Stability study provide a means for checking the physical and chemical stability of the drug product at various storage conditions,

including the compatibility of the formulation with the components of the device, as well as performance of nasal and inhalation spray drug products. The physical instability could be due to interaction of drug with the excipient used in the formulation. The degradation of drug may occur due to its inherent instability or due to its interaction with excipient used in the formulation. Formulation batch AZ5 was placed for stability study. There were no significant changes in visual appearance, pH, drug content and viscosity of the formulation was found (Table 3).

Table 3: Stability data of optimized batch AZ5

Sr no.	Test	Time interval (month)	Initial	40^0C/75%RH
1	Appearance	3	Clear	clear
2	pH	1	6.58±0.05	6.58
		2		6.59
		3		6.58
3	% Drug Content	1	98.18±0.32	98.14
		2		98.1
		3		97.98
4	Viscosity(cps)	1	55.55±0.99	53.48
		2		54.68
		3		56.12

Conclusion

In the present study, gellan gum with HPMC E4M was used for the nasal drug delivery system of the antihistaminic drug azelastine hydrochloride. Owing to its increased viscosity after gelation and mucoadhesive characteristics, the formulation displays prolonged nasal residence time. Among all formulations, AZ5, containing 0.4% gellan gum and 0.1% HPMC E4M, was found to be optimized formulation. It can be concluded that the optimized in situ nasal gel of azelastine hydrochloride appears to be suitable in seasonal allergic rhinitis, with prolonged residence time.

Author's contributions

VC and PS have carried out studies mentioned in manuscript. IF and IBP has guided this project and made substantial contributions for data interpretation and involved in drafting the manuscript. All authors read and approved the final manuscript.

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