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Formulation and Evaluation of Nasal In Situ Gel for Cyproheptadine HCl

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ABSTRACT

The main aim of the formulation was to prepare Nasal in situ gel for cyproheptadine HCl using an admixture of pH sensitive polymer i.e. carbopol 940 and viscosifying agent i.e. HPMC K100 M in order to achieve a sustained release of drug. The Nasal in situ gel containing cyproheptadine HCl was prepared by taking carbopol 940 and HPMC K100 M in different ratios. The concentrations of carbopol 940 and HPMC K100 M were investigated using 3^2 full factorial design. The parameters determined were pH, physical appearance, drug content, gelling capacity, Mucoadhesive strength, viscosity, in vitro drug release. The drug excipient compatibility study was carried out by using Fourier transform infrared spectroscopy(FTIR). The pH values in situ gels were between 5.0 to 6.0. Drug content values were between 98% to 100%. The release profile of in situ gels exhibited a sustained release of cyproheptadine HCl. Drug release was dependent on the concentration of carbopol 940 and concentration of HPMC K100 M. Cyproheptadine HCl was successfully formulated as an nasal in situ gel to deliver drug for 8 h. The drug release of the nasal in situ gel decreased with decrease in concentration carbopol 940 and viscosity increased with increasing levels of HPMC K100 M. The drug release and viscosity could be adjusted and modified by varying the ratio of polymer and viscosifying agent. The optimized formulation F8 (0.6 % w/v carbopol 940 and 0.4 % w/v of HPMC K100 M) provide drug release of 8 h and release drug immediately after it is instilled into the nose. Formulation F8 was seen to be stable after one month of stability study.

Keywords: Cyproheptadine HCl, Nasal in situ gel, carbopol 940

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INTRODUCTION

Intranasal administration is a approach for rapid-onset delivery of medications and to circumvent their first-pass elimination when taken orally. It is a needle free, alternative to the parentral and mucoadhesive is the best tool for nasal drug delivery system.[1]

In situ gel is referred to a kind of preparation in a form of solution condition; and with the changes of physiological environment of administration position, the phase changes and forms a gelatinous semi-solid preparation. Because it is benefited from merits of both solution and gel, it has a broad application prospect in research of drug carrier.[2,3,4]

The poor bioavailability and therapeutic response exhibited by the conventional nasal solution due to short residence time is the basic problem of highly efficient nasal route. This drawback can be overcome by the use of in situ gelling systems, which upon instillation as liquid droplets, undergo to gel transition in to nasal cavity, which ultimately leads to increased residence time of drug and aids drug absorption with rapid onset of action. For this method of preparation industrialization and optimization will be easy to achieve.

Antihistamines are used to relieve or prevent the symptoms of hay fever and other types of allergy. They work by preventing the effects of a substance called histamine, which is produced by the body. Histamine can cause itching, sneezing, runny nose, and watery eyes. Also, in some persons histamine can close up the bronchial tubes (air passages of the lungs) and make breathing difficult. Some of the antihistamines are also used to prevent motion sickness, nausea, vomiting, and dizziness. Antihistamines are available as conventional solid and liquid oral dosage forms, nasal in situ gel as well.[5,6]

An ideal nasal drug candidate should possess the following attributes:

1. Appropriate aqueous solubility to provide the desired dose in a 25–150 ml volume of formulation administration per nostril.
2. Appropriate nasal absorption properties.
3. No nasal irritation from the drug.
4. A suitable clinical rationale for nasal dosage forms, e.g. rapid onset of action.
5. Low dose. Generally, below 25 mg per dose.
6. No toxic nasal metabolites.
7. No offensive odors/aroma associated with the drug.
8. Suitable stability characteristics.

In our present work, study was focused to develop a ideal nasal in situ gelling system using a potent and effective anti histamine as a model drug, various polymers and by simple method. The study includes the preformulation studies and the formulations will be subjected for its physicochemical and release studies. Stability studies as per ICH Guidelines.

The aim of the present study is to develop and evaluate pH triggered in situ gel of Cyproheptadine HCl. In which aqueous solution will be converted into gel due to pH transition. Such an in situ gel gives prolong nasal residence time and increased bioavailability of drug.

MATERIALS AND METHOD:

Cyproheptadine HCl was gifted from the Camphor Pharmaceutical Ltd.and other polymenr was purchased from S.D. Fine chemicals, Mumbai

Preparation of SNS (simulated nasal solution)

Weigh accurately 7.45mg/ml NaCl, 1.29mg/ml KCl and 0.32mg/ml CaCl2.2H2O and dissolve in 1000 mL of distilled water to produce simulated nasal solution.

Preparation of standard curve:

Standard cyproheptadine HCl solution 100µg/ml (0.5 ml, 1ml, 1.5 ml, 2 ml ,2.5 ml, 3 ml) was pipette out in to the series of 10 ml volumetric flask and volume was adjusted by SNS to get concentration of 5 µg/ml , 10 µg/ml ,15µg/ml, 20 µg/ml , 25 µg/ml, 30 µg/ml Absorbance was read at 286 nm against blank SNS. The values of absorbance was plotted graphically against the concentration of standard solution.

A. Determination of melting point of cyproheptadine HCl.

The pharmacopeias regard the capillary method as the standard technique for melting point determination. In this methodology, a thin glass capillary tube a compact column of the substance to be determined is introduced into a heated stand (liquid bath or metal block) in close proximity to a high accuracy thermometer. The temperature in the heating stand is ramped at a user-programmable fixed rate until the sample in the tube transition into the liquid state**.**

B. Drug-excipient compatibility studies.

Drug-excipient compatibility study: In the preparation of the formulation, drug and polymer may interact as they are in close contact with each other , which could lead to the instability of drug. Preformulation studies regarding the drug-polymer interaction are therefore very critical in selecting appropriate polymers.

FT-IR spectroscopy:

The compatibility study carried out using Fourier transform infrared spectroscopy (FTIR).The IR study were carried out by the pressed pellet technique using KBR press. Potassium bromide was taken and kept in hot air oven for two hour for the removal of any moisture if present. The drug

powder sample was mixed with dried KBR crystal and mixture was pressed to form pellets using KBR press. The prepared pellet was placed in the sample holder and kept in the

instrument to record the IR peaks. The same process is repeated with the physical mixture sample of drug and polymers and IR peaks were recorded. FTIR absorption spectra of pure drug and physical mixture were recorded in the range of 400 to 4000 cm-1 by KBr disc method using FTIR spectrophotometer.

EVALUATION OF FORMULATIONS[7]

pH

The pH of the formulations was determined by using pH meter.

In vitro gelling capacity

The gelling capacity was determined by freshly prepared drop of system in a vial containing 2 ml of freshly prepared simulated nasal fluid and equilibrated at 37ºC. The visual assessment of gel formation was carried out. Time required for gelation as well as time taken for the formed gel to dissolve were also noted. Different grades were rate of formation of gel with respect to time. The grades were given as no gelation (-), gelation after few minutes and remains few h (+), gelation immediate and remains few h (++), and gelation immediate and remain extended time (+++).

3^2 full factorial design

A full factorial 3^2 design was used for optimization procedure. It is suitable for investigating the quadratic response surfaces and for constructing a second-order polynomial model, thus enabling optimization of in situ gelling system. Mathematical modelling was employed for the evaluation of the ability to fit to the model and response surface modeling were performed with employing sigma plot software (Version 11.0). The studied factors (independent variables) were concentration of HPMC K100M (X1) and concentration of carbopol 940 (X2). Preliminary studies provided a setting of the levels for each formulation variable. The response (dependent variables) studied was mucoadhesive strength (Y1) and viscosity(Y2)

The independent and dependent variables along with their levels. The resulted formulations (testing runs) are listed in Table .The factorial formulations were coded as F1 to F9. A statistical model incorporating interactive and polynomial term was used to evaluate the response

$$Y = \beta0 + \beta1X1 + \beta2X2 + \beta12X1X2 + \beta11X1^2 + \beta22X2^2$$

Where, Y1 and Y2 are the dependent variables, β0 is the arithmetic mean response of the nine runs, and β1 is the estimated coefficient for the factor β1. The main effects (X1 and X2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X1X2) show how the response changes when two factors are

simultaneously changed. The polynomial terms ($X1^2$ and $X2^2$) are included to investigate non-linearity.

Table 1 : Formulation layout for factorial formulations

Formulation	X1 HPMC K 100 M (%)		X2 Carbopol 940 (%)	
F1	-1	0.3	-1	0.4
F2	0	0.4	-1	0.4
F3	+1	0.5	-1	0.4
F4	-1	0.3	0	0.5
F5	0	0.4	0	0.5
F6	+1	0.5	0	0.5
F7	-1	0.3	+1	0.6
F8	0	0.4	+1	0.6
F9	+1	0.5	+1	0.6

Preparation of Nasal In Situ Gel

Composition of in situ gel were shown in table. The formulations were prepared by dispersing carbopol 940 in distilled water with continuous stirring (Thermostatic hot plate with magnetic stirrer) until completely dissolved and allowed to hydrate overnight. HPMC K 100 M was dissolved in distilled water using magnetic stirrer and allowed to hydrate. Then the carbopol solution was sprinkled over this solution and allowed to hydrate overnight. After the complete hydration of polymers, a separate solution of Cyproheptadine HCl in water with propylene glycol was added to the polymeric solution. mixed, benzalkonium chloride was then added and mixing was confirmed until a uniform and clear solutions were formed. Final volume was made by adding required amount of distilled water.

Table 2: Formulation Nasal In Situ Gel of Cyproheptadine HCl

BATCH	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9
Cyproheptadin HCl (%)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
HPMC K 100 M (%)	0.3	0.4	0.5	0.3	0.4	0.5	0.3	0.4	0.5
Carbopol 940 (%)	0.4	0.4	0.4	0.5	0.5	0.5	0.6	0.6	0.6
Propylene Glycol (ml)	5	5	5	5	5	5	5	5	5
Benzakonium Chloride(%)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Water	100ml	100ml	100ml	100ml	100ml	100ml	100ml	100ml	100ml

EVALUATION PARAMETERS[7]

Physical appearance

The appearance of the formulation after and before gelling was determined by visual examination of the formulation under light alternatively against white and black backgrounds.

In vitro gelling capacity

The gelling capacity was determined by freshly prepared drop of system in a vial containing 2 ml of freshly prepared artificial nasal fluid and equilibrated at 37ºC. The visual assessment of gel formation was carried out. Time required for gelation as well as time taken for the

formed gel to dissolve were also noted. Different grades were allotted as the gel integrity, weight, and rate of formation of gel with respect to time. The grades were given as no gelation (-), gelation after few minutes and remains few h (+), gelation immediate and remains few h (++), and gelation immediate and remain extended time (+++).

Gel-strength.

A sample of 50g of the nasal gel was put in a 100 ml graduated cylinder and gelled in a thermostatically controlled water bath at 37°C. A weight of 35 g was placed onto the gelled solution. The gel strength, which is an indication for the viscosity of the nasal gel at physiological temperature, was determined by the time in seconds required by the weight to penetrate 5 cm into the gel.

Viscosity

Residence time of in situ gel formulation mainly depends on viscosity. Viscosities of all formulations were measured by using Brookfield viscometer . Also the effect of pH condition on viscosity of gel was determined. The viscosity of the gel at respective pH and after adjusting the pH to 7.4 was determined.

pH measurement

1ml quantity of each formulation was transferred to a beaker and diluted by using distilled water to make 25ml. pH of the resulting solution was determined using digital pH meter.

Drug content.

1ml of formulation was taken in 10ml volumetric flask, diluted with distilled water and volume adjusted to 10ml. 1ml quantity from this solution was again diluted with 10ml of distilled water. Finally the absorbance of prepared solution was measured at 286 nm by using UV visible spectrophotometer.

IN-VITRO DRUG RELEASE STUDY.

Preparation of Simulated Nasal Solution

Weigh accurately 7.45mg/mL NaCl, 1.29mg/mL KCl and 0.32mg/mL CaCl2. 2H2Oand dissolve in 1000 mL of distilled water to produce simulated nasal solution. In vitro release study of the formulation was carried out using laboratory designed diffusion cell through egg membrane. 1mL of gel were placed in donor compartment and freshly prepared simulated nasal solution. Egg membrane was mounted between donor and receptor compartment. Temperature of receiver compartment was maintained at 37±2°C during experiment and content of the receiver compartment was stirred using magnetic stirrer. The position of donor compartment was adjusted so that egg membrane just touches the diffusion fluid. An aliquot of 1 mL was withdrawn from receiver compartment after 30 min, 1, 2, 3, 4, 5, 6, 7, and 8 hr and same volume of fresh medium was replaced. Aliquot so withdrawn were suitably diluted

and analyzed using UV visible spectrophotometer at 286 nm. Natural membranes are utilized to determine in-vitro permeation study to mimic the in-vivo permeation patterns. In this experiment goat nasal mucosa was utilized because the respiratory area of goat is large and it is easy to get. Fresh mucosal tissue was removed from the nasal cavity of goat. The tissue was placed on the diffusion cell with permeation area 0.786 cm2. The acceptor chamber of the diffusion cell (laboratory designed) with a volume capacity 100mL was filled with simulated nasal fluid (SNF) contained accurately 7.45mg/mL NaCl, 1.29mg/mL KCl and 0.32mg/mL CaCl2.2H2O. 1mL (10 mg equivalent) of formulation was placed in donor compartment. At predetermined time point of 30 min, 1, 2, 3, 4, 5, 6, 7, and 8 hrs 1mL of sample was withdrawn from the acceptor compartment replacing the sample removed with simulated nasal fluid after each sampling for period of 8 hrs. Then samples were specifically diluted and absorbance was noted at 286nm.

Permeability coefficient (p) was calculated by the following formula:

$$P = (dQ/dt) / (C0 \times A)$$

Where, dQ/dt is the flux or permeability rate (mg/h), C0 is the initial concentration in the donor compartment, and A is the effective surface area of nasal mucosa.

Determination of mucoadhesive strength

The mucoadhesive strength was determined. The mucoadhesive potential of each formulation was determined by measuring a force required to detach the formulation from nasal mucosal tissue. A section of sheep nasal mucosa was fixed on each of two glass slides using thread. 50mg of gel was placed on first slide and this slide placed below the height adjustable pan. While another slide with mucosal section was fixed in inverted position to the underside of the same pan. Both the slides with gel formulation between them held in contact with each other, for 2min to ensure intimate contact between them. Then weight was kept rising in second pan until slides get detached from each other. The mucoadhesive force expressed as the detachment stress in dynes/cm2 was determined from the minimal weight that detached the mucosal tissue from surface of each formulation.

$$\text{Mucoadhesive Strength (dynes/cm2)} = mg/A$$

Where, m = weight required for detachment in gram,

g= Acceleration due to gravity (980cm/s2),

A = Area of mucosa exposed.

Stability study as per ICH guideline

Stability studies were conducted according to ICH guidelines $40^0C \pm 2^0C$ 75%± 5% RH to test the physical and chemical stability of the developed in situ nasal gel. A sufficient

quantity of pH sensitive in situ gel, in screw capped vials was stored at different stability condition.

Release kinetic

In order to understand the mechanism and kinetics of drug release, the results of the in vitro drug release study were fitted with various kinetic equations namely zero order (% release vs t), first order (log% unreleased vs. t), higuchi matrix (% release vs. square root of time). In order to define a model which will represent a better fit for the formulation, drug release data further analyzed by Korsmeyer Peppas equation, $Mt/M\infty=ktn$, where Mt is the amount of drug released at time t and $M\infty$ is the amount released at time ∞, the $Mt/M\infty$ is the fraction of drug released at time t, k is the kinetic constant and n is the diffusional exponent, a measure of the primary mechanism of drug release. R2 values were calculated for the linear curves obtained by regression analysis of the above plots. Comparison with various models

Zero order kinetics

A zero order release would be predicated by the following equation

$At = A0 - K0t$

Where, At = Drug released at time t

A0 = Initial drug concentration

K0 = Zero order rate constant (h -1).

When the data was plotted as cumulative percent drug release versus time it yields a straight line indicating that the release obeys zero order kinetics, with a slope equal to K0.

First order kinetics

A first order release would be predicated by the following equation

$Log\ C = Log\ C0 – kt/2.303$

Where, C = Amount of drug remained at time t

C0 = Initial amount of drug

K = First order rate constant (h -1)

When the data was plotted as cumulative percent drug remaining versus time it yield a straight line, indicating that the release follows first order kinetics. The constant ‘k’ can be obtained by multiplying 2.303 with slope values.

Higuchi’s model

Drug release from the matrix devices by diffusion has been described by Higuchi’s classical diffusion equation

$Q = [De / t\ x\ (2A – eCst)]$

Where, Q = Amount of drug released at time‘t’.

D = Diffusion coefficient of the drug in the matrix

A = Total amount of drug in unit volume of matrix

Cs = The solubility of the drug in the matrix.

e = Porosity of the matrix.

t = Tortuosity.

t = Time (h.)

The equation may be simplified, if one assumes that D,e, T, Cs and A are constant.

Then the equation becomes

Q = kt1/2

When the data is plotted according to equation i.e. cumulative drug released versus square root of time yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to 'k'

Korsmeyer Peppas model

In order to understand the mode of release of drug from swellable matrices, the data were fitted to the following equation

$M_t / M_\infty = kt^n$

Where,

M_t / M_∞ = The fraction of drug released

k = Kinetic constant

n = Diffusional exponent for drug release

The equation 4.10 can be simplified by applying log on both sides, we get

$\log M_t / M_\infty = \log k + n \log t$

When the data plotted as log percentage drug released versus log time, yields a straight line with a slope equal to 'n' and the 'k' can be obtained from y-intercept. The value of 'n' gives an indication of the release mechanism. When n = 1, the release rate is independent of time (zero order case II transport); n = 0.5 for fickian diffusion and when $0.5 < n < 1$, diffusion and non-fickian transport are implicated. When n > 1.0 super case II transport is apparent.

RESULTS AND DISCUSSION

Standard calibration curve of Cyproheptadine HCL :

The linear regression analysis was done on absorbance data points. The result for standard curve in SNS is given.

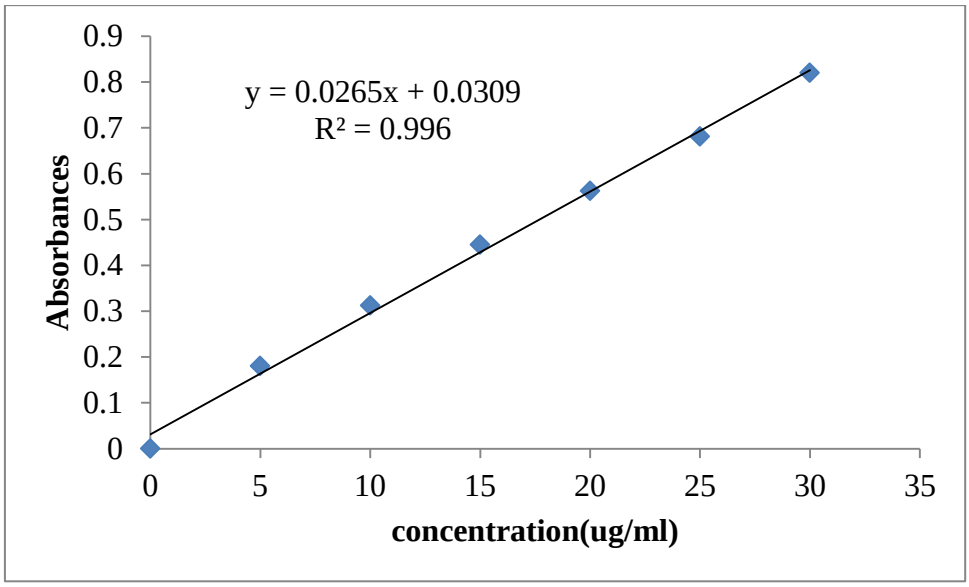

Figure 1 : Standard calibration curve of cyproheptadine HCl in SNS.

Compatibility Study:

FTIR:

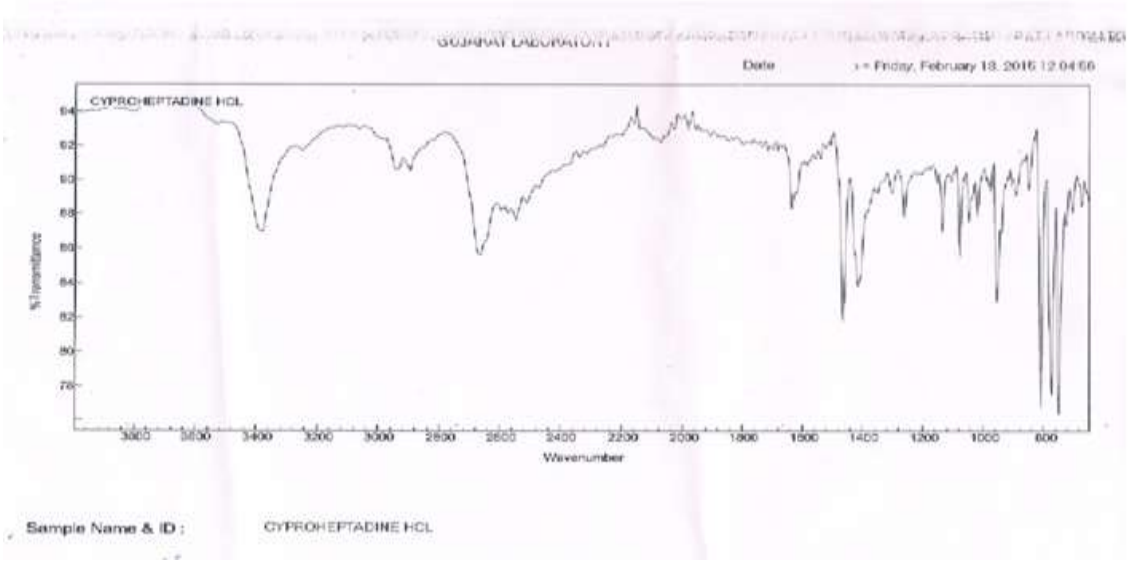

Figure 2 : FT-IR study of cyproheptadine HCl

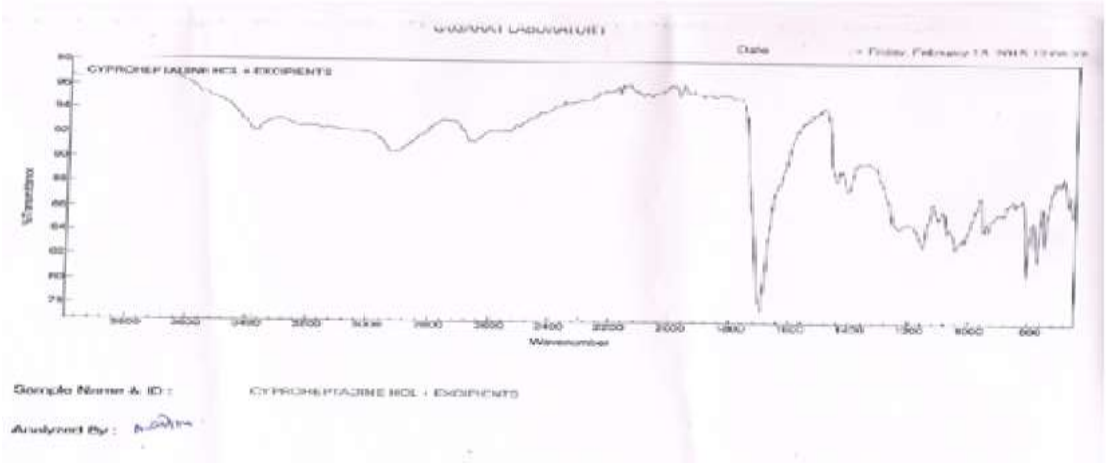

Figure 3 : FT-IR study of cyproheptadine HCl+ Excipient

Table 3 : Evaluation of factorial batch :

Code	pH	Physical appereance	Gelling Capacity	Gel strength (sec)	Drug content (%)	Mucoadhesive strength(dyne/cm^2)
F1	5.4±0.12	Transparent	++	12.6±0.52	98.32±0.798	1044.37
F2	5.1±0.11	Transparent	++	19.5±2.61	99.20±0.625	1395.11
F3	5.3±0.05	Transparent	+++	21.28±2.34	97.35±0.823	1709.97
F4	5.6±0.10	Transparent	++	25.72±0.23	97.12±0.702	2259.65
F5	5.0±0.12	Transparent	+++	31.25±1.25	99.73±0.546	2305.26
F6	5.4±0.10	Transparent	++	36.20±2.36	98.47±0.682	2557.80
F7	5.3±0.11	Transparent	++	39.52±0.59	98.36±0.847	2690.22
F8	5.1±0.05	Transparent	+++	42.28±2.24	99.64±0.520	2811.45
F9	5.0±0.15	Transparent	+++	45.69±2.41	98.89±0.74	3130.14

+ Gelation after few minutes & remain few hrs

++ Gelation immediate & remain few hrs

+++ Gelation immediate & remain extended time

Table 4: % In vitro drug release from cyproheptadine HCl in situ gel

Time in Hr.	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	13.02 ± 0.56	34.00 ±051	41.22 ±0.51	57.11 ±0.29	30.33 ±0.68	25.29 ±0.74	15.45 ±0.56	26.07 ±0.83	22.63 ±0.45
2	81.38 ± 0.48	51.98 ±0.33	55.70 ±0.50	74.17 ±0.74	40.95 ±0.74	43.86 ±0.80	67.28 ±0.64	40.74 ±0.70	38.83 ±0.44
3	86.44 ± 0.52	57.76 ±0.71	67.26 ±0.50	87.71 ±0.45	51.30 ±0.61	59.91 ±0.17	72.67 ±0.40	55.58 ±0.34	56.31 ±0.22
4	89.06 ± 0.57	68.92 ±0.02	72.40 ±0.54	92.78 ±0.59	76.65 ±0.39	70.07 ±0.48	79.36 ±0.56	68.73 ±0.46	65.79 ±0.26
5	90.25 ±0.71	74.44 ±0.16	76.14 ±0.43	96.84 ±0.35	87.56 ±0.36	79.69 ±0.55	85.34 ±0.51	77.64 ±0.35	75.53 ±0.49
6	92.06 ±0.76	82.05 ±0.26	84.73 ±0.30	98.26 ±0.24	95.14 ±0.66	88.10 ±0.46	95.56 ±0.27	86.06 ±0.38	84.11 ±0.56
7	93.56 ±0.44	86.44 ±0.56	89.92 ±0.64	101.25 ±0.19	99.25 ±0.89	93.86 ±0.60	98.56 ±0.18	97.77 ±0.47	90.56 ±0.48
8	94.89 ±0.32	89.25 ±0.49	93.26 ±0.30			96.29 ±0.17		100.87 ±0.27	95.70 ±0.34

RELEASE KINETIC MODEL DATA

To gain a better insight into the mechanism underlying the release of cyproheptadine hcl from in situ gel forming system the release kinetic of cyproheptadine hcl was investigated. Diffusion data was given higuchi,zero order,first orde,first ordeand korsmeyer peppas kinetic treatment for all formulation. These different kinetic equation were applied to interpret the release rate from all the formulation. The best with higher correlation (r^2 0.998)was found with korsmyer peppas model as shon in table. The value of diffusion of diffusion exponent n for formulation f8 was found to be 0.47. The value of release exponent is more than 0.45 and less than 0.89 indicating fickian release from F8.

Table 5: Kinetic data of drug release

Formulation Code	Parameters	Model			
F8		Zero Order	First Order	Higuchi	Korsmyer-Peppas
	R^2	0.9778	0.8986	0.9964	0.9980
	Slope	10.82	10.079	42.58	0.67
	Intercept	20.48	1.44	-17.61	1.41

Table 6 : Viscosity of formulations at respective pH

RPM	F1	F2	F3	F4	F5	F6	F7	F8	F9
3	341	343	337	337	383	365	392	423	467
6	293	295	292	289	322	312	346	384	324
12	210	232	241	243	251	267	282	303	317

STATISTICAL ANALYSIS OF THE DATA AND VALIDATION OF THE MODEL

The statistical analysis of the factorial design formulations was performed by multiple linear regression analysis carried out in Microsoft Excel 2007. The Mucoadhesive Strength and Viscosity for the 9 formulations (F-1 to F- 9) showed a wide variation; the results are shown in Table. The data clearly indicate that the values of Mucoadhesive Strength and Viscosity are strongly dependent on the independent variables.

The fitted full model equation relating the response Y1 (Mucoadhesive Strength) and Y2 (Viscosity) to the transformed factor are shown in following equation.

Y1 (Mucoadhesive Strength) = 233.29 + (233.94X1) + (747.06X2) - (56.42X1X2) + (61.41X1X1) + (244.02 X2X2)

Y2 (Viscosity) = 255 + (15X1) + (36.5X2) + (1X1X2) - (2X1X1) + (10.5X2X2)

The P value for X1, X2, X22 were found to be 0.0095,0.00032, and 0.03 respectively which is less than 0.05. Thus X1, X2, and X22 has significant effect on dependent variable (Y1) Mucoadhesive Strength while other term, X12 and X11 was rendered insignificant having P value greater than 0.05.

The P value for X1, X2, X22 were found to 0.0043,0.00031 ,0.050 respectively which is less than 0.05. Thus X1, X2 and X22 has significant effect on dependent variable (Y2) Viscosity while other term X12 and X11 were rendered insignificant having P value greater than 0.05.

So, the reduced model equation is as follows:

Y1 (Mucoadhesive Strength) = 233.29 + (233.94X1) + (747.06X2) - (244.02 X2X2)

Y2 (Viscosity) = 255 + (15X1) + (36.5X2) + (10.5X2X2)

The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries in Table shows the results of the analysis of variance, which was performed to identify insignificant factors. The high values of

correlation coefficient for Mucoadhesive Strength and Viscosity indicate a good fit, i.e. good agreement between the dependent and independent variables. The significance test for regression coefficients was performed by applying the student F test. A coefficient is significant if the calculated F value is less than the critical value of F.

Table 7 : Summary of results of regression analysis

Regression Statistics	**Y_1 (Mucoadhesive Strength)**		**Y_2 (Viscosity)**	
Multiple R	0.9963		0.9965	
R Square	0.9927		0.9931	
Adjusted R Square	0.980		0.9817	
Standard Error	96.46		4.69	
Observations	9		9	
Y1 (Mucoadhesive Strength)				
	FM Coefficients	**P-value**	**RM Coefficients**	**P-value**
Intercept	2333.29	0.000064	2374.23	0.000014
X1	233.94	0.0095	233.94	0.0020
X2	747.06	0.00032	747.06	0.000081
X1.X2	-56.42	0.32	-	-
X1.X1	61.41	0.43	-	-
X2.X2	-244.02	0.03	-244.027	0.017
Y2 (Viscosity)				
	FM Coefficients	**P-value**	**RM Coefficients**	**P-value**
Intercept	255	0.000056	253.66	0.000011
X1	15	0.0043	15	0.00024
X2	36.5	0.00031	36.5	0.000031
X1.X2	1	0.6985	-	-
X1.X1	-2	0.5890	-	-
X2.X2	10.5	0.050	10.5	0.013

Where, FM = Full model and RM = Reduced model

Table 8: F-value calculations for testing the model in portions

(Mucoadhesive Strength)						
	DF	**SS**	**MS**	**F**	**R^2**	
Regression						
FM	5	3816349	763269.8	82.030	0.9927	Fcal=1.24
RM	3	37960	1265	131.28	0.9874	
	Residual					Fcri=19
FM	3	27913.97	9304.65	-	-	DF=(2,2)
RM	5	4891.26	9638.25	-	-	
Viscosity						
	DF	**SS**	**MS**	**F**	**R^2**	
Regression						
FM	5	9576	1915.2	87.05	0.9931	Fcal=0.27
RM	3	9564	3188	204.35	0.9919	
	Residual					Fcri=19
FM	3	66	22	-	-	DF=(2,2)
RM	5	78	15.6	-	-	

DF: degree of freedom, SS: sum of squares, MS: mean of squares, F: Fischer's ratio, R^2: regression coefficient, FM: full model, RM: reduced model.

Full and reduced model for Mucoadhesive Strength and Viscosity

The full model for of Mucoadhesive Strength and Viscosity was developed by using the coefficients. The results of statistical analysis are shown in For Mucoadhesive Strength the significance level of coefficient β12 and β11 was found to be p = 0.32 and 0.43, hence it was omitted from the full model to generate the reduced model. The coefficients β1, β2, and β22 were found to be significant at $p < 0.05$; hence they were retained in the reduced model. The reduced model was tested in portions to determine whether the coefficient β12 and β11 contribute significant information for the prediction of lag time of rupture or not. The results for testing the model in portions are shown in Table. The critical value of F for $\alpha = 0.05$ is equal to 19 (df = 2, 2). Since the calculated value (F =1.24) is less than critical value, it may be concluded that the interaction term β12 and quadratic term β11 does not contribute significantly to the prediction Mucoadhesive Strength and therefore can be omitted from the full model.

For Viscosity the significance level of coefficient β12 and β11 was found to be p = 0.6985,0.5890, hence it was omitted from the full model to generate the reduced model. The coefficients β1, β2, and β22 were found to be significant at $p < 0.05$; hence they were retained in the reduced model. The reduced model was tested in portions to determine whether the coefficient β12 and β11 contribute significant information for the prediction of Viscosity. The results for testing the model in portions are shown in Table. The critical value of F for $\alpha = 0.05$ is equal to 19 (df = 2, 2). Since the calculated value (F =0.27) is less than critical value, it may be concluded that the interaction term β12 and quadratic term β11 does not contribute significantly to the prediction of Viscosity and therefore can be omitted from the full model.

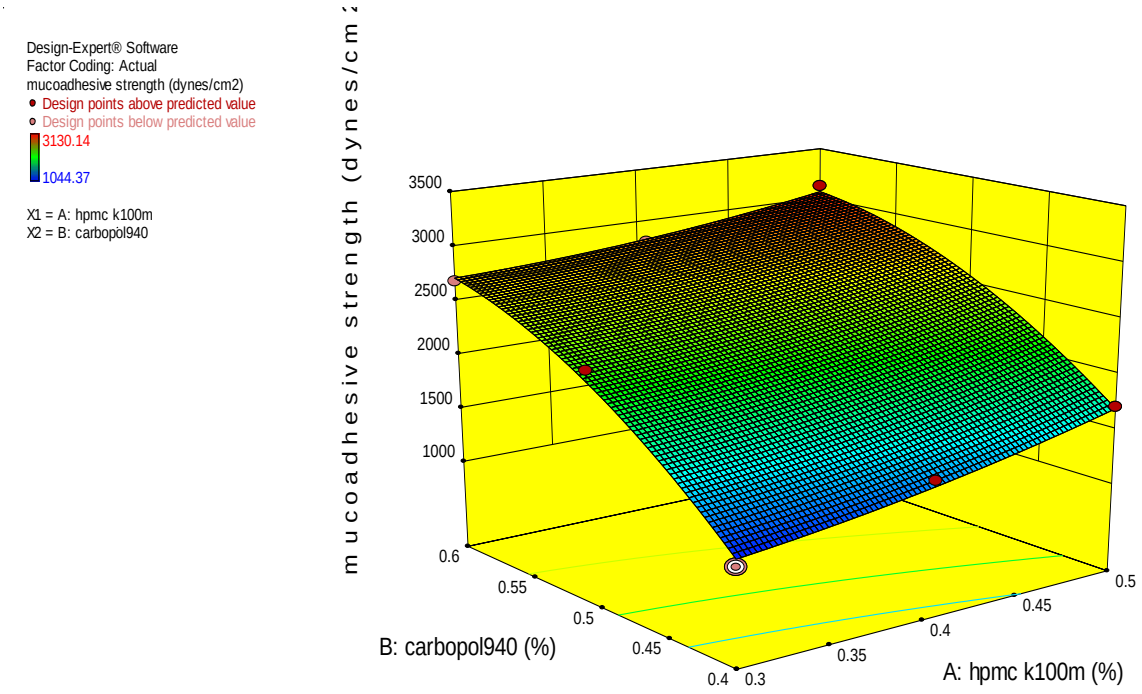

Figure 4 : Response surface plot showing the effect of amount of HPMC K 100 M (X1) and CARBOPOL 940 (X2) on the response Mucoadhesive Strength (Y1)

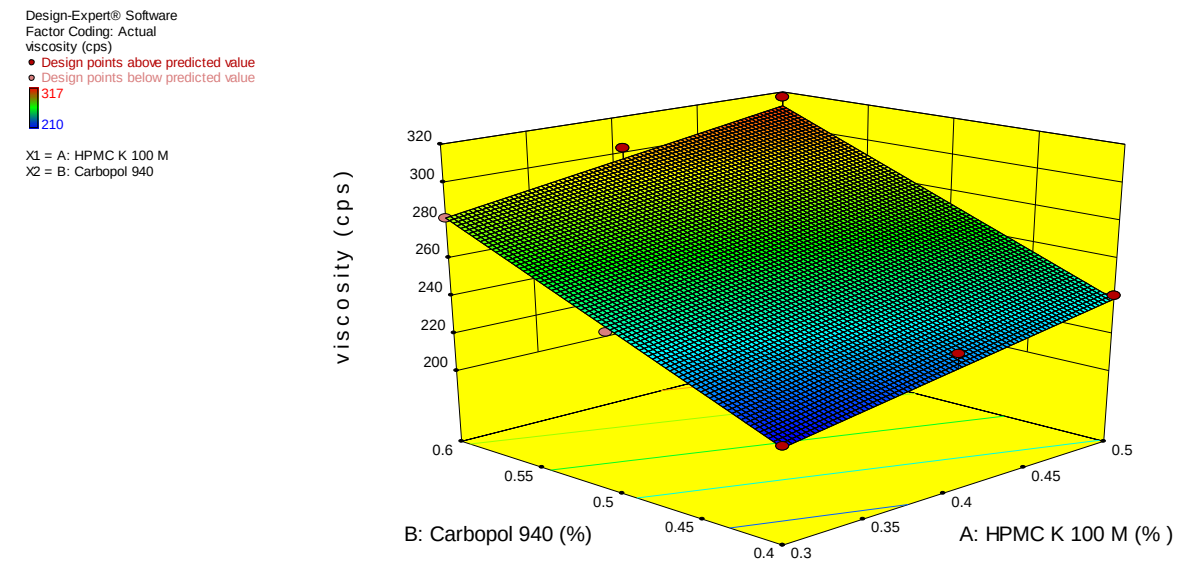

Figure 5: **Response surface plot showing the effect of amount of HPMC K 100 M (X1) and CARBOPOL 940 (X2) on the response viscosity(Y2)**

Validation of Optimized batch (F8)

Formulation F8 containing 0.4%w/v HPMC K 100 M and 0.6 %W/V CARBOPOL 940 was found to maximum desirability found in the experimental region of the overlay plot. So, it was selected as the optimized batch.

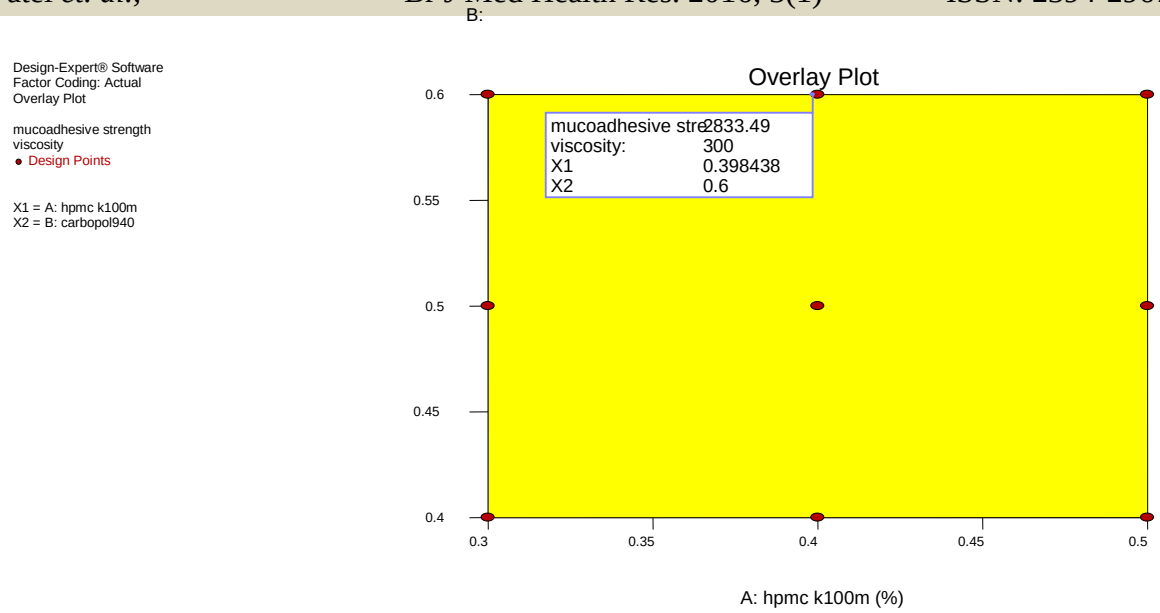

Figure 6: Overlay Plot showing combined effects of HPMC K 100 M and CARBOPOL 940 on Mucoadhesive Strength And Viscosity (Y1, Y2)

Table 9 : Composition of Optimized Formulation (F8)

Ingredient	F-8
Cyproheptadin HCl (%)	0.4
HPMC K 100 M (%)	0.4
Carbopol 940 (%)	0.6
Propylene Glycol (ml)	5
Benzakonium Chloride (%)	0.01
Water	100ml

Stability Study:

Stability study was carried out at 40 ± 2°C and 75 % RH and at Room temperature for one month storage condition. Batch F8 was taken for stability study and various parameters were compared for stability study.

Table 10: Stability Study

SR NO.	Observation	Initial	After Stability (1 Month)
1	Appearance	Transparent	Transparent
2	pH	5.1	5.2
3	Drug Content	99.64	99.80
4	In Vitro Drug Release Study(%)	100.87	100.56

CONCLUSION

Cyproheptadine HCl was successfully formulated as an nasal *in situ* gel to deliver drug for 8 h. The drug release of the nasal *in situ* gel decreased with decrease in concentration carbopol 940 and viscosity increased with increasing levels of HPMC K100 M. The drug release and viscosity could be adjusted and modified by varying the ratio of polymer and viscosifying agent. The optimized formulation F8 (0.6 % w/v carbopol 940 and 0.4 % w/v of HPMC K100

M) provide drug releae of 8 h and release drug immediately after it is instilled into the nose. Formulation F8 was seen to be stable after one month of stability study.

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