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Formulation, Development and Evaluation of pH Sensitive *In-Situ* Fexofenadine Hydrochloride gel for Nasal Administration

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ABSTRACT

Nasal mucosa has been considered as a potential administration route to achieve faster and higher levels of drug absorption because it is permeable to more compounds than the gastrointestinal tract. It is an attractive route of administration due to lack of pancreatic and gastric enzymatic activity, neutral pH of the nasal mucus and less dilution by gastrointestinal contents. In-situ gel forming systems have been developed to prolong the nasal residence time of a drug and improve nasal bioavailability. Fexofenadine hydrochloride is one of the most widely used drugs for allergic rhinitis. It is an anti-histaminic and is available in oral dosage forms. The present work is aimed at designing a gel formula for nasal administration. To enhance therapeutic effect of the nasal formulation of fexofenadine hydrochloride pH sensitive polymers were used. Xanthan gum and Carbopol 934 were selected as independent variables in the factorial design. Solubility of the drug in the formulation was enhanced by using Tween 80. Evaluation parameters of the formulation like pH, clarity; rheological study both formulation i.e. solution and performed gel, gelling time, drug content, in-vitro drug diffusion study, stability studies were performed. The formulations so designed shall enhance bioavailability of drug and offer better therapeutic outcomes, offer aesthetically appealing dosage form to increase patient compliance and shall overcome side effects and limitations of other dosage forms of marketed preparations.

Keywords: Nasal drug delivery, Fexofenadine Hydrochloride, pH sensitive, gels.

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INTRODUCTION

Antihistamines play an important role in clinical treatment of allergic rhinitis. Especially, among the second-generation antihistamines.[1]Fexofenadine hydrochloride, the major active metabolite of terfenadine, is an antihistamine with selective peripheral H_1-receptor antagonist activity that is devoid of sedative effects.[2]Fexofenadine is unique in that it appears to be purely no sedating, even at higher doses. Since fexofenadine is the substrate of P-glycoprotein and several organic anion transporting polypeptide (OATP), food and co-administration of drugs will have significant effect on its oral bioavailability.[3] The pharmacokinetics of Fexofenadine hydrochloride has been studied. It is completely absorbed from the gastro-intestinal tract following oral administration, but bioavailability is reported to be only about 45% due to hepatic first pass metabolism. Most of the drug is eliminated in liver as metabolites and only 1% of the intact drug is excreted from the kidney. Therefore, Fexofenadine hydrochloride might be designed as a suitable delivery system with long term effect and bypass the liver, such as nasal drug delivery system. [4] Conventionally the nasal cavity is used for the treatment of local diseases, such as rhinitis and nasal congestion. However, in the past few decades nasal drug delivery has been paid much more attention as a promising drug administration route for the systemic therapy. This is due to the anatomy and physiology of the nasal passage, such as the large surface area, highly vascularised epithelium, porous endothelial membrane, and the avoidance of first-pass metabolism. [5] The nasal route is one of the most permeable and highly vascularized site for drug administration ensuring rapid absorption and onset of therapeutic action. It has been potentially explored as an alternative route for drugs with poor bioavailability and for the delivery of biosensitive and high molecular weight compounds such as proteins, peptides, steroids, vaccines etc.[6]Intranasal delivery is a non-invasive and convenient method that could provide efficient systemic delivery for certain therapeutic compounds. The nasal route might also avoid the first-pass metabolism if the nasal drug could be retained and absorbed in the nasal cavity, thereby reducing the biotransformation of the parent drug to metabolites. [7]

MATERIALS AND METHOD

Fexofenadine Hydrochloride was purchased by Balaji Chemicals (Surat, Gujarat, India),Carbopol 934 was obtained from Reliance Cellulose and Xanthan gum obtained from Signet Chemicals. All other chemicals used were of analytical grade.

Method

3^2 factorial design was used for composition of different formulations (Table 1). All different

formulations were prepared as per Table 2. Gel was prepared by using cold method. Accurately weighed quantity of the Fexofenadine Hydrochloride was dissolved in Tween 80.The Carbopol 934 and Xanthan gum was sprinkled over of deionised water and was allowed to hydrate for 12 hours to produce a clear solution. The Benzalkonium chloride was added to the above polymer dispersion.The dispersions were then stored in a refrigerator until clear solutions were obtained and polymer dispersion was slowly added to the drug solution under aseptic condition.

Table 1: Composition of formulation batches as per 3^2 factorial design

Formulation	Fexofenadine Hydrochloride	Carbopol 934	Xanthan gum	Tween 80	Benzalkonium chloride
F1	1	0.1	0.1	10	0.01
F2	1	0.2	0.1	10	0.01
F3	1	0.3	0.1	10	0.01
F4	1	0.1	0.15	10	0.01
F5	1	0.2	0.15	10	0.01
F6	1	0.3	0.15	10	0.01
F7	1	0.1	0.2	10	0.01
F8	1	0.2	0.2	10	0.01
F9	1	0.3	0.2	10	0.01

Table 2: Experimental Design as per 3^2 factorial design

Formulation Code	Coded Values			
	X_1	%	X_2	%
F1	-1	0.1	-1	0.1
F2	0	0.2	-1	0.1
F3	+1	0.3	-1	0.1
F4	-1	0.1	0	0.15
F5	0	0.2	0	0.15
F6	+1	0.3	0	0.15
F7	-1	0.1	+1	0.2
F8	0	0.2	+1	0.2
F9	+1	0.3	+1	0.2

Evaluation of Nasal *in situ* Gel Formulation

Determination of clarity, pH[8]

The clarity was determined visually. The pH of each formulation was determined by using Digital pH meter (Digital pH meter 335).

Drug content[9, 10]

1mL of gel was taken in 10 mL of volumetric flask; in that flask upto 10 mL of pH7.4 Phosphate buffer solution was added. From this solution 1mL was pipette out and diluted upto 10 with pH 7.4 Phosphate buffer solution. Then given 1 mL solution was diluted again in 10 of volumetric flask

and diluted upto 10 mL with pH7.4 Phosphate buffer solution to get final concentration of 10µg/mL. The absorbance of prepared solution was measured at 238 nm by using UV visible spectrophotometer.

Compatibility study[11]

Compatibility study was carried out by using Fourier transform infrared spectrophotometer (Cary 630, Agilent technology USA). FTIR study was carried on pure drug and physical mixture of drug and polymers. Physical mixtures were prepared and samples kept for 1 month at 40°C. The infrared absorption spectrum of Fexofenadine Hydrochloride and physical mixture of drug and polymers were recorded using a KBr disc over the wave number 4000 to 650 cm^{-1}.

Rheological study[12]

The rheological properties of gels were determined by the Brookfield viscometer; type DV-II+PRO using spindle no- 61, 62 and 63.Viscosity of formulations was determined at two different pH, formulation at respective pH and at pH 7.4 with varying shear rate.

Gel Strength[13]

A sample of 50 g of the gel was put in a 50 mL graduated cylinder. A weight of 14.32 g was placed on the gel surface. The gel strength, which is an indication for the nasal gel at physiological temperature, was determined by the time in seconds required by the weight to penetrate 5 cm into the gel. All measurements were performed in triplicate (n=3). The apparatus used for measuring gel strength is shown in (Figure 1). The Mucoadhesive strength of each formulation was determined by measuring the force required to detach the formulation from sheep nasal mucosal tissue by using a modified mucoadhesion test apparatus that is modified physical balance. *In vitro* mucoadhesion studies were conducted using modified mucoadhesion test assembly described by Kassem et.al [14]

Fabrication of equipment

The equipment was fabricated by us in the laboratory as shown in (Figure 2). A double beam physical balance was taken, both the pans were removed. The left pan was replaced with a brass wire, to which was hanged a Teflon disc (A), also locally fabricated. The dimensions are 2 cm height and include an expanded cap of diameter 3.8 cm and thickness 2cm. Another Teflon of 2 cm height and 1.5 cm diameter was placed right below the suspended disc upon the base of the balance. The right pan (C) was replaced with a lighter pan so that, the left pan weighs 5.25 gm more than the right pan. The lower Teflon block was intended to hold the mucosal tissue (D) of goat nasal mucosa and to be placed in a beaker containing simulated nasal solution pH 6.7. (E).

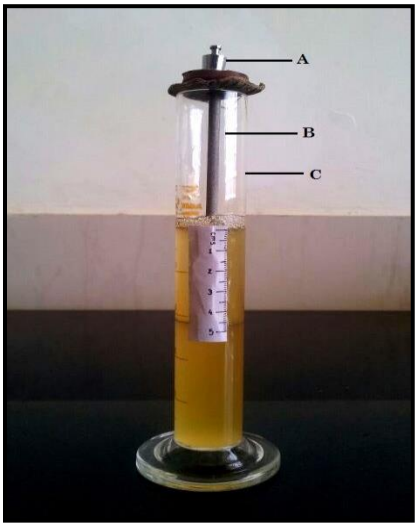

Figure 1:Gel strength measuring device (A) weights (B) device (C) Graduated cylinder

Mucoadhesive strength

Measurement of adhesion force

Goat nasal mucosa was obtained commercially; the nasal mucosa was collected into a sterile container containing sterile buffer solution of pH 6.7. The nasal mucosa brought was stored in a refrigerator until use. The following procedure was used for all the test formulations using the above equipment. The nasal mucosa was removed from refrigerator and allowed to attain equilibrium with ambient conditions in the laboratory. The goat nasal mucosa was carefully excised, without removing connective and adipose tissue and washed with simulated nasal solution. The tissue was stored in fresh simulated nasal solution. Immediately afterwards the membrane was placed over the surface of lower Teflon cylinder (B) and secured. This assembly was placed into beaker containing simulated nasal solution pH 6.7 at 37 ± 2°C. From each batch, some quantity of gel was taken and applied on the lower surface of the upper Teflon cylinder. The beaker containing mucosal tissue secured upon lower cylinder (B), was manipulated over the base of the balance so that, the mucosal tissue is exactly below the upper cylinder (A). The exposed part of the gel was wetted with a drop of simulated nasal solution, and then a weight of 10 gm was placed above the expanded cap, left for 10 minutes. After which the gel binds with mucin. The weight was removed. Then slowly and gradually weights were added on the right side pan till the gel separates from the mucosal surface/ membrane. The weight required for complete detachment is noted (W1 (W1-5.25G)) gives force required for detachment expressed in weight in grams. Procedure was repeated for two more times. Average was computed and recorded.

Calibration of test equipment

Initially, a gel from the same batch was taken ten times and individual force required for complete detachment was noted and S. D. was calculated.

Force of adhesion (N) = (bioadhesive strength/1000)X 9.81

Bond strength (N/m^2) = Force of adhesion (N)/Surface area of disc (m^2)

The Apparatus for Mucoadhesive study is shown in (Figure 2).

Figure 2: Modified bioadhesion test apparatus (Fabricated)

***In-vitro* drug release study**

Preparation of Simulated Nasal Solution

Weigh accurately7.45mg/mL NaCl, 1.29mg/mL KCl and 0.32mg/mL $CaCl_2.2H_2O$and dissolve in 1000 mL of distilled water to produce simulated nasal solution; finally adjusted the pH with phosphoric acid to 6.75. *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 (The simulated nasal fluid (SNF) contained 0.87% NaCl, 0.088% $CaCl_2$. $2H_2O$, 0.31% KCl) in receptor compartment (100mL). 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 238 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 cm^2. 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 $CaCl_2.2H_2O$. 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 238nm.

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

$$P = (dQ/dt) / (C_0 \times A)$$

Where, dQ/dt is the flux or permeability rate (mg/h), C_0 is the initial concentration in the donor compartment, and A is the effective surface area of nasal mucosa.

Stability studies [15]

The formulations were stored at room temperature with RH 60± 5%. The formulations were evaluated mainly for their physical characteristics at the predetermined intervals of 3 months and after 6 months like appearance/clarity, pH, viscosity and drug content.

RESULTS AND DISCUSSION

On careful visual inspection against dark and white background, all the prepared nasal gel formulations were found to be free from any suspended particulate matter. All the formulations were found to be clear. This also indicates that the Tween 80 can be a good solubilizing agent at 10% v/v concentration for solubilizing 1% Fexofenadine Hydrochloride in prepared formulation. The pH of all the formulations from F1 to F9 was found to be in the range of 5.7 to 6.0 pH values of formulations shown in Table 3. Ideally, the ophthalmic solutions should possess pH in the range of 4.5-6.5, so as to minimize discomfort or excessive tear flux causing faster drainage of the instilled dose due to nasal irritation.

Table 3. Evaluation Parameters

Sr. No	Formulation code	Observed pH (±S.D.)	Gel strength (sec) (±S.D.)	Detachment force (Newton) (±S.D.)	Drug content (%) (±S.D.)	Cumulative Drug Release (%) (±S.D.) after 8hrs
1	F1	5.91±0.010	0.800±0.010	0.135±0.026	98.55±0.33	92.79±0.164
2	F2	5.74±0.006	0.863±0.015	0.465±0.002	98.27±0.19	89.32±0.201
3	F3	5.92±0.006	0.893±0.015	0.649±0.049	100.6±0.53	88.24±0.050
4	F4	5.75±0.010	0.810±0.020	0.291±0.035	98.44±1.58	95.02±0.065
5	F5	5.82±0.015	0.853±0.015	0.677±0.049	102.53±1.77	87.67±0.069
6	F6	5.84±0.020	0.903±0.021	0.955±0.057	99.75±1.72	85.30±0.049

7	F7	5.71±0.020	0.870±0.010	0.471±0.012	98.80±0.35	98.34±0.165
8	F8	5.95±0.020	0.897±0.006	0.649±0.049	98.98±1.63	87.31±0.075
9	F9	6.03±0.015	0.907±0.012	1.323±0.117	102.73±1.33	83.53±0.099

Compatibility Study

Infra-red spectra of drug and polymers showed matching peaks with the drug spectra. The characteristic peaks of drug were also present in the spectra of all drug- polymer combinations. Spectra of drug with physical mixture is shown in (Figure 3).

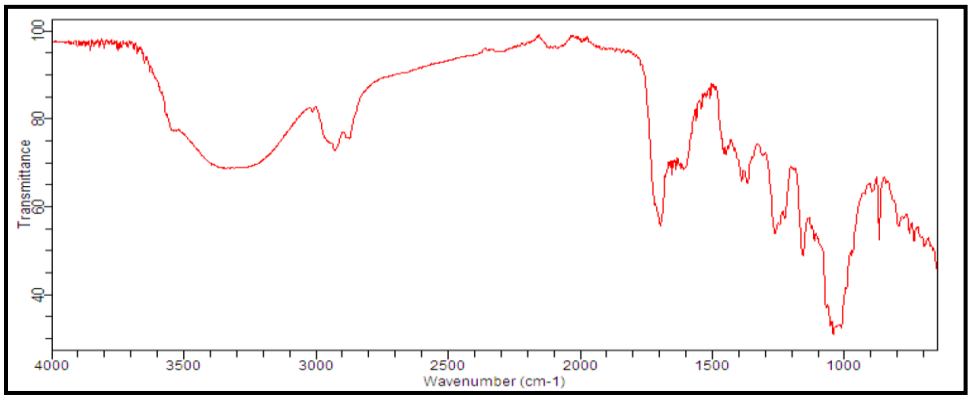

Figure 3: Fourier Transform Infra-red spectrum of Drug and Polymer mixture

Rheological study

The viscosity of formulations at respective pH and at pH 7.4 are shown in Table 4 and 5 respectively

Table 4: Viscosity of formulations at respective pH

RPM	Viscosity (cp) at respective pH								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
25	52.21	93.55	279.53	131.93	176.83	383.55	274.80	367.60	589.67
50	40.77	70.67	140.33	94.35	123.53	262.59	135.77	275.40	313.33
75	32.98	53.51	112.57	66.72	87.73	182.41	101.89	195.15	249.47
100	21.71	43.95	99.67	55.24	73.93	131.85	88.62	123.67	201.50

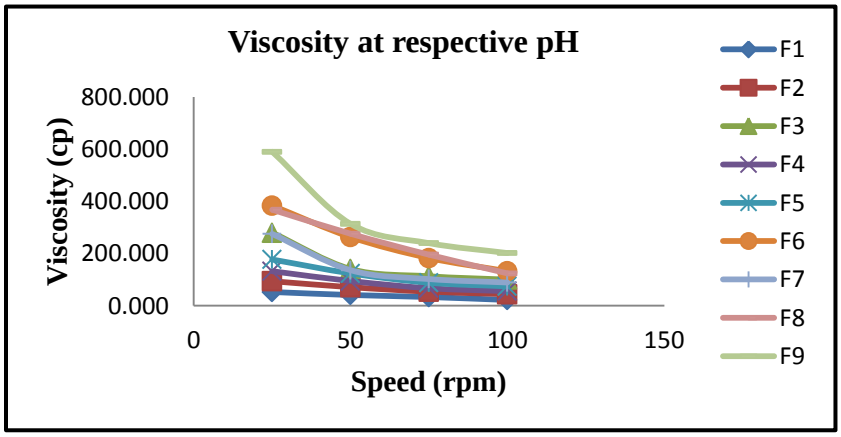

Figure 4: Viscosity profile of formulations at respective pH

Table 5:Viscosity of formulations at pH 7.4

RPM	Viscosity (cp) at pH 7.4								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
25	146.70	151.52	452.33	215.37	344.47	925.37	850.97	948.60	1040.10
50	89.79	112.74	243.17	179.53	181.33	613.13	486.65	614.53	728.63
75	78.88	86.67	160.20	101.40	138.30	401.77	269.18	386.30	470.20
100	69.13	75.12	135.57	82.33	115.43	232.13	125.20	185.67	288.33

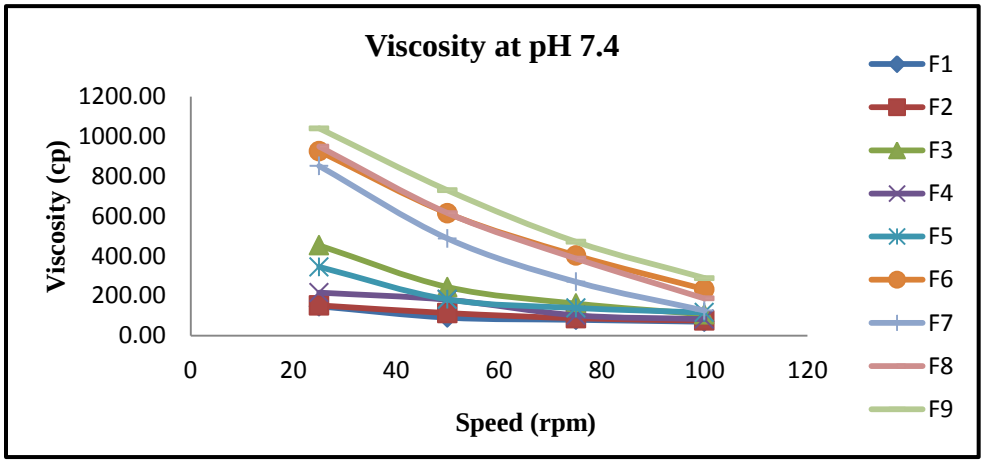

Figure 5: Viscosity profile of formulations at pH 7.4

The viscosity profile of formulations at respective pH and at pH 7.4 are shown in Figure 4 and 5 respectively. Viscosity v/s rpm plots for all formulations shows decrease in viscosity as shear rate (rpm) was increased. Concentration of Xanthan gum and Carbopol 934 was a major factor affecting viscosity of formulations. As pH was increased the increased in viscosity was observed. Which indicate that gel has the pseudoplastic flow. In combination with Carbopol 934 Xanthan gum has shown considerable increases in viscosity when concentration of Carbopol 934 is 0.1% w/v to 0.3%w/v and Xanthan gum is 0.1% w/v to 0.2% w/v.

Gel strength

The gel strength was found to be affected by concentrations of gelling and mucoadhesive polymers. Optimal mucoadhesive gel must have suitable gel strength so as to be administered easily and can be retained at nasal mucosal region without leakage after administration. Gel strength of all formulations showed comparable results as that of viscosity results. (Table 3)

Mucoadhesive strength

Mucoadhesive force means the force with which gels bind to nasal mucosa. Greater mucoadhesion is indicative of prolonged residence time of a gel and thus prevents its drainage from nasal cavity. The mucoadhesion force increased significantly as the concentration of mucoadhesion polymers increased. The Detachment Stress was determined for nasal gels. Results of this test indicate that

the variable Xanthan gum and Carbopol 934 both are having effect on mucoadhesive strength. It shows that mucoadhesive force was increased with the increasing concentration of the Xanthan gum and Carbopol 934. Shown in Table 3.

***In-vitro* drug release study**

Out of nine formulations maximum release after 8 hrs was found for F7 formulation. This indicates release of 98.33% drug available for antihypertensive activity of the drug. F7 formulation showed steady state release up to 8hrs which also indicates that this formulation would show better contact with biological membrane. *In-vitro* drug release profile of formulations shown in (Figure 6).

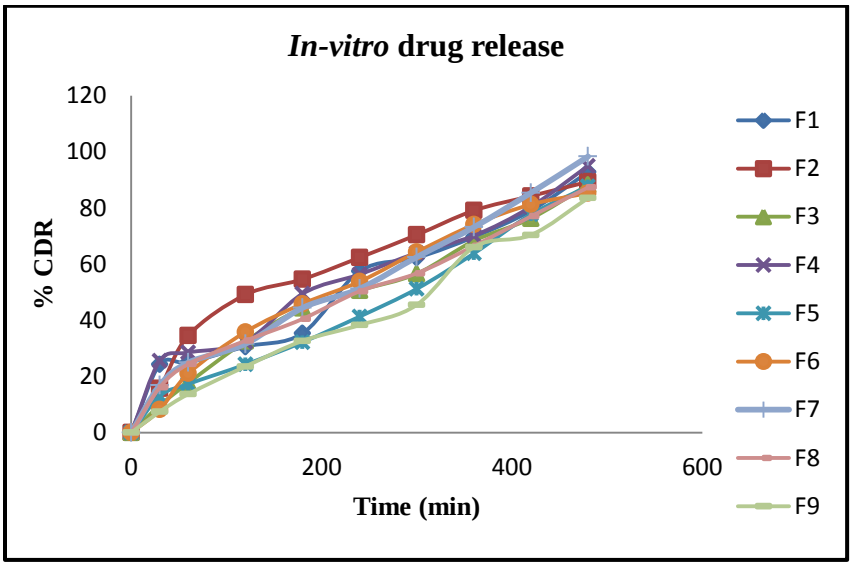

Figure 6: *In-vitro* drug release profile of formulations

Optimization[16]

A 3^2 full factorial design was selected and the 2 factors were evaluated at 3 levels, respectively. The percentage of Carbopol 934 (X_1) and Xanthan gum (X_2) were selected as independent variables and the dependent variable was% drug release, viscosity and Mucoadhesive strength. The data obtained were treated using Design expert version 9.0.2.0software and analyzed statistically using analysis of variance (ANOVA). The data were also subjected to 3-D response surface methodology to study the interaction of Carbopol 934 (X_1) and Xanthan gum(X_2) on dependent variable. ANOVA for the dependent variable % drug release. The values of X_1 and X_2 were found to be significant at $p < 0.05$, hence confirmed the significant effect of both the variables on the selected responses. From this data optimum concentration of Carbopol 934 0.1%w/v and Xanthan gum 0.2%w/v was found.3-D response surface Shown in (Figure 7) and (Figure 8).

Y1 (% CDR)= 103.35+ (-44.6833)*(A)-25.7333*(B)

Y2 (Mucoadhesive strength) = -65.2638+ (360.2833)*(A)+370.9667*(B)

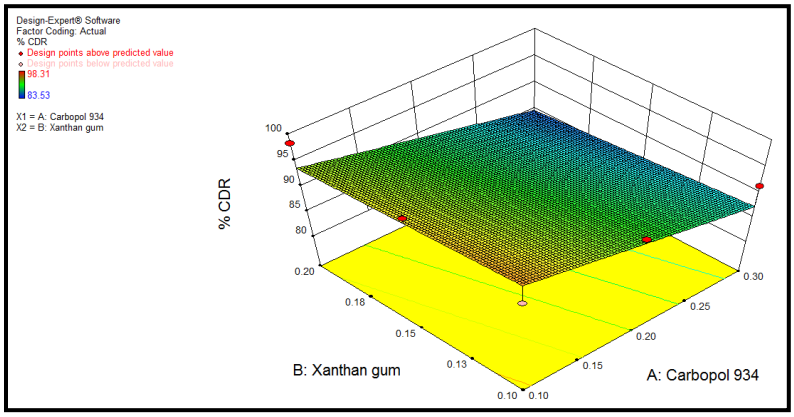

Figure 7: Surface response plot showing effect of Carbopol 934 and Xanthan gum on drug release.

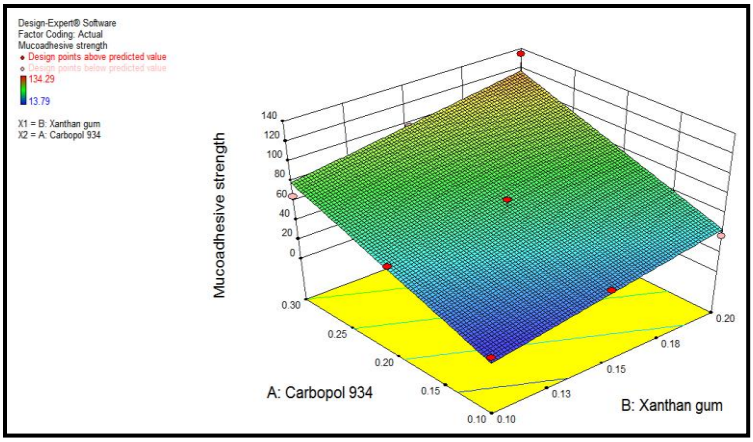

Figure 8: Surface response plot showing effect of Carbopol 934 and Xanthan gum on

From design expert version 9.0.2.0 thirty nine solutions were found in which optimum batch Carbopol 934(0.1% w/v) and Xanthan gum (0.2% w/v) with desirability was found to be optimum. From this data F7 batch was selected as optimum formulation.

Mucoadhesive strength.

***In-vitro* permeation study**

The permeation study of optimized batch F7 was carried out by using laboratory designed diffusion cell in which goat nasal mucosa was use as a diffusion membrane and simulated nasal fluid was used as a diffusion medium. Drug release profile was obtained by plotting percent drug release against time Figure 9 and result of permeation study is given in Table6.

Table 6: *In-vitro* permeation study for optimized batch F7

Sr. No.	Time (hrs)	Drug permeation rate (mg/cm/hr) (± S.D.)	% Cumulative drug permeation (±S.D.)
1	30 min	0.5064±0.002	11.95±0.049
2	1	0.4173±0.001	19.68±0.049
3	2	0.2776±0.001	26.19±0.064

4	3	0.2733±0.001	38.61±0.010
5	4	0.2427±0.002	45.55±0.049
6	5	0.2344±0.024	58.52±0.049
7	6	0.2416±0.001	68.16±0.082
8	7	0.2421±0.001	79.72±0.051
9	8	0.2349±0.001	88.41±0.057

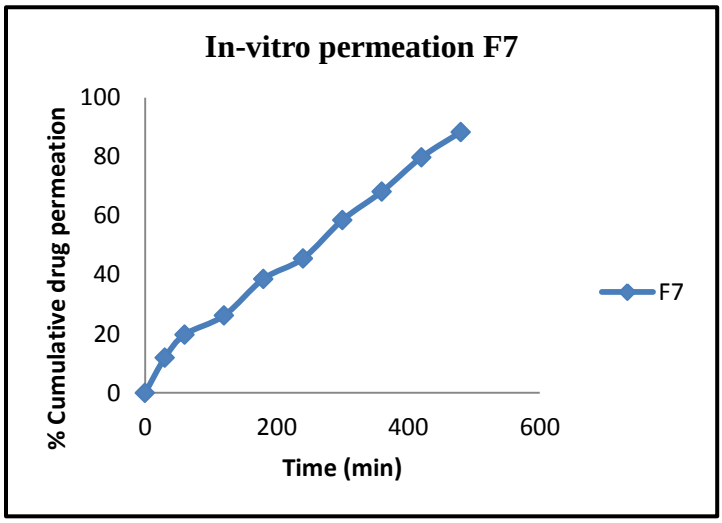

Figure 9: In-vitro permeation release of optimized batch F7

In vitro permeation study was performed for the optimized batch using goat nasal mucosa. The percent drug permeated after 8 h was found to be 88.41% from nasal gel formulation. The results showed that the formed gels had the ability to retain Fexofenadine hydrochloride for the duration of 480 min. During gel formation, formulation got converted into the gel phase and thus drug release became slow.

Release Kinetics[17, 18]

In the present study, the release was analyzed by PCP Disso version v3 software to study the kinetics of drug release mechanism. The results showed that the factorial design batches followed korsmeyer-peppas model kinetics. The release exponent $0.5 < n < 1.0$ this indicates that it is following Non-Fickian release (anomalous), this means that drug release followed both diffusion and erosion controlled mechanisms.

Stability study

Formulations at room temperature were found to be stable upto 6 months. There is no change in drug content, pH, clarity and viscosity.

CONCLUSION

On the basis of the fact that Carbopol 934 and Xanthan gum can enhance the intranasal permeation of drug moiety**,** I formulated an optimized pH sensitive in-situ Fexofenadine hydrochloride for

nasal administration. Permeation study suggested that such formulation can be an alternative route to the conventional therapy of Fexofenadine Hydrochloride and it can also be a part replacement/supportive therapy to the conventional oral administration of Fexofenadine Hydrochloride. Thus Carbopol 934 based pH sensitive nasal gel could open a way to design of new controlled delivery for Fexofenadine Hydrochloride administration via nasal route. This formulation can also be effective against, the patient suffering from food interaction and also drug interaction.

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