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FORMULATION AND EVALUATION OF *IN-SITU* NASAL GEL OF RAMIPRIL

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ABSTRACT

Nasal mucosa has been considered as a potential administration route to achieve faster and higher level of drug absorption because it is permeable to more compounds than the gastrointestinal tract due to lack of pancreatic and gastric enzymatic activity, neutral pH of the nasal mucus and less dilution by gastrointestinal contents . In recent years many drugs have been shown to achieve better systemic bioavailability through nasal route than by oral administration. Hence in-situ nasal mucoadhesive gel of Ramipril was formulated by cold method using Carbopol 940 as a pH sensitive polymer, xanthan gum as a mucoadhesive polymer and PEG 400 as the penetration enhancer. A 32 full factorial design was applied to study effect of varying concentration of independent variables carbopol 940 (X1) and Xanthan gum (X2) on dependent variables in vitro drug release, viscosity and mucoadhesive strength. In vitro drug release kinetics was studied using different kinetic models to know exact mechanism of drug release. It was found that formulation additives shows effect on drug release, viscosity and mucoadhesive strength, as the concentration of polymers increases mucoadhesive strength and viscosity increases, drug release was also increases.

INTRODUCTION

Intranasal drug delivery is now recognized to be useful and reliable alternative to oral and parenteral route since nasal mucosa offers numerous benefits as a target tissue for the delivery of the wide variety of therapeutic compounds. This is due to large surface area, porous endothelial membrane and high total blood flow with avoidance of hepatic first-pass elimination, gut wall metabolism and/or destruction in the gastrointestinal tract [1].

Nasal cavity offers a number of unique advantages as increased bioavailability, good permeability, and direct delivery to brain. Recently many drugs have been shown to achieve better systemic bioavailability through the nasal route than by oral administration [2].

Treatment for hypertension is a long term therapy and requires higher doses of drug with high dose frequency. The model drug Ramipril undergoes hepatic first pass metabolism orally and only 23-28% bioavailable for antihypertensive effect. Hence to avoid side effects associated with these oral formulations we can formulate the in situ nasal gel formulation to increase the contact time of the drug with nasal surface and increase absorption of the drug and produce an antihypertensive effect.

Ramipril is angiotensin converting enzyme inhibitor used in the management of hypertension, angina pectoris, cardiac arrhythmias, myocardial infarction, and heart failure. The half-life of drug is relatively short approximately 2-4 hrs. Orally it is absorbed about 95% but its oral bioavailability is only 23-28% due to hepatic first pass metabolism. In the present investigation in situ nasal mucoadhesive gel of Ramipril was formulated, in attempt to increase residence time of drug, increase permeation and to reduce first pass metabolism [3].

Carbopol 940 is a mucoadhesive polymer produced from acrylic acid monomers. It is having high viscosity and pH dependent property. It is having drug release retarding effect with increasing concentration. Xanthan gum is a high molecular weight hydrophilic polymer obtained as a result of microbial fermentation of glucose by bacterium Xanthomonascampestris, which not only retards the drug release but also provides the time dependent release kinetics with advantages of biocompatibity and inertness [4].

MATERIALS AND METHODS

MATERIALS

Ramipril was provided by IPCA research lab, Mumbai. Xanthan gum was provided by Glenmark Pharmaceuticals limited, Nasik. Carbopol 940 (Loba Chemicals Pvt. Ltd.) and polyethylene glycol 400. Benzalkonium chloride was used of analytical grade.

METHODS

Determination of λ_{max} of Ramipril[6]

The UV spectrum of Ramipril was obtained using UV Jasco V630. Ramipril (10mg) was accurately weighed and transferred to 100 ml volumetric flask. It was then dissolved in small quantity of methanol and diluted up to 100 ml with methanol. The above made solution was further diluted to obtain concentration of 25µg/ml. The resulting solution was scanned from 200-400 nm and the spectrum was recorded to obtain the value of maximum wavelength. The λ_{max} was found to be 207 nm .

Drug excipients compatibility study [7]

Infra-red spectrum

The Infra-red spectrum of Ramipril was recorded with KBr disc over the wave number of 4000 to 400 cm^{-1} by using Fourier Transform Infra-red spectrophotometer [84005 Shimadzu. Japan].

Differential scanning calorimetric studies

Thermal analysis was performed using a differential scanning calorimetric equipped with a computerized data station. The sample of pure drug was weighed and heated at a scanning rate of 10°C/min between 40 and 200°C and 40 ml/min of nitrogen flow. The differential scanning calorimetric analysis gives an idea about the interaction of various materials at different temperatures. It also allows us to study the possible degradation of the material [Mettlar Toledo] [7]

Preparation of in situ nasal mucoadhesive gel of Ramipril [8]

In situ gels were prepared by cold technique, reported by Schmolka. To the 2%w/v, solution of drug in small quantity of ethanol then in distilled water, carbopol 940 was added in the quantity of 0.3, 0.4, 0.5, and w/v. This solution was then stirred until carbopol940 completely swells in it. After the complete swelling of carbopol, Xanthan gum was added in the quantity 0.15, 0.20, 0.25%w/v. After the complete hydration of both the polymers PEG 400(10%) and Methylparaben (0.033%) were added to it. This resulting formulation was then kept at 40^0C overnight until clear gel is obtained.

Formulation optimization[9]

3^2 full factorial design was applied to the formulation that showed the satisfactory results to see the effect of varying the concentration of variables carbopol 940 (X1)and Xanthan gum (X2) on various responses like % cumulative drug release, viscosity, mucoadhesive strength.

For the carbopol 940 lower levels was 0.3 mg, middle was 0.4mg and higher level was 0.5mg. Similarly for the Xanthan gum lower level was 0.15mg, middle was 0.20 mg and higher level was 0.25mg. Composition of all the batches is shown in table 1.

Table 1: Composition of Formulation

Composition formulation code	Ramipril (%w/v)	Carbopol 940(%w/v)	Xanthan gum (%w/v)	PEG 400 (%v/v)	Methyl paraben (%v/v)	Distilled water Upto (ml)
F1	2	0.1	0.15	10	0.033	100
F2	2	0.2	0.15	10	0.033	100
F3	2	0.3	0.15	10	0.033	100
F4	2	0.1	0.20	10	0.033	100
F5	2	0.2	0.20	10	0.033	100
F6	2	0.3	0.20	10	0.033	100
F7	2	0.1	0.25	10	0.033	100
F8	2	0.2	0.25	10	0.033	100
F9	2	0.3	0.25	10	0.033	100

Evaluation of *in situ* nasal gel

1] pH[10]

pH of each formulation was determined by using Digital pH meter (systronics digital pH meter 335).The pH meter was calibrated using pH 4 and pH 7 buffer by using standard buffer tablet.

2]Viscosity[11]

Viscosity (rheological properties of prepared gel was determined with the help of Brookfield Viscometer; type DV-II+PRO using spindle no-62 and 63.Viscosity of formulations were determined at two different pH, formulation pH and at pH 7.4 with varying shear rate.

3]Measurement of gel strength[12]

A sample of 50g of the nasal gel was put in a 50 ml graduated cylinder. A weight of 5 g was placed onto the gel surface. 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 deep into the gel.

4]Mucoadhesive force (detachment stress)[13,14]

The mucoadhesive strength of each formulation was determined by measuring the force required to detach the formulation from goat nasal mucosal tissue by using a modified bioadhesion test apparatus that is modified physical balance. *In vitro* mucoadhesion studies were conducted using modified bioadhesion test assembly described by Mohammad et.al

Fabrication of equipment:

The equipment was fabricated by us in the laboratory as shown in figure 1. 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 block (A), also locally fabricated. The dimensions are a Teflon block of 3.8 cm diameter and 2 cm height was fabricated with an upward position of 2 cm height and 1.5 cm diameter on one side. The right pan (C) was replaced with a lighter pan so that, the left pan weighs 5.20gm 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 6.4. (E).

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 block (B) and secured. This assembly was placed into beaker containing simulated nasal solution pH 6.4 at 37 ± 20 ○ C. From each batch, some quantity of gel was taken and applied on the lower surface of the upper Teflon block. 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) (W1-5.20gm)) gives force required for detachment expressed in weight in grams. Procedure was repeated for two more times. Average was computed and recorded.

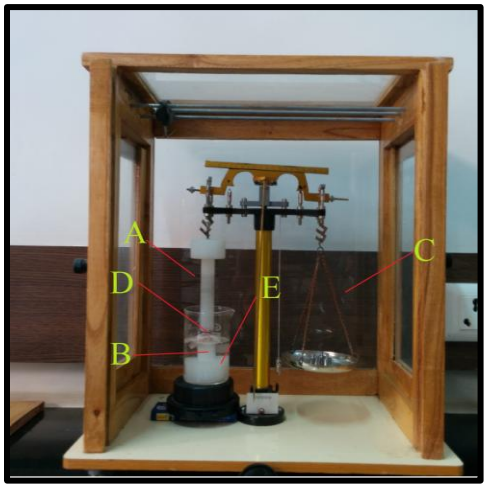

Fig 1: Modified mucoadhesion test apparatus (Fabricated)

5] Drug content [15]

Drug content was determined by taking 1ml of formulation was taken in 100 ml volumetric flask. It was dissolved in distilled water properly and final volume was made to 100 ml with distilled water. 1ml quantity from this solution was transferred into the 10ml volumetric flask and final volume was made to 10ml by using distilled water Finally the absorbance of prepared solution was measured at 207nm by using UV visible spectrophotometer. By using absorbance value % drug content in the formulation was calculated.

6] In Vitro Drug Release study [16]

A) Preparation of simulated nasal solution:

Weigh accurately 0.87% NaCl, 0.31%KCl and 0.088% CaCl2·2H2O and dissolve in 1000 ml of distilled water to produce simulated nasal solution; finally adjusted the pH with phosphoric acid to 6.4. [17]

B]In vitro release study

The formulation was carried out using laboratory designed diffusion cell through egg membrane. From the gel 1 ml was placed in donor compartment and freshly prepared simulated nasal solution (The simulated nasal fluid (SNF) contained in receptor compartment (100ml)). Egg membrane was mounted between donor and receptor compartment. Temperature of receiver compartment was maintained at 37 ± 2^0C 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 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 hrs. And same volume of fresh medium was replaced. Aliquot so withdrawn were suitably diluted and

analyzed using UV visible spectrophotometer at 207 nm. The concentration of drug was determined from a previously constructed calibration curve.(y = 0.03629x + 0.059, R2 =0.996).

7] Drug release kinetics [18]

It is generally understood that the release of the drug from gels can be considered as mass transport phenomenon involving diffusion of the molecules from a region of higher concentration to a region of lower concentration in the surrounding environment. The in vitro drug release data was fitted to different models, i.e. zero order, first order, Higuchi and Connor's and Korsemeyer's Peppas to study the drug release mechanism of the formulation.

8] *In Vitro* permeation study [19]

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 0.75cm2. The acceptor chamber of the diffusion cell (laboratory designed) with a volume capacity 100ml was filled with simulated nasal fluid (SNF) contained accurately 0.87%NaCl, 0.31% KCl and 0.088%CaCl2·2H2O. From the gel formulation 1ml (25mg equivalent) ml of 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 207nm. Permeability coefficient (p) was calculated by the following formula:

$$P = \frac{dQ/dt}{C0.A}$$

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.

9] Accelerated stability study [20]

Stability studies were conducted according to ICH guidelines 400C ± 20C/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.

RESULTS AND DISCUSSION

Compatibility study

Infrared Spectroscopy

The IR spectra of Ramipril, polymers and physical mixture were generated. The IR absorption bands observed in the IR spectrum of drug and polymers resembles with that of found in the physical mixture proves compatibility of drug with polymers.

pH

The normal physiological pH of the nasal mucosa ranges from 4.5-6.5. But the nasal cavity has the capability to tolerate pH between 3-10. pH of all formulations was found to be between 5.2 to 5.8 that is within the range, which are presented in the Table 2.

Viscosity

Viscosities of all the formulations were noted at formulation pH and pH 7.4. It was observed that as the pH increases viscosity also increases. Mucoadhesive polymer Xanthan gum is also having synergistic effect with pH .All the formulations showed psudoplastic flow. Viscosities of all the formulations at 25rpm are shown in table 2 shows viscosity profile of all formulations.

Gel strength

Gel strength was recorded for all the formulations by using laboratory designed apparatus. It was observed that gel strength is showing synergistic effect with the viscosity, as the polymer concentration and pH increases gel strength also increases. Gel strength for the formulations is noted in Table 2.

Drug content

Drug content found in the in situ nasal gel formulations resembled that of literature value. Range of drug content was 99-101%. Therefore uniformity of content was maintained in all formulation. Drug content of all the formulations is listed in Table 2.

Mucoadhesive strength

Mucoadhesive strength was determined by measuring the force required to detach the formulation from mucosal surface that is detachment stress. Results reveal that increase in carbopol 940 and Xanthan gum concentration increases the mucoadhesive strength. This was due to interaction of polymeric chains with the mucin strands to form weak chemical bonds due to stronger mucoadhesive force. Mucoadhesive strength is listed in Table 2.

In vitro drug release study

Out of nine formulations maximum release after 8 hrs was found for F5 formulation. This indicates release of 97.27% drug available for antidepressant activity of the drug. F5 formulation showed steady state release up to 8hrs which also indicates that this formulation would show better contact with biological membrane. Drug release of all the formulations is listed in Table 2.*In-vitro* drug release profile of formulations shown in (Figure 2).

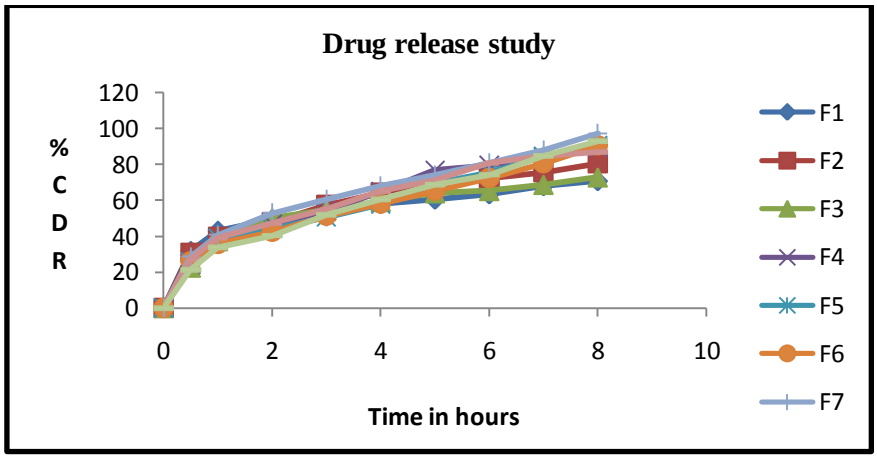

Fig. 2: *In-vitro* drug release profile of all formulations

Table 2: *In-vitro* drug release profile of all formulations

Formulation code	pH	Gel strength [sec]	Viscosity [cps] at 25rpm	Drug content %	Mucoadhesive strength [gm]	In-vitro drug release study
F1	5.66±0.01	1.07±0.025	263.9	98.08 ±0.00068	24.82 ±0.026	74.64±0.00022
F2	5.61±0.017	1.17±0.026	316	100.83±0.00023	30.69±0.026	80.44±0
F3	5.49±0.012	1.24±0.017	602.3	101.77±0.00036	51±0.26	72.77±0.00026
F4	5.65±0.01	1.40±0.041	552.3	99.02±0.00025	64..03±0.057	89.59±0
F5	5.51±0.015	1.42±0.026	572.8	100.57±0.00036	71.56±0.05	90.54±0.00017
F6	5.55±0.017	1.45±0.028	800.8	99.77±0.00024	99.53±0.01	90.39±0.0001
F7	5.40±0.026	1.50±0.022	606.18	98.78±0.00046	69.27±0.02	97.27±0.0002
F8	5.29±0.015	1.57±0.026	723.5	98.32±0.00015	111.17±0.01	86.77±0.00017
F9	5.51±0.022	2.04±0.046	1497	100±0.00015	149.49±0.01	92.87±0.00017

Drug release kinetics

In vitro drug release kinetics was studied for all the formulations using different kinetic models. From the regression value it can be predicted that formulation follows Zero order because regression value was greater than 0.9 (concentration independent mechanism) Higuchi and Connor's and Korsemeyer's Peppas release kinetics (r2 value greater than 0.9),

the n value of Korsemeyer's Peppas release kinetics was near to 0.5 from which we can conclude that formulation follows fickian release mechanism that is release by swellable polymeric matrix.

***In vitro* permeation study**

In vitro drug release was observed for the optimized formulation by using goat nasal mucosa. Permeation of the drug from goat nasal mucosa was studied for 8 hrs. It was found to be 90.89% at 8th hr. Permeation of the drug shows synergistic mechanism with that of in vitro drug release.

Table 2: *Ex-vivo*permeation study for optimized batch F7. (n=3)

Sr no.	Time (hrs.)	Drug permeation rate (mg/cm/hr.) (± S.D.)	% Cumulative drug permeation (±S.D.)
1	0.5	0.5693 ±0.017	21.39±0.085
2	1.00	0.4353±0.025	34.87±0.081
3	2.00	0.255±0.022	43.72±0.078
4	3.00	0.1844±0.012	50.78±0.036
5	4.00	0.148±0.01	57.81±0.08
6	5.00	0.1306±0.012	66.89±0.057
7	6.00	0.1206±0.01	72.4±0.133
8	7.00	0.1055±0.007	83.87±0.01
9	8.00	0.0947±0.01	90.89±0.01

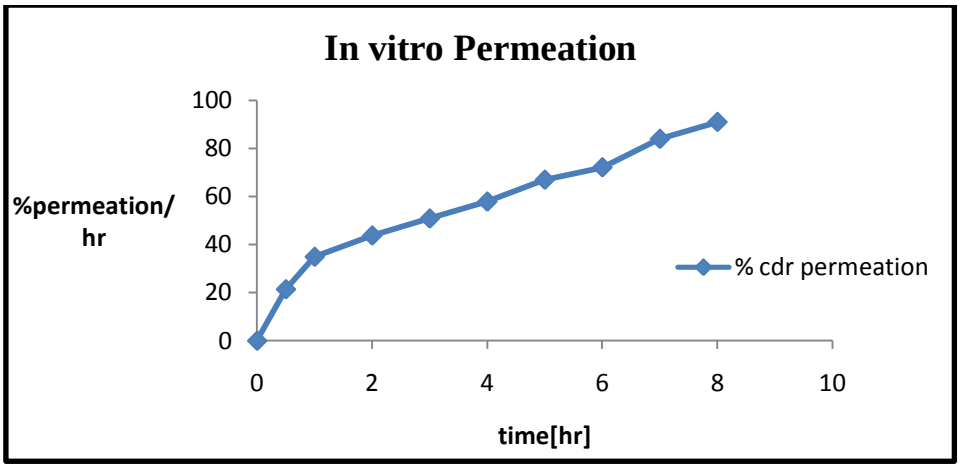

Fig 3: *In- vitro* permeation study for optimized batch F7

Accelerated stability study

Results of the stability studies showed that there is no change in the physical parameters of the formulation. Drug content of the formulation was also found to be same as that before stability testing.

Statistical analysis

The purpose of using 32 full factorial design was to conduct comprehensive study of effect of process parameters like carbopol 940 (X1) and Xanthan gum(X2) and their interactions using a suitable statistical tool (Design expert software version 7.0) by applying one way ANNOVA at 0.05 levels. A mathematical modeling was carried out. Polynomial equation obtained depending on significant influences among 2 factors on their experimental design.

Statistical analysis

A 32 full factorial design was selected and the 2 factors were evaluated at 3 levels, respectively. The percentage of Carbopol 940 (X1) and Xanthan gum (X2) 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 7.0.software 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 940 (X1) and Xanthan gum(X2) on dependent variable. ANOVA for the dependent variable % drug release. The values of X1 and X2 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 940 0.3%w/v and Xanthan gum 0.25%w/v was found.3-D response surface Shown in Fig. 4 , Fig.5 and Fig. 6

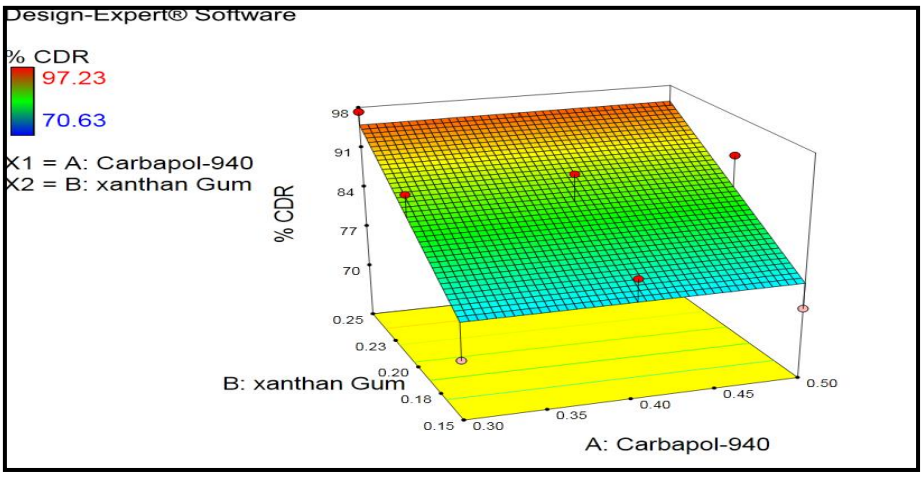

Fig.4: Surface response plot showing effect of carbopol 940 and xanthan gum on drug release.

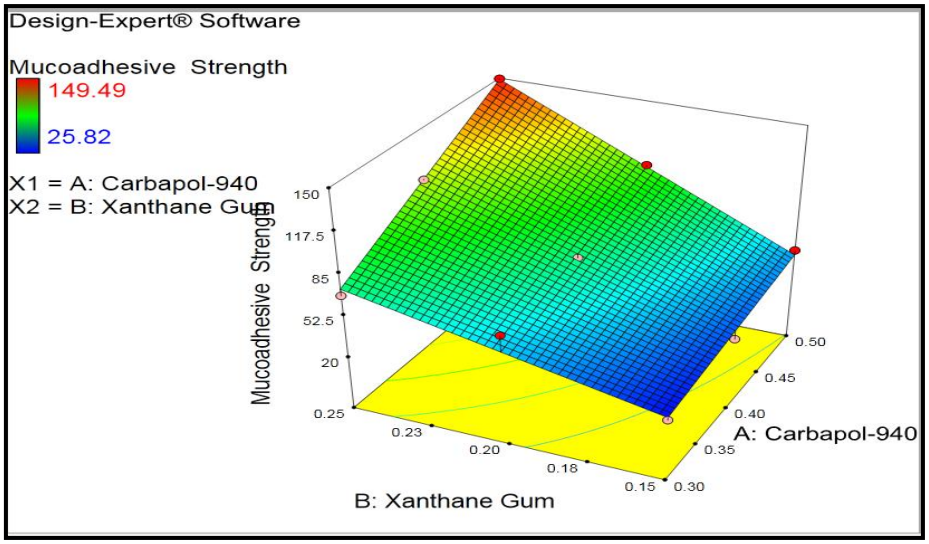

Fig.5: Surface response plot showing effect of carbopol 940 and xanthan gum on mucoadhesive strength

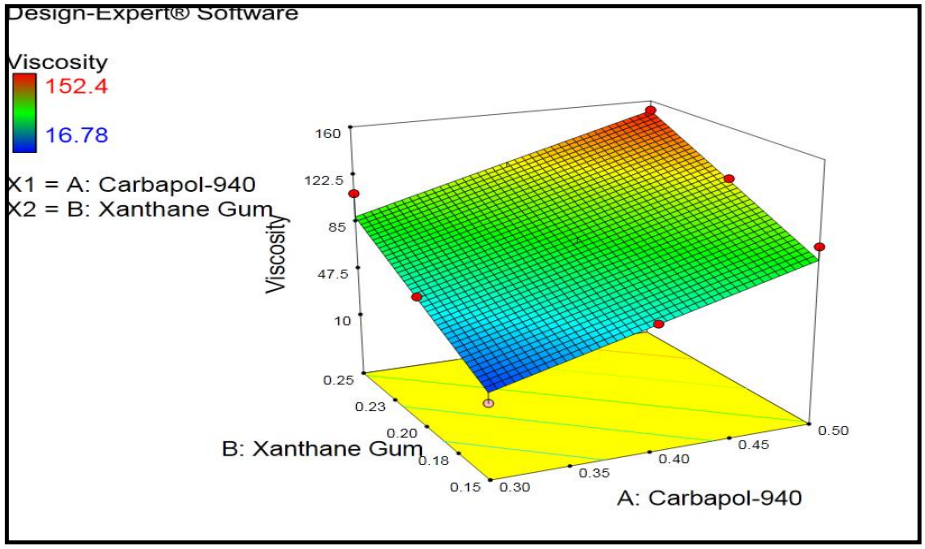

Fig.6: Surface response plot showing effect of carbopol 940 and xanthan gum on viscosity.

Optimized formula

After generating model equations relating main effects and responses, various gel formulations containing Ramipril were optimized based on in vitro drug release (Y1), Viscosity (Y2), mucoadhesive strength(Y3). The optimal values for responses were obtained by numerical analysis based on the criteria of desirability, and optimal batch was selected. Optimized batch was having highest drug release, optimal viscosity and mucoadhesive strength. This reveals that mathematical model obtained by factorial design to produce optimized responses was well fitted.

CONCLUSION

The formulation and development *in-situ* gelling system for nasal administration for an antihypertensive drug Ramipril by using Carbopol 940 and Xanthan gum achieves the systemic delivery of drug through the nasal route and thereby reducing the dose of drug, avoiding its first pass metabolism. Evaluation of gel for nasal gel the *in situ mucoadhesive* gelling system for shows the good the physicochemical properties and *in-vitro* drug release profile of the formulations. The nasal gel dosage forms have application in pharmaceutical industries and research laboratories.

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