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Original Article

DEVELOPMENT AND EVALUATION OF IN SITU NASAL MUCOADHESIVE GEL OF METOPROLOL SUCCINATE BY USING 3^2 FULL FACTORIAL DESIGN

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ABSTRACT

Objective: To develop and evaluate in situ nasal mucoadhesive gel of metoprolol succinate.

Methods: In situ nasal mucoadhesive gel 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 3^2 full factorial designs was successfully applied for the optimization. All the formulated gels were evaluated for different chemical and physical characteristics. *In vitro* release data were fitted to different models to know exact mechanism of drug release.

Results: All the prepared in situ gel showed good *In vitro* drug release profile. Carbopol 940 and xanthan gum in higher concentrations were effective in sustaining the release of drug up to 8 hrs. *In vitro* permeation study showed that optimized F4 batch permeates 95% at 8^{th} hr.

Conclusion: In situ nasal mucoadhesive gel was fond to be effective in sustaining the release of the drug up to 8 hrs hence it could be a useful formulation as a alternative therapy in management of hypertension.

Keywords: Metoprolol succinate, Mucoadhesive strength, Carbopol 940, Nasal gel.

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 metoprolol succinate undergoes hepatic first pass metabolism orally and only 40-50% 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.

Metoprolol succinate is selective adrenergic receptor blocking agent used in the management of hypertension, angina pectoris, cardiac arrhythmias, myocardial infarction, heart failure, and in the prophylactic treatment of migraine. The half-life of drug is relatively short approximately 4-6 hrs. Orally it is absorbed about 95% but its oral bioavailability is only 40-50% due to hepatic first pass metabolism. In the present investigation in situ nasal mucoadhesive gel of metoprolol succinate 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 Xanthomonas campestris, 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

Metoprolol succinate 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

Solubility study of metoprolol succinate

Excess amount of metoprolol succinate was placed in the different study media (10 ml) and shaken for 24 hrs at 25^0 C. From each sample1 ml of aliquot was taken out and filtered through Whatman filter paper. After making the dilutions, absorbance was measured and calculations for solubility were done [5].

Determination of λ_{max} of metoprolol succinate

The UV spectrum of metoprolol succinate was obtained using UV Jasco V630. Metoprolol succinate (10mg) was accurately weighed and transferred to 100 ml volumetric flask. It was then dissolved and diluted up to 100 ml with distilled water. 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 221 nm [6].

Drug excipients compatibility study

Infra red spectrum

The infra red spectrum of metoprolol succinate 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 metoprolol succinate

In situ gels were prepared by cold technique, reported by Schmolka. To the 2%w/v, solution of drug in distilled water, carbopol 940 was added in the quantity of 0.1, 0.2, 0.3, and w/v. This solution was then stirred until carbopol 940 completely dissolves in it. After the complete dissolving of carbopol, xanthan gum was added in the quantity 0.05, 0.1, 0.15%w/v. After the complete hydration of both the polymers PEG 400(10%) and benzalkonium chloride (0.02%) was added to it. This resulting formulation was then kept at 4^0C until clear gel is obtained. Composition of all the formulations is shown in table 1[8].

Formulation optimization

3^2fullfactorial design was applied to the formulation that showed the satisfactory results to see the effect of varying the concentration of independent variables C940 (X_1) and xanthan gum (X_2) on dependent variables i. e.% cumulative drug release, viscosity, mucoadhesive strength [9].

Independent variables	Levels(-1) Lower	(0)Middle	(+1)Upper
Carbopol 940(X_1)	0.1	0.2	0.3
Xanthan gum (X_2)	0.05	0.1	0.15

Characterization of in situ nasal mucoadhesive gel

pH

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 [10].

Viscosity

Viscosity (rheological properties of prepared gel was determined with the help of Brookfield Viscometer; type DV-II+PRO using spindle no- 61, 62 and 63. Viscosity of formulations were determined at two different pH, formulation pH and at pH 7.4 with varying shear rate [11].

Measurement of gel strength

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 [12].

Mucoadhesive force (detachment stress)

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 [13].

In vitro mucoadhesion studies were conducted using modified bioadhesion test assembly described by Mohammad et. al

i) Fabrication of equipment

The equipment was fabricated by us in the laboratory. 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. Equipment is shown in fig 1.

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 5.25 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).

Fig. 1: Modified mucoadhesion test apparatus (Fabricated)

ii) 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 the 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 the force required for detachment expressed in weight in grams. Procedure was repeated for two more times. Average was computed and recorded.

Drug content

Drug content was determined by taking 1 gm of gel in 100 ml volumetric flasks. It was dissolved in distilled water properly and the final volume was made to 100 ml with distilled water. 1 ml quantity from this solution was transferred into the 10 ml volumetric flask and final volume was made to 10 ml by using distilled water Finally the absorbance of prepared solution was measured at 221 nm by using UV visible spectrophotometer. By using absorbance value % drug content in the formulation, was calculated [14].

***In vitro* drug release study**

A) Preparation of 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; finally adjusted the pH with triethanol amine to 6.75.

In vitro release study of the formulation was carried out using laboratory designed diffusion cell through egg membrane. From the formulation 0.5 ml of gel was placed in donor compartment and freshly prepared simulated nasal solution in receptor compartment (100 ml). Egg membrane was mounted between donor and receptor compartment. Temperature of receiver compartment was maintained at 37±2^{0}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 0.25, 0.5, 0.75, 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 vis spectrophotometer at 221 nm. The concentration of drug was determined from a previously constructed calibration curve. (y = 0.0027x + 0.009, R2 =0.995) [15].

Drug release kinetics

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 [16]. 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.

***In-Vitro* permeation study**

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 100 ml was filled with simulated nasal fluid (SNF) contained accurately7.45mg/ml NaCl, 1.29mg/ml KCl and 0.32mg/ml CaCl2·2H2O.

From the gel formulation 0.5(10 mg equivalent) ml of formulation was placed in donor compartment. At predetermined time point of 0.25, 0.5.0.75, 1,2,3,4,5,6,7, and 8 hrs 1 ml of sample was withdrawn from the acceptor compartment replacing the sample removed with simulated nasal fluid after each sampling for the period of 8 hrs. Then samples were specifically diluted and absorbance was noted at 221 nm. Permeability coefficient (p) was calculated by the following formula [17].

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

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.

Accelerated stability study

Stability studies were conducted according to ICH guidelines 40^{0}C ± 2^0 C 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 [18].

RESULTS AND DISCUSSION

Solubility of metoprolol succinate in different solvents is listed in table 2.

Table 1: Composition of formulation

Composition And formulation code	Metoprolol succinate (%w/v)	Carbopol 940(%w/v)	Xanthan gum (%w/v)	PEG 400(%v/v)	Benzal Konium Chloride (%v/v)	Distilled water up to (ml)
F1	2	0.1	0.05	10	0.02	100
F2	2	0.2	0.05	10	0.02	100
F3	2	0.3	0.05	10	0.02	100
F4	2	0.1	0.1	10	0.02	100
F5	2	0.2	0.1	10	0.02	100
F6	2	0.3	0.1	10	0.02	100
F7	2	0.1	0.15	10	0.02	100
F8	2	0.2	0.15	10	0.02	100
F9	2	0.3	0.15	10	0.02	100

Table 2: Solubility of metoprolol succinate in different solvents

Solvent	Solubility(mg/ml)
Distilled water	243.71
pH6.5 phosphate buffer	24.58
pH6.8 phosphate buffer	217.92
pH7 phosphate buffer	77.51
pH7.5 phosphate buffer	190.72
pH 6.8 phosphate buffer: Distilled water.	166.16

The IR spectra of metoprolol succinate, polymers and physical mixture is shown in fig. 2. 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. In figure, A is the absorption spectra of drug, B is the absorption spectra of physical mixture, C is the absorption spectra of xanthan gum and D is the absorption spectra of carbopol 940.

DSC thermogram of drug shows strong endothermic peak at 138.26^{0}C and physical mixture exhibited the characteristic peak at 138.43^{0}C. From the results, it can be concluded that there is no interaction between drug and polymers because there is no significant shifting of peaks. In fig. A is the DSC thermogram of drug, B is the DSC thermogram of physical mixture, C is the DSC thermogram of xanthan gum and D is DSC thermogram of carbopol 940.

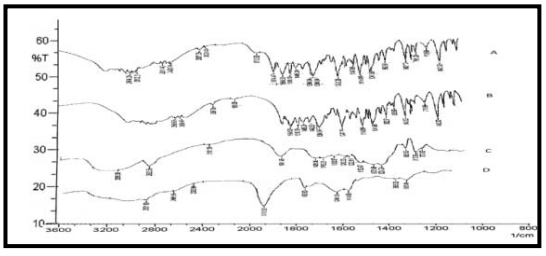

Fig. 2: Overlay IR spectra of drug with polymers

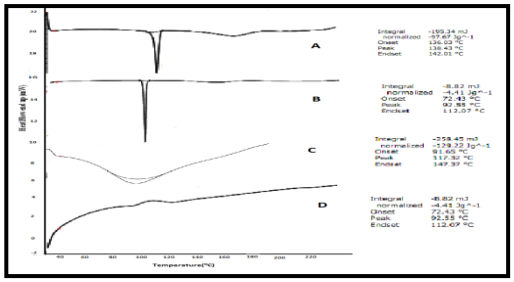

Fig. 3: Overlay DSC thermogram of drug with polymers and physical mixture

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 5.1 to 5.7 that is within the range, which are presented in the table 3.

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 is also having synergistic effect with pH. All the formulations showed psudoplastic flow. Viscosities of all the formulations are shown in table 3 and fig 4 shows viscosity profile of all formulations.

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 3.

Drug content found in the in situ nasal gel formulations resembling that of literature value. Range of drug content was 98-102%. Therefore uniformity of content was maintained in all formulation. Drug content of all the formulations is listed in table 3

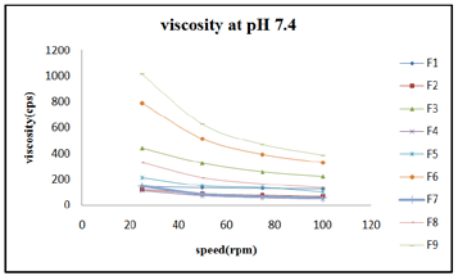

Fig. 4: Viscosity profile of formulations at pH 7.4

Mucoadhesive strength was determined by measuring the force required to detach the formulation from the 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 3.

Table 3: Evaluation parameters for all the batches of gel formulation

Formulation code	pH	Gel strength (sec)	Viscosity (cps) at 10 rpm	Drug content (%)	Mucoadhesive strength(gm)	*In vitro* drug release (%)
F1	5.59±0.01	1.35±0.05	267.80	100.46±0.26	22.70 ±1.050	92.23±0.02
F2	5.47±0.01	1.94±0.06	298.13	100.041±0.23	31.69±1.86	86.86±0.10
F3	5.25±0.15	2.62±0.00	677.86	102.28±0.028	47.5±0	78.67±0.04
F4	5.47±0.00	3.87±0.01	274.56	98.85±0.440	22±0	98.14±0.02
F5	5.47±0.01	3.98 ±0.85	432.66	98.36±0.028	62.14±1.91	89.25±0.04
F6	5.33±0.00	3.26 ±0.07	618.66.66	100.90±0.412	99.25±1.20	75.42±0.24
F7	5.77±0.01	2.05±0.04	375.31	98.4.42±0.17	62.69±0.9620	90.47±0.04
F8	5.51±0.00	2.91±0.87	572.7	100.01±0.115	110.62±1.32	85.32±0.02
F9	5.37±0.00	3.96 ±0.45	930.4	99.66±0.1858	146.25±0	70.34±0.07

Table 4: Regression co-efficient of the model equations on *In vitro* diffusion kinetics

Formulation code	Zero order	First order	Higuchi and Connors	Korsemeyers Peppas.	
	R2	R2	R2	R2	n
F1	0.821	0.970	0.988	0.948	0.707
F2	0.810	0.966	0.955	0.982	0.705
F3	0.838	0.969	0.965	0.932	0.65
F4	0.784	0.974	0.935	0.912	0.657
F5	0.857	0.943	0.974	0.861	0.647
F6	0.790	0.952	0.933	0.924	0.646
F7	0.865	0.974	0.972	0.884	0.647
F8	0.873	0.976	0.981	0.940	0.680
F9	0.805	0.959	0.945	0.950	0.652

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 95.39% at 8th hr. Permeation of the drug shows synergistic mechanism with that of *In vitro* drug release. *In vitro* permeation profile of the optimized formulation is shown in fig. 6.

Table 5: Results of stability study (n=3)

S. No.	Observation	Before stability testing	After stability testing			
			1 month	2 months	3 months	6 months
1	Clarity	Clear	Clear	Clear	Clear	Clear
2	visual appearance	Transparent	Transparent	Transparent	Transparent	Transparent
3	pH	5.47	5.49	5.45	5.45	5.42
4	drug content	98.85±0.440	98.80±0.003	98.85±0.045	98.80±0.24	98.78±0.23

The release profile of metoprolol succinate mucoadhesive nasal *in – situ* gel shown in fig 5. In these formulations as the level of Carbopol 940 and xanthan gum increases drug release of the formulation decreases. It suggests that decrease in drug release may be due to higher viscosity of the formulation, which increases with increase in carbopol content. The retarding effect of the mucoadhesive polymer xanthan gum could be attributed to their ability to increase the overall product viscosity as well their ability to distort or squeeze the extramiceller aqueous channel through which drug diffuses thereby delaying the release process.

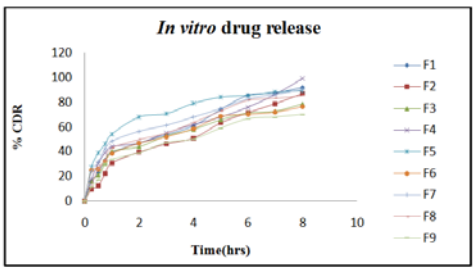

Fig. 5: *In-vitro* drug release profile of formulations

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 first order ((conc. dependent mechanism) Higuchi and Connor's and Korsemeyer's Peppas release kinetics, the n value of Korsemeyer's Peppas release kinetics is greater than 0.5 from which we can conclude that formulation follows non fickinian release mechanism that is release by diffusion and erosion of swellable polymeric matrix. Regression values for all the models are shown in table 4.

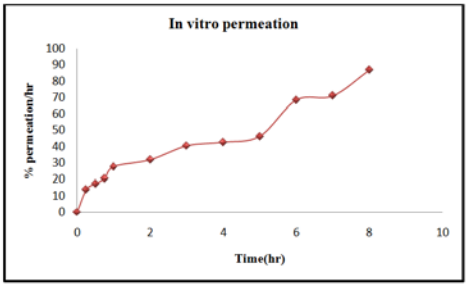

Fig. 6: *In vitro* permeation study for optimized batch F4

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. Stability data is shown in table 5

The purpose of using 3^2 full factorial design was to conduct comprehensive study of effect of process parameters like carbopol 940 (X_1) and xanthan gum(X_2) and their interactions using a suitable statistical tool (Design expert software version 9.0.2.0) by applying one way ANNOVA at 0.05 levels. Mathematical modelling was carried out. Polynomial equation was obtained depending on significant influences among 2 factors on their experimental design.

The influence of the main effects on responses was further elucidated by response surface methodology. It is widely used tool in the development and design of the dosage form. The three dimensional response surface plot and corresponding two dimensional contour plots were generated by the software. The response surface plot is very useful for determination of the main and interaction effects of the independent variables whereas two dimensional plot gives visual representation of values of responses.

In case of *In vitro* drug release the three dimensional response surface plot depicted the decrease in drug release as polymer concentration increases. The two dimensional contour plot relating X_1X_2 (interaction between carbopol 940 and xanthan gum was non linear indicating interaction between two variables.

In case of viscosity the three dimensional response surface plot depicted the increase in viscosity as polymer concentration increases. The two dimensional contour plot relating X_1X_2 (interaction between carbopol 940 and xanthan gum was non linear indicating interaction between two variables.

In case of mucoadhesive strength the three dimensional response surface plot depicted the increase in mucoadhesive strength as polymer concentration increases. The two dimensional contour plot relating X_1X_2 (interaction between carbopol 940 and xanthan gum was non linear indicating interaction between two variables.

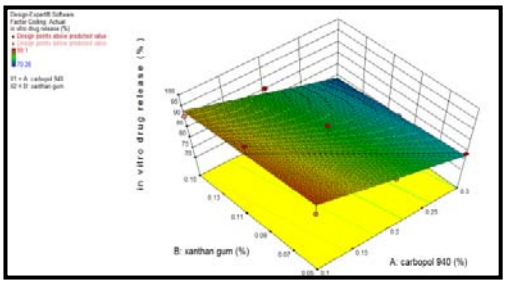

Fig. 7: Surface response plot showing effect of carbopol 940 and xanthan gum on drug release

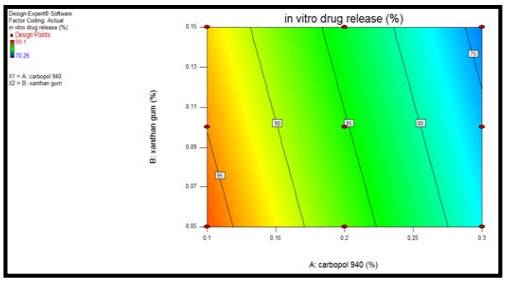

Fig. 8: Contour plot showing effect of carbopol 940 and xanthan gum on drug release

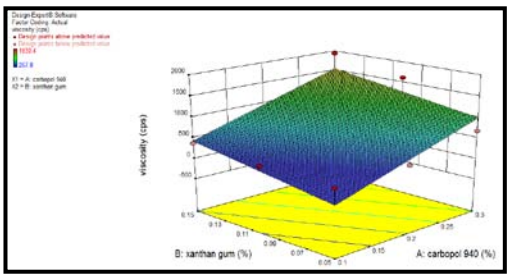

Fig. 9: Surface response plot showing effect of carbopol 940 and xanthan gum on viscosity

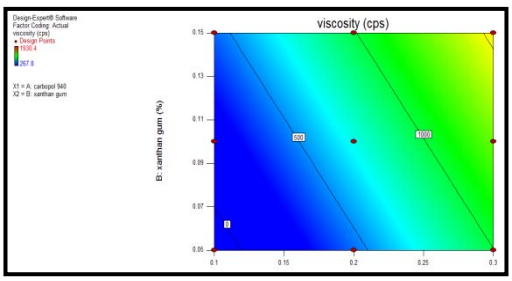

Fig. 10: Contour plot showing effect of carbopol 940 and xanthan gum on viscosity.

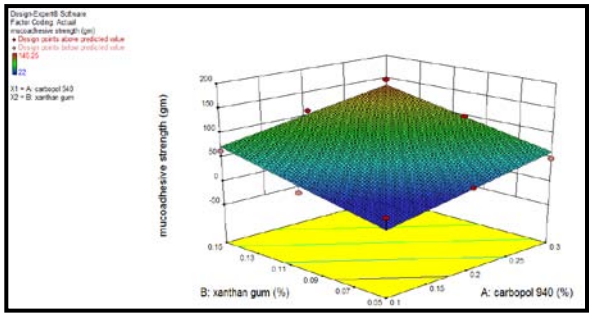

Fig. 11: Surface response plot showing effect of carbopol 940 and xanthan gum on mucoadhesive strength

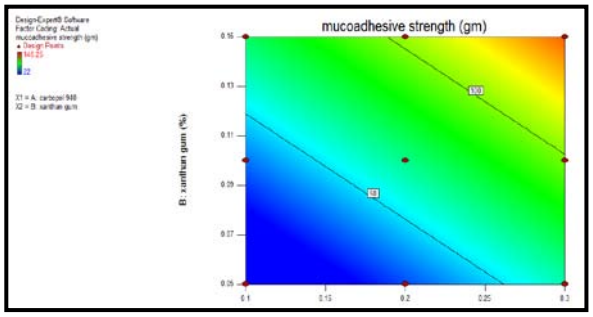

Fig. 12: Contour plot showing effect of carbopol 940 and xanthan gum on Mucoadhesive strength

After generating model equations relating main effects and responses, various gel formulations containing metoprolol succinate 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

In situ nasal mucoadhesive gel of metoprolol succinate was successfully formulated using carbopol 940 and xanthan gum as pH sensitive and mucoadhesive polymers respectively. The formulated system provides sustained *In vitro* release of drug for 8 hrs. The nasal residence time could be significantly improved owing to higher viscosity and mucoadhesive strength. Nasal administration will give increased bioavailability due to absence of hepatic first pass metabolism, thus enhancing better patient compliance.

CONFLICT OF INTERESTS

Declared None

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