

## Research Article



## Design, Development and Evaluation of in situ Nasal Mucoadhesive Gel of Metoprolol Succinate by Using Guar gum as a Natural Mucoadhesive Polymer

Pagar Swati Appasaheb\*, Shinkar Dattatraya Manohar<sup>1</sup>, Saudagar Ravindra Bhanudas<sup>2</sup>

<sup>1</sup>Department of Pharmaceutics, KCT'S RGS College of Pharmacy, Anjaneri, Nashik, Maharashtra, India.

<sup>2</sup>Department of Pharmaceutical Chemistry, KCT'S RGS College of Pharmacy, Anjaneri, Nashik, Maharashtra, India.

\*Corresponding author's E-mail: [Swati.pagar2210@gmail.com](mailto:Swati.pagar2210@gmail.com)

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### ABSTRACT

Delivery of drugs through nasal route is the emerging alternative to oral and parenteral route as nasal mucosa offers numerous benefits like increased surface area for absorption, increased bioavailability, fast absorption and reduced hepatic first pass metabolism. Hence it was planned to formulate in situ nasal gel of Metoprolol succinate for systemic delivery. Guar gum was used as a natural mucoadhesive polymer to formulate nasal in situ gel of Metoprolol succinate to sustain the release of drug, to reduce mucociliary clearance thereby increasing the contact of formulation with nasal mucosa and hence improving the absorption of drug. Carbopol 940 was key ingredient which gives pH induced sol gel conversion of formulations. These formulations were evaluated for pH, drug content, viscosity, gel strength, mucoadhesive strength, *in vitro* drug release and *in vitro* permeation profile. A 3<sup>2</sup> full factorial design was applied to study effect of varying concentration of independent variables carbopol 940 (X<sub>1</sub>) and guar gum (X<sub>2</sub>) 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 but drug release was found to decrease.

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

### INTRODUCTION

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.<sup>1,2</sup> 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 nasal route than by oral administration.<sup>3</sup> Transmucosal nasal delivery is a promising drug delivery option where common drug administrations, such as intravenous, intramuscular, or oral are inapplicable.<sup>4</sup>

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 4-6hrs. 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.<sup>5,6</sup>

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. Guar gum is a high molecular weight polysaccharide obtained from the ground endosperm of *Cymopsis tetragonolobus* which not only retards the drug release but also provides the time dependent release kinetics with advantages of biocompatibility and inertness.<sup>7</sup>

### MATERIALS AND METHODS

#### Materials

Metoprolol succinate was provided by IPCA research lab, Mumbai. Guar gum was provided by Glenmark Pharmaceuticals limited, Nashik. Carbopol 940 (Loba Chemicals Pvt. Ltd.) and polyethylene glycol 400, benzalkonium chloride of analytical grade were used.

#### 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<sup>0</sup> C. From resulting solution 1 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.<sup>8</sup>

##### Determination of $\lambda_{max}$ 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  $\lambda_{max}$  was found to be 221 nm.<sup>9, 10</sup>

### Drug excipients compatibility study<sup>11</sup>

#### 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].

### Preparation of in situ nasal mucoadhesive gel of Metoprolol succinate<sup>12</sup>

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 swells in it. After the complete swelling of carbopol, guar 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%) were added to it. This resulting formulation was then kept at 4°C overnight until clear gel is obtained. Composition of all the formulations is shown in table 1.

### Formulation optimization<sup>13</sup>

3<sup>2</sup> 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 ( $X_1$ ) and guar gum ( $X_2$ ) on various responses like % cumulative drug release, viscosity, mucoadhesive strength. For the carbopol 940 lower levels was 0.1 mg, middle was 0.2 mg and higher level was 0.3mg. Similarly for the guar gum lower level was 0.05 mg, middle was 0.1 mg and higher level was 0.15mg. Composition of all the batches is shown in table 1.

### Characterization of in situ nasal mucoadhesive gel

#### pH<sup>14</sup>

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.

### Viscosity<sup>15</sup>

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.

### Measurement of gel strength<sup>16</sup>

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.

### Mucoadhesive force (detachment stress)<sup>17, 18</sup>

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 5.25 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: Modified mucoadhesion test apparatus (Fabricated)

### 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 6.7 at  $37 \pm 2^\circ\text{C}$ .

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) (W1-5.25G) gives force required for detachment expressed in weight in grams. Procedure was repeated for two more times. Average was computed and recorded.

### Drug content<sup>19</sup>

Drug content was determined by taking 1gm of gel 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 221nm by using UV visible spectrophotometer. By using absorbance value % drug content in the formulation was calculated.

### In Vitro Drug Release study<sup>20</sup>

A) Preparation of simulated nasal solution: Weigh accurately 7.45mg/ml NaCl, 1.29mg/ml KCl and 0.32mg/ml CaCl<sub>2</sub>·2H<sub>2</sub>O and dissolve in 1000 ml of distilled water to produce simulated nasal solution; finally adjusted the pH with phosphoric acid to 6.75.<sup>21</sup>

B) In vitro release study of the formulation was carried out using laboratory designed diffusion cell through egg membrane. From the gel 0.5 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  $37 \pm 2^\circ\text{C}$  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. ( $y = 0.0027x + 0.009$ ,  $R^2 = 0.995$ ).

### Drug release kinetics<sup>22</sup>

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.

### In Vitro permeation study<sup>23</sup>

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 0.786cm<sup>2</sup>. 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<sub>2</sub>·2H<sub>2</sub>O. From the gel formulation 0.5 (10 mg equivalent) ml of was placed in donor compartment. At predetermined time point of 15, 30 min, 45 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 221nm. Permeability coefficient (p) was calculated by the following formula:

$$p = \frac{dQ/dt}{C_0 \times A}$$

Where, dQ/dt is the flux or permeability rate (mg/h), C<sub>0</sub> is the initial concentration in the donor compartment, and A is the effective surface area of nasal mucosa.

### Accelerated stability study<sup>24</sup>

Stability studies were conducted according to ICH guidelines  $40^\circ\text{C} \pm 2^\circ\text{C}/75\% \pm 5\% \text{RH}$  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

### Solubility study

Solubility of Metoprolol succinate in different solvents is 243.71mg/ml in distilled water, 24.58mg/ml in pH 6.5 phosphate buffer, 217.92mg/ml in pH 6.8 phosphate buffer, 77.51mg/ml in pH 7 phosphate buffer,

89.89mg/ml in pH 7.5 phosphate buffer, 166.16mg/ml in pH 6.8 phosphate buffer: Distilled water.

### Compatibility study

#### Infrared Spectroscopy

The IR spectra of Metoprolol succinate, 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.

**Table 1:** Composition of formulation (MCG= Metoprolol + carbopol 940 + guar gum)

Composition And formulation code	Metoprolol succinate (%w/v)	Carbopol 940 (%w/v)	Guar gum (%w/v)	PEG 400 (%v/v)	Benzalkonium Chloride (%v/v)	Distilled water up to (ml)
MCG1	2	0.1	0.05	10	0.02	100
MCG2	2	0.2	0.05	10	0.02	100
MCG3	2	0.3	0.05	10	0.02	100
MCG4	2	0.1	0.1	10	0.02	100
MCG4	2	0.2	0.1	10	0.02	100
MCG6	2	0.3	0.1	10	0.02	100
MCG7	2	0.1	0.15	10	0.02	100
MCG8	2	0.2	0.15	10	0.02	100
MCG9	2	0.3	0.15	10	0.02	100

**Table 2:** Evaluation parameters for all the formulations

Formulation code	pH	Gel strength (sec)	Viscosity (cps) at 10 rpm	Drug content (%)	Mucoadhesive strength (gm)	In vitro drug release (%)
MCG1	5.5±0.05	1.64 ±0.05	212.26	99.42±0.37	22.00 ±0.05	92.51±0.028
MCG2	5.3±0.01	1.76 ±0.002	282.29	101.26±0.4	28.44±1.43	86.18±0.028
MCG3	5.1±0.007	1.78 ±0.42	572.6	100.2±0.11	64.38±1.95	76.79±0.056
MCG4	5.5±0.01	1.90 ±0.025	251.16	98.5±0.025	63.34±0.0	90.75±0.028
MCG4	5.3±0.01	1.53 ±0.012	343.41	98.41±0.25	89.96±1.17	80.48±0.021
MCG6	5.2±0.01	1.59 ±0.678	932.32	99.2±0.23	11.25±0.0	75.19±0.049
MCG7	5.1±0.011	1.87 ±0.132	365.31	101.0±0.18	101.4±1.20	98.55±0.101
MCG8	5.6±0.005	1.89 ±0.002	527.99	98.8±0.42	40.83±0.80	89.59±0.021
MCG9	5.3±0.05	2.06 ±0.89	1799.29	102.1±0.23	135±1.30	70.78±0.028

### Differential scanning calorimetric analysis

DSC thermogram of drug shows strong endothermic peak at 138.26°C and physical mixture exhibited characteristic peak at 138.10°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.

### 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 5.1 to 5.7 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 guar gum is also having synergistic effect with pH. All the formulations showed pseudoplastic flow. Viscosities of all the formulations at 10 rpm 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 98-102%. 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 guar 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

In these formulations as the level of carbopol 940 and guar 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 940 and guar gum content. The retarding effect of the mucoadhesive polymer guar gum could be attributed to their ability to increase the overall product viscosity as well their ability to distort or squeeze the extracellular aqueous channel through which drug diffuses thereby delaying the release process.

### 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 first order because regression value was greater than 0.9 (concentration dependent mechanism) Higuchi and Connor's and Korsmeyer's Peppas release kinetics ( $r^2$  value greater than 0.9), the  $n$  value of Korsmeyer's Peppas release kinetics was greater than 0.5 from which we can conclude that formulation follows non fickian release mechanism that is release by diffusion and erosion of 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 77.13% at 8<sup>th</sup> hr. Permeation of the drug shows synergistic mechanism with that of in vitro drug release.

### 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

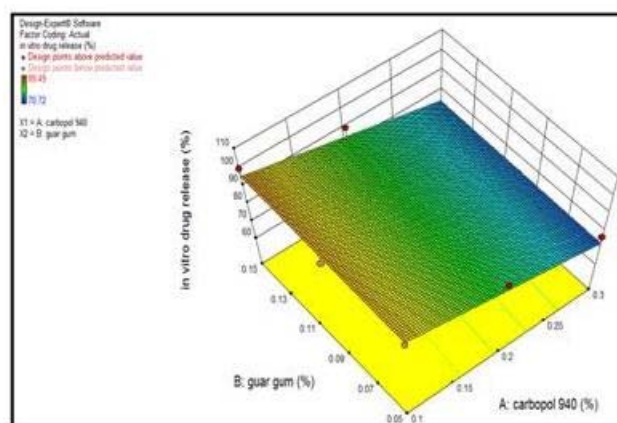
The purpose of using  $3^2$  full factorial design was to conduct comprehensive study of effect of process parameters like carbopol 940 ( $X_1$ ) and guar gum( $X_2$ ) and their interactions using a suitable statistical tool (Design expert software version 9.0.2.0) by applying one way ANNOVA at 0.05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

### Response surface methodology

The influence of main effects on responses was further elucidated by response surface methodology. It is widely used tool in the development and design of 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 main and interaction effects of the independent variables whereas two dimensional plots 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 guar gum was non linear indicating interaction between two variables.

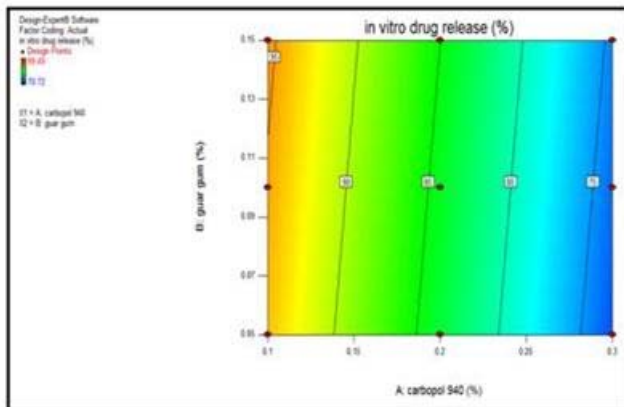
In case of viscosity the three dimensional response surface plot depicted the increase in viscosity as polymer concentration increases. The two dimensional contour plot relating  $X_1X_2$  (interaction between carbopol 940 and guar gum was non linear indicating interaction between two variables.



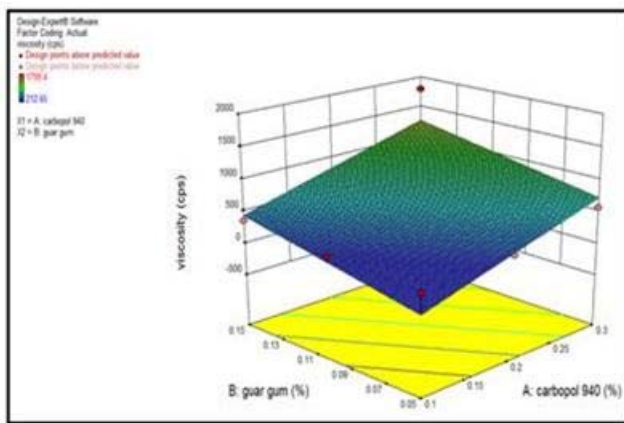
**Figure 2:** Surface response plot showing effect of carbopol 940 and guar gum on drug release.

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 guar gum was non linear indicating interaction between two variables.

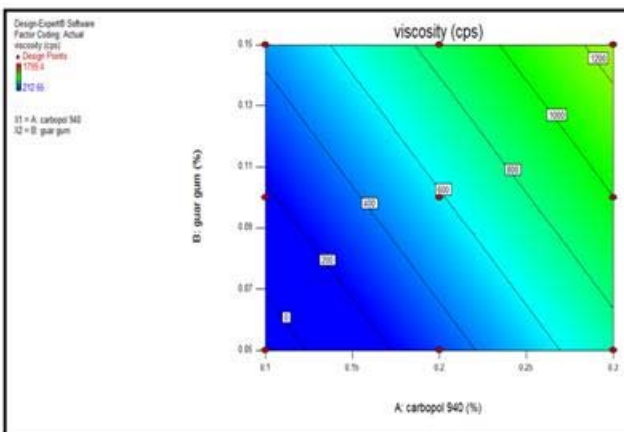
Response surface plots are shown in figures 4, 6 and 8 and contour plots are shown in figures 5, 7 and 8 for in vitro drug release, viscosity and mucoadhesive strength respectively.



**Figure 3:** Contour plot showing effect of carbopol 940 and guar gum on drug release



**Figure 4:** Surface response plot showing effect of carbopol 940 and guar gum on viscosity

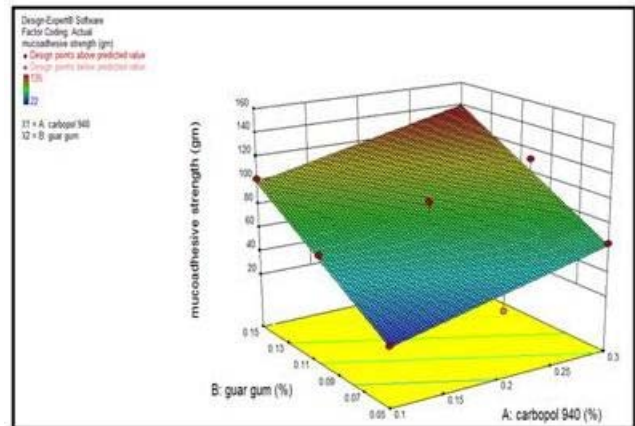


**Figure 5:** Contour plot showing effect of carbopol 940 and guar gum on viscosity

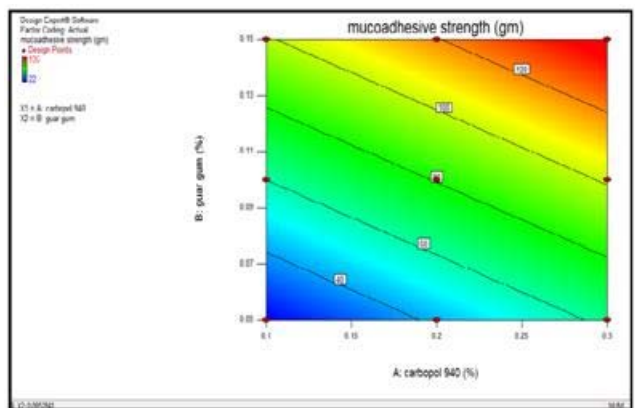
**Optimized formula**

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.



**Figure 6:** Surface response plot showing effect of carbopol 940 and guar gum on mucoadhesive strength.



**Figure 7:** Contour plot showing effect of carbopol 940 and guar gum on mucoadhesive strength

**CONCLUSION**

In situ nasal mucoadhesive gel of Metoprolol succinate was successfully formulated using carbopol 940 and guar 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.

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