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Research Article

DESIGN AND OPTIMIZATION OF NASAL *IN SITU* GEL OF ONDANSETRON USING FACTORIAL DESIGN

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ABSTRACT

In the recent years one of the big problems with cancer chemotherapy is cancer induced nausea and vomiting (CINV). In the treatment of CINV the use of $5HT_3$ receptor antagonist is most popular. One of such $5HT_3$ antagonist is Ondansetron Hydrochloride. But Ondansetron has a low oral bioavailability along with a patient suffering from vomiting problem it is difficult to deliver the drug through oral route. So our objective is to prepare a *in situ* nasal gel of the Ondansetron using PF-127 as the thermoreversible polymer. We used HPMC E15 and Chitosan as the mucoadhesive polymer to increase the nasal residence time of the formulation. To increase the permeation we used Polyethylene Glycol 400 and Propylene glycol as the permeation enhancer. A 3-factor, 2-level full factorial design (2^3) was employed for optimization of Ondansetron gels with PF 127 amount (%, X_1), permeation enhancers (PEG 400 1%/PPG 1%, X_2) and polymers (HPMC E15 1 %/Chitosan 0.5 %, X_3) as the prime selected independent variables, which were varied at 2 different levels (low and high). The effect of formulation variables on the response variables were statistically evaluated by using a commercially available software package Design-Expert® version 8.0 (Stat-Ease, Inc.).

1.INTRODUCTION

Cancer induced nausea and vomiting is one of the major side effects of the cancer chemotherapy. For the treatment of the CNIV the use $5HT_3$recepter antagonist is the most effective. Ondansetron has a oral bioavailability of 60% due to the first pass metabolism. In a patient suffering from nausea & vomiting it is difficult to deliver the drug through oral route. So to bypass the oral route, we have delivered the drug through nasal route which have bioavailability tense to the I.V route due to high vascularity[1].

One of the major disadvantages to deliver drug through nasal route is the mucocilliary clearance. To avoid this problem there is so many strategies one of this is the use of the mucoadhesive polymer to increase the nasal residence time. Therefore we use mucoadhesive polymer Chitosan and hydroxy propyl methyl cellulose to increase the nasal residence time[2,3].

We used PF-127 which is a block copolymer consisting of polyoxyethelene and polyoxypropylene unit as it has thermoreversible character due to the hydrophobic interaction in warm water. The temperature of the gelation is dependent on the concentration of the PF-127. So by adjusting the concentration of the PF-127 concentration we can prepare the insitu nasal gel with Ondansetron hydrochloride.

For better patient compliance it is desirable to deliver the drug quickly through the nasal mucosa because it is difficult to hold the gel in the nasal cavity for more than 6-7 hrs. So we

have used PEG 400 and Propylene Glycol as the permeation enhancer.

2.MATERIALS AND METHOD

2.1Materials

Ondansetron Hydrochloride (fig 1.1) was a generous gift from Albert David Ltd, Kolkata, India. PF-127 and chitosan were also provided by Albert David Ltd. HPMC E15of analytical grade from Loba Chemie Pvt. Ltd, PEG 400 and Propylene Glycol from Merck. Sodium chloride, Potassium chloride and Calcium chloride used were of analytical grade.

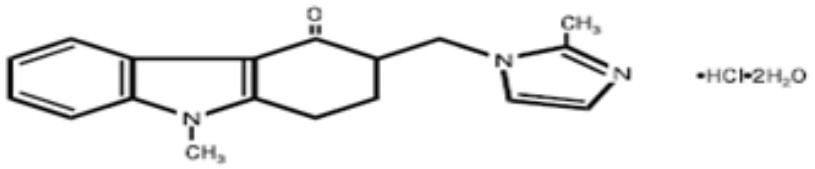


Fig. 1.1: Ondansetron hydrochloride, dihydrate

2.2Method

IR study

To study the possible interaction between Ondansetron hydrochloride and polymeric materials (PF-127, chitosan and hydroxylpropylmethyl cellulose E 15) of the gel formulations, infrared (IR) spectroscopy was carried out on pure substances and their physical mixtures. The IR spectra were recorded using IR Spectrophotometer (Alpha - A4 size FT-IR, BRUKER. Germany) and found compatible.

Experimental design

A 3-factor, 2-level full factorial design (2^3) was employed for optimization of Ondansetron gel with PF-127 amount (%, X_1), permeation enhancers (PEG 400 1% **/**PPG 1%, X_2) and polymers (HPMC E15 1% **/**Chitosan 0.5 %, X_3) as the prime selected independent variables, which were varied at 2 different levels (low and high). Here, we have considered PF-127 amount (%, X_1) in numerical value; whereas permeation enhancers (PEG 400 1% **/**PPG 1%, X_2) and polymers (HPMC E15 1 %**/** Chitosan 0.5 %, X_3) were considered as categorical value in the above factorial matrix. The drug release in 5 hrs (Y_1) and mucoadhesive strength (Y_2) were used as dependent variables. Design-Expert 8.0.3 software (Stat-Ease Inc., Minneapolis, USA) was used for the generation and evaluation of the statistical experimental design. The matrix of the design including response obtained as drug load is shown in Table I.

Table 1: Matrix of the design including response

		V A R I A B L E S		
		X_1	X_2	X_3
		PF 127 amount (%)	Permeation enhancers (PEG 400 1%/PPG 1%)	Polymers (HPMC E15 1 %/Chitosan 0.5 %)
LEVELS	High	20 %	1	1
	Low	15 %	0	0

Preparation of the *In-situ* Gel

For the preparation of the in-situ, the technique described by Schmolka et.al., was used[4, 5]. 1% of Ondansetron hydrochloride was dissolved in distilled water. Then propylene glycol and PEG 400 were included as permeation enhancer at 1% concentration. Muco-adhesive polymer, 1% HPMC E 15 and 0.5% Chitosan were added and stirred completely till to get the clear solution. Then the solution was kept into the refrigerator and cooled to 4°C. Then PF- 127 was added in the concentration range of 20% and15% along with a mild stirring and kept overnight at 4°C.

Table 1.1: Combination of the eight Formulations

Formulation code		Thermo-reversible Polymer(w/v)	Permeation enhancer(w/v)	Muco-adhesive polymer(w/v)
			PEG 400/ PPG	CHITOSAN / HPMC E15
BF1	DRUG	PF- 127 20 %	PEG 400 1 %	HPMC E15 1%
BF2			PPG 1 %	HPMC E15 1 %
BF3			PEG 400 1 %	CHITOSAN 0.5%
BF4			PPG 1 %	CHITOSAN 0.5%
BF5		PF- 127 15 %	PEG 400 1 %	HPMC E15 1%
BF6			PPG 1 %	HPMC E15 1 %
BF7			PEG 400 1 %	CHITOSAN 0.5%
BF8			PPG 1 %	CHITOSAN 0.5%

Physical characterization

Clarity

To check the clarity of the formulation we have used the technique of visual inspection in front of the black & white background & distinguished in terms of clear & very clear which were denoted as '++' & '+++' respectively.

pH

To check the pH of the formulation, a 5% solution of the prepared gel was made and the pH was checked using digital pH meter (Systronics pH System 362).

Content Uniformity

1ml of the gel in a 25 ml volumetric flask, then serial dilutions were made using distilled water to make the concentration of the solution 10mcg/ml. Then the absorbance of the final solution was examined using UV-VIS spectrophotometer (Shimadzu UV-VIS1800, Japan).

Gelation Temperature[6]

To evaluate the gelation temperature, the technique proposed by Choi et al., was referred. The gel was first cooled to 4°C. Then from it, 10 ml of the gel was taken in a 20 ml beaker. After that the gel was placed on a hot plate magnetic stirrer and a magnetic bid (1x5/16 inch octagonal) was inserted into it. The gel was constant stirred at 100 rpm with an increase in temperature at 1°C /min. The temperature at which the magnetic bid stopped its rotation was noted as the gelation temperature.

Determination of Mucoadhesive Force[7]

The mucoadhesive force of the formulation was determined using goat nasal membrane. Two cylindrical plastic vials with 2cm diameter were taken. A hook was attached on one side of both the vial. The goat nasal membrane was then tied to the other side of both the vial. After that 50 micro liter of the gel was applied on one of the membrane side of one vial then the other vial was applied at the membrane side on the first. The two vials were held for 2min after that the unit was hanged from a hook and at the bottom of the system a plastic container was placed. Water was poured drop by drop into the container until the two vials got detached from each other. Then the weight of the container with water was noted along with the bottom vial from which the container was hanged.

The bioadhesive force, expressed as the detachment stress in dyne/cm2, was determined from the minimal weights that detached the tissues from the surface of each formulation using the following equation.

Detachment stress (dyne/cm2) = m x g /A,

Where, m =Weight required for detachment of two vials in grams,

g = Acceleration due to gravity [980cm/s2],

A = Area of tissue exposed

The nasal mucosa was changed for each measurement. Measurements were repeated six times for each of the gel preparations.

Viscosity Measurement[8,9,10]

The viscosities of various formulations were measured with increase in temperature by using Cone and Plate viscometer (Brookfield viscometer Model Cap 2000 +2).

***In-vitro* Permeation Study**

In-vitro permeation study of the gel was performed with goat nasal membrane collected from the local Municipal approved slaughter house ,using Keshary Chein cell. The mucosa was stored in normal saline with few drops of gentamycin sulphate injection to avoid bacterial growth. After the removal of blood and bony cartilage from the mucosal membrane it was ready for use. 67 ml of the Nasal Electrolyte solution (pH 5.5) was placed in to the acceptor

chamber. The temperature within the chamber was maintained at 34^0C by circulating hot water. Then formulation equivalent to 2mg was placed in the donor compartment & sampling was done at predetermined interval from the acceptor compartment & equal amount of fresh SNES solution was replaced. Then the absorbance was examined using UV-VIS spectrometer at 249 nm.

Statistical Analysis

The effect of formulation variables on the response variables were statistically evaluated by using a commercially available software package Design-Expert® version 8.0 (Stat-Ease, Inc.). This software is able to evaluate each factor's importance based on the formulation responses. Moreover, it examined the interactions between the variables affecting the drug and mucoadhesive strength. Finally, according to the final results, this program suggested some formulations and also predicted their responses containing a probability factor named "Desirability" that ranged between 0 - 1.

ANOVA was applied to estimate the significance of the model ($p < 0.05$). The fitted regression equations relating the responses of drug release in 5 hrs and mucoadhesive strength were shown in the equations, respectively.

RESULTS AND DISCUSSION

pH, Clarity and Content uniformity

pH of all the formulation were found to be within 5 to 5.2 . There was no such distinct effect of the change of the formulations on the pH of the final formulations.

Again, from the clarity test it can be said that all the formulations are clear. The formulations with HPMC E15 were found to be clearer than formulations containing Chitosan. The formulations which are very clear are denoted by +++ & the formulations are clear not very clear denoted by ++.

The percentage drug content of all prepared nasal formulations were checked and found to be in the range of 97-101% (table 1.2).

Table 1. 2: Clarity, pH, Content Uniformity of the Eight Formulations

Formulation Code	Clarity	pH ± S.D	Content Uniformity % ± S.D
BF1	+++	5.11±0.094	98.5 % ± 0.03
BF2	+++	5.23±0.054	97.6% ± 0.042
BF3	++	5.22± 0.088	98.4% ± 0.067
BF4	++	5.2 ± 0.10	101.1% ± 0.023
BF5	+++	5.17 ± 0.04	98.2% ± 0.031
BF6	+++	5.2 ± 0.008	97.3% ± 0.021
BF7	++	5.21 ± .089	98.7% ± 0.087
BF8	++	5.10 ± .082	99.54% ± 0.067

Gelation Temperature

The gelation temperature is one of the important phenomena of this formulation. The in-situ gelling of the formulation was designed to occur near to the nasal temperature. The gelation temperature of the various formulations varied greatly with the combinations of the formulations (table 1.3). We have studied them differently.

First the effects of PF-127 concentration were studied on the gelation temperature. The formulations with 20% of PF- 127 showed gelation temperature within the range of 32 – 29°C. But the formulations with 15% of PF- 127 showed gelation at higher temperature from graph.

Again, while studying the different formulations with same PF-127 concentration, we saw that the formulation with HPMC E15 as mucoadhesive polymer showed higher gelation temperatures than the formulations with Chitosan as mucoadhesive polymer in both the higher and lower PF127 containing gel. That means the gels with 20% PF-127 BF1, BF2 has higher gelation temperature than BF 3, BF4. Similarly, the gels with 15% PF-127, BF5, BF6 shows higher gelation temperature than BF 7, BF8 (fig 1.3).

Now, if the formulations were evaluated with respect to the permeation enhancer. We see that formulation with same PF- 127 concentration , same mucoadhesive polymer containing PEG 400 shows slightly lower gelation temperature than the formulation containing propylene glycol as permeation enhancer. That means BF1 shows gelation temperature lower than BF2, similarly gelation

temperature of BF3 is lower than the gelation temperature of BF4, gelation temperature of BF5 is less than gelation temperature of BF 6 and gelation temperature of BF 7 is lower than that of BF 8.

Table 1.3: Gelation temperature, Mucoadhesive force of eight formulations

Formulation Code	Gelation Temperature (°C) ± S.D	Mucoadhesive Strength (dyne/cm^2)±S.D
BF1	30.3 ± 0.37	11607.4± 0.45
BF2	32.07 ± 0.37	10322.92± 25.34
BF3	28.2 ± 0.36	13635.53± 8.72
BF4	29.43 ± 0.17	12676.78± 0.52
BF5	66.73 ± 0.39	707.9967 ± 0.28
BF6	67.73 ± 0.31	698.0567± 0.66
BF7	59.1± 0.045	815.7933 ± 0.93
BF8	62.7 ± 0.54	794.02 ± 0.62

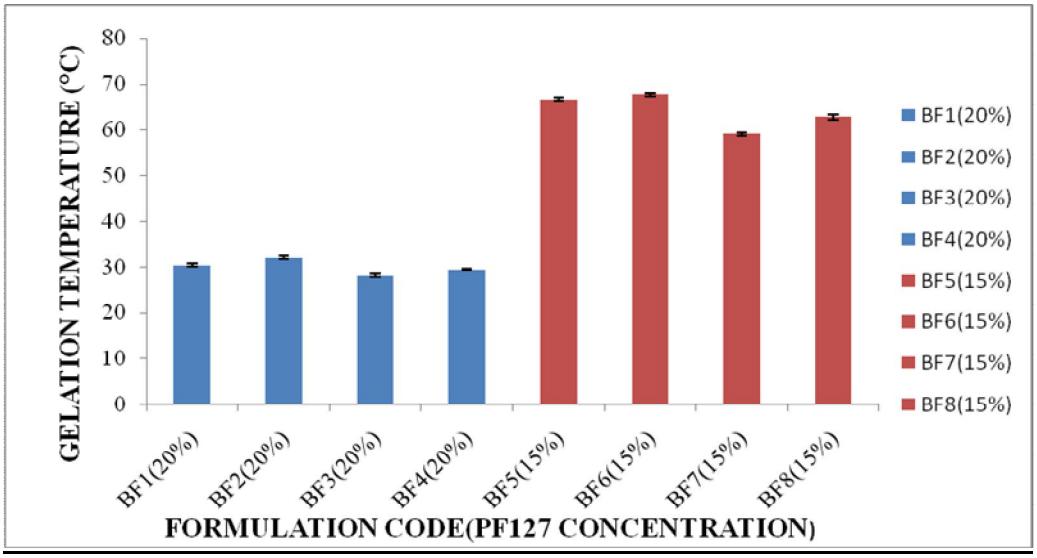


Fig. 1.2: Effect of PF 127concentration on gelation temperature

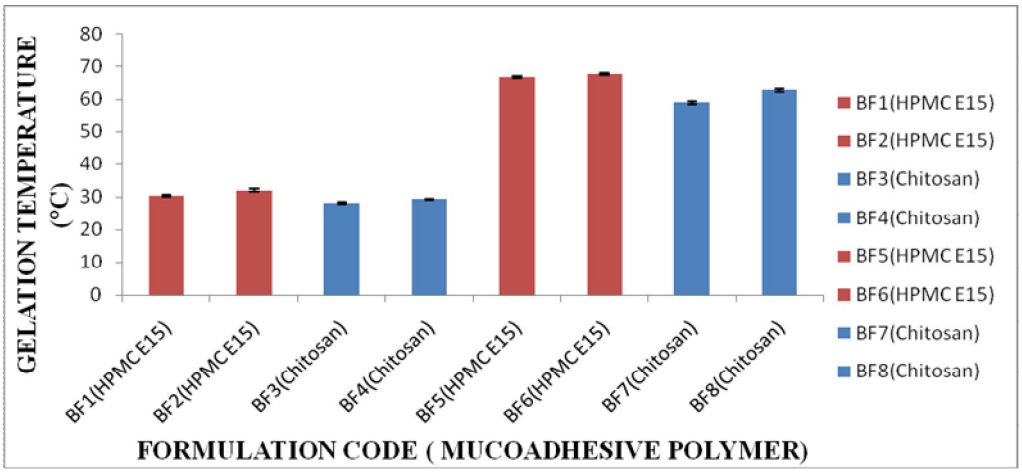


Fig. 1.3: Effect of Mucoadhesive polymer on Gelation Temperature

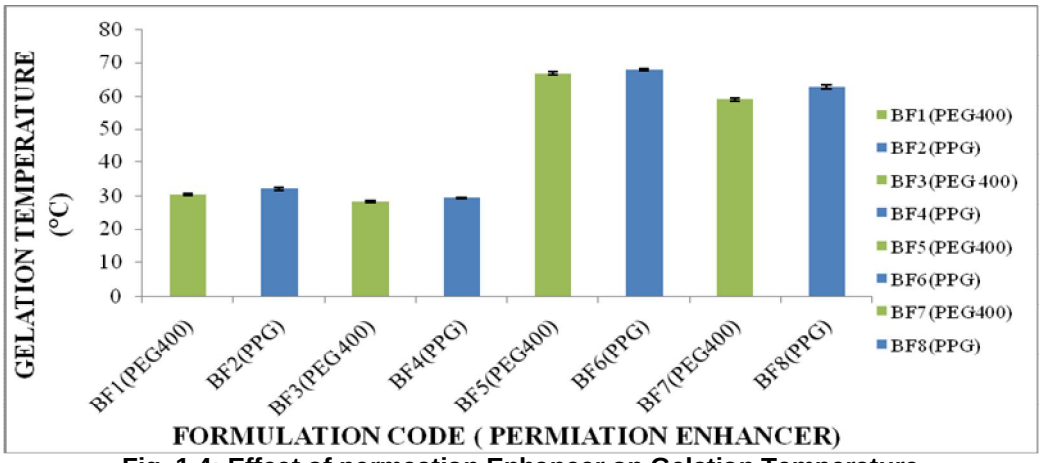


Fig. 1.4: Effect of permeation Enhancer on Gelation Temperature

Mucoadhesive Force

Mucoadhesive force is required to increase the nasal residence time of the gel. So mucoadhesive force is also an important parameter for the nasal gel. The formulation should have an optimum mucoadhesive force to provide optimum resistance to the mucocilliary clearance of the gel. The formulations have a distinct effect on the mucoadhesive force of the gel. The mucoadhesive polymer itself is not only the mucoadhesive force provider. There is a distinct effect of the PF- 127 on the mucoadhesive force. Not much but the permeations enhancers also have effect on the mucoadhesive force of the gel (Table 1.3).

If studying in respect to the PF- 127 concentration, it was found that the first 4 formulations BF1, BF2, BF3, and BF4 with 20% PF-127 showed quite higher mucoadhesive force than the formulations with 15% PF127 i.e., BF5, BF6, BF7, BF8(fig 1.5).

Again, in both case of the 15% and 20% PF 127 containing gel, it has seen that between the formulations with same amount of PF-127 the formulations with Chitosan as mucoadhesive polymer shows higher mucoadhesive force than the formulations with HPMC E15 as mucoadhesive polymer.

While studying the effect of the permeation enhancer, we have seen that the formulations with same amount of PF-127 and same adhesive polymer the formulation containing PEG 400 as permeation enhancer show lower mucoadhesive force than the formulations with propylene glycol as the permeation enhancer (fig 1.6 & 1.7).

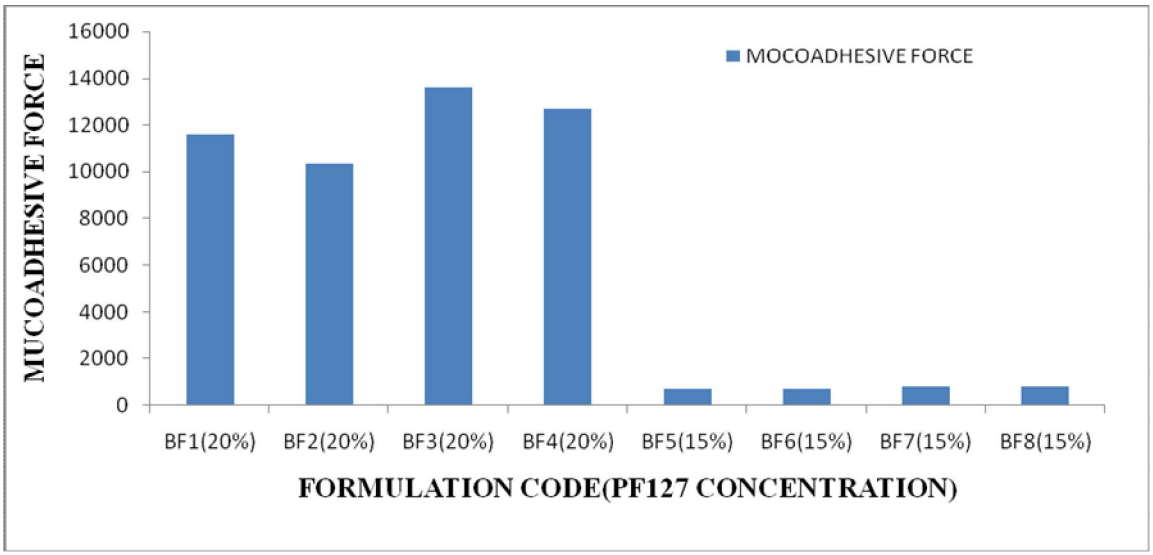


Fig. 1.5: Effect of PF-127 concentration on Mucoadhesive force

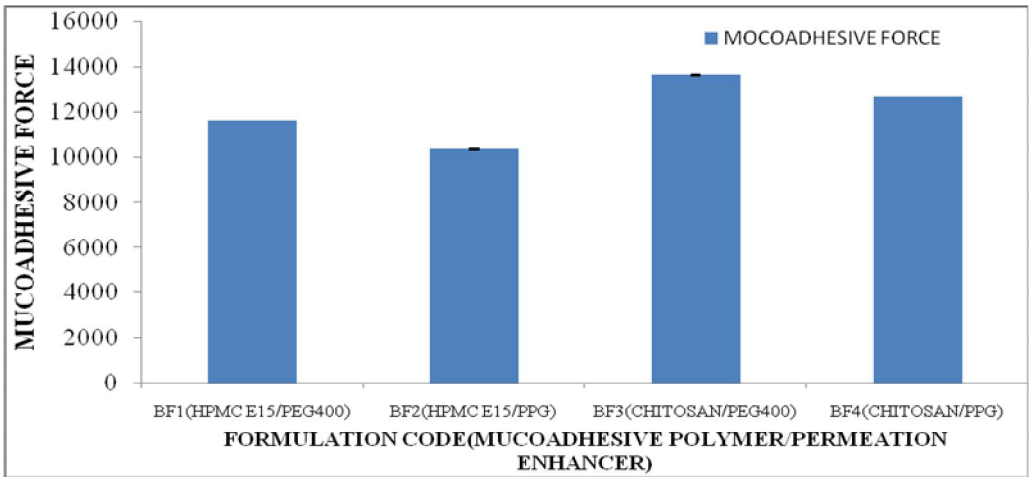


Fig. 1.6: Effect of Mucoadhesive polymer & Permeation Enhancer on the Mucoadhesive force of the formulations contain 20% PF-127

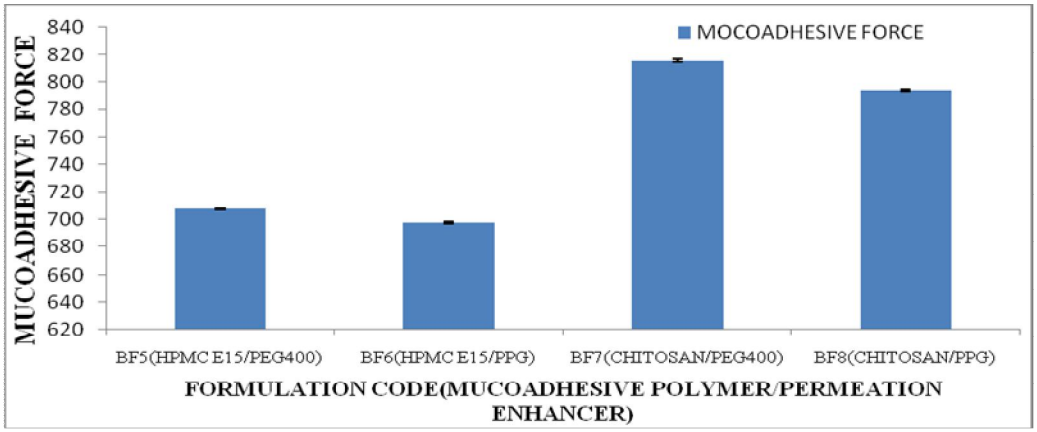


Fig. 1.7: Effect of the Mucoadhesive polymer & Permeation Enhancer on the Mucoadhesive force of the formulations contain 15% PF-127

Viscosity

The viscosity of the formulations remains lower up to a certain temperature then a sudden rise in the viscosity occurred with the increase in the temperature (fig 1.8).

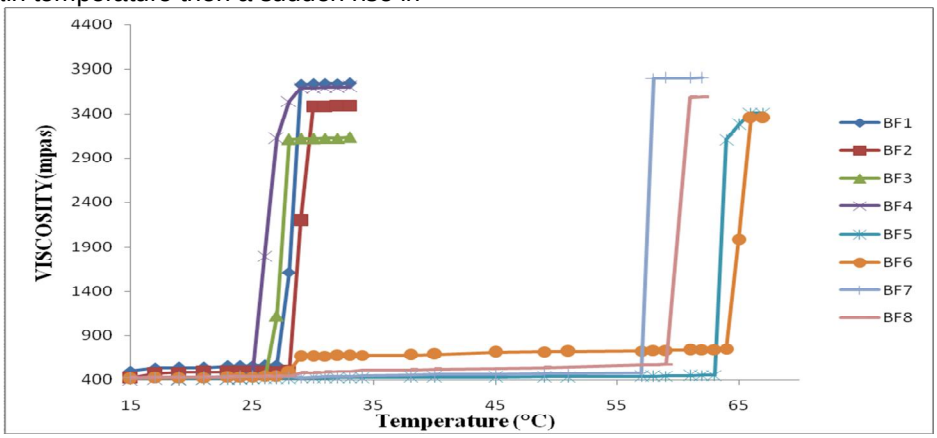


Fig. 1.8: Viscosity of the various formulation

***In- Vitro* drug permeation Study**

The *in- vitro* drug permeation study of the various formulations are studied using Goat Nasal Membrane, collected from the local slaughter house approved by the Municipal Corporation, Durgapur, W.B. Cumulative % release of drug from 8 formulations at 5 hr is tabulated in Table – 1.4. The formulations containing 15 % PF-127 showed higher % release at 5 hr than the formulations containing 20% PF-127. Further, from the values of the permeability co-efficient (table 1.4) it has been observed that the formulations with same concentration of PF-127 & mucoadhesive polymer containing propylene glycol as permeation enhancer showed higher values of permeability co-efficient than the formulation containing PEG400 as the permeation enhancer.

Table 1.4: *In-Vitro* Percentage Cumulative Permeation ,Flux & Permeability co-efficient of Ondansetron Hydrochloride through Goat Nasal Membrane from the formulations containing 20 % & 15 % of PF-127 at 5hr.respectively

Formulation code	% Release in 5 hr	Flux	Permeability coefficient
BF1	79.69	5.067	2.53
BF2	85.08	5.413	2.70
BF3	42.73	3.20	1.60
BF4	46.47	3.542	1.77
BF5	98.61	7.957	3.98
BF6	96.74	8.068	8.068
BF7	97.33	7.251	7.251
BF8	98.98	7.447	7.447

Analysis of the Release Mechanism

From R^2 value we can state all the formulations show highest linearity to the Korsmeyer-Peppas Model (table 1.5) & from the n value it was seen that the drug is diffused from the formulations following non-fickian diffusion mechanism (table 1.6).

Table 1.5: Regression co-efficient of the Model equations on the *in-vitro* diffusion kinetics

KINETIC MODEL	BF1	BF2	BF3	BF4	BF5	BF6	BF7	BF8
	r^2 value	r^2 value	r^2 value	r^2 value	r^2 value	r^2 value	r^2 value	r^2value
HIGUCHI MODEL	0.927	0.962	0.907	0.848	0.852	0.717	0.967	0.963
ZERO ORDER MODEL	0.46	0.201	0.86	0.971	-0.100	-0.700	0.721	0.646
1^{ST} ORDER MODEL	0.978	0.945	0.902	0.929	0.745	0.509	0.846	0.773
KORSMEYER-PEPPAS MODEL	0.982	0.984	0.941	0.977	0.924	0.884	0.979	0.975

Table 1.6: Table of 'n' values of Korsmeyer-Peppas model

FORMULATIONS CODE	BF1	BF2	BF3	BF4	BF5	BF6	BF7	BF8
'n' value	0.520	0.470	0.550	0.740	0.470	0.480	0.590	0.570

DISCUSSION

pH , Clarity & content uniformity

The pH of the formulations was maintained within the range of 5 - 5.2 to activate the lysozyme in the nasal secretions, which is responsible for destroying certain microbes at acidic pH. Under alkaline pH lysozyme is inactive and nasal tissue is susceptible to microbial infection.

Again all the formulation remained clear & content uniformity remained within 97-101%. This signified that the polymer along with the drug was homogeneously mixed with water to from clear solution.

Gelation Temperature

The increase in the PF 127 concentration resulted in decrease of gelation temperature. This is because of the strengthening of the lattice structure of the PF 127 in the solution at higher concentration which are become closely packed as a result higher number and volume occupied by micelles at low temperature to form the gel[11].

The lower gelation temperature of the Chitosan containing formulations than the HPMC E15 containing formulation is because Chitosan has greater ability to increase viscosity & to produce more extensive intermolecular hydrogen bonding to produce a close alignment in the gel structure.

Again, the increased gelation temperature of the Propylene glycol containing formulation than the PEG 400 containing formulation is because of more distortion of the lattice structure of the gel by Propylene glycol than PEG 400. So the gel is formed at slightly higher temperature.

Mucoadhesive Force

The mucoadhesiveness of the gel is due to the formation of the hydrogen bonding between the gel and the mucus membrane.

The increase in PF-127 concentration increases the mucoadhesive strength of the gel. This is because as the concentration is increased more compact lattice structure is produced as well as density is increased. For that reason more no of mucoadhesive polymer remains within a fixed volume of gel to produce more hydrogen bonding than the low PF-127 containing gel.

Again, the higher mucoadhesive force of Chitosan than HPMC E15 is because of its ability to form more condensed hydrogen bonding than HPMC E15 which provides higher mucoadhesive force to the formulations.

The mucoadhesive force reducing effect of the Propylene glycol than PEG 400 is due to increased formation of the mixed micelle by Propylene glycol than PEG 400.

Viscosity

The viscosity of the formulation remains low up to a certain temperature. This is because the formulation remains in liquid state up to that temperature. Then with the increase with temperature the formulation change into gel. As a result the viscosity of the formulation gets increased.

Release Study

The release of the formulation is evaluated at 32° C. As a result the formulation containing 15% PF-127 remains liquid in that temperature. But the formulation containing 20% PF-127 transfer to gel at that temperature. As a result release is retarded for the formulation containing 20% PF-127 due to the close matrix structure of the gel.[12]

Again, from the permeability co-efficient values it is clear that propylene glycol provides higher % release across the nasal membrane than the PEG 400 that proves the better permeation enhancing effect of the propylene glycol than PEG 400.

Analysis of the Release Mechanism

From the R^2 value it is clear that all the formulation shows release by following Korsmeyer-Peppas Model and from the 'n' value we see that the release followed the Non-Fickian release mechanism. That means here the release is occurred by diffusion as well polymeric chain erosion.

Statistical Analysis

The purpose of using a full 2^3 factorial experimental design was to conduct a comprehensive study of the effect of the process parameters like PF 127 amount (%, X_1), permeation enhancers (PEG 400 1%**/**PPG 1%, X_2) and polymers (HPMC K4M 1 %**/**Chitosan 0.5 %, X_3) and their interactions using a suitable statistical tool (Design-Expert 8.0.3 software) by applying one-way ANOVA at 0.05 levels. A mathematical modeling was carried out by using Equation-I to obtain a first–order polynomial equation depending on significant influences among three factors (X_1, X_2 and X_3) and their interaction factors (X_1X_2, X_2X_3, and X_1X_3) of the factorial design model.

Y = bo + b1 X1 + b2 X2 + b3X3+ b4 X1X2+ b5X1X3 + b6X2X3 …… (I)

Where Y = the dependent variable, while bo = the intercept, b1, b2, b3, b4, b5, b6 and b7 = regression coefficients; X1, X2 and X3 = main factors; X_1X_2, X_2X3, and X1X3 = interactions between main factors.

Table 1.7: Factor VS Response of different formulations

Run	Factor 1 PF 127(%X_1)	Factor 2 Permeation Enhancer (X_2)	Factor 3 Mucoadhesive Polymer(X_3)	Response 1 (Y_1) Release in 5 Hr (%)	Response 2(Y_2) Mucoadhesive Force (dynes)
1	15.00	1.00	0.00	98.33	815.793
2	20.00	1.00	0.00	42.73	13635.5
3	20.00	1.00	1.00	79.69	11607.4
4	15.00	1.00	1.00	98.61	707.997
5	20.00	0.00	0.00	47.47	12676.8
6	20.00	0.00	1.00	85.08	10322.9
7	15.00	0.00	0.00	98.98	794.02
8	15.00	0.00	1.00	96.74	698.057

ANOVA: Release in 5 hours

Table 1.8 : Aalysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-Value Prob > F	
Model	3787.41	6	631.24	502.53	0.0341	
X1	2369.82	1	2369.82	1886.63	0.0147	
X2	9.92	1	9.92	7.90	0.2176	Significant
X3	659.03	1	659.03	524.66	0.0278	
X1X2	16.10	1	16.10	12.82	0.1734	
X1X3	732.11	1	732.11	582.83	0.0264	
X2X3	0.44	1	0.44	0.35	0.6607	

The Model F-value of 502.53 implies the model is significant. There is only a 3.41% chance that a "Model F-Value" this large could occur due to noise.
Values of "Prob > F" less than 0.0500 indicate model terms are significant.
In this case A, C, AC are significant model terms.
Values greater than 0.1000 indicate the model terms are not significant.

Table 1.9: Results showing standard parameters

Std. Dev.	**1.12**	**R-Squared**	**0.9997**
Mean	**80.95**	**Adj R-Squared**	**0.9977**
C.V. %	**1.38**	**Pred R-Squared**	**0.9788**
PRESS	**80.39**	**Adeq Precision**	**53.790**

The "Pred R-Squared" of 0.9788 is in reasonable agreement with the "Adj R-Squared" of 0.9977.
"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. ratio of 53.790 indicates an adequate signal.

Mucoadhesive force

Table 1.10 : Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of squares	df	Nean square	F value	p-value Prob >F	
Model	$2.618E^{+008}$	6	$4.363E^{+007}$	3062.94	0.0138	
X1	$2.557E^{+005}$	1	$2.557E^{+006}$	17950.67	0.0048	
X2	$6.469E^{+005}$	1	6.469^{+005}	45.42	0.0938	Significant
X3	$2.629E^{+006}$	1	$2.629E^{+006}$	184.55	0.0468	
X1X2	$6.114E^{+005}$	1	$6.114E^{+005}$	42.92	0.0964	
X1X3	$2.182E^{+006}$	1	$2.182E^{+006}$	153.21	0.0513	
X2X3	12316.39	1	12316.39	0.86	0.5231	

The Model F-value of 3062.94 implies the model is significant. There is only a 1.38% chance that a "Model F-Value" this large could occur due to noise.
Values of "Prob > F" less than 0.0500 indicate model terms are significant.
In this case A, C are significant model terms.
Values greater than 0.1000 indicate the model terms are not significant.

Table 1.11: Results of RSM values

Std.Dev. 0.9999	119.35	R-Squared
Mean 0.9996	6407.31	Adj. R-Squared
CV% 0.9965	1.86	Pred R-Squared
PRESS 116.643	$9.116E^{+006}$	Adeq Precision

The "Pred R-Squared" of 0.9965 is in reasonable agreement with the "Adj R-Squared" of 0.9996.
"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. ratio of 116.643 indicates an adequate signal.

The values of the drug release in 5 hrs (Y_1) and mucoadhesive strength (Y_2) data in 2^3 factorial design (Table 1.7) were fitted to a first–order polynomial model. From the ANOVA results (Table 1.7) of the model relating responses, it can be noticed that all the coefficients of these model equations had statistic significances ($p < 0.05$) with the model *F*-values of 502.53 for the drug release in 5 hrs (Y_1) and 3062.94 for mucoadhesive strength (Y_2). The R^2 values of these models were obtained 0.9997 and 0.9999, respectively. The ANOVA results showed that the PF-127 amount (%, X_1), and polymers (HPMC E151 %*/*Chitosan 0.5 %, X_3) and interaction between them(X_1 X3) significantly influenced the drug release in 5 hrs (Y_1). But, the effect of permeation enhancers (PEG 400 1% *I* PPG 1%, X_2) the drug release in 5 hrs (Y_1) was not significant. In case of mucoadhesive force, ANOVA results showed that the PF-127 amount (%, X_1), and polymers (X_3) significantly influenced the mucoadhesivity but, the effect of permeation enhancers (X_2) and interactions between all the factors not significantly influence the mucoadhesiveness.

Model equations involving the individual main effects and interaction factors was selected based on the estimation of several statistical parameters, such as multiple correlation coefficient (R^2), adjusted multiple correlation coefficient (adjusted R^2), and predicted residual sum of squares (PRESS), provided by the Design-Expert® Software (V.8, Stat-Ease Inc., USA). The factorial model was selected as a suitable statistical model for optimization, because it had smallest value of PRESS. PRESS is measure of the fit of the model to data points in the design. The smaller the PRESS statistic is, the better the model fits to the data points.

The model equations relating drug release in 5 hrs (Y_1) as response given by the statistical tool are:

when, X2 = 0 and X3 = 0; $Y_1 = + 250.73 - 10.14\ X1$

when, X2 = 1 and X3 = 0; $Y_1 = + 267.90 - 11.27\ X1$

when, X2 = 0 and X3 = 1; $Y_1 = + 134.49 - 2.49\ X1$

when, X2 = 1 and X3 = 1; $Y_1 = + 152.59 - 3.62\ X1$

The model equations relating mucoadhesive strength (Y_2) as response given by the statistical tool are:

when, B = 0 and C = 0; $Y_2 = -34558.89211 + 2359.67384 * A$

when, B = 1 and C = 0; $Y_2 = -37938.78469 + 2580.82551 * A$

when, B = 0 and C = 1; $Y_2 = - 28471.90109 + 1941.85082 * A$

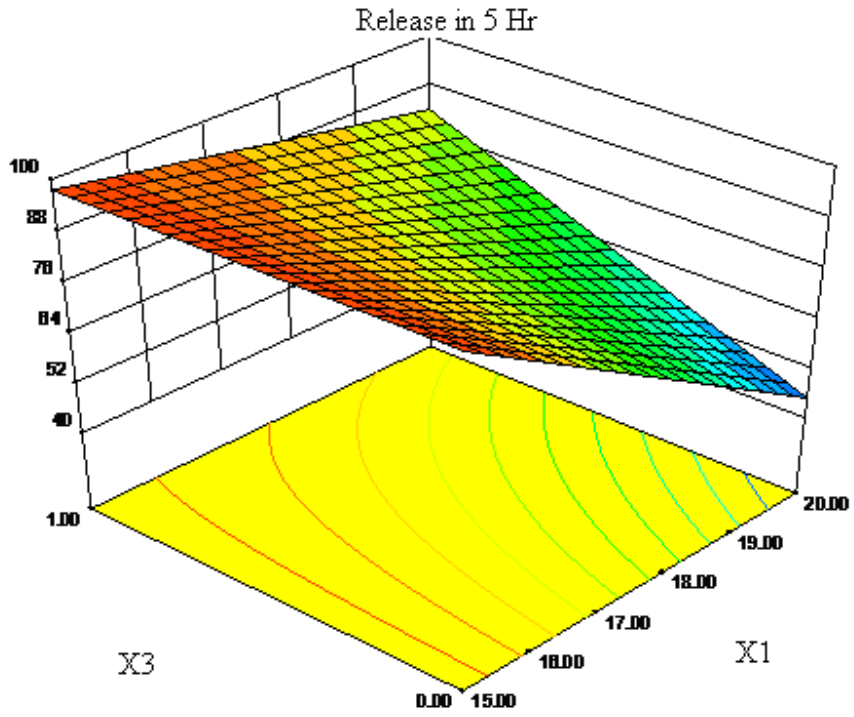
when, B = 1 and C = 1; $Y_2 = - 31694.84531 + 2163.00250 * A$

Response Surface Methodology

The influence of main effects on response (X1 is PF-127 Conc., X1 is permeation Enhancer, X3 is Mucoadhesive Polymer) was further elucidated by response surface methodology. Response surface methodology is a widely proficient approach in the development and optimization of drug delivery devices[13-16]. The three-dimensional

response surface graph and corresponding two-dimensional contour plot were generated by the Design-Expert 8.0.3 software. The three-dimensional response surface graph is very useful in learning about the main and interaction effects of the independent variables (factors), whereas two-dimensional contour plot gives a visual representation of values of the response[17] The three-dimensional response surface graphs depict the increase in drug release in 5 hrs (Y_1) with the decrease of PF-127 amount (%), and addition of HPMC E15 1% as polymer in the Ondansetron gel formulations. They also depict that the increase in drug release in 5 hrs (Y_1) with the addition of PPG 1% as permeation enhancer; but, the effect of permeation enhancer on the drug release was not significant analyzed by ANOVA. The two-dimensional contour plots relating X_1X_2 (interaction between PF-127 amount and permeation enhancer) and X_2 X_3 (interaction between polymers and permeation enhancer) were found to be linear, which indicate that there were absence of interactions between these variables. However, contour plot relating X_1 X_3 (interaction between PF-127 amount and polymers) was found to be nonlinear indicating the interaction between these variables.

In case of mucoadhesive force, the three-dimensional response surface graphs depict its increased value with the increase in PF-127 amount (%), and addition of Chitosan 1 % as polymer in the Ondansetron gel formulations. They also depict that the increase in mucoadhesive force (Y_2) with the addition of PPG 1% as permeation enhancer; but, the effect of permeation enhancer on the drug release was not significant analyzed by ANOVA. The two-dimensional contour plots relating X_1 X_2 (interaction between PF-127 amount and permeation enhancer), X_1 X_3 (interaction between PF-127 amount and polymers) and X_2 X_3 (interaction between polymers and permeation enhancer) were found to be linear, which indicates there were absence of interactions between these all variables.



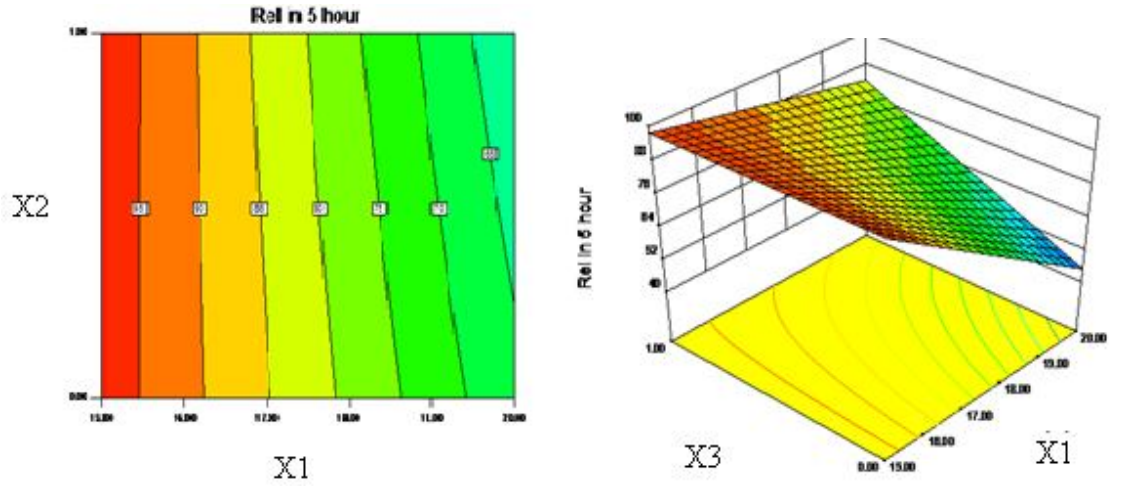
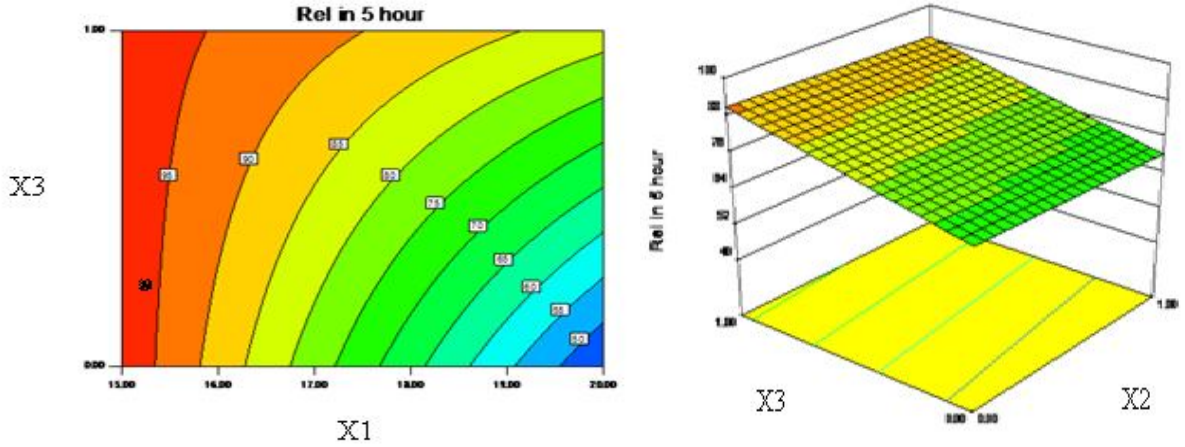
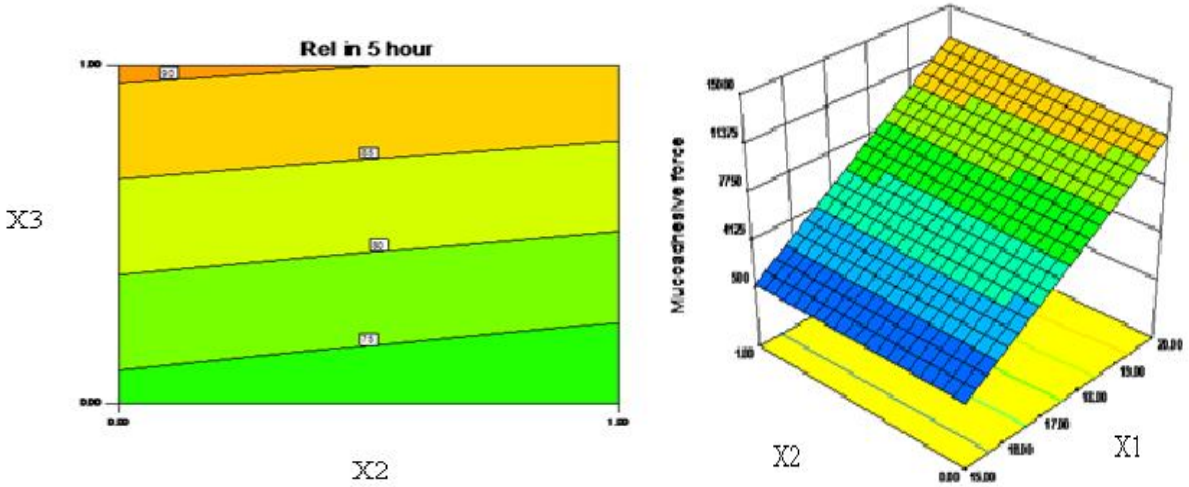


Fig. 1.9: RSM of formulations





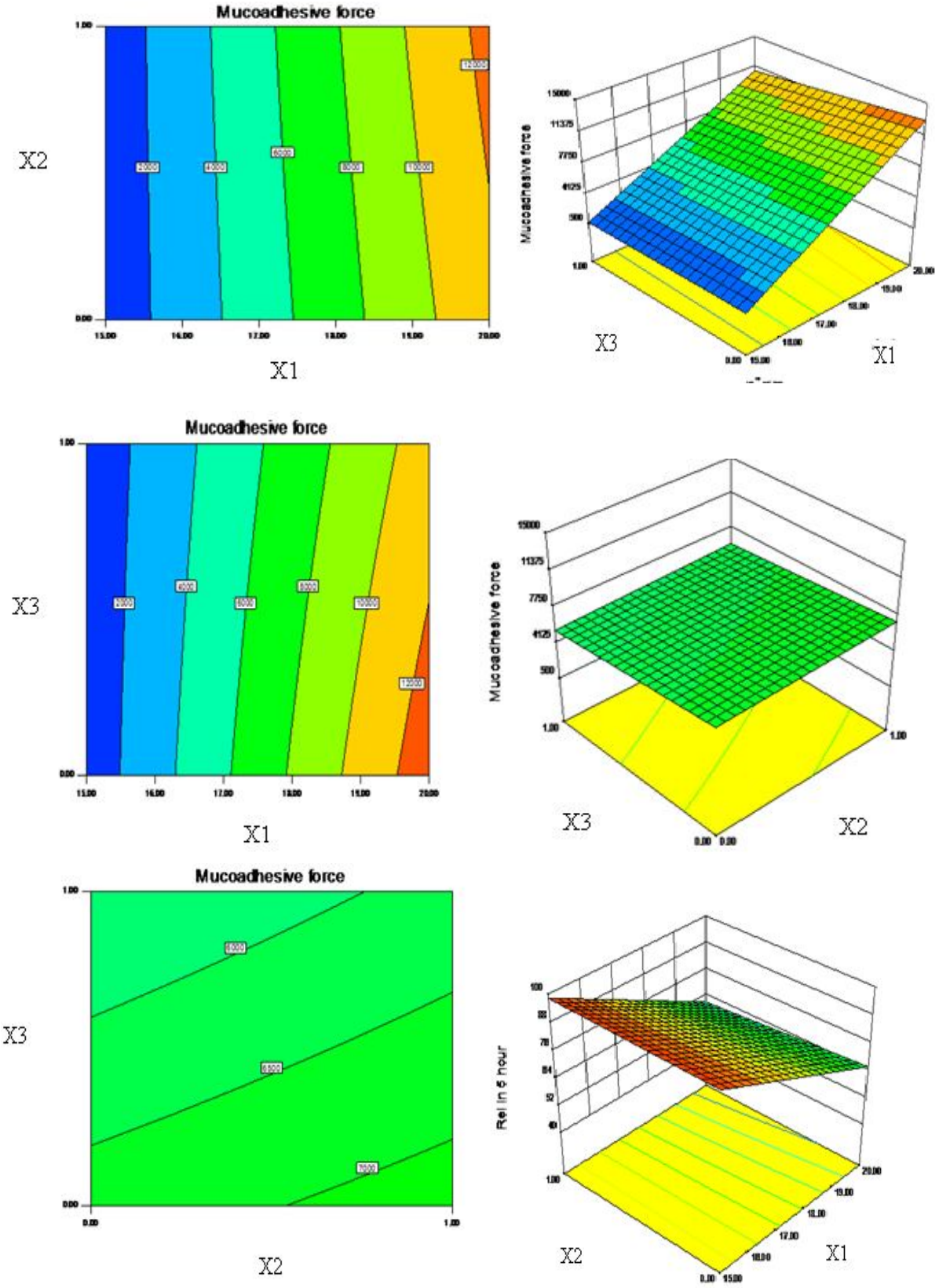


Fig. 1.10: RSM of formulations

Optimised Formula

After generating the model equations relating the main effects (factors) and responses, the various gel formulations containing Ondansetron HCL were optimized for the response Y1 (drug release in 5 hrs) and Y2 (mucoadhesive strength). The desirable range of these responses were restricted to 70 % ≤ Y1 ≤ 90 %, and 12000 ≤ Y2 ≤ 14000 %, respectively. The optimal values of responses were obtained by numerical analysis using the Design-Expert® software (V.7.0, Stat-Ease Inc., USA) based on the criterion of desirability[18]. In order to evaluate optimization capability of models generated according to the results of the factorial design, gel formulation was prepared using the optimal process variable settings. The optimized formulations of Ondansetron HCl (O-1, O-2, and O-3) were evaluated for drug release in 5 hrs (Y_1) and mucoadhesive strength (Y_2). Lists the results of experiments with predicted responses by the mathematical model and those observed. The observed responses of the optimized formulations (O-1, O-2, and O-3) vs. its predicted values showed the in table . This reveals that the mathematical model obtained by factorial design to produce optimized responses was well fitted.

Table 1.12 : Results of RSM of formulations

Om 1	Om 2	Om 3	Release in 5hr	Mucoadhesive force	Desirability
20.84	1	1	77.03	13387.10	1
21	1	1	76.45	13733.60	1
21.67	0	1	80.51	13616.3	1

Table 1.13 :Results showing optimized parameters

Code Number	Om1		Om2		Om3	
	Optimized	Actual	Optimized	Actual	Optimized	Actual
Release in 5 Hr	77.03	75.87	76.45	71.62	80.51	82.58
Mucoadhesive Force	13387.10	13108.36	13733.60	13571.37	13616.3	13609.38

RESULT

Om2 is the best composition formula based on statistical finding, while conformation experiment also proves this result.

CONCLUSION

Ondansetron hydrochloride was successfully formulated as an *in-situ* gelling system using HPMC E15 and chitosan. The formulated system provided a sustained release of the drug over a 5- hour period *in-vitro* and the developed formulations showed marked increase in permeation rate. The nasal residence time has significantly improved, and this can be viewed as viable alternative to conventional nasal drops. The ease of administration coupled with its ability to provide sustained release could probably result in less frequent administration, thus enhancing better patient compliance.

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