RESEARCH ARTICLE

Design and optimization of mucoadhesive nasal *in situ* gel containing sodium cromoglycate using factorial design

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Nasal *in situ* gel of sodium cromoglycate was prepared for sustained release and improvement of drug bioavailability. Carbopol 940 was used as a key ingredient which gives pH-induced sol to gel conversion of the formulations. Different formulations were prepared by varying the concentrations of carbopol 940 and different grades of Hydroxyl Propyl Methyl Cellulose (HPMC K100, HPMC K4M and HPMC K15M). These formulations were evaluated for parameters like pH, drug content, viscosity, gel strength, *in vitro* drug release and drug excipient compatibility. In this study, the release profile depends on the concentration of carbopol 940 and grade HPMC. A 3^2 factorial was applied to check the effect of varying the concentration of carbopol 940 (X_1) and different grades of HPMC (X_2) on the dependent variable i.e. viscosity, mucoadhesive strength, cumulative percentage drug released in 1 h (Q_1), 4 h (Q_4) and 8 h (Q_8) as dependent variables**.** *In vitro* release data was fitted to various models to ascertain the kinetic of drug release. Regression analysis and analysis of variance were performed for dependent variables. The results of the F-statistics were used to select the most appropriate model. Formulation F6 was considered optimum which contained carbopol 940 (0.75%) and HPMC K4M (0.50%) and was more similar to the theoretical predicted dissolution profile (f_2=70.99). The studies indicate that the formulation was effective in providing *in vitro* release, *in vitro* permeation of drug and the mucoadhesion which increases the residence time of drug.

Key words: *Carbopol 940, HPMC, mucoadhesive, pH-dependent nasal in situ gel, sodium cromoglycate*

INTRODUCTION

In recent decades, the nasal mucosa has become an established administration site for systemic drug delivery and a desirable alternative to parenteral medication since it is amenable to self-medication, has potential for direct-to-central nervous system delivery, no first-pass metabolism, non-invasiveness and virtually painless. From a pharmacokinetic standpoint, intranasal administration circumvents first-pass elimination and drug absorption is rapid due to the existence of a rich vasculature and a highly permeable structure within the nasal membranes.[1,2]

Allergic rhinitis is an inflammation of the nasal passages, usually associated with watery nasal discharge and itching of the nose and eyes. It occurs when an allergen such as pollen or dust is inhaled by an individual with a sensitized immune system and triggers antibody production. The specific antibody is immunoglobulin E (IgE) which binds to mast cells and basophils containing histamine. IgE bound to mast cells are stimulated by pollen and dust, causing the release of inflammatory mediators such as histamine.[3]

Sodium cromoglycate is a mast cell stabilizer, use in the treatment of allergic rhinitis and anti-asthmatic. Orally, it is poorly absorbed from the gastrointestinal tract with a reported bioavailability of 1%. The biological half life of the drug is only 45-100 min.[4] About 7% drug adsorption has been reported in nasal route.[5]

Nasal delivery of a drug has many advantages over other delivery still possess some drawbacks like nasal

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mucociliary clearance which results in reduced absorption of the drug. Due to mucociliary clearance, the formulation will be excreted within 15 mins in case of conventional nasal drug delivery (nasal drops, inhaler, aerosols etc). Several strategies were tested to improve nasal absorption of drug. To improve the nasal absorption of the drug, it is necessary to increase the nasal residence time. One method to lengthen nasal residence time has been to include a bioadhesive in the formulation. The use of bioadhesive polymers can increase nasal absorption of drugs with several ways such as increasing the residence time of the drug in the nasal cavity. Carbopol derivatives, which have pH-dependent *in situ* gelling property, are bioadhesive polymers used in an attempt to formulate a mucoadhesive drug delivery system for application to the nasal mucosa.[6]

The purpose of the present work was to develop a nasal formulation which increases the residence time of the formulation and also the sustained drug release to increase the bioavailability of the drug having a short biological half-life. The formulation gets retained for a longer time at the site which increases the drug absorption.

MATERIALS AND METHODS

Materials

Sodium cromoglycate, HPMC K100, HPMC K4M and HPMC K15M were purchased from Yarrow Chem, Mumbai, India. Carbopol 940 was obtained from Sd fine chem. Limited, Mumbai. Benzalkonium chloride was obtained from Merck Specialties Pvt. Ltd. Mumbai, India.

Methods

Preparation of mucoadhesive polymer-based in situ gels

Formulations as shown in Table 1 were prepared by dispersing carbopol 940 as pH-dependent *in situ* gelling agent and HPMC as gel-strengthening agent in distilled water as per the required quantity with continuous stirring until completely dissolved and allowed to hydrate overnight. After complete hydration of polymers, sodium cromoglycate was added to the mixture. The resultant solution was thoroughly mixed. Another excipient like benzalkonium chloride was added as a preservative to the above solution and mixing was confirmed until uniform and clear solutions were formed. Final volume was made by adding required amount of distilled water.[7]

Preliminary formulations

A preliminary screening was performed to optimize the concentration of carbopol 940 and different grades of HPMC as shown in Table 1. Batches (P1 to P3) were formulated using carbopol 940 and HPMC K100, Batches (P4 to P6) were formulated using carbopol 940 and HPMC K4M and Batches (P7 to P9) were formulated using carbopol 940 and HPMC K15M. All these batches were evaluated on the basis of viscosity, mucoadhesive strength, *in vitro* drug release profile and gel strength to optimize the concentration of different grades of HPMC.

Evaluation of Gel

pH measurement

pH of each formulation was determined by using pH meter (Shakti Chem.,Mehsana). The pH meter was first calibrated using buffer solutions of pH 4 and pH 7.[7]

Content uniformity

Nasal *in situ* gel of sodium cromoglycate was assayed by Ultra-Violet (UV) spectrophotometric analysis. In this study, each formulation (1 ml) was taken in a 100-ml volumetric flask diluted with phosphate buffer saline (PBS) pH 7.4 and was shaken to dissolve the drug in PBS pH 7.4. The solution was filtered through Whatman Grade 1 filter paper; this filtrate was further diluted if necessary with PBS pH 7.4. Drug content was determined using a Shimadzu UV 1800 double-beam spectrophotometer at 327 nm. The concentration of the drug was determined from a previously constructed calibration curve ($y=0.0150\ X+0.0030$, $R^2=0.9981$); the method was validated and the results for validation parameters are shown in Table 2.

Viscosity measurement

Viscosity of the prepared formulations was measured by using Brookfield digital Viscometer DV-II+ Proviscometer (Shakti Chem.,Mehsana). The gel under study was placed in the spindle S64 at 100 RPM for liquid formulations and gels.[6,7]

Determination of mucoadhesive strength

The mucoadhesive strength was determined by using the modified method as described by Gaikwad V[7] (2010). The mucoadhesive potential of each formulation was determined by measuring the force required to detach the formulation from the nasal mucosal tissue. A section of sheep nasal mucosa

Table 1: Preliminary formulations

Ingredients	Formulations								
	P1	P2	P3	P4	P5	P6	P7	P8	P9
Sodium Cromoglycate (%w/v)	2	2	2	2	2	2	2	2	2
Carbopol 940 (%w/v)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
HPMC K100 (%w/v)	0.25	0.50	0.75	-	-	-	-	-	-
HPMC K4M (%w/v)	-	-	-	0.25	0.50	0.75	-	-	-
HPMC K15M (%w/v)	-	-	-	-	-	-	0.25	0.50	0.75
Benzalkonium chloride (%w/v)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Distilled water (ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

q.s. = quantity sufficient

Table 2: Results for validation parameters

Parameter		Observation
Linearity range		15-55 µg/ml
Correlation coefficient		0.9981
Intraday precision (%RSD), *n*=3	15 µg/ml	0.22%
	30 µg/ml	0.37%
	45 µg/ml	0.14%
Interday precision (%RSD), *n*=3	15 µg/ml	1.64%
	30 µg/ml	0.65%
	45 µg/ml	0.82%
Accuracy (%Recovery)		98.63%-101.29%
LOD		0.42 µg/ml
LOQ		1.26 µg/ml

*n=number of replicates

was fixed on each of two glass slides using thread. Fifty milligrams of gel was placed on the first slide and this slide was placed below the height-adjustable pan. While another slide with mucosal section was fixed in inverted position to the underside of the same pan. Both the slides with the gel formulation between them were held in contact with each other for 2 mins to ensure intimate contact between them. Then weight was kept rising in second pan until the slides got detached from each other. The mucoadhesive force expressed as the detachment stress in dyne/cm^2 was determined from the minimal weight that detached the mucosal tissue from the surface of each formulation.

$$\text{Mucoadhesive strength (dyne/cm}^2) = \frac{m \times g}{A} \times 100 \qquad (1)$$

Where, m=weight (in grams) required for detachment,
g=Acceleration due to gravity (980cm/s^2),
A=Area of mucosa exposed.
The nasal mucosa was changed for each measurement.

Gel strength determination

A sample of 50 g of the nasal gel was put in a 100-ml graduated cylinder. A weight of 35 g was placed on the gelled form. The gel strength, which is an indication of the viscosity of the nasal gel at physiological temperature, was determined by the time in seconds required by the weight to penetrate 5 cm into the gel.[6,7]

In vitro release study

Drug release from the gel was tested with nasal diffusion cell, using dialysis membrane (mol.wt.12, 000-14,000) with permeation area of 0.785 cm^2. Twenty milliliters of phosphate buffer pH 7.4 was added to the acceptor chamber. Gel containing drug equivalent to 10 mg was placed in donor compartment. At predetermined time points, a 1-ml sample was withdrawn from the acceptor compartment, replacing the sampled volume with phosphate buffer pH 7.4 after each sampling up to 8 h. The samples were suitably diluted and measured spectrophotometrically at 327 nm. The concentration of the drug was determined from a previously constructed calibration curve (y=0.0150 X+0.0030, R^2=0.9981).[6,7]

In vitro permeation study

Fresh nasal tissue was removed from the nasal cavity of sheep obtained from a local slaughter house. Tissue was inserted in the diffusion cell having permeation area of 0.785 cm^2. Similar way as in drug release study, gel containing drug equivalent to 10 mg was kept in donor compartment. At a predetermined time point, sampling was done. Amount of drug permeated was determined by UV spectrophotometer.[7,8,9]

Histopathological evaluation of mucosa

Histopathological evaluation of tissue incubated in PBS (pH 7.4) after collection was compared with tissue incubated in the diffusion chamber with gel formulation (F6). Tissue was fixed in 10% buffered formalin (pH 7.4), routinely processed and embedded in paraffin. Sections were cut on glass slides and stained with hematoxylin and eosin (HE). Sections were examined under a light microscope, to detect any damage to the tissue during *in vitro* permeation by a pathologist blinded to the study.

Optimization of variables using full factorial design

A 3^2 randomized full factorial design was used in the present study. In this design, two independent factors were evaluated, each at three levels and experimental trials were performed for all nine possible combinations. The Concentration of Carbopol 940% (X_1) and different grades of HPMC (X_2) were chosen as independent variables in 3^2 full factorial design. Viscosity, mucoadhesive strength, cumulative % drug release at 1 h (Q_1), cumulative % drug release at 4 h (Q_4) and cumulative % drug release at 8 h (Q_8) were taken as dependent variables. *In vitro* diffusion profiles of F1 to F9 are shown in Figure 1. The formulation layout for the factorial design batches (F1 to F9) are shown in Table 3.

Kinetic modeling of drug release data

The release profiles of all batches were fitted to various models such as zero order, first order, Higuchi,[10] Hixon Crowell,[11] Korsemeyer and Peppas,[12] to ascertain the kinetics of drug release. The method described by Korsemeyer and Peppas was used to describe the mechanism of drug release.

Comparison of drug release profiles for selection of optimum batch

The similarity factor (f_2) given by Scale Up and Post Approval Changes (SUPAC) guidelines for a modified release dosage form was used as a basis to compare release profiles. The release profiles are considered to be similar when f_2 is between 50 and 100. The release profiles of products were compared using an f_2 which was calculated from the following formula,

$$f_2 = 50\text{X}\log\left\{\left[1+\left(\frac{1}{n}\right)\sum_{t=1}^{n} w_t\left(R_t - T_t\right)^2\right]^{-0.5} \times 100\right\} \qquad (2)$$

where n is the release time and R_t and T_t are the reference (theoretical and test value of sodium cromoglycate) at time t.[13]

Table 3: Formulation layout

Ingredients	Formulations								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Sodium Cromoglycate (%w/v)	2	2	2	2	2	2	2	2	2
Carbopol 940 (%w/v)	0.25	0.50	0.75	0.25	0.50	0.75	0.25	0.50	0.75
HPMC K100 (%w/v)	0.50	0.50	0.50	-	-	-	-	-	-
HPMC K4M (%w/v)	-	-	-	0.50	0.50	0.50	-	-	-
HPMC K15M (%w/v)	-	-	-	-	-	-	0.50	0.50	0.50
Benzalkonium chloride (%w/v)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Distilled water (ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

q.s. = quantity sufficient

Table 4: Evaluation parameters of F1 to F9

Formulation cord	pH (Mean±S.D.)	Drug Content (Mean±S.D.)	Mucoadhesive strength (dyne/cm^2)	Gel strength (sec)	Viscosity (cps)
F1	6.0±0.34	99.15±2.21	3730.10±1.55	5.67±1.55	60.08
F2	5.9±0.12	99.76±1.33	3988.75±1.86	13.56±0.24	524.07
F3	5.7±0.31	99.45±1.96	4218.18±2.87	17.37±1.87	833.00
F4	6.1±0.24	98.84±1.12	4386.67±1.09	14.65±2.21	590.08
F5	5.9±0.55	99.64±1.15	5215.00±1.61	28.17±1.50	1890.00
F6	5.6±0.18	100.11±1.38	5798.33±1.81	26.78±1.35	2525.00
F7	6.2±0.24	97.97±1.02	4211.65±1.23	25.19±1.40	749.04
F8	6.2±0.10	98.98±1.31	4632.15±2.98	38.08±1.31	2075.00
F9	5.7±0.32	99.12±1.13	5013.32±2.43	54.78±1.99	3012.00

Values expressed as Mean±S.D., *n*=3

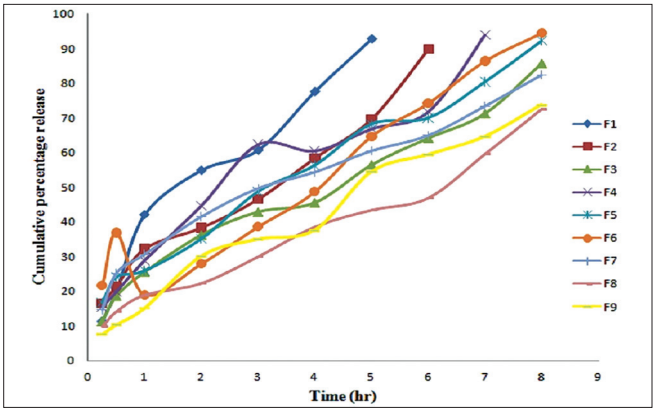

Figure 1: *In vitro* drug release profile of prepared formulations (F1 to F9)

Drug excipients' interaction study

Fourier transform infrared (FTIR) technique had been used to study the physical and chemical interaction between drug and excipients used. The FTIR spectrum of sodium cromoglycate and a physical mixture of sodium cromoglycate: HPMC: carbopol 940: benzalkonium chloride was recorded using KBr mixing method on the FTIR instrument available at the central instrument laboratory of the institute (FTIR-1700, Shimadzu, Kyoto, Japan).

RESULTS AND DISCUSSION

Preliminary screening

Preliminary study batches P2 to P5 and P7-P8 provided promising results in terms of *in situ* gelation, viscosity, *in vitro* drug release and mucoadhesive strength. Batch P1 had low viscosity (100 cps), three times lower mucoadhesive strength (1203.98 dyne/cm^2) than the average mucoadhesive strength and the drug was released within 3.5 h. Batch P9 showed vefi times higher viscosity due to the higher concentration of HPMC K15M. The viscosity of the sol form (1224 cps) was too high to pour the formulation, and the gel form was also too viscous (4771 cps). Batches P2, P5 and P8 were used for further study.

pH of formulation

It is known that the normal physiological pH of the nasal mucosa is 4.5–6.5 and pH increases in rhinitis in the range of 7.2-8.3;[14] however, the nasal mucosa can tolerate solutions within a pH range of 3-10. pH of all the formulations (F1 to F9) was found to be within 5.6 to 6.2, that was between physiological range of pH of nasal mucosa which is shown in Table 4.

Drug content

The percentage drug content of all the prepared gel formulations was checked and found to be in the range of 97.97-100.11%.

Mucoadhesive strength

All the formulations were subjected to mucoadhesion study. The mucoadhesion force is an important parameter for *in situ* gelling nasal formulations since it prolongs the nasal clearance of gels and increases its residence time in the nasal cavity. The reinforcement of the mucoadhesive forces in the nasal *in situ* gels by the use of mucoadhesive

polymers could be explained by the fact that secondary bond forming groups (hydroxy, ethoxy and amine) are the principle sources of mucoadhesion. The bioadhesive force generally depends on the nature and concentration of bioadhesive polymers. The stronger the bioadhesive force, longer is the nasal residence time. But if the mucoadhesion is too strong, the gel can damage the mucosal membrane. As per Table 4, the concentration of carbopol 940 and HPMC increased the mucoadhesive strength but in case of different grades of HPMC, HPMC K4M gave the highest mucoadhesion among the three of them.

In vitro permeation study

In vitro permeation study[8] was done for the comparison of permeation between formulation and pure drug solution which is shown in Figure 2. The formulation had higher permeation than the pure drug solution because anionic polymers such as polycarbophil or carbopol are reported to demonstrate permeation-enhancing properties. These polymers were shown to express a high Ca^{2+} binding ability. Carbopol enhanced the permeation of the drug from the gel significantly. This result could be attributed to the increase in concentration of the ionized carboxyl group to a level required to cause conformational changes in the polymer chain. Electrostatic repulsion of the ionized carboxyl group results in decoiling of the polymer chain, resulting in the relaxation of the polymer network. At this stage, the drug is rapidly dissolved and released from the gels as a result of very high swelling (or fast dissolution) of the ionized carbopol.

Histological evaluation of mucosa

The microscopic observations indicate that the optimized formulation had no significant effect on the microscopic structure of the mucosa. As shown in Figure 3, neither cell necrosis nor removal of the epithelium from the nasal mucosa was observed after permeation of F6. The epithelium layer was intact and there were no alteration in the basal membrane and superficial part of the submucosa as compared with PBS-treated mucosa. Thus, gel formulations seem to be safe with respect to nasal administration.

Full factorial design

A statistical model incorporating interactive and polynomial terms was used to evaluate the responses.

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \qquad (3)$$

where Y is the dependent variable, b_0 is the arithmetic mean response of the nine runs, and b_i is the estimated coefficient for the factor X_i. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high values. The two-way interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed. The *in vitro* release profile for nine batches showed a variation, i.e. cumulative drug release at 1 h (Q_1) (from 15.05 to 42.14). The data indicates that the release profile of the drug is strongly dependent on the selected independent variables. The fitted equations (full and reduced) relating the responses, viscosity, mucoadhesive strength, Q1, Q4 and Q8 to the transformed factor are shown in Table 5. The polynomial equations can be used to draw conclusions after considering the magnitude of the coefficient and the mathematical sign it carries (i.e. negative or positive). Table 6 shows the results of the analysis of variance (ANOVA), which was performed to identify insignificant factors. Data was analyzed using Microsoft Excel.

R^2 values for viscosity, mucoadhesive strength, Q1, Q4 and Q8 are 0.9906, 0.9464, 0.9559, 0.9335 and 0.9373 respectively indicating good correlation between dependent and independent variables. The reduced models were developed for response variables by omitting the insignificant terms with $P>0.05$. The terms with $P<0.05$ were considered statistically significant and retained in the reduced model. The coefficients for full and reduced models for response variables are shown in Table 6.

Full and reduced model for viscosity

The significance level of the coefficients b_1^2 was found to be $P=0.179$, hence they were omitted from the full model

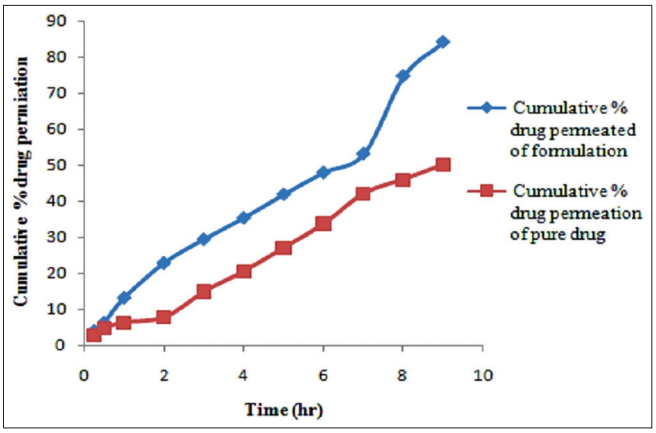

Figure 2: *In vitro* permeation of pure drug solution and formulation F6

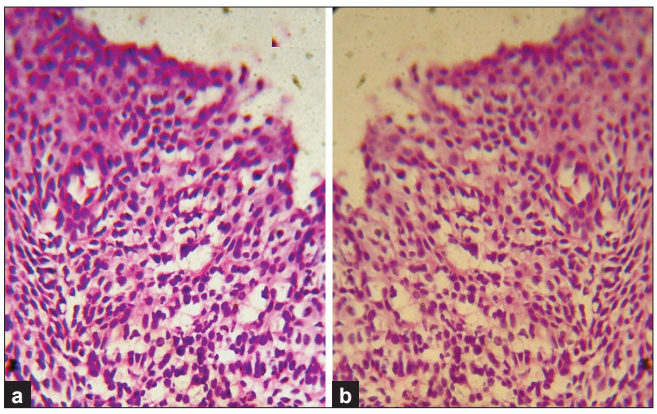

Figure 3: Histopathological evaluation of section of sheep nasal mucosa (a) mucosal layer after incubation with PBS (pH 7.4) (b) mucosal layer after incubation with gel formulation

Table 5: Formulation and evaluation of batches in 3^2 full factorial design

Batch code	X_1	X_2	Y_1	Y_2	Y_3	Y_4	Y_5	F2
F1	-1	-1	60.08	3730.1	42.14	77.54	92.69	37.06
F2	0	-1	524.07	3988.75	32.25	58.34	89.69	57.36
F3	1	-1	833	4218.43	25.65	45.59	85.64	48.73
F4	-1	0	590.08	4386.67	29.00	60.50	93.90	50.77
F5	0	0	1890	5215	26.01	56.39	92.22	61.27
F6	1	0	2525	5748.33	19.01	48.72	94.43	70.99
F7	-1	1	749.04	4211.65	30.72	54.40	82.43	47.75
F8	0	1	2075	4632.15	19.03	38.60	72.50	33.96
F9	1	1	3012	5013.32	15.05	37.54	73.69	39.56

Actual values

Coded values	X_1 Concentration of carbopol 940 (%)	X_2 Different grades of HPMC
-1	0.25	HPMC K100
0	0.50	HPMC K4M
1	0.75	HPMC K15M

Y_1 Viscosity (cps), Y_2 Mucoadhesive strength (dyne/cm^2), Y_3 Cumulative drug release in 1 h (Q1), Y_4 Cumulative drug release in 4 h (Q4), Y_5 Cumulative drug release in 8 h (Q8)

Table 6: Summary of results of regression analysis

Viscosity						
Response(Viscosity)	b_0	b_1	b_2	b_{12}	b_1^2	b_2^2
FM	1802.69	828.47	736.48	372.51	-201.49	-459.50
RM	166.36	828.47	736.48	372.51		-459.50
Mucoadhesive strength						
Response (Mucoadhesive strength)	b_0	b_1	b_2	b_{12}	b_1^2	b_2^2
FM	5157.03	441.94	319.97	78.33	-60.55	-817.60
RM	5116.67	441.94	319.97	-	-	-817.60
Q1						
Response (Q1)	b_0	b_1	b_2	b_{12}	b_1^2	b_2^2
FM	23.90	-7.03	-5.87	0.21	1.17	1.99
RM	26.54	-7.03	-5.87	-	-	-
Q4						
Response (Q4)	b_0	b_1	b_2	b_{12}	b_1^2	b_2^2
FM	53.24	-10.10	-8.49	3.77	2.94	-3.20
RM	53.07	-10.10	-8.49	-	-	-
Q8						
Response (Q8)	b_0	b_1	b_2	b_{12}	b_1^2	b_2^2
FM	91.97	-2.54	-6.57	-0.42	2.32	-10.74
RM	93.52	-	-6.57	-	-	-10.74

FM=Full model, RM=Reduced model

to generate a reduced model. The results of the statistical analysis are shown in Table 6. The coefficients b_1, b_2, b_{12} and b_2^2 were found to be significant at $P<0.05$; hence they were retained in the reduced model. The reduced model was tested in proportion to determine whether the coefficient b_1^2 contributed significant information to the prediction of viscosity.[15] The results of model testing are shown in Table 7. The critical value of F for $\alpha=0.05$ is equal to 7.71 (df=1,4). Since the calculated value (F=3.057) is less than the critical value (F=7.71), it may be concluded that the term b_1^2 did not contribute significantly to the prediction of viscosity and can be omitted to generate the reduced model. Response surface plot for viscosity is shown in Figure 4 which was plotted using Design Expert 8.

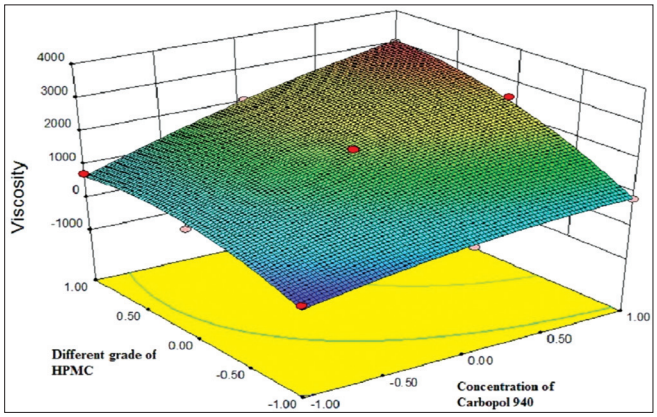

Figure 4: Three-dimensional response surface plot for viscosity

Full and reduced model for mucoadhesive force

The significance levels of the coefficients b_{12} and b_1^2 were found to be $P=0.567$ and 0.749 respectively, so they were omitted from the full model to generate a reduced model. The results of the statistical analysis are shown in Table 6. The coefficients b_1, b_2 and b_2^2 were found to be significant at $P<0.05$; hence they were retained in the reduced model. The reduced model was tested in proportion to determine whether the coefficients b_{12} and b_1^2 contributed significant information to the prediction of mucoadhesive force. The results of the model testing are shown in Table 7. The critical value of F for $\alpha=0.05$ was equal to 9.55 (df=2, 3). Since the calculated value (F=0.2674) was less than the critical value (F=9.55), it may be concluded that the terms b_{12} and b_1^2 did not contribute significantly to the prediction of mucoadhesive force and can be omitted to generate the reduced model. Response surface plot for mucoadhesive force is shown in Figure 5.

Full and reduced model for Q_1

The significance levels of the coefficients b_{12}, b_1^2 and b_2^2 were found to be $P=0.894$, 0.601 and 0.256 respectively, so they were omitted from the full model to generate a reduced model. The results of the statistical analysis are shown in Table 6. The coefficients b_1 and b_2 were found to be significant at $P<0.05$; hence they were retained in the reduced model. The reduced model was tested in proportion to determine whether the coefficients b_{12}, b_1^2 and b_2^2 contributed significant information to the prediction of Q_1. The results of model testing are shown in Table 7. The critical value of F for $\alpha=0.05$ was equal to 19 (df=2, 2). Since the calculated value (F=13.19) was less than the critical value (F=19), it may be concluded that the terms b_{12}, b_1^2 and b_2^2 did not contribute significantly to the prediction of Q_1 and can be omitted to generate the reduced model. Response surface plot for Q_1 is shown in Figure 6.

Table 7: Calculation for testing the model in portions

	DF	SS	MS	F	R^2	
Viscosity						
Regression						
FM	5	8431096.13	1686219.23	63.50	0.9906	F_{cal}=3.057
RM	4	8349899.69	2087474.92	51.91	0.9811	F_{tab}=7.71
Error						
FM	4	79669.68	26556.56	-	-	DF(1,4)
RM	4	160866.12	40216.53	-	-	-
Mucoadhesive strength						
Regression						
FM	5	3154998.68	630999.74	10.59	0.9464	F_{cal}=0.2674
RM	3	3123120.58	1041040.20	24.71	0.9368	F_{tab}=9.55
Error						
FM	3	178769.77	59589.92	-	-	DF(2, 3)
RM	5	210647.8591	42129.57	-	-	-
Q1						
Regression						
FM	5	521.64	104.33	13.02	0.9559	F_{cal}=13.19
RM	3	503.08	251.54	35.43	0.9219	$F_{tab=}$19
Error						
FM	2	24.04	8.013	-	-	DF(2, 2)
RM	6	42.60	7.10	-	-	-
Q4						
Regression						
FM	5	1138.76	227.75	8.42	0.9335	F_{cal}=1.16
RM	3	1044.04	522.02	17.81	0.8559	F_{tab}=19
Error						
FM	2	81.12	27.038	-	-	DF(2,2)
RM	6	175.84	29.31	-	-	
Q8						
Regression						
FM	5	539.86	107.97	8.97	0.9373	F_{cal}=1.39
RM	3	489.51	244.76	16.98	0.8499	F_{tab}=19
Error						
FM	2	36.13	12.04	-	-	DF(2,2)
RM	6	86.48	14.41	-	-	

DF=degree of freedom; SS=sum of squares; MS=mean of squares; R^2=regression coefficient; FM=Full model; RM=Reduced model

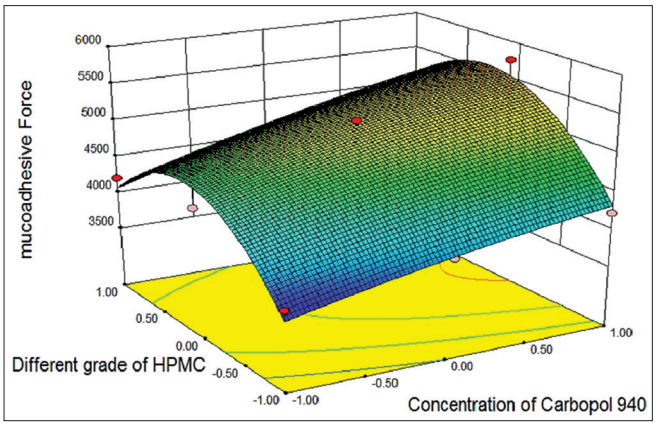

Figure 5: Three-dimensional response surface plot for mucoadhesive strength

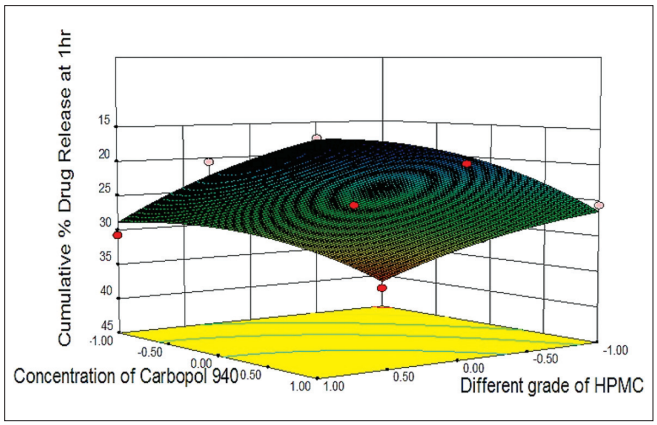

Figure 6: Three-dimensional response surface plot for Q1

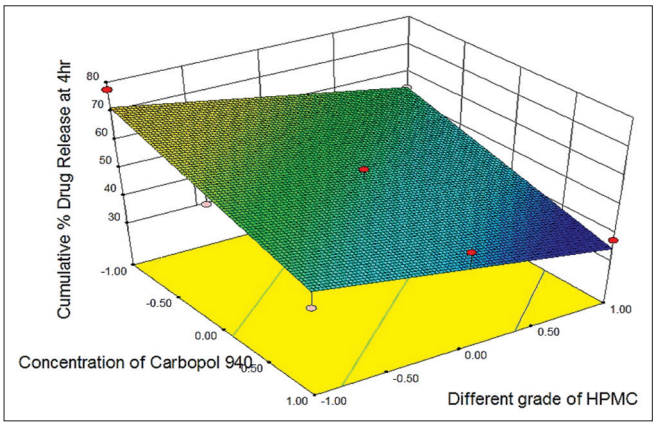

Figure 7: Three-dimensional response surface plot for Q4

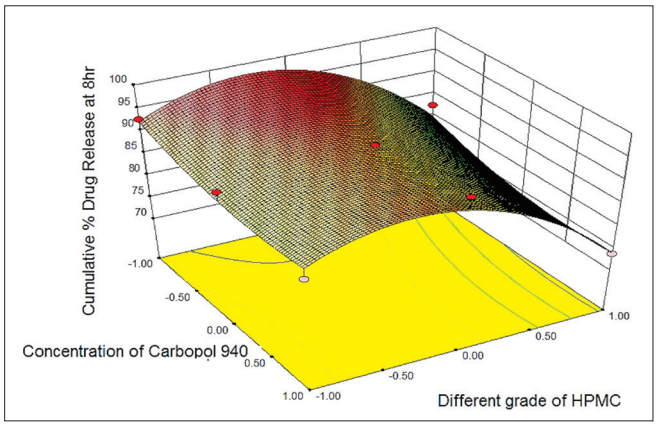

Figure 8: Three-dimensional response surface plot for Q8

Full and reduced model for Q_4

The significance levels of the coefficients b_{12}, b_1^2 and b_2^2 were found to be $P=0.242$, 0.482 and 0.448 respectively, so they were omitted from the full model to generate a reduced model. The results of the statistical analysis are shown in Table 6. The coefficients b_1 and b_2 were found to be significant at $P<0.05$; hence they were retained in the reduced model. The reduced model was tested in proportion to determine whether the coefficients b_{12}, b_1^2 and b_2^2 contributed significance information to the prediction of Q_4. The results of model testing are shown in Table 7. The critical value of F for $\alpha=0.05$ was equal to 19 (df=2, 2). Since the calculated value (F=1.16) was less than critical value (F=19), it may be concluded that the terms b_{12}, b_1^2 and b_2^2 did not contribute significantly to the prediction of Q_4 and can be omitted to generate the reduced model. Response surface plot for Q_4 is shown in Figure 7.

Full and reduced model for Q_8

The significance levels of the coefficients b_1, b_{12} and b_1^2 were found to be $P=0.170$, 0.823 and 0.413 respectively, so they were omitted from the full model to generate a reduced model. The results of the statistical analysis are shown in Table 6. The coefficients b_2 and b_2^2 were found to be significant at $P<0.05$; hence they were retained in the reduced model. The reduced model was tested in proportion to determine whether the coefficients b_1, b_{12} and b_1^2 contributed significant information to the prediction of Q_8. The results of model testing are shown in Table 7. The critical value of F for $\alpha=0.05$ was equal to 19 (df=2, 2). Since the calculated value (F=1.39) was less than critical value (F=19), it may be concluded that the terms b_1, b_{12} and b_1^2 did not contribute significantly to the prediction of Q_8 and can be omitted to generate the reduced model. Response surface plot for Q_8 is shown in Figure 8.

Kinetic modeling of *in vitro* permeation data

The kinetics of the dissolution data were well fitted to zero order, Higuchi model and Korsemeyer-Peppas model as is evident from regression coefficients [Table 8]. In case of the controlled or sustained release formulations, diffusion, swelling and erosion were the three most important rate-controlling mechanisms. Formulations containing swelling polymers show swelling as well as diffusion mechanism because the kinetics of swelling include relaxation of polymer chains and imbibition of water, causing the polymer to swell and changing it from a glassy to a rubbery state. The diffusion exponent *n* is indicative of the mechanism of drug release from the formulation. For a swellable cylindrical (tablet) drug delivery system, the *n* value of 0.45 is indicative of Fickian diffusion controlled drug release, *n* value between 0.5-0.85 signifies anomalous (non-Fickian)

transport, *n* value of 0.85 indicates case II transport, and n value greater than 0.85 indicates super case II transport.[16,17] The value of diffusion exponent *n* for all factorial formulations F2, F5, F6 and F7 was found to be <0.5 [Table 8] indicating Fickian transport drug release while remaining F1, F3, F4, F8 and F9 have *n* value between 0.5-0.85 so they follow anomalous (non-Fickian) transport drug release.

Comparison of *in vitro* release profiles for selection of optimum batch

The values of similarity factor (f_2) for batches F1 to F9 are shown in Table 5. The batch F6 showed maximum value of f_2 (70.99), hence was selected as optimum batch.

Drug excipients' interaction study

Drug excipients' interactions play a vital role in the release of the drug from the formulation. The pure sodium cromoglycate and its mixture with each of different HPMC, carbopol 940 were mixed separately with KBr (moisture free) and were scanned over a range of 400-4500 cm^{-1} using FTIR instrument (FTIR-1700, Shimadzu, Kyoto, Japan). The drug exhibits peaks due to ketonic group, broad peak of alcohol group and C=C stretching which is shown in Figure 9. It was observed that there were no changes in these main peaks in the IR spectra of a mixture of drug and polymers [Figures 10-12]. The FTIR study revealed no physical or chemical interactions of sodium cromoglycate with HPMC and carbopol 940 as evident from Figures 10-12.

CONCLUSION

Sodium cromoglycate was successfully formulated as a pH-induced *in situ* nasal gelling system using carbopol 940. The optimized formulation F6 provided sustained *in vitro* release of the drug over an extended period of 8 h. The optimized formulation can be a competent alternative to

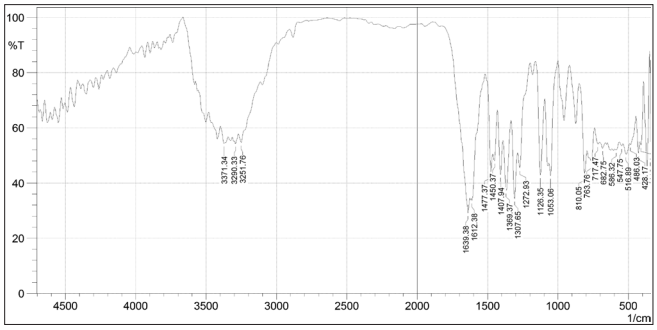

Figure 9: FTIR spectrum of sodium cromoglycate

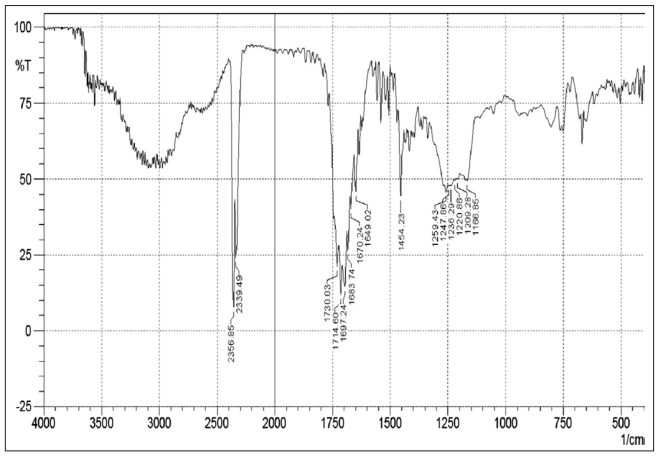

Figure 10: FTIR spectrum of physical mixture (sodium cromoglycate, carbopol 940, HPMC K100)

Table 8: Kinetic treatment of dissolution data

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Zero order									
R^2	0.9743	0.9894	0.9882	0.9687	0.9925	0.9506	0.9506	0.9506	0.9506
A	15.6771	11.486	8.203	10.299	9.233	9.223	9.223	9.223	9.223
B	16.0837	15.340	15.20	18.744	17.869	16.998	16.998	16.998	16.998
First order									
R^2	0.8881	0.9689	0.9257	0.9222	0.9617	0.9255	0.9152	0.9630	0.9366
A	0.1604	0.113	0.0989	0.101	0.093	0.0876	0.0772	0.1018	0.1304
B	1.3	1.3	1.24	1.33	1.32	1.33	1.37	1.11	1.02
Higuchi									
R^2	0.9887	0.9688	0.9928	0.9798	0.9889	0.8962	0.9940	0.9789	0.9862
A	43.88	33.4	26.22	33.14	29.26	27.66	26.30	21.09	27.19
B	-8.75	-3.803	-1.37	-2.43	-0.384	1.331	3.723	-2.828	-9.52
Hixon-crowell									
R^2	-0.9249	-0.9894	-0.9882	-0.9687	-0.9925	-0.9506	-0.9831	-0.993	-0.9885
A	-0.4169	-3.829	-2.73	-3.4329	-3.078	-3.074	-2.569	-2.24	-2.86
B	2.012	28.22	28.27	27.08	27.38	27.67	26.18	29.97	30.84
Korsemeyer and peppas									
R^2	0.9852	0.9836	0.9956	0.9915	0.98411	0.8160	0.9931	0.9867	0.9931
N	0.665	0.494	0.515	0.526	0.462	0.374	0.451	0.504	0.669
B	-0.4879	-0.512	-0.604	-0.515	-0.522	-0.503	-0.518	-0.720	-0.766

B=Intercept, A=Slope, R^2=Correlation coefficient, N=Diffusion exponent

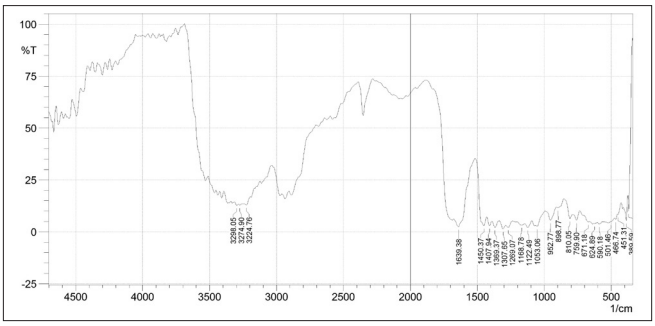

Figure 11: FTIR spectrum of physical mixture (sodium cromoglycate, carbopol 940, HPMC K4M)

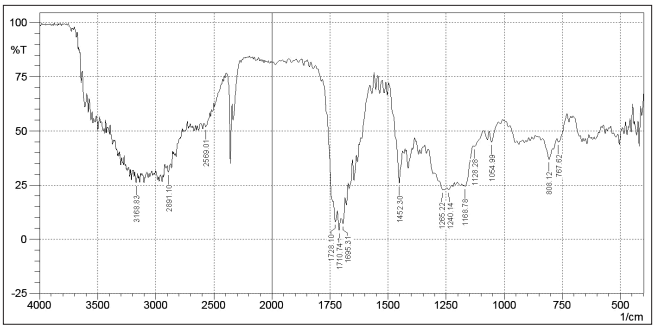

Figure 12: FTIR spectrum of physical mixture (sodium cromoglycate, carbopol 940, HPMC K15M)

conventional nasal drops. As a consequence of its enhanced permeation due to free carboxylic group in carbopol and absorption due to longer residence time, it avoids the first-pass effect, mucociliary clearance and reduces the dosing frequency as well.

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