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Formulation development and evaluation of *in situ* ophthalmic gel of sodium cromoglycate

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ABSTRACT

The poor bioavailability of ophthalmic solutions caused by dilution and drainage from the eye can be overcome by using In situ forming ophthalmic drug delivery system prepared from polymer that exhibit reversible liquid–gel phase transition. The objective of the study was to develop optimized formulation of in situ ophthalmic gel of Sodium cromoglycate, anti-allergic drug, using ion activated polymer, gelrite (gellan gum) as a gelling polymer and HPMC E-15LV (hydroxyl propyl methyl cellulose) as release retardant. The 3^2 full factorial design was employed to optimize the formulation considering Gelrite and HPMC as independent variables. The formulations were assessed for appearance, gelling ability, sterility, pH, drug content, viscosity, rheology, release through cellophane membrane & corneal membrane of goat, ocular irritation study & stability study as per ICH guidelines. Formulations F_4 and F_8 were selected and again analysed by grid analysis and F_4 was found to be the best formulation from the nine formulation developed by 3^2 factorial design. The study revealed that the In situ system of sodium cromoglycate sustained the effect of drug to 12 hours. The formulation F_4 extended the release of drug upto 12 hours as compare to marketed preparation of sodium cromoglycate, Cromal(solution), which gives 98.11% drug release within 6 hours.

Keywords: Sodium cromoglycate, Gelrite, HPMC E-15LV, Rheometer, Draize test.

INTRODUCTION

Ophthalmic preparations are defined in the USP as “sterile dosage forms, essentially free from foreign particles, suitably compounded and packed for instillation into the eye” [1]. In eye drug is administered at various site such as cornea, conjunctiva and sclera for better achievement of bioavailability and required effects related with the therapy. The drugs for allergies, glaucoma, bacterial infections, conjunctivitis, keratitis, local anaesthetics and viral infection can be administered at suitable sites in the eye.

Before reaching the anatomical barrier of the cornea, any drug molecule administered by the topical route has to cross the precorneal barriers. The medication, upon instillation, stimulates the protective physiological mechanisms, i.e., blinking and tears production, which exert defense against ophthalmic drug delivery. Thus the bioavailability of ocular delivery systems is affected by low residence volume i.e. only 7-10 µl, further it is affected by loss of administered amount due to rapid clearance by lachrymation, non- productive absorption through conjunctiva and through naso-lachrymal drainage.

Following characteristics are required to optimize ocular drug delivery system:

- Good corneal penetration.
- Prolong contact time with corneal tissue.
- Simplicity of instillation for the patient.
- Non-irritant and comfortable form (viscous solution should not provoke lachrymal secretion and reflex blinking).
- Appropriate rheological properties.

Consequently, it is imperative to optimize ophthalmic drug delivery. One of the ways to do so is by addition of polymers of various grades, development of viscous gel, development of colloidal suspension or using erodible or non erodible insert to prolong the pre-corneal drug retention. A significant increase in pre-corneal residence time of drugs and consequently increase in bioavailability can be achieved by using delivery systems based on concept of *In situ* gel formation. These systems consist of polymers, which undergo reversible sol to gel phase transition in response to physiological stimuli. The sol-gel transition can be induced by a shift in pH, temperature or ion activated systems. This type of gel combines the advantage of a solution (accurate and reproducible administration of drug) and gels (prolonged residence time) for enhancing ocular bioavailability [2, 3].

Sodium cromoglycate, an antiallergic drug, is achromone complex that acts by inhibiting the release of chemical mediators from sensitized mast cells. Sodium cromoglycate eye drops (2% and 4%) are used for symptomatic treatment of vernal kerato-conjunctivitis, vernal conjunctivitis, and vernal keratitis. In the present study, an attempt is made to develop ion induced *In situ* gelling ophthalmic delivery system of Sodium cromoglycate using ion sensitive polymer, gelrite and viscosity modifier such as HPMC E15LV for sustained drug delivery by enhancing residence time of drug in eye.

MATERIALS AND METHODS

Material

The sodium cromoglycate, gelrite and HPMC E15LV were gifted by Cipla R&D, Mumbai, India. All chemicals used were of analytical grade.

Method

Sodium cromoglycate was characterized by determining its solubility, melting point, UV curve, IR spectrum and DSC graph.

UV method development

The method development was done by determining λmax, precision, recovery, LOD, LOQ, linearity and by preparing calibration curve [4].

Table I. Formulation of Preliminary Batches and its Evaluation

Sr. No.	Batch code	Gelrite (%)	HPMC E15LV (%)	Gelling capacity	Viscosity (cps)
1	P_1	-	0.8	No gelation	16.12
2	P_2	-	1	No gelation	28.9
3	P_3	-	2	No gelation	35.32
4	P_4	-	3	No gelation	48.04
5	P_5	-	4	No gelation	65.78
6	P_6	0.2	0.6	-	23.15
7	P_7	0.4	0.8	+	32.5
8	P_8	0.5	1	++	39.42
9	P_9	0.6	1.5	+++	54.31
10	P_{10}	0.8	2	Highly viscous liquid	104.06
11	P_{11}	1	2.5	Highly viscous liquid	226.13
12	P_{12}	1.2	3	Direct gelling	322.43
13	P_{13}	1.4	3.5	Direct gelling	496.18
14	P_{14}	1.6	4	Direct gelling	561.84

(–): The solutions which did not undergo phase transition at all. (+): The solutions which exhibited phase transition only after 60 sec. and the formed gels which collapsed within 1-2 hrs. (++): The solutions which formed the gels after 60 sec. however, the gels formed did not remain stable for more than 3 hrs. (+++): The solutions which exhibited phase transition within 60 sec. and the gels so formed remained stable for more than 7-8 hrs.

Preliminary study

The preliminary compatibility study for sodium cromoglycate and gelrite was carried out by differential scanning calorimetry (DSC).

Further the preliminary batches of 2% Sodium cromoglycate were formulated using gelrite, HPMC E15LV, benzalkonium chloride and mannitol [5]. The batches were evaluated for in vitro *In situ* gelation in artificial tear fluid (ATF) and viscosity to optimize the concentration of gelrite and HPMC E15LV for final formulation, as per table no I.

The above preliminary batches indicated that:

1. HPMC alone does not possess any *In situ* gelling properties (P_1 to P_5).
2. Gelrite above the concentration of 0.4% forms *In situ* gel in artificial tear fluid (P_7 to P_9).
3. Gelrite above 1.0% concentration produce formulation of high viscosity that is not suitable for instillation into eyes (P_{11} to P_{14}).
4. Gelrite above the concentration of 1.2% produce direct gel formulation (P_{12} to P_{14}) so level of Gelrite was decided based on *In situ* gelling capacity.

Optimization by 3^2 factorial design

The 2% w/v solutions of Sodium cromoglycate were prepared using different concentrations of gelrite as per table I. The two independent variables selected were HPMC E15LV (X_1) and Gelrite (X_2), and the dependent variables were viscosity (Y1) and release (Y2) variables. The factorial designed batches are shown in Table II.

Table II. Formulation of Factorial Batches of Sodium cromoglycate

Sr. No.	Formulation code	Sodium cromoglycate (%w/v)	Gelrite (%w/v)	HPMC E15LV (%w/v)	BKC (%w/v)	Mannitol (%w/v)
1	F_1	2	0.4	1	0.01	5
2	F_2	2	0.4	1.5	0.01	5
3	F_3	2	0.4	2	0.01	5
4	F_4	2	0.5	1	0.01	5
5	F_5	2	0.5	1.5	0.01	5
6	F_6	2	0.5	2	0.01	5
7	F_7	2	0.6	1	0.01	5
8	F_8	2	0.6	1.5	0.01	5
9	F_9	2	0.6	2	0.01	5

Evaluation of *In situ* gelling formulations

The ophthalmic formulations were evaluated for various physical and performance characteristics i.e. for appearance/ clarity, pH gelling ability sterility, stability and viscosity before and after gel formation.

The test for sterility was confirmed by method B described in USP and the end point was judged visually noting the presence of turbidity in the inoculated media. Both positive and negative controls were also maintained simultaneously. The method of detection was visual inspection of turbidity. The test for gelling ability was conducted using artificial tear fluid (ATF) [6]. The transition of solution to viscous gel was observed visually and numerical scores were assigned depending upon the quickness of gel formation and time taken for collapse of gel structure on shaking the vials. The drug content was determined spectrophotometrically. The viscosity and rheological behavior of gel was studied using rheometer.

In vitro drug release study

In vitro release was performed through cellophane membrane (pore size 0.45µm) using modified dissolution testing apparatus, figure1. The glass cylinder was attached to the shaft of USP apparatus I (Basket type) in place of basket. The dissolution media was ATF (50 ml) maintained at 37 ± 0.5 °C. The sample (1 ml) was withdrawn at regular interval of 1 hr for 12 hrs and was replaced immediately with the same volume of ATF. The samples withdrawn were observed spectrophotometrically at 326.8 nm [7].

Figure 1: Modified dissolution test apparatus

In vitro Release Test Through Corneal Membrane

All the test conditions of in vitro drug release were followed except the cellophane membrane was replaced with the biological membrane i.e. freshly excised cornea of goat. The study was carried out upto 8 hrs for the selected formulation F4 and F8.

Preparation of Corneal Membrane (Goat): The goat eyeballs was procured from a nearby slaughter house and carefully transported to the laboratory in an airtight container containing cold saline solution (0-2°C). Carefully separated cornea along with surrounding sclera tissue (5-6 mm) was washed with cold saline and preserved in freshly prepared ATF (maintained at 0-2°C).

Ocular irritation study in rabbits

The selected formulations F4 & F8 were instilled (0.1ml) into the eye of rabbit. The eyeball was observed for acute toxicity symptoms i.e. redness, inflammation & tear flux at an interval of 1, 24, 48, 72 hrs and after 1 week. The ocular irritation study was measured on a scale of 0 to 4 [8, 13].

Stability study

The formulations F4 and F8 were subjected to stability studies as per ICH guidelines. The formulations were assessed for appearance, gelation ability, sterility, pH, drug content and viscosity [12].

RESULTS AND DISCUSSION

The drug, Sodium cromoglycate was characterized by observing its UV, DSC and IR spectrum. The λ_{max} of drug in ATF was found to be 326.8nm. The IR spectrum obtained is given in figure 2.

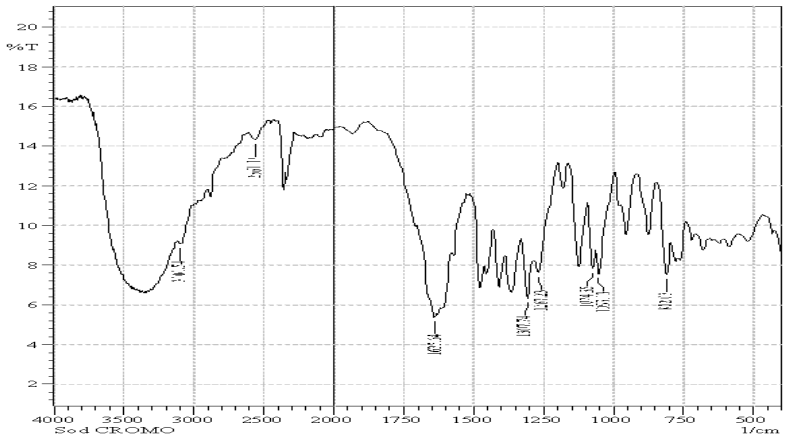

Figure 2: IR spectrum of sodium cromoglycate

The formulated ophthalmic formulations were evaluated for various physical and performance characteristics. The ophthalmic formulations were observed carefully for color, odour and presence of suspended particulate matter if any. The clarity of solutions was further assessed by observing them against a dark and white background as described in the USP. The pH of all the formulations was found to be in the range of 4.5 to 7.4. Viscosity increased with the increase in the concentration of gelrite from 0.4 to 0.6%. Similarly viscosity increased with the increase in the concentration of HPMC E-15LV for concentration from 1.0 to 2.0%. Increase in viscosity of ophthalmic solutions after instillation in eye was a desired feature for the purpose of sustaining therapeutics actions of sodium alginate by providing increased pre-corneal residence time. The increase in viscosity was achieved due to the inclusion of gelrite which undergoes gelation when it comes in contact of calcium or sodium ions of tear fluid. The test for sterility for the selected formulations indicated no tubidity after incubation at specified conditions upto 14 days, while the positive controls revealed dense turbidity. Viscosities of all formulation were recorded, as in table III using Brookfield viscometer and Rheometer before and after gelling respectively.

Table III. Viscosity Values for Ophthalmic Formulation

Sr. No.	Formulation code/ RPM	Viscosity (cps) after gelling in ATF*						Viscosity (cps) before gelling**
		10	20	30	50	60	100	60
1	F1	4051	2608	1792	1216	1024	966	23.94
2	F2	6798	2941	1927	1433	1243	1098	34.6
3	F3	5515	3933	2712	1784	1565	1424	41.23
4	F4	7001	3441	2320	1298	917	783	37.16
5	F5	2854	1550	981	718	666	464	45.05
6	F6	2192	977	764	558	417	369	56.88
7	F7	3151	1872	629	603	551	390	47.19
8	F8	2828	1462	872	614	450	351	54.87
9	F9	2885	1665	1394	1033	949	250	68.98

**Using R/S CPS+ Rheometer, **Brooke Field LVF Viscometer- spindle LV1 at 60 rpm.*

The graph of shear rate verses shear stress, as shown in figure 3 and 4, were obtained from Rheometer for ophthalmic solution as well as preformed ophthalmic gel in ATF [9].

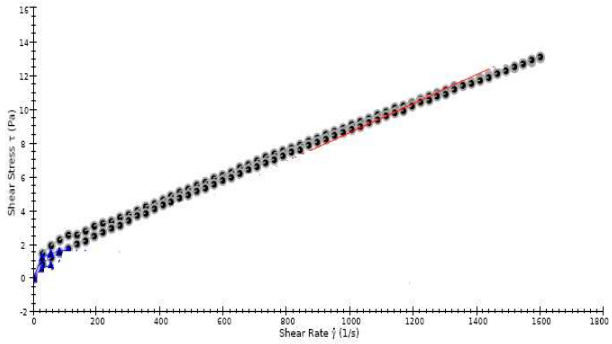

Figure 3: Newtonian flow of ophthalmic solution (F_4)

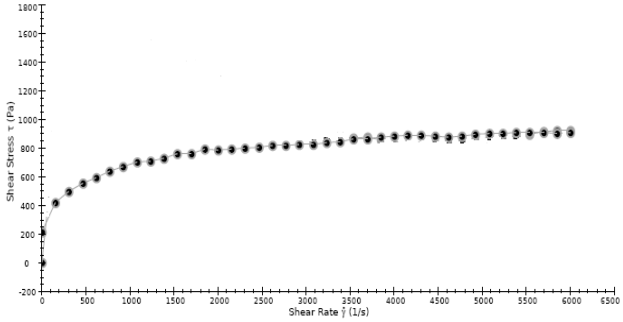

Figure 4: Pseudoplastic behavior of preformed gel in ATF (F_4)

The test for in vitro gelation ability was performed to assess the gel characteristics which would affect drug release in the ATF. The numerical scores for gelling ability of solutions were found to vary with change in the concentration of gelrite as shown in table IV.

Table IV. Gelling Capacity of Ophthalmic Formulation

Sr. No.	Formulation code	Gelrite (%w/v)	HPMC E15LV (%w/v)	Gelling ability
1	F1	0.4	1	+
2	F2	0.4	1.5	+
3	F3	0.4	2	++
4	F4	0.5	1	+
5	F5	0.5	1.5	++
6	F6	0.5	2	++
7	F7	0.6	1	++
8	F8	0.6	1.5	+++
9	F9	0.6	2	+++

(+) Gels after a, dissolves rapidly (within 1-2 hrs), (++) Gelation immediately, remains for few hrs (3-4 hrs), (+++) Gelation immediately, remains for extended period (more than 6-8 hr)

The phase transition of the ophthalmic formulations containing gelrite was found to be concentration dependent. Thus, the increased concentration of gelrite caused decrease in the time taken for gelation. The drug content determined spectrophotometrically was in the range of 97.61 to 102.10% of labeled content.

The ocular irritation study (Draize test), revealed the numerical score for the formulation F_4 and F_8 read zero, an indication of safe and non irritant for administration. The findings of the stability study suggested no significant change in the selected physical parameters for the formulations F_4 and F_8.

In vitro release through cellophane membrane revealed that with the increase in the concentration of HPMC E-15LV the release decreased due to the formation of gel structure. As the conc. of gelrite increased from 0.4% to 0.6% there was further retardation in the release (batches F4, F5, F6 and F7, F8, F9).This may be accounted for the reduction in number and dimensions of the channels in the gel structure due to enhanced viscosity of gel. The kinetics of drug release mechanism (PCP Disso version 2.08 software) revealed matrix model kinetics.

The release of Sodium cromoglycate through goat cornea was slower as compared to the release through cellophane membrane. Hence, the formulation containing gelrite is most effective in sustaining the release of Sodium cromoglycate (over the period 12 hours).

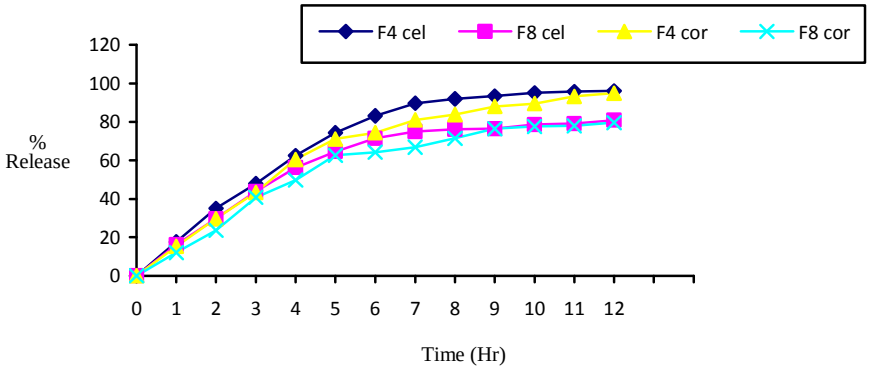

Figure 5: Drug release from formulation F_4 and F_8 (F_4 cel, F_8 cel: release through cellophane; F_4 cor, F_8 cor: through corneal membrane)

The 3^2 full factorial design was selected to study the effect of independent variables gelrite (X_1) and HPMC E15LV (X_2) on dependent variables viscosity and % release. The viscosity and % release values are strongly dependent on the selected independent variables. The equation conveyed the basis to study of the effects of variables. The

regression coefficient values are the estimates of the model fitting. The r^2 was high indicating the adequate fitting of the quadratic model. The polynomial equations can also be used to draw conclusions considering the magnitude of co-efficient and the mathematical sign it carries; i.e. positive or negative.

The negative coefficient of variable X_2 i.e. HPMC E15LV in case of response release indicates that as the HPMC concentration was increased, release value decreased. However, the positive coefficient for viscosity shows opposite effect indicating that the increased concentration of HPMC E15LV leads to increased viscosity value.

Similarly, the variable X_1 showed positive coefficient for both responses i.e. release and viscosity.

Final Equations in Terms of Coded Factors (Release)

$$Release=92.72-3.55X_1-5.05X_2+0.49X_1X_2-4.99X_1^2-0.66X_2^2$$

Final equations in Terms of Actual Factors (Release)

$$Release=92.72-3.55Gelrite-5.05HPMC+0.49Gelrite.HPMC-4.99Gelrite^2-0.66HPMC^2$$

Final Equations in Terms of Coded Factors (Viscosity)

$$Viscosity=45.66+10.13X_1+9.86X_2+1.13X_1X_2-1.23X_1^2+1.06X_2^2-0.090X_1^2X_2+2.62X_1X_2^2$$

Final equations in Terms of Actual Factors (Viscosity)

$$Viscosity=45.66+10.13Gelrite+9.86HPMC+1.13Gelrite.HPMC-1.23Gelrite^2+1.06HPMC^2-0.090Gelrite^2HPMC+2.62Gelrite.HPMC^2$$

Evaluation and interpretation of research findings are almost important and the p-value serves a valuable purpose in these findings. The coefficients of X_1 and X_2 were found to be significant at $p < 0.05$, hence confirmed the significant effect of both the variables on the selected responses. The increase in concentration of the Gelrite resulted in decrease in the drug release. Similarly, increasing the concentration of the HPMC E15LV resulted in the decrease in the release of Sodium cromoglycate. Overall both the variables caused significant change in the responses. ANOVA and Multiple regression analysis were done using Stat-Ease Design Expert 7.1.4 software.

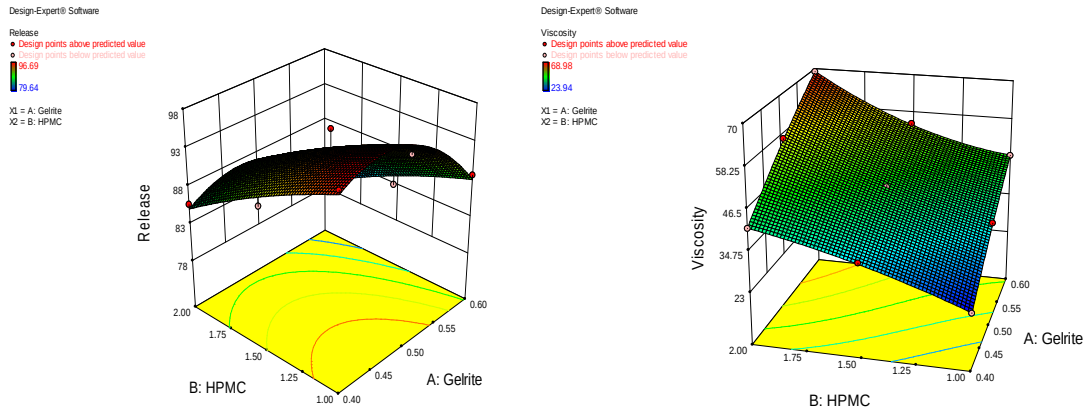

Figure 6: Response surface plot of release and viscosity

The Variance Inflation Factor (VIF) measured how much the variance of that model coefficient was inflated by the lack of orthogonality in the design and was calculated for release and viscosity respectively. It was found to be near one, indicating good estimation of the coefficient. Similarly Ri-squared was near to zero which led to good model. The values of Prob>F were less than 0.05, which indicated model terms were significant. In all cases A, B, AB, A^2

and B^2 were significant model terms. There were 2.87%, 2.63% and 1.23% chances of "Model F-value" which could occur due to noise. In all cases "Pred R-squared" values were in reasonable agreement with the "Adj R-squared" values. The Adeq-Precision was the measure of the signal to noise ratio. A ratio > 4 was desirable. In our case the Adeq-Precision values were 10.681 and 29.139 for release and viscosity respectively, which indicated an adequate signal. However, both the variables favor the preparation of controlled release *In situ* gelling system of Sodium cromoglycate.

The response surface plot, figure 6, showed that various combinations of independent variables X_1 and X_2 may satisfy any specific requirement (i.e. maximum drug release with floating upto12 hrs) while taking into consideration of various factors involved in dosage form.

The release profile of the F_4 and F_8 were compared with that of marketed preparation (CROMAL), as shown in figure 7.

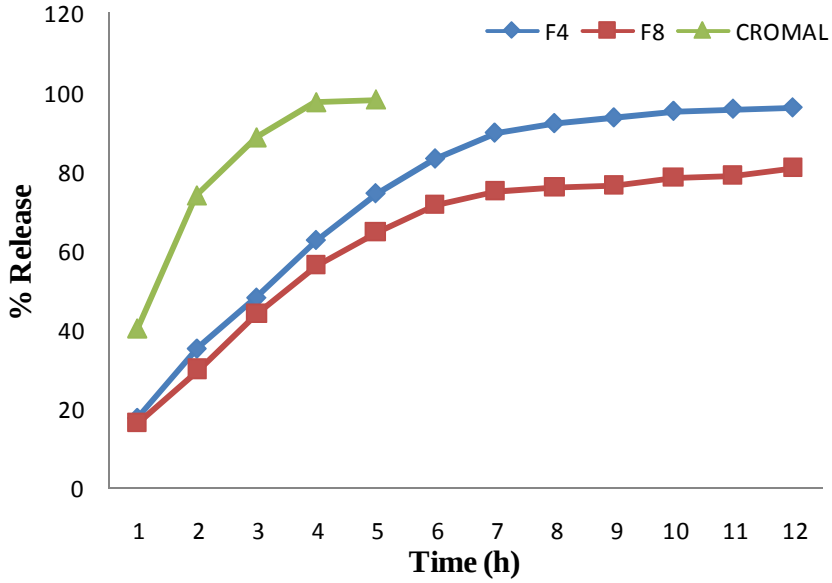

Figure 7: Sodium cromoglycate release from marketed, F_4 and F_8

The grid analysis was performed for selection of the optimized level for release and viscosity. The best results for the above response were obtained at the middle value of gelrite and lower value of HPMC. Thus, the formulation F_4 was selected as optimized formulation.

CONCLUSION

An attempt has been made to develop *In situ* gelling systems of Sodium cromoglycate, an antiallergic drug, to increase the ocular residence time. The variables gelrite and HPMC E15LV exhibited significant effect on the responses viscosity and release of the formulation. A stable *In situ* gelling system of Sodium cromoglycate has been successfully developed using gelrite and HPMC E15LV.

The statistical approach for formulation optimization is useful tool, particularly when two or more variables are to be evaluated simultaneously. The variables Gelrite and HPMC E15LV evaluated in this study exhibited significant effect on the responses release and viscosity of the formulation.

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