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QBD APPROACH IN FORMULATION AND EVALUATION OF GELRITE BASED *IN SITU* OPHTHALMIC GEL OF NEPAFENAC

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ABSTRACT

The present investigation was focused on application of QbD approach to see the effect of formulation variables on *in situ* ophthalmic gel containing a newer NSAID drug, nepafenac. Risk assessment of critical material and process parameters are linked to critical quality attributes (CQAs) of the product with respect to obtain target quality product profile (TQPP). The effects of critical parameters (concentration of Gelrite, Hypromellose METHOCEL E 15 Premium LV) were investigated by executing design of experimentation (DoE) using 3^2 factorial designs. Drug release, viscosity at non physiological condition (NP) and viscosity at physiological condition (P) were considered critical quality attributes (CQAs). Gelrite based ophthalmic Nepafenac *in situ* gels were prepared and evaluated. Multiple regression analysis and ANOVA were employed to identify and estimate the effect of important parameters and establish their relationship with CQAs and to obtain design space for optimization purpose. The best *in vitro* drug release profile, viscosity at non physiological condition (NP), viscosity at physiological condition (P) and desired product quality was achieved with the formulation prepared in the region of design space. 3D response graph and overlay plots were successfully implemented to interpret effects and selection of significant parameters on CQAs. Formulation parameters which affect the nepafenac *in situ* ophthalmic gel can be successfully optimized.

KEYWORDS: *In situ* ophthalmic gel, Nepafenac, Gelrite, Ion sensitive gelling system, HPMC E 15LV.

INTRODUCTION

Nonsteroidal Anti inflammatory Drugs (NSAIDs) have been used to treat various diseases for over 100 years. These drugs show anti-inflammatory, anti-allergic, analgesic and antipyretic activity and widely used to treat chronic inflammatory states, such as arthritis, psoriases and asthma. Since the introduction of topical Indomethacin for use in ophthalmic disease, several generations of NSAID have been brought to market. One of the more recent products of the NSAID class approved for topical opthalmic use is Nepafenac, a prodrug of Amfenac for the treatment of post-operative inflammation after cataract surgery. Nepafenac is described chemically as 2-amino-3-benzoylbenzeneacetamide, and is preferred over the other NSAID drugs as is having an excellent ability to penetrate corneal epithelium. The only available formulation is 0.1%w/v suspension and after administration of which, less of the drug reaches the posterior segment of the eye because of the long diffusion distance and the rapid clearance by aqueous humor flow results in poor bioavailability.[2-4] So to increase precorneal residence time with reduced drug elimination in *situ* gelling systems are widely useful.

This novel drug delivery system promotes the ease and convenience of administration, deliverance of accurate dose as well as to prolong residence time of drug in contact with mucosa, that problems generally encountered in semisolid dosage forms.[2-4] *In situ* gel formation occurs due to one or combination of different stimuli like pH change, temperature modulation and solvent exchange. Smart polymeric systems represent promising means of delivering the drugs; these polymers undergo sol-gel transition, once administered.[3,4] *In situ* forming gels are liquid upon instillation and undergo phase transition in the ocular cul-de-sac to form viscoelastic gel and this provides a response to environmental changes.[4]

The application of quality-by-design (QbD) approach in formulation development has provided an opportunity for a harmonized pharmaceutical quality system based on continuous quality improvement which can yield safer, more efficacious product. Design of experimentation, selection of appropriate model is important and criteria for selection can vary based on number and type of factors, number of levels for factor, type of study, time and cost for experiments. In this paper, we used QbD approach for better understanding of relationship of critical formulation and process parameters to CQAs relating to quality product profile of in situ ophthalmic gel of nepafenac. Formulations were prepared using Gelrite (gellan gum) and Hypromellose METHOCEL E 15 Premium LV. Based on risk assessment understanding for formulations, high risk variables were selected and 3^2 factorial

designs was employed for design of experimentation. Formulations were evaluated for *in vitro* drug release, viscosity at non physiological condition (NP) and viscosity at physiological condition (P). We presented different graphs, polynomial equations, ANOVA and P (Probe >F) value to understand correlation and significance of critical parameters on QTPP. Based on effects of critical formulation variables on QTPP, proposed design space to obtain robust formulation.[23-25]

MATERIALS AND METHOD

Nepafenac was received as gift sample from Ajanta Pharma Ltd Mumbai, Gelrite and Hypromellose METHOCEL E 15 Premium LV were procured as gift samples from Signet and Dow chemical, Mumbai.

All other reagents and chemicals used of analytical grade.

Risk assessment of Critical material and process attributes

Risk assessment for the experiment was carried out by basic risk management facilitation. Polymer concentration (Gelrite and Hypromellose METHOCEL E 15 Premium LV) were considered for design of experimentation of the Nepafenac in situ ophthalmic gels.

Preliminary study

Method

Nepafenac was characterized by determining its solubility, melting point, UV curve, IR spectrum.

The preliminary compatibility study for nepafenac and gelrite was carried out by IR spectroscopy.

Further the preliminary batches of 0.1% Nepafenac were formulated using Gelrite, Hydroxypropyl methylcellulose (Hypromellose 2910 /or Hypromellose METHOCEL E 15 Premium LV), SBE-β-cyclodextrin/ or hydroxypropyl-gamma-cyclodextrin, boric acid, mannitol, and Benzalkonium chloride. The batches were evaluated for in vitro in situ gelation in artificial tear fluid (ATF) and viscosity to optimize the concentration of gelrite and Hypromellose METHOCEL E 15 Premium LV for final formulation, as per table 1.

Table 1: Formulation of preliminary batches and their evaluation

Sr. No.	Batch Code	Gelrite (%)	METHOCEL E15 PREMIUM LV (%)	Gelling capacity	Viscosity (cps)
1	**S1**	-	0.8	No gelation	17.14
2	**S2**	-	1.0	No gelation	29.95
3	**S3**	-	2.0	No gelation	35.30
4	**S4**	-	3.0	No gelation	49.84
5	**S5**	-	4.0	No gelation	67.20
6	**S6**	0.2	0.6	-	25.45
7	**S7**	0.4	0.8	+	37.05
8	**S8**	0.5	1.0	++	41.75
9	**S9**	0.6	1.5	+++	50.25
10	**S10**	0.8	2.0	Highly viscous liquid	114.20
11	**S11**	1.0	2.5	Highly viscous liquid	250.13
12	**S12**	1.2	3.0	Direct gelling	352.20
13	**S13**	1.4	3.5	Direct gelling	489.59
14	**S14**	1.6	4.0	Direct gelling	554.20

The above preliminary batches indicated that:

1. Hypromellose METHOCEL E 15 Premium LV alone does not possess any *in situ* gelling properties (S1 to S5).
2. Gelrite above the concentration of 0.4% forms *in situ* gel in artificial tear fluid (S7 to S9).
3. Gelrite above 1.0% concentration produce formulation of high viscosity that is not suitable for instillation into eyes (S11 to S14).
4. Gelrite above the concentration of 1.2% produce direct gel formulation (S12 to S14) so level of Gelrite was decided based on *in situ* gelling capacity.

Optimization by 3^2 factorial design

The % w/v solutions of nepafenac were prepared using different concentrations of gelrite as per table I. The two independent variables selected were Gelrite (X1) and Hypromellose METHOCEL E 15 Premium LV (X2) and the dependent variables were release (Y1), viscosity at NP (Y2) and viscosity at P (Y3). The factorial designed batches are shown in table 2.

Formulation of *in situ* ophthalmic gels of Nepafenac

Preparation of solution A: Accurately weighed quantity of SBE-β-cyclodextrin was dissolved in 30 ml deionized water followed by the addition of accurately weighed quantity of nepafenac. The mannitol, Boric acid and Benzalkonium chloride was added to above mixture with continuous stirring.

Preparation of solution B: The Gellan gum and Hypromellose METHOCEL E 15 Premium LV were sprinkled over 50 ml of boiling water and was allowed to hydrate for 15 min to produce a clear solution.

Compounding of ophthalmic solution: The solution B was mixed slowly to solution A with continuous mechanical stirring to produce clear and transparent solution. The pH of formulation was checked and adjusted with 0.1 N NaOH and volume was made up with deionized water to 100ml.

Sterilization /Filtration of ophthalmic formulation: The final formulation was sterilized by autoclaving at 121°C for 15 min or by filtration through 0.22µ PVDF filter 47mm (make: Millipore)

Table 2: Formulation of factorial batches

Ingredients (% w/v)	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
*Nepafenac	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
SBE-β-cyclodextrin	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Gelrite	0.4	0.4	0.4	0.5	0.5	0.5	0.6	0.6	0.6
Hypromellose METHOCEL E 15 Premium LV	1	1.5	2	1	1.5	2	1	1.5	2
Mannitol	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3
Boric acid	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Benzalkonium chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Water for injection	q.s to 100mL	q.s to 100mL	q.s to 100mL	q.s to 100mL	q.s to 100mL	q.s to 100mL	q.s to 100mL	q.s to 100mL	q.s to 100mL

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). 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.[9,10,14-16,28]

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.[28]

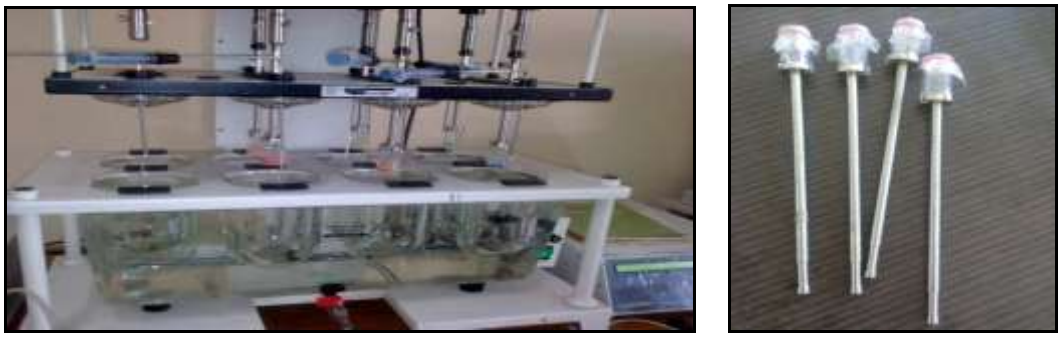

Figure 1: Dissolution apparatus with modified assembly

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 238 nm.

Stability study

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

RESULTS AND DISCUSSION

Based on QbD approach, risk assessment was carried and high risk parameters, based on their strong correlation to Critical Quality Attributes (CQAs) were considered for Design of experimentation to ensure a predefined quality of the product. In order to define the ‘‘design space’’ the critical formulation variables (independent variables) and the responses able to measure the product quality were defined based on prior knowledge and preliminary studies.

Table 3: Risk assessment of the drug product CQAs

Drug product CQA’s	Impact of Gelrite (Gelllan gum)	Impact of Hypromellose METHOCEL E 15 Premium LV
Drug Release	High	Medium
Viscosity at NP	Low	High
Viscosity at P	High	High

Table 4: Risk assessment of the drug product CQAs

Formulation Variables	CQA's	Justification
Gelrite Concentration	Viscosity at P	Change in concentration may have impact on viscosity at physiological condition, the risk is high.
Polymer concentration	Dissolution	Release of drug depends on the amount of polymer in formulation so the risk is high.
Hypromellose METHOCEL E 15 Premium LV level	Viscosity at NP	Change in concentration may have impact on viscosity at non physiological condition, the risk is high.

The independent variables considered for formulations are concentration of Gelrite (gellan gum) and Hypromellose METHOCEL E 15 Premium LV since they were considered critical in determining responses i.e. % Drug release, viscosity at nonphysiological (NP) and viscosity at physiological (P) conditions. Based on the nature of variables, number of formulation variables, levels of variables, optimization study, to estimate the main as well as interactive effects of variable and minimum number of experimental trials, 3^2 factorial design with 9 runs was selected to see the effect of formulation variables on nepafenac ophthalmic in situ gel.

The drug, Nepafenac was characterized by observing its UV and IR spectrum. The λmax of drug in ATF (Artificial tear fluid) was found to be 238 nm.

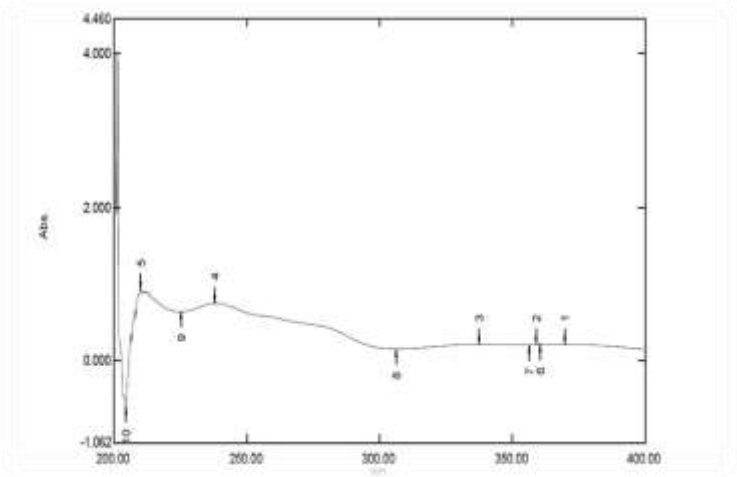

Figure 2: UV spectrum of Nepafenac

The IR spectrum of pure drug and with excipients is given in figures 3 and 4.

Table 5: Peaks observed in IR spectrum of Nepafenac

Sr. No.	IR frequency (cm^{-1})	Group
1	**3325.28**	N-H stretching
2	**2910.58**	Aromatic C-H stretching

3	**1676.14**	C=O carboxylic acid
4	**1193.94**	C-O stretching (ester)
5	**960.55**	Aromatic C-H
6	**761.88**	C-Br

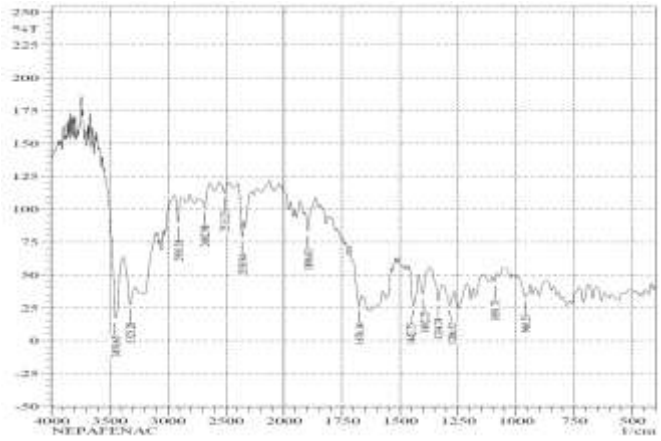

Figure 3: FTIR spectrum of Nepafenac

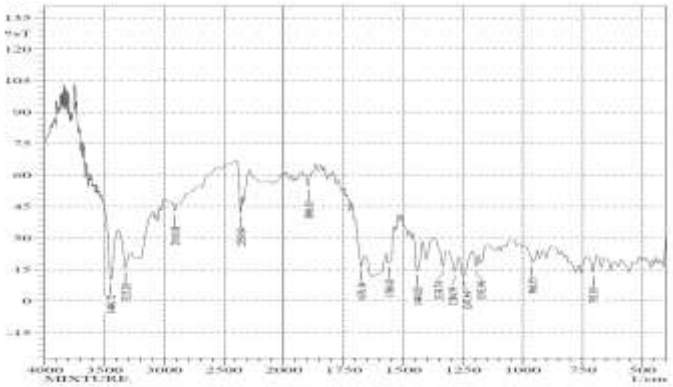

Figure 4: FTIR spectra of Nepafenac and excipients mixture

The formulations were prepared by using Gelrite and Hypromellose METHOCEL E 15 Premium LV in different concentration. 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 6.9 to 7.8. 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 Hypromellose METHOCEL E 15 Premium LV 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 6 using Brookfield viscometer and Rheometer before and after gelling respectively.

Table 6: Viscosity of ophthalmic solutions and preformed gels

Sr. No.	Formulation code	Viscosity at NP (cps) (ophthalmic solutions) at 60 rpm	Viscosity at P(cps) (preformed gels) at 60 rpm
1	F1	22.21	890
2	F2	39.20	920
3	F3	48.20	970
4	F4	35.12	1054
5	F5	47.07	1076
6	F6	56.27	1024
7	F7	40.23	1120
8	F8	59.67	1160
9	F9	70.21	1100

The graph of shear rate verses shear stress, as shown in figure 5 and 6, were obtained from Rheometer for ophthalmic solution as well as preformed ophthalmic gel in ATF.

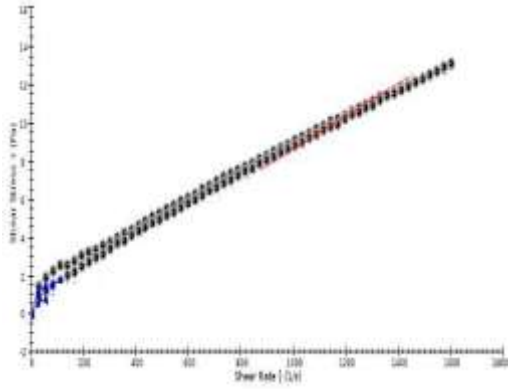

Figure 5: Newtonian flow of ophthalmic solution (F_4)

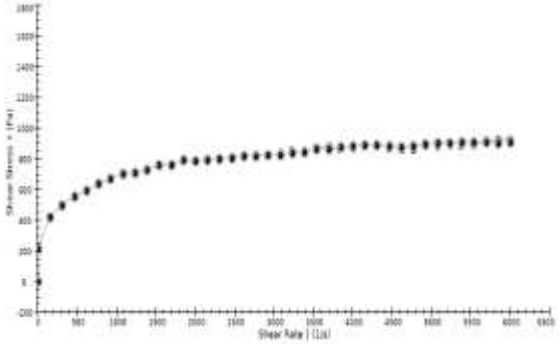

Figure 6: Pseudoplastic behavior of preformed gel in ATF (F_4)

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 7.

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 97.60 to 101.10% of labeled content.

Table 7: Gelling capacity of ophthalmic formulation

Sr. No.	Formulation code	Gelrite (% w/v)	Hypromellose METHOCEL E 15 Premium LV (% w/v)	Gelling ability
1	F1	0.4	1.0	+
2	F2	0.4	1.5	+
3	F3	0.4	2.0	++
4	F4	0.5	1.0	+
5	F5	0.5	1.5	++
6	F6	0.5	2.0	++
7	F7	0.6	1.0	++
8	F8	0.6	1.5	+++
9	F9	0.6	2.0	+++

In vitro release through cellophane membrane revealed that with the increase in the concentration of Hypromellose METHOCEL E 15 Premium LV 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. Zero order plots for all the formulations were found to be linear. The regression coefficients predict that the release from the formulations best fit the Zero order plots. Hence it can be concluded that all the drug release from all the formulations follow the Zero order kinetics.

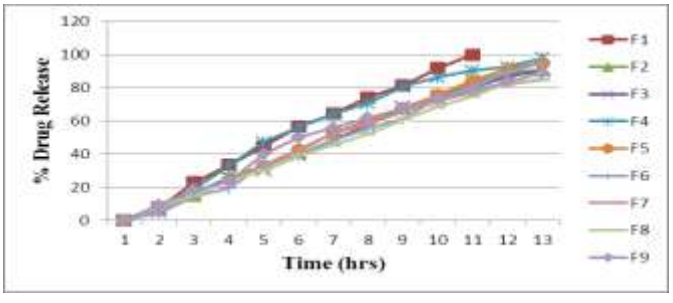

Figure 7: Diffusion of Nepafenac from F1 to F9

The drug release data was also plotted for Korsmeyer Peppa's model. These plots were also linear and the slope values (n) were more than 0.5 indicating that the drug release is by Non-Fickian diffusion. The highest release of 98.003% was shown by formulation F4.

The 3^2 full factorial design was selected to study the effect of independent variables gelrite (X1) and Hypromellose METHOCEL E 15 Premium LV (X2) on dependent variables % release and viscosity (At NP and P). The % release and viscosity 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 negative coefficient of variable X2 i.e. Hypromellose METHOCEL E 15 Premium LV 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 Hypromellose METHOCEL E 15 Premium LV leads to increased viscosity value. Similarly, the variable X1 showed positive coefficient for both responses i.e. release and viscosity.

STATISTICAL DESIGN AND ANALYSIS

Prepared formulations were evaluated in a randomized order for %Drug release, and viscosities at nonphysiological and physiological conditions. Analysis of variance (ANOVA) was applied for testing the significance, P value <0.05 indicated that the assumed regression model was significant and valid for the examined responses.

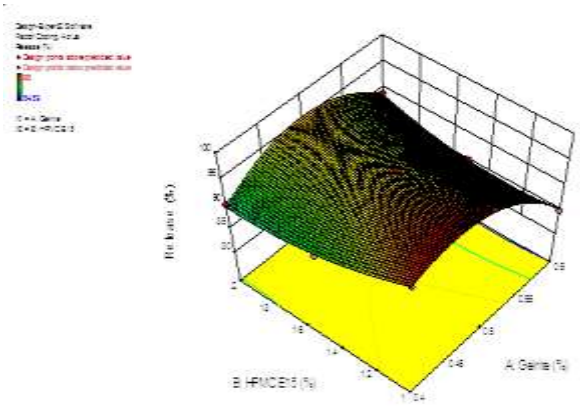

Figure 8: Response surface plot of drug release

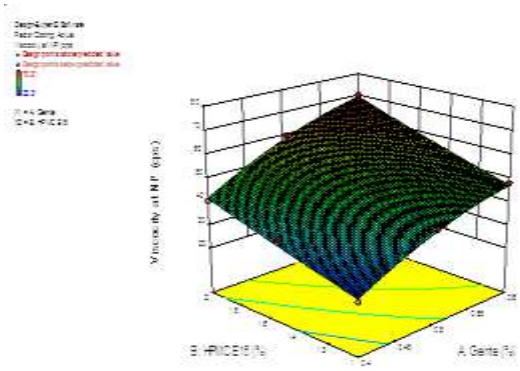

Figure 9: Response surface plot of viscosity at NP

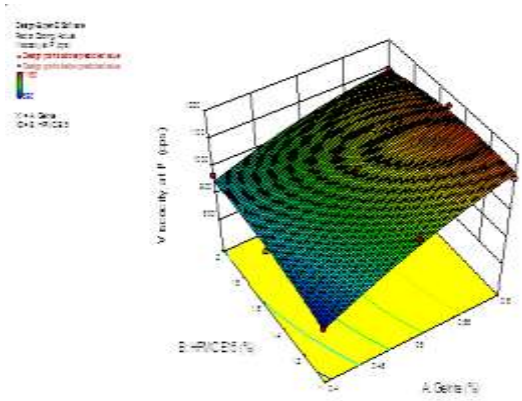

Figure 10: Response surface plot of viscosity at P

Establishing Design Space and Control Strategy

In general, the knowledge space within the QbD approach represents the whole range of interactions between critical parameters and their effects on CQAs that has been examined during process characterization studies. Whereas, "design space" is space within which desired quality of product can be built. Regulatory point of view changes within design space are not considered as changes, but changes outside design space would normally initiate regulatory post approval process. Concentration of polymer (Gelrite and Hypromellose METHOCEL E 15 Premium LV) were found to be critical on responses Drug release and viscosities at nonphysiological and physiological conditions. The variables ranked as high risk in the initial risk assessment are included in the control strategy. Based on the requirement of product quality the criteria considered for responses were minimum of 85% drug release in 12 hrs, 22.21 and 1160 cps viscosity at non physiological and physiological conditions respectively. This study leads to the design space from multidimensional combination of Gelrite and Hypromellose METHOCEL E 15 Premium LV to the acceptable operating ranges for formulating ophthalmic *in situ* gel with respect to target product profile. When critical variables operated within the established design space compliance to CQAs

would be assured. Design space shown in figure also called as overlay plot which is shaded region with yellow color indicates that region of successful operating ranges.

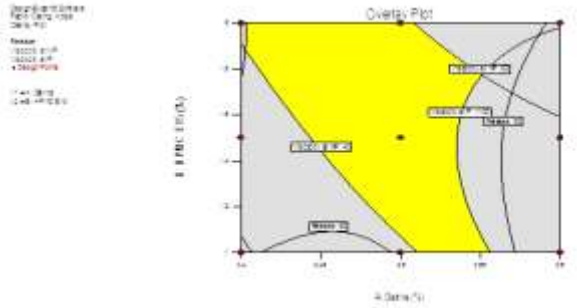

Figure 11: Design space

Validation of optimized formulations

The composition of the checkpoints, the predicted and experimental values of all the response variables drug release and viscosities at nonphysiological and physiological conditions (At NP and P) were as shown in table 9. This indicates statistical equivalence between experimental and predicted values, demonstrating the validity of the selected formulation variables, their levels and applied 3^2 factorial design to conduct design of experimentation. We could conclude that, if we keep the selected parameters within design space we would be able to achieve desired QTPP for Nepafenac ophthalmic in situ gels.

Table 8: Formulation for model validation

Sr. No.	Ingredients (% w/v)	MV1
1	Nepafenac	0.1
2	SBE-β-cyclodextrin	2.5
3	Gelrite (X_1)	0.5
4	Hypromellose METHOCEL E 15 Premium LV (X_2)	1
5	Benzalkonium chloride	0.01
6	Mannitol	4.3
7	Boric acid	0.3
8	Water for injection	Q.S to 100mL

Table 9: Comparison of predicted and experimental values of MV1

Responses	MV1	
	Predicted	Experimental
Drug Release (%)	97.673	96.90
Viscosity at NP(cps)	38.719	39.10
Viscosity at P(cps)	1036.899	1049.20

CONCLUSION

It can be concluded that QbD approach can be successfully implemented to see the effect of formulation parameters on in situ gel formulation with predictable % Drug release and viscosities at nonphysiological and physiological conditions. All critical parameters ranked as high risk in the initial risk assessment were included in the design of experimentation. Amount of Gelrite and Hypromellose METHOCEL E 15 Premium LV were identified as critical parameters to achieve desired QTPP. Based on selection criteria, 3^2 factorial design (RSM) was employed to conduct design of experimentation. Polynomial equations, ANOVA, different statistical values were utilized to interpret significance of formulation parameters on responses and design space was proposed with desired QTPP. From the experiments, it can be concluded that if formulation parameters were operated within the proposed design space, high risk can be lowered to low level of risk. From this study it can be concluded that formulation prepared within design space can produce formulation with acceptable *in vitro* drug release and viscosities at nonphysiological and physiological conditions.

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