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FORMULATION AND EVALUATION OF GELRITE BASED PHASE CHANGE OPHTHALMIC SOLUTION OF NEPAFENAC- As QBD APPROACH

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ABSTRACT

The present investigation was focused on application of QbD approach to seethe effect of formulation variables on *in situ* ophthalmic gel containing a newer NSAID drug, nepafenac. Riskassessment of critical material and process parameters are linked to criticalquality attributes (CQAs) of the product with respect to obtain target qualityproduct profile (TQPP). The effects of critical parameters (concentration of Gelrite, Hypromellose METHOCEL E 15 LV) were investigated by executing design ofexperimentation (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 andestimate the effect of important parameters and establish their relationshipwith 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) anddesired product quality was achieved with the formulation prepared in theregion of design space.3D response graph and overlay plots were successfullyimplemented to interpret effects and selection of significant parameters onCQAs. 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] *Insitu*forming gels are liquid upon instillation and undergo phasetransition in the ocular cul-de-sac to form viscoelastic gel and this provides a response toenvironmental changes.[4]

The application of quality-by-design (QbD) approachin formulation development has provided anopportunity for a harmonized pharmaceutical qualitysystem based on continuous quality improvementwhich can yield safer, more efficacious product. Designof experimentation, selection of appropriate model isimportant and criteria for selection can vary based onnumber and type of factors, number of levels for factor,type of study, time and cost for experiments. In thispaper, we used QbD approach for better understandingof relationship of critical formulation and processparameters to CQAs relating to quality product profileof in situ ophthalmic gel of nepafenac. Formulations were prepared using Gelrite (gellan gum) and Hypromellose METHOCEL E15LV. Based on risk assessment understanding for formulations, high risk variables were selected and 3^2 factorialdesigns was

employed for design of experimentation. Formulations were evaluated for *invitro* 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 understandcorrelation and significance of critical parameters on QTPP. Based on effects of critical formulation variableson QTPP, proposed design space to obtain robustformulation.[23-25]

MATERIALS AND METHOD

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

All other reagents and chemicals used ofanalytical grade.

Risk assessment of Critical material and processattributes

Risk assessment for the experiment was carried out bybasic risk management facilitation. Polymer concentration (Gelrite and Hypromellose METHOCEL E15 LV) were considered for design of experimentation of theNepafenacin 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 of0.1% Nepafenac were formulated using Gelrite, Hydroxypropyl methylcellulose (Hypromellose 2910 /or Hypromellose METHOCEL E 15 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 tearfluid (ATF) and viscosity to optimize the concentration of gelrite and Hypromellose METHOCEL E 15 LV for final formulation, as pertable 1.

Table 1: Formulation of preliminary batches and their evaluation.

Sr. No.	Batch Code	Gelrite (%)	METHOCEL E15 LV (%)	Gelling capacity	Viscosity (cps)
1	P1	-	0.8	No gelation	18.14
2	P2	-	1.0	No gelation	28.95
3	P3	-	2.0	No gelation	34.70
4	P4	-	3.0	No gelation	45.25
5	P5	-	4.0	No gelation	77.20
6	P6	0.2	0.6	-	20.45
7	P7	0.4	0.8	+	33.25
8	P8	0.5	1.0	++	42.88
9	P9	0.6	1.5	+++	51.22
10	P10	0.8	2.0	viscous liquid	110.20
11	P11	1.0	2.5	Highly viscous liquid	226.13
12	P12	1.2	3.0	Direct gelling	350.80
13	P13	1.4	3.5	Direct gelling	482.20
14	P14	1.6	4.0	Direct gelling	556.80

The formulations were graded as per follows:

Table 2: Grades And Inference.

Grade	Inference
-	No Gelation
+	Gels after few seconds, dissolves rapidly (within 1-2 hr)
++	Gelation immediately, remains for few hours (3-4 hr)
+++	Gelation immediately, remains for extended period (more than 6-8 hr)

The above preliminary batches indicated that:

1) HPMC alone does not possess any *in situ* gelling properties (P1 to P5).
2) Gelrite above the concentration of 0.4% forms *in situ* gel in artificial tear fluid (P6 to P9).
3) Gelrite above 1.0% concentration produce formulation of high viscosity that is not suitable for instillation into eyes (P11 to S14).

The formulations showed in vitro gelation above the concentration of 0.4% Gelrite and no gelation observed below 0.4%.of Gelrite. The increase in concentration of Gelrite above 0.6 then increased the viscosity of the formulation making it difficult to instill into the eyes. The Gelrite produced direct gelling above the concentration of 1.0%.

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 Hypromellose METHOCEL E 15 LV(X1) and Gelrite (X2) and the dependent variables were release at 4 hr (Y1) Release at

10hr (Y2) and Release at 12hr (Y3), viscosity at NP (Y4) and viscosity at P (Y5). The factorial designed batches are shown in table 5

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 waterfollowed 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 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 3: Concentration Ranges of Selected Variables.

HPMC E15	1.0 to 2.0 %
Gellan gum (Gelrite)	0.5 to 0.7 %

Table 4: Variables in Optimization Study.

Variables	**Factor**
Independent	
X1	HPMC E15LV
X2	Gellan gum (Gelrite)
Dependent	
Y1	Release at 4 hr
Y2	Release at 10 hr
Y3	Release at 12 hr
Y4	Viscosity at NP
Y5	Viscosity at P

Depending upon these ranges the nine formulations were formulated as per experimental design Table 7.

Table 5: Experimental Design as per 3^2 Factorial Design.

Formulation Code	Coded Values	
	X_1	X_2
F1	-1	-1
F2	0	-1
F3	+1	-1
F4	-1	0
F5	0	0
F6	+1	0
F7	-1	+1
F8	0	+1
F9	+1	+1

Table 6 Translational Coded Factor Level.

Coded Values	Actual Values (%)	
	X_1 (HPMC E15LV)	X_2 (Gellan gum)
-1	1.0	0.5
0	1.5	0.6
+1	2.0	0.7

Table 7: 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
Hypromellose	1.0	1.5	2	1	1.5	2	1	1.5	2
Gelrite	0.5	0.5	0.5	0.6	0.6	0.6	0.7	0.7	0.7
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
NaoH/Hc l (0.1N)	Q.s to pH 7.00	Q.s to pH 7.00	Q.s to pH 7.00	Q.s to pH 7.00	Q.s to pH 7.00	Q.s to pH 7.00	Q.s to pH 7.00	Q.s to pH 7.00	Q.s to pH 7.00
Water for injection	q.s to 100 mL	q.s to 100 mL	q.s to 100 mL	q.s to 100 mL	q.s to 100 mL	q.s to 100 mL	q.s to 100 mL	q.s to 100 mL	q.s to 100 mL

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 thepresence of turbidity in the inoculated media. Both positive and

negative controls were also maintainedsimultaneously. The method of detection was visual inspection of turbidity. The test for gelling ability wasconducted using artificial tear fluid (ATF). The transition of solution to viscous gel was observed visually andnumerical scores were assigned depending upon the quickness of gel formation and time taken for collapse of gelstructure on shaking the vials. The drug content was determined spectrophotometrically. The viscosity andrheological 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 testingapparatus, figure1. The glass cylinder was attached to the shaft of USP apparatus I (Basket type) in place of basket.[28]

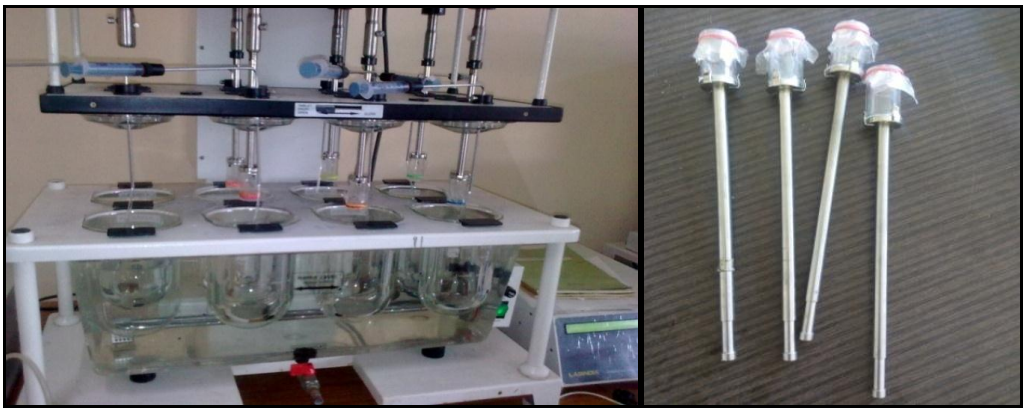

Figure 1: Dissolution apparatus with modified assembly.

The dissolution media was ATF (50 ml) maintained at 37 ± 0.5 °C. The sample (1 ml) was withdrawn at regularinterval of 1 hr for 12 hrs and was replaced immediately with the same volume of ATF. The samples withdrawnwere observed spectrophotometrically at 238 nm.

STABILITY STUDY

The formulation F5 was subjected to stability studies as per ICH guidelines. The formulations wereassessed for appearance, gelation ability, sterility, pH, drug content and viscosity.

COMPATIBILITY STUDY

Follows various compatibility studies was performed

- ✓ Selection of appropriate container
- ✓ Selection of appropriate plug or Nozzle with different drop size
- ✓ Selection of specific filters to perform the filtration of the product
- ✓ Selection of proper tubing's to transfer the product solution during filtration & filling

- ✓ The effect of Freeze thaw cycles
- ✓ The effect of Hold time study at Room Temp and 2-8°C

RESULTS AND DISCUSSION

Based on QbD approach, risk assessment was carriedand high risk parameters, based on their strongcorrelation to Critical Quality Attributes (CQAs) wereconsidered for Design of experimentation to ensure apredefined quality of the product. In order to definethe ‘‘design space’’ the critical formulation variables(independent variables) and the responses able tomeasure the product quality were defined based onprior knowledge and preliminary studies.

Table 8: Risk assessment of the drug product CQAs.

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

The independent variables considered for formulations are concentration of Gelrite (gellan gum) and Hypromellose METHOCEL E 15 LVsince they were considered critical indetermining responses i.e. % Drug release,viscosity at nonphysiological (NP) and viscosity at physiological (P) conditions. Basedon the nature of variables, number of formulationvariables, levels of variables, optimization study, toestimate the main as well as interactive effects ofvariable and minimum number of experimental trials, 3^2 factorial design with 9 runs was selected to see theeffect of formulation variables on nepafenacophthalmic 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 238nm.

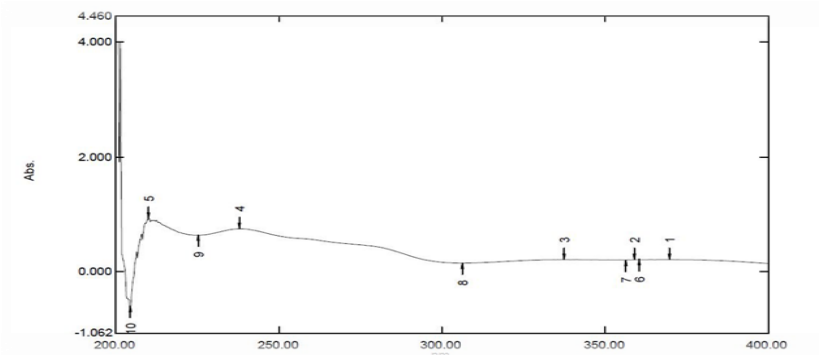

Figure 2: UV spectrum of Nepafenac.

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

Table 9: 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

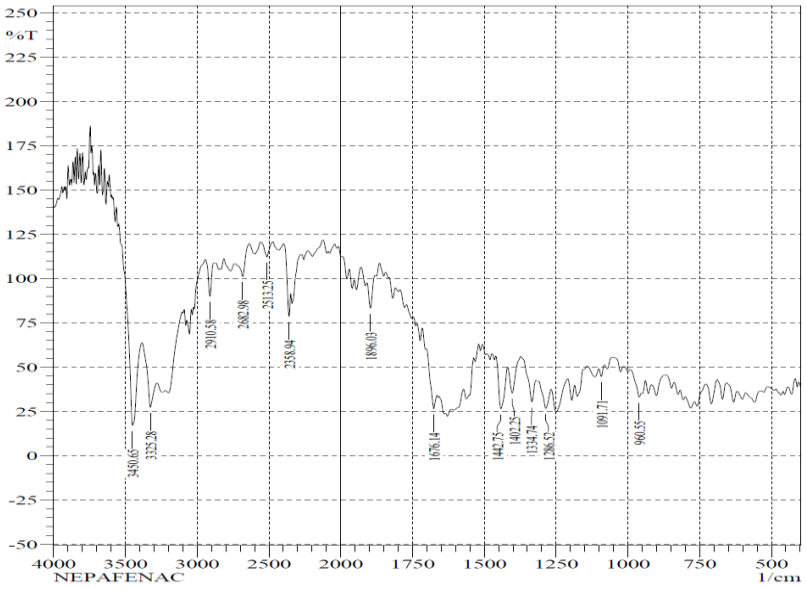

Figure 3: FTIR spectrum of Nepafenac.

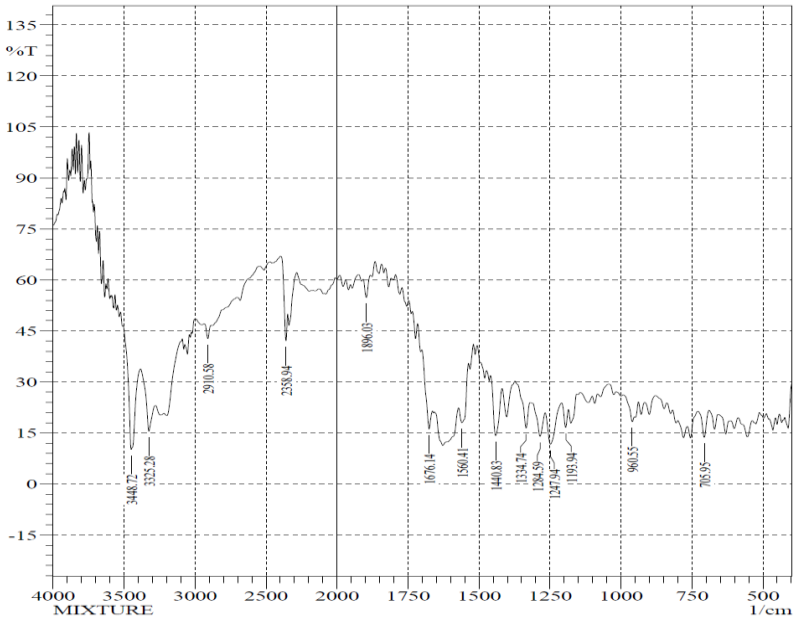

Figure 4: FTIR spectra of Nepafenac and excipients mixture.

The formulations were prepared byusing Gelrite and Hypromellose METHOCEL E 15 LVin different concentration. The formulated ophthalmic formulations were evaluated for various physical and performance characteristics. Theophthalmic formulations were observed carefully for color, odour and presence of suspended particulate matter ifany. The clarity of solutions was further assessed by observing them against a dark and white background asdescribed in the USP. The pH of all the formulations was found to be in the range of 6.9 to 7.4. Viscosity increasedwith the increase in the concentration of gelrite from 0.5 to 0.7%. Similarly viscosity increased with the increase in theconcentration of Hypromellose METHOCEL E 15 LVfor concentration from 1.0 to 2.0%. Increase in viscosity of ophthalmic solutionsafter instillation in eye was a desired feature for the purpose of sustaining therapeutics actions of sodium alginate byproviding increased pre-corneal residence time. The increase in viscosity was achieved due to the inclusion of gelritewhich undergoes gelation when it comes in contact of calcium or sodium ions of tear fluid. The test for sterility forthe 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 usingBrookfield viscometer and Rheometer before and after gelling respectively.

Table 10: 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	24	750
2	F2	42	910
3	F3	88	988
4	F4	33	889
5	F5	49	1020
6	F6	94	996
7	F7	37	920
8	F8	57	1150
9	F9	106	1200

The test for in vitro gelation ability was performed to assess the gel characteristics which would affect drug releasein the ATF. The numerical scores for gelling ability of solutions were found to vary with change in the concentrationof gelrite as shown in table 11.

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 contentdetermined spectrophotometrically was in the range of 97.60 to 101.10% of labeled content.

Table 11: Gelling capacity of ophthalmic formulation.

Sr. No.	Formulation code	Gelrite (% w/v)	Hypromellose METHOCEL E 15 LV(% w/v)	Gelling ability
1	F1	0.5	1.0	+
2	F2	0.5	1.5	+
3	F3	0.5	2.0	+
4	F4	0.6	1.0	++
5	F5	0.6	1.5	+++
6	F6	0.6	2.0	+++
7	F7	0.7	1.0	+++
8	F8	0.7	1.5	++++
9	F9	0.7	2.0	++++

The formulations were graded as follows

Grade	Inference
-	No Gelation
+	Gels after few seconds, dissolves rapidly (within 1-2 hr)
++	Gelation immediately, remains for few hours (3-4 hr)
+++	Gelation immediately, remains for extended period (more than 6-8 hr)
++++	Gelation immediately, remains for extended period (more than 12 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 time taken for gelation.

In vitro release through cellophane membrane revealed that with the increase in the concentration of Hypromellose METHOCEL E 15 LVthe release decreased due to the formation of gel structure. As the conc. of gelrite increased from 0.5% to 0.7% there was further retardation in the release This may be accounted for the reduction innumber 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 belinear. 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.

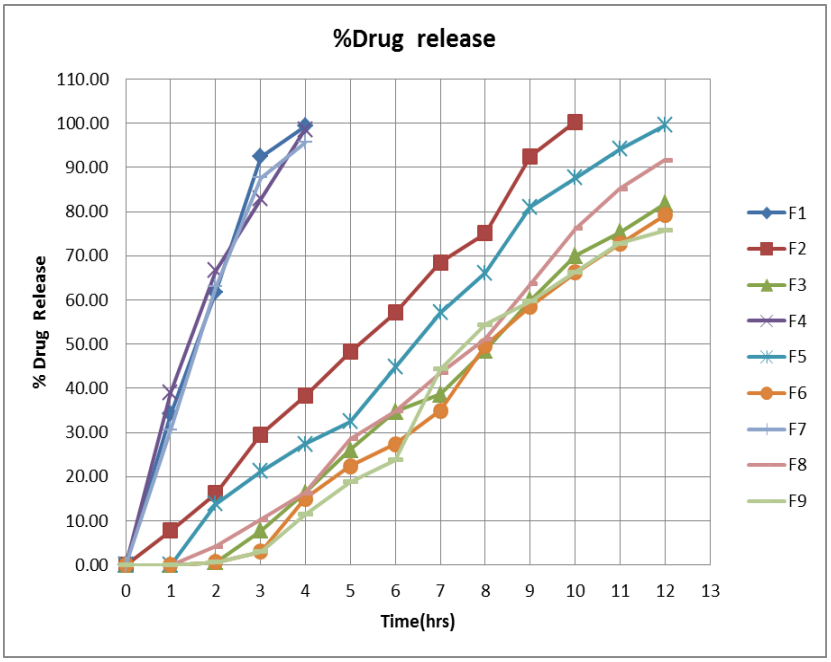

Figure 5: Diffusion of Nepafenac from F1 to F9.

The 3^2 full factorial design was selected to study the effect of independent variables Hypromellose METHOCEL E 15 LV(X1) and gelrite (X2) and on dependent variables % releaseandviscosity (At NP and P). The % release and viscosity values are strongly dependent onthe selected independent variables. The equation conveyed the basis to study of the effects of variables. Theregression coefficient values are the estimates of the model fitting. The r^2 was high indicating the adequate fitting ofthe quadratic model. The negative coefficient of variable X1 i.e. Hypromellose METHOCEL E 15 LVin case of response release indicates that as the HPMCconcentration was increased, release value decreased. However, the positive coefficient for viscosity shows oppositeeffect indicating that the increased concentration of Hypromellose METHOCEL E 15 L Vleads 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 arandomized order for %Drug release, and viscosities at nonphysiological and physiological conditions. Analysis of variance(ANOVA) was applied for testing the significance, Pvalue <0.05indicated that the assumed regressionmodel was significant and valid for the examinedresponses.

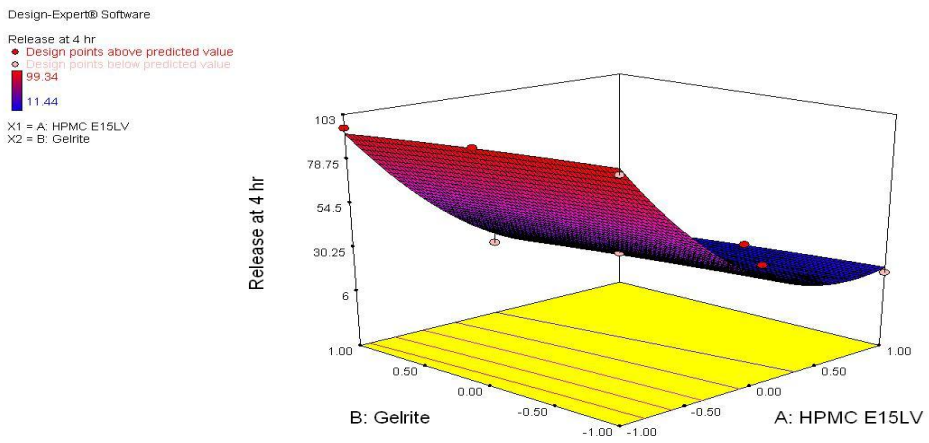

Figure 6: Response surface plot of drug release at 4 hr.

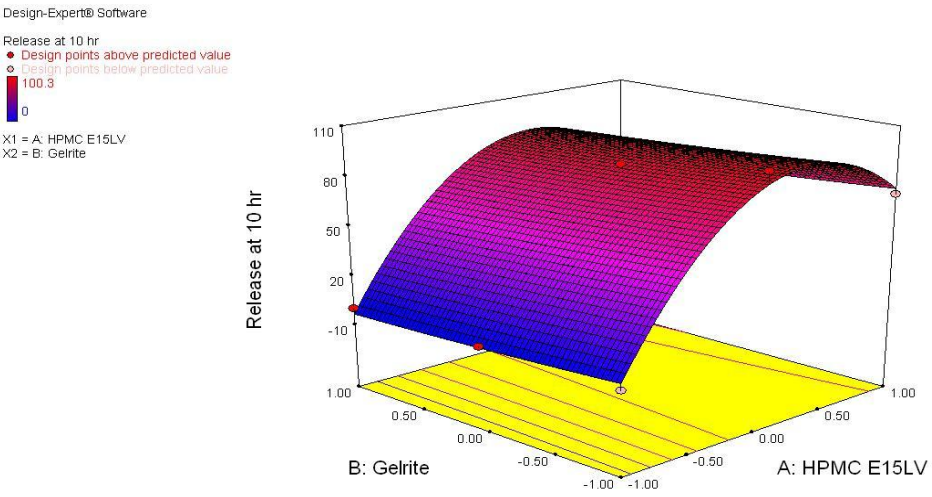

Figure 7: Response surface plot of drug release at 10 hr.

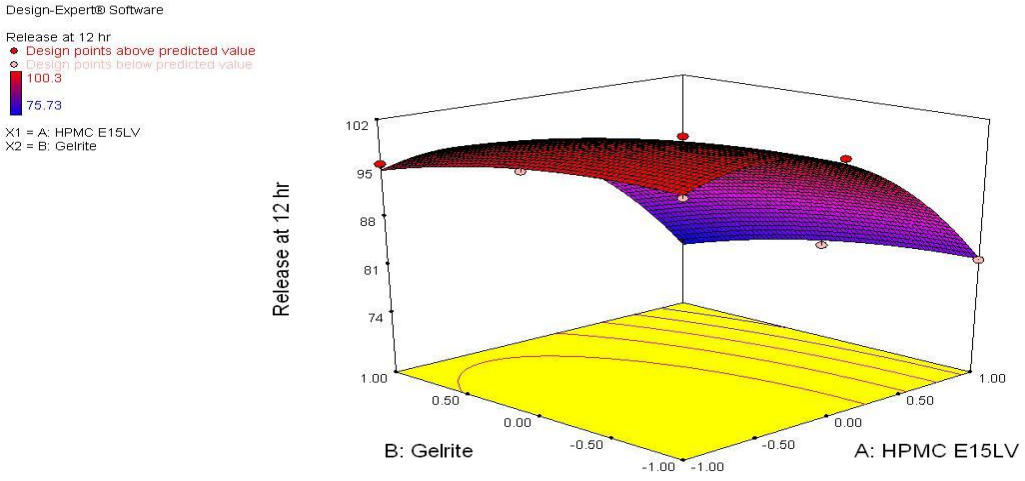

Figure 8: Response surface plot of drug release at 12 hr

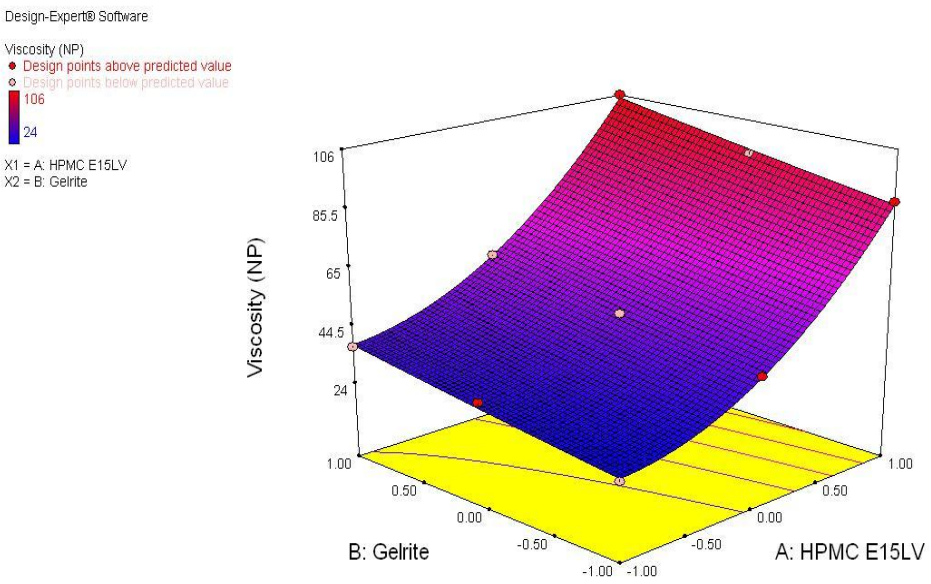

Figure 9: Contour plot of viscosity at NP.

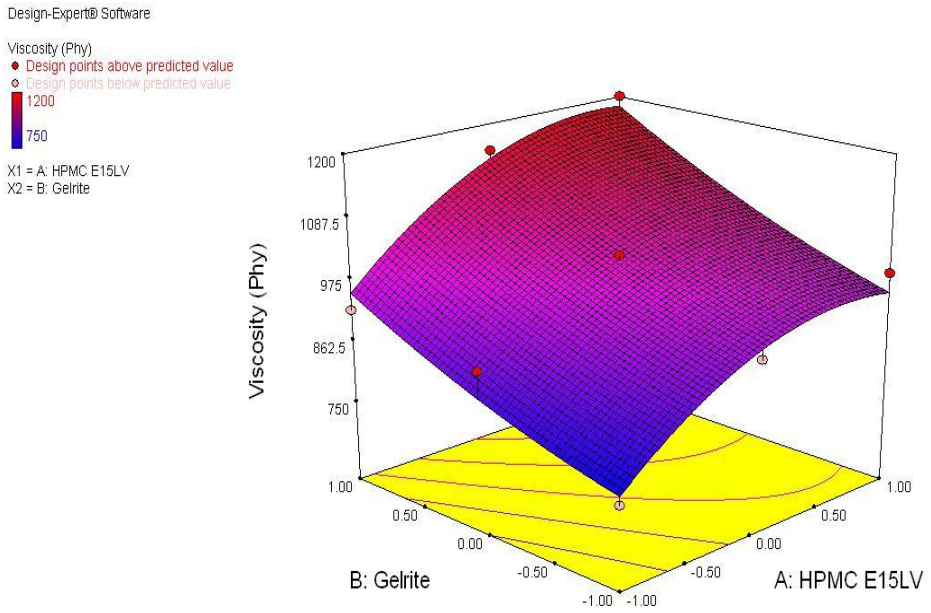

Figure10: Response surface plot of viscosity at P.

COMPATIBILITY STUDY WITH FINAL FORMULATION

Selection of Container

Table 12: Container compatibility results.

Sr No	Test	Results			
		ETO sterilized container		Gamma sterilized container	
		Initial	1M 40ºC/75%RH	Initial	1M 40ºC/75%RH
1	Description	pale yellow to yellowish solution free from extraneous matter	pale yellow to yellowish solution free from extraneous matter	pale yellow to yellowish solution free from extraneous matter	pale yellow to yellowish solution free from extraneous matter
2	pH	7.20	7.17	7.20	7.25
3	Osmolality	302	300	302	299
4	Density	0.976	0.978	0.976	0.979
5	viscosity	50	47	50	45
6	% drug content	99.66	99.06	99.66	86.34
7	% drug release	96.44	95.48	96.44	82.98

Discussion

The data shows that the product, Nepafenac Ophthalmic solution 0.1%, having loss or binding of drug in gamma sterilsed container in 1months 40ºC/75%RH, accordingly %drug release give 82.98%.

The ETO sterilsed container result are better than gamma sterilsed container Hence, we have decided to select the ETO sterilsed container for further batches of Nepafenac Ophthalmic solution 0.1%.

Selection of Plug or Nozzle

Our RLD sample having 30 micro liter of drop size, hence we were used different type of plug to produce desired.

Table 13: Plug drop size results.

Sr No	Test	Results			
		RLD	with 30 µL plug	with 45 µL plug	with 60 µL plug
1	Drop size	28µL	28 µL	43 µL	54 µL

Selection of Filter

Table 14A: Filter compatibility results.

Sr No	Test	Results				
		Filter Static Study				
			PVDF (Millipore)	PVDF (Pall)	PES (Millipore)	PES (Pall)
		Initial	10 hr sample	10 hr sample	10 hr sample	10 hr sample
1	Description	pale yellow to yellowish solution free from extraneous matter	pale yellow to yellowish solution free from extraneous matter	pale yellow to yellowish solution free from extraneous matter	pale yellow to yellowish solution free from extraneous matter	pale yellow to yellowish solution free from extraneous matter
2	Filter membrane physical test	White color with smooth texture	Slight yellow color with smooth texture	Dark yellow with shredding of filter with rough texture	Dark yellow with shredding of filter with rough texture	Dark yellow with shredding of filter with rough texture
3	pH	7.20	7.22	7.26	**7.54**	**7.60**
4	Density	0.976	0.979	0.999	0.979	0.987
5	viscosity	50	49	Not Analyzed	Not Analyzed	Not Analyzed
6	%drug content	99.66	98.94	Not Analyzed	Not Analyzed	Not Analyzed
7	% drug release	96.44	94.86	Not Analyzed	Not Analyzed	Not Analyzed

Table 14B: Filter compatibility results.

Sr No	Test	Results	
		Filter Dynamic Study	
			PVDF (Millipore)
		Initial	2hr circulation
1	Description	pale yellow to yellowish solution free from extraneous matter	pale yellow to yellowish solution free from extraneous matter
2	Filter membrane physical test	White color with smooth texture	Slight yellow color with smooth texture
3	pH	7.20	7.22
4	Density	0.976	0.979
5	viscosity	50	49
6	%drug content	99.66	96.78
7	% drug release	96.44	93.87

Discussion

The data shows that the product, Nepafenac Ophthalmic solution 0.1%, stored in 10 hr with different filter and with different supplier contact.

According to filter membrane physical observation, only Millipore supplied PVDF filter having no significant changes observed in 10 hr solution and 2hy circulation of rodcut contact and no drug binding observed, however other pall supplied PVDF and PES filter (Pall and Millipore) was not suitable with product.

Hence Millipore supplied PVDF filter result are better than other filter Hence, we have decided to select the Millipore supplied PVDF filter for further batches of Nepafenac Ophthalmic solution 0.1%.

Selection of Tubing's

Table 15: Tubings compatibility results

Sr No	Test	Results				
		Tubing Static and dynamic Study				
			TYGON 3350 SILICON		Pharma 50	
		Initial	10 hr sample static	2 hr Dynamic	10 hr sample static	2 hr Dynamic
1	Description	pale yellow to yellowish solution free from extraneous matter	pale yellow to yellowish solution free from extraneous matter	pale yellow to yellowish solution free from extraneous matter	pale yellow to yellowish solution free from extraneous matter	pale yellow to yellowish solution free from extraneous matter
2	pH	7.20	7.28	7.24	7.30	7.32
3	Density	0.976	0.999	0.998	0.979	0.987
4	viscosity	50	48	49	54	54
5	%drug content	99.66	97.98	96.90	**83.94**	**86.58**
6	% drug release	96.44	96.20	94.89	Not analyzed	Not analyzed

Discussion

The data shows that the product, Nepafenac Ophthalmic solution 0.1%, stored in 10 hr with different tubings and with different supplier contact.

According to above results of % drug content, only Tygon 3350 tubing having no significant changes observed in 10 hr solution and 2 hr circulation of product contact and no drug binding observed, however Pharma 50 tubing found adsorption of drug, hence it was not suitable with product.

Hence, we have decided to select the Tygon 3350 tubing for further batches of Nepafenac Ophthalmic solution 0.1%,

The effect of Freeze thaw cycles

Table 16: Freeze thaw cycles results.

Sr No	Test	Results			
		Freeze thaw cycles Study			
		Initial	First cycle	Second cycle	Third cycle
1	Description	pale yellow to yellowish solution free from extraneous matter	pale yellow to yellowish solution free from extraneous matter	pale yellow to yellowish solution free from extraneous matter	pale yellow to yellowish solution free from extraneous matter
2	pH	7.20	7.24	7.25	7.22
3	Density	0.976	0.989	0.971	0.970
4	viscosity	50	53	51	53
5	%drug content	99.66	98.58	98.33	96.78
6	% drug release	96.44	95.32	95.208	95.42

Discussion

The samples that had undergone the III rd freeze thaw cycle were analysed.

The results shown in Table 41 indicates that there was no significant difference observed in the values of chemical parameters like assay of drug and % drug release etc. after carrying out the freeze thaw study for the Nepafenac Ophthalmic solution 0.1%, Hence, these formulations in their immediate packs are able to withstand three freeze thaw cycles without getting affected product Appearance as well as quality

Hold Time Study

Table 17A: Hold Time study results.

Sr No	Test	Results		
		Unfiltered Hold Time Study		
		Initial	12 hrs At Room Temperature	12 hr At 2-8
1	Description	pale yellow to yellowish solution free from extraneous matter	pale yellow to yellowish solution free from extraneous matter	pale yellow to yellowish solution free from extraneous matter
2	pH	7.20	7.20	7.22
3	Density	0.990	0.990	0.993
4	viscosity	50	54	50
5	%drug content	99.42	99.18	98.34
6	% drug release	98.48	97.5	96.08

Table 17: B Hold Time study results.

Sr No	Test	Results		
		Filtered Hold Time Study		
		Initial	12 hrs At Room Temperature	12 hr At 2-8
1	Description	pale yellow to yellowish solution free from extraneous matter	pale yellow to yellowish solution free from extraneous matter	pale yellow to yellowish solution free from extraneous matter
2	pH	7.20	7.24	7.25
3	Density	0.976	0.989	0.971
4	viscosity	50	52	50
5	%drug content	98.10	97.98	98.10
6	% drug release	96.42	95.16	93.98

DISCUSSION

The data shown in Table 42a,42b shows that the 12 Hrs hold does not affect the chemical stability of the product at 2-8°C as well room temperature Hence, it is concluded that the product can be held for unforeseen reasons maximum up to 12 hrs only at 2-8°C or room temperature.

CONCLUSION

It can be concluded that QbD approach can besuccessfully 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 rankedas high risk in the initial risk assessment were includedin the design of experimentation. Amount of Gelrite and Hypromellose METHOCEL E 15 LVwere identified as criticalparameters to achieve desired QTPP. Based onselection criteria, 3^2 factorial design (RSM) wasemployed to conduct design of experimentation.Polynomial equations, ANOVA, different statisticalvalues were utilized to interpret significance offormulation parameters on responses and designspace was proposed with desired QTPP. From theexperiments, it can be concluded that if formulationparameters were operated within the proposed designspace, high risk can be lowered to low level of risk.From this study it can be concluded that formulationprepared within design space can produce formulationwith acceptable *invitro* drug release and viscosities at nonphysiological and physiological conditions.

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