Original Research Article **Novel drug delivery system**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

A 3^2 FULL FACTORIAL DESIGN FOR OPTIMIZING THE OCULAR RETENTION OF BROMFENAC SODIUM USING THERMO SENSITIVE *IN SITU* GEL

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ABSTRACT

The main aim of the present work is to formulate and evaluate anophthalmic drug delivery system of bromfenac sodium using suitable gelling polymers by cooling technique. For elucidating the effect of formulation factors of press coated tablets a 3^2 full factorial design was employed. The effect of two factors, amount of gelling agent (X_1) and amount of viscosity enhancer (X_2) as independent variables were studied on dependent variables Y_1 (gelling capaciy), Y_2(gelation temperature) and Y_3 (Cumulative % drug release). The prepared *in situ* gelwere evaluated for clarity, pH, gelling capacity, gelation temperature, viscosity, drug content, *in vitro* drug release and kinetics studies.All the formulations were clear, transparent and yellow.The pH of the formulations ranges from 7.01-7.34 which is an acceptable range when compared with the eye pH of 7.4.Gelation temperature was between 37-43.5°C for all the formulations.The gelling capacity of the formulations was found to be between 4-10 hr. All the formulations follow pseudo plastic behaviour (shear thinning systems). This property is very useful during the inter blinking of the eye. A shear thinning ophthalmic system does not cause any inconvenience to the patient.Drug content of all the formulations was in between 95.13-98.55 %.*In vitro* drug release of the formulations was decreased when the amount of gelling agent and viscosity enhancer was increased.The optimum formulation consisting of bromfenac (90mg), poloxamer 188 (15g) and HPMC E5 (1.75g) exhibited gelling capacity of 7.12 h, gelling temperature of 37.02 °C and cumulative % drug release of 98.19 % in 12 h. Bromfenac sodium *in situ* gel exhibited biphasic release profile obeying zero order release kinetics following non-Fickian diffusion mechanism.

KEYWORDS: ***Bromfenac sodium, Factorial design, In situ gel, Ocular retention, Rheology, thermo-sensitive.***

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Received on: 16-06-2017
Revised and Accepted on: 05-10-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.4.p200-211

INTRODUCTION

Eye is a unique interesting organ with respect to its drug disposition characteristics. The ocular inflammatory disease is frequently occurring disorder may be due to natural cause or in ophthalmic therapy especially after cataract surgery[1,2]. Non-steroidal anti-inflammatory drugs (NSAIDs) are mostly targeted for topical route used in the management and prevention of ocular inflammation and cystoid macular edema (CME) and the maintenance of mydriasis during cataract surgery.[3,4] Though steroids are considered as the major scope in the treatment of eye diseases since a very long time, their usage has come into a run since two decades.[5-9] Despite of their ability to treat ocular diseases, the steroids have many disadvantages like the decreased immunological response to infection, cataract formation, steroid induced raised intraocular pressure (IOP) and inhibition of re-epithelisation following epithelial denudation.[10] Ocular drug delivery is a suggested route for NSAIDs used in the treatment of ophthalmic diseases as their oral administration may result in gastrointestinal disturbances.[11] A significant challenge to the formulator is to protect the barriers of the eye without causing permanent tissue damage. Conventional ophthalmic formulations like solutions, suspensions, and ointments have many disadvantages which result in to poor bioavailability of drug in the ocular cavity. Accordingly, a therapeutic system could be designed achieving an optimal concentration of drug at the active site for an appropriate duration.[12]A successful design of a drug delivery system requires an integrated knowledge of the drug molecule and the constraints offered by the ocular route of administration.[13]The various approaches that have been attempted to increase the bioavailability and the duration of the therapeutic action of ocular drugs can be divided into two categories. The primarytarget is to employ either sustained drug delivery systems such as erodible/non-erodible inserts, liposomes, and micro/nano-carrier systems or to maximize corneal drug absorption and minimizing the precorneal drug loss. Inorderto optimize the ophthalmic drug delivery, it is indispensable to prolong the precorneal drug retentionby exploiting various sustained release polymers or development of *in situ* gel or colloidal suspension on account of increased cost of aforementioned modified drug delivery systems. Bromfenac sodium is a non-steroidal anti-inflammatory drug (NSAID) having lower t½, poor bioavailability and solubility, which are unable to reach the therapeutic concentration, sustained release formulations are designed. The conventional eye drops of bromfenac have been commercialized as NSAID for the management of postoperative ocular pain and inflammation. The maximum proposed dose of one drop to each eye is required to be administered at a dose of 0.09 mg 4-5 times a day after the cataract surgery.Generally only 5% of the installed dose is being absorbed and the remaining will be drained out by the tear fluid. Several researchers have reported the applicability of *in situ* ophthalmic gel systems in overcoming the drawbacks of conventional ophthalmic dosage forms.[14-20] Sustained drug delivery can be provided by the use of temperature sensitive polymers that change from sol to gel at the temperature of the ocular cavity viz., pluronics, poloxamer, hydroxyl propyl methyl cellulose, hydroxyl ethyl cellulose, methyl cellulose, chitosan, carbopol and eudragit etc.[21-23]To improve the precorneal resident time and ocular bioavailability, it is desired to develop a twice-a-day thermo-sensitive ocular *in situ* gel of bromfenac sodium. The *in situ* formulation will have better patient compliance owing to reduced side effects caused by the application of sols, which up on contact will form the corresponding gels. Therefore in order to sustain its effect in the body and to decrease draining out by artificial tear fluid (ATF) a 3^2 full factorial experimentation[24] was designed for elucidating the effect of formulation factors on the thermo reversible gel. Thermo reversible ocular gels with good bioadhesion properties of bromfenac sodium were prepared by cold method using poloxamer and HPMC. HPMC undergoes gelation at higher temperature due to interaction between hydrophobic components of the poloxamer to from stable gels. The phase transition temperature of HPMC can be lowered to ~ 40 °C by adding buffering agents, which reduce the hydroxyl propyl molar substitution of HPMC. The effect of two factors, concentration of gelling agent, poloxamer (X_1) and concentration of viscosity enhancer, HPMC (X_2) as independent variables were studied on dependent variables Y_1 (gelling capacity), Y_2 (% gelation temperature) and Y_3 (% drug release). The prepared *in situ* gel was evaluated for clarity, pH, gelation temperature, gelling capacity, rheological properties, drug content, *in vitro* drug release and kinetic studies. The experimental values were cross validated with the predicted values of optimized formula and compared with marketed formulation.

MATERIALS AND METHODS

Bromfenac sodium was purchased from Sigma Aldrich, Mumbai. Poloxamer and HPMC E5 were obtained from Yarrow chemicals, Mumbai. sodium chloride, calcium chloride, sodium bicarbonate and citric acid were procured from Qualigens fine chemicals, Hyderabad. All other ingredients used in the present investigation are of HPLC and analytical grade and generally regarded as safe.

Drug-excipients compatibility studies using FT-IR

Drug-polymer interactions were studied by FTIR spectroscopy. The spectra were recorded for pure drug, pure polymers, and physical mixture of drug and polymers. The spectrum of sample was obtained by using FTIR- BRUKER. The procedure used was KBr disc method[25]. In this method, 100 mg of the sample was mixed with KBr and triturated in a mortar & pestle to ensure uniform mixing. This mixture was pressed into discs using hydraulic press at 7 tons pressure for 3 minutes. The pellet was placed in the light path and the spectrum was recorded in the range of 4000-500 cm^{-1}.

Formulation of in situocular gel of bromfenac sodium using 3^2 full factorial design

A 3^2 full factorial design was used for the development and optimization of the bromfenac sodium thermo sensitive ocular *in situ* gel, two factors were evaluated each at three levels and experimental trials were

performed using all the nine possible combinations and middle value was performed in quadruplicate. The concentration of gelling agent (X_1) and concentration of viscosity enhancer (X_2) were selected as independent variables. Gelling capacity (Y_1), gelling temperature (Y_2) and cumulative percent drug release (Y_3) were selected as dependant variables to optimize the response data[24]. Experimental range and levels of independent variables are represented in Table 1.

Table 1
Experimental range and levels of the independent variables in a 3^2 full factorial design

Run No.	Variable level in coded form	
	X_1	**X_2**
1	-1	-1
2	0	-1
3	+1	-1
4	-1	0
5	0	0
6	+1	0
7	-1	+1
8	0	+1
9	+1	+1
10	0	0
11	0	0
12	0	0
13	0	0
	Actual values	
Coded values	**X_1 (gelling agent, mg) Poloxamer**	**X_2 (viscosity enhancer, mg) HPMC**
-1	10	1.0
0	15	1.75
+1	20	2.5

The polynomial equation below given was used to study the effect of variables on different evaluation responses (Y), where the coefficients in the equation ($\beta_0, \beta_1, \beta_2, \beta_{12}$) were related to the effects and interactions of the factors.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1 X_1 + \beta_{22} X_2 X_2$$

Where, Y is the dependent variable, β_0 is the arithmetic mean response of the 9 runs, and β_1& β_2 are the estimated coefficients for the factors X_1& X_2 respectively. The main effect (X_1& X_2) represents the average result of changing one factor at a time from its low to high value. The interaction term (X_1X_2) shows how the response changes when two factors are changed simultaneously. The polynomial terms (X_1X_1, X_2X_2) are included to investigate nonlinearity. Design Expert (9.0.1 trial version) was used for ANOVA, multiple regression analysis (to obtain coefficient values in the equation), generate response surface plots and to optimize the data. A factor is considered to influence the response if the effects significantly differ from zero and the *p*-value is less than 0.05. Preparation of bromfenac sodium thermo sensitive gel: In the present investigation, *in situ gel*s were prepared by cooling technique (Figure 1).By using different concentrations of polymer and viscosity enhancer *in situ gels*were prepared as per statistical design[26]. Total 13 formulations were prepared by maintaining constant amount of drug (0.09g), citric acid (0.407g) and disodium hydrogen phosphate (1.125g). Accurately weighed amount of citric acid and disodium hydrogen phosphate were dissolved in 70 ml of distilled water.To this solution HPMC E5 was added in required quantities and dissolved to form a clear solution. Weighed amount of poloxamer 188 was taken and sprinkled in to the above solution. It was allowed to stand for overnight swelling in refrigerator. The drug solution was then added to the polymeric solution under constant magnetic stirring until a uniform solution was obtained.The final volume is made up to 100 ml and subjected to terminal sterilization by autoclaving at 121°C and 15 psi for 20 mins.

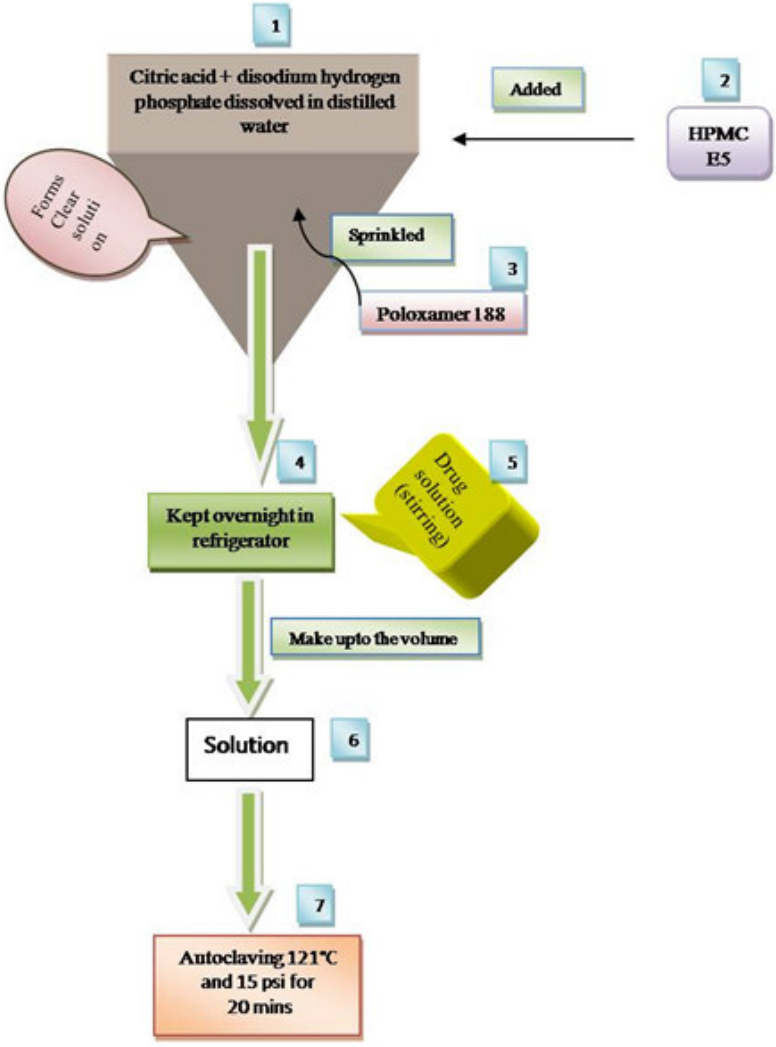

Figure1
Schematic procedure for the preparation of ocular in situ gel

Evaluation of bromfenac sodium in situ ocular gel[27]
The prepared ocular *in-situ* gel was evaluated for clarity, pH, gelation temperature, gelling capacity, rheological properties, drug content, *invitro* drug release and kinetic studies.

Clarity
All the prepared bromfenac sodium ocular *in situ* gels were visually inspected for colour and clarity.

Determination of pH
pH is one of the most important factors involved in the formulation process. Two areas of critical importance are the effects of pH on solubility and stability profile of the drug. The pH of ophthalmic formulation should be such that the formulation will be stable at that pH and at the same time there will be no irritation to the patient upon administration of the formulation. The pH of the prepared formulations was checked by using ElicopH meter.

Gelling capacity
Gelling capacity was determined by placing two drops of formulation in a test tube containing two ml of freshly prepared ATF solution. The time required for gelation and time taken for formed gel to dissolve was recorded at temperature 37±0.5°C. The same procedure was triplicated and recordings are noted.

Gelation temperature
An aliquot of one ml of the prepared formulation sample was taken in to a beaker containing two ml of ATF. The solution was heated at a rate of 2°C/min with continuous stirring. The temperature at which the gel was formed was noted. The same procedure was triplicated and recordings are noted.

Drug content
Uniform distribution of active ingredient is important to achieve dose uniformity. The drug content was determined by taking one ml of the prepared formulation and diluting it to 100 ml with distilled water. The concentration of bromfenac sodium was determined at 230 nm by using UV-Visible spectrophotometer. The same procedure was triplicate and recordings are noted.

Invitro drug release studies
Franz diffusion cellwas used for performing the *in vitro*drugrelease studies of thermo sensitive *insitu* gel. An aliquot of one ml of the prepared formulation was placed in the donor compartment and freshly prepared artificial tear fluid was placed in the receptor compartment. A dialysis membrane soaked overnight in

ATF was placed between the donor and receptor compartment. The whole assembly was placed on a thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37±0.5 °C. An aliquot of one ml of sample was withdrawn at predetermined time intervals, for every 1h until 6 hr and from then for every 2 hr up to 12 hr. The cell was replaced by the same volume of fresh ATF medium. The withdrawn samples were suitably diluted and analyzed at 230 nm using UV-Visible spectrophotometer. The same procedure was triplicated and recordings are noted[28].

Comparison of diffusion data

The analysis of drug release kinetics and mechanism from a pharmaceutical dosage form is an important but complicated process. By mathematical modeling, the release data was fitted into different kinetic models. According to model dependent approach, the order of drug release was described by zero order [29] or first order kinetics [30, 31]. The mechanism of drug release is studied by using Higuchi diffusion model[32]and Hixson-Crowell erosion model[33]. Korsmeyer-Peppas support the drug release mechanism for further judgment[34, 35]

Rheological studies

The viscosity was determined by using cone and plate viscometer. An aliquot of one ml of the prepared formulation was placed directly on a plate and cone was adjusted to the position. The instrument was adjusted to zero reading. The viscosity of all the prepared formulations was determined at different angular velocities from 10 to 100 rpm and 100 to 10 rpm. The same procedure was repeated by placing one drop of formulation in ATF medium. The viscosity of all the formulations was determined in ATF from 10 to 100 rpm and 100 to 10 rpm. Each viscosity estimation procedure was done in triplicate.

Data analysis, optimization and cross validation

STATISTICAL ANALYSIS OF THE DATA

Responses, Y_1 (gelling capacity), Y_2 (gelation temperature), Y_3 (cumulative percent drug release) were used for statistical analysis and optimization. Responses obtained from the all runs were simultaneously fitted to linear, interactive and quadratic models using the design expert software[36]. The software will select and suggest the highest order polynomial model as a suitable model based on coefficient of determination (R^2) and predicted residual sum of squares (PRESS) values where the additional terms are significant. Analysis of variance (ANOVA) was performed on the suggested model for the responses Y_1, Y_2 and $Y_3$to identify significant effect. Multiple regression analysis was performed on the dependent variables to know the significance of the regression coefficients on the model. A multi-criteria decision approach, numerical optimization technique (desirability) and graphical optimization technique (overlay plots) were employed to optimize the formulations with the desired responses (responses from theoretical profile values). Optimization was performed with constraints of gelling capacity (Y_1) = 7.1 hr, gelling temperature (Y_2) =37.02 °C and % drug release (Y_3) = 98.18 which were obtained from the theoretical profile. For finalizing the optimum formulation, targets were set for these constraints for getting respective desirability function response and overlay plots. The formulation with maximum desirability was chosen as the optimized formula and the model was further cross validated.

Cross validation of model

The chosen experimental design was validated by comparison of experimental optimized formulation with the optimized formulation as per the procedure described using predicted optimal independent values. Optimized formulation was evaluated for gelling capacity, gelling temperature and %drug release. The experimental values of the responses Y_1, Y_2 and Y_3 were determined for the optimized formulation. The percentage relative error between predicted values and experimental values of each response was calculated using the below equation.

$$\%\ Prediction error = \frac{Predicted value - Experimental value}{Predicted value} X\ 100$$

Comparison with marketed formulation

The marketed formulation of bromfenac (Megabrom®) was taken and drug release studies were performed in ATF at 37±0.5°C using Franz diffusion cell. The *in vitro* drug release profile of the optimized formulation (test) was compared with the theoretical release profile (reference) of marketed formulation.

RESULTS AND DISCUSSION

Drug-excipients compatibility studies using FTIR

The presence of incompatibility between drug and excipients result in the disappearance of original peaks of the active ingredient or a shift in its band position or appearance of new peaks in the mixture spectra. There was no disposition/disappearance in the FTIR spectra of bromfenac sodium in combination with HPMC E5 and poloxamer 188mixturesas observed in Figure 2. The characteristic Br, C=O stretch, aromatic C-H stretch, and primary amine in plane of bromfenac sodium observed at 529, 1717, 2966 and 3446cm^{-1} respectively, were not shifted/disappeared in the polymer mixtures; indicating the absence of solid-state interactions between drug and polymer matrix blend. The characteristic absorption bands of N-H and C=O stretch of bromfenac sodium observed at 3418 cm^{-1} and 2973 cm^{-1} showed no disposition/disappearance in the spectra of its polymer mixtures indicating the drug-excipients compatibility.

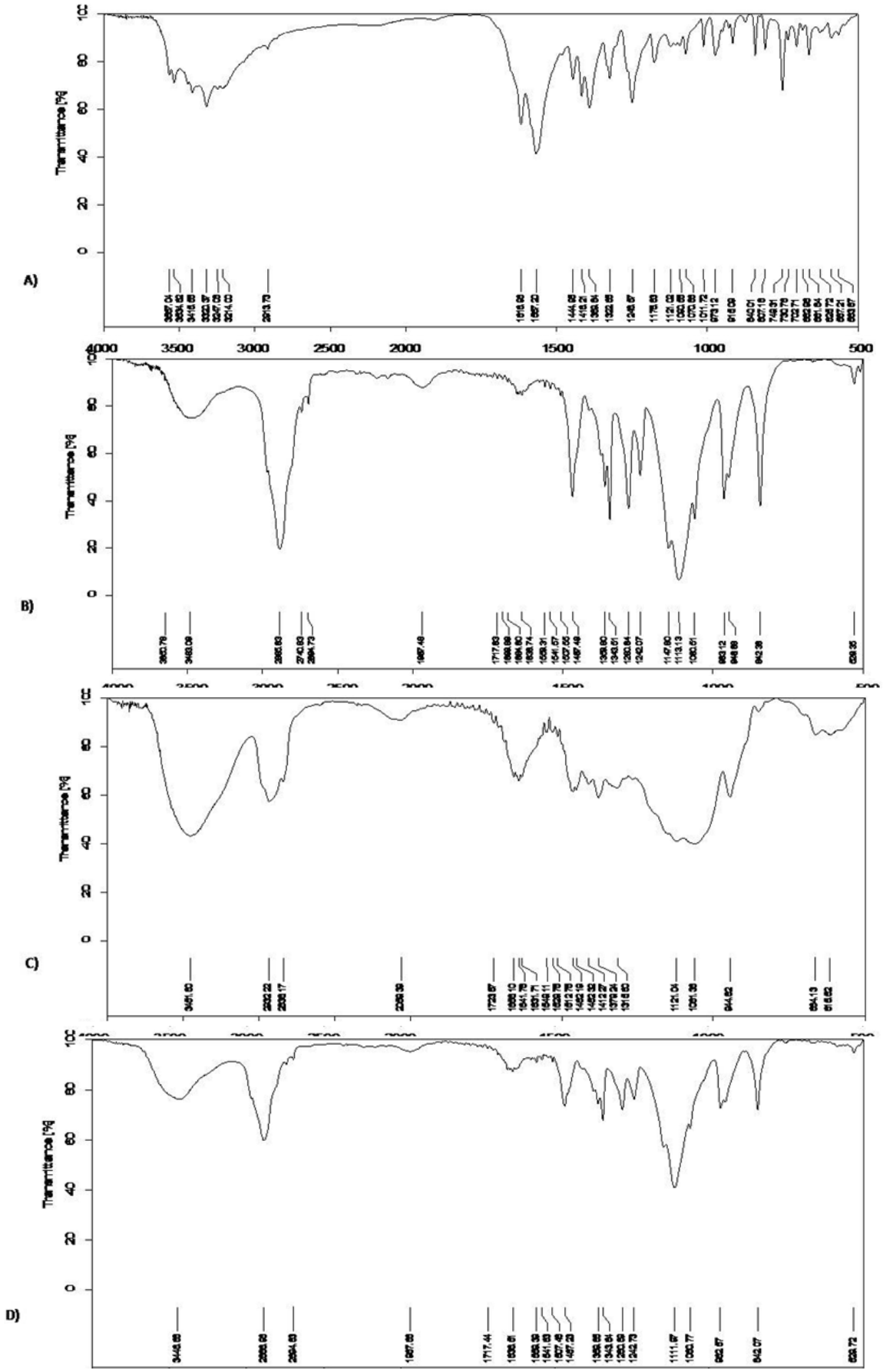

Figure 2
FTIR spectra of (A) bromfenac sodium, (B) poloxamer 188 (C) HPMC E5 and (D) physical mixture of drug and polymer

Evaluation of bromfenac sodium in situ gel

Clarity

Clarity of all formulations found was to be satisfactory. All the prepared formulations were clear, transparent and yellow in colour with good patient compliance.

pH of the formulations

pH of all the formulations was observed to be between 7.06 - 7.34.This pH was found to be within the acceptable range and hence it may be concluded that would not cause any irritation upon administration of the formulation.

Gelling capacity

Gelling capacity of the formulations was found to be between 4-10 hr. The two main prerequisites of gelling system are viscosity and gelling capacity (speed and extent of gelation). The formulation should have an optimum viscosity, which will allow its easy instillation into the eye as a liquid (drops), which will then undergo rapid sol to gel transition due tochange in temperature. Moreover, to facilitate sustained release of the drug to the ocular tissue, the *insitu*formed gel should preserve its integrity without dissolving or eroding for a prolonged period of time. All the formulations gelled instantaneously (less than a minute) on contact with ATF. By visual inspection, the formulations formed a translucent matrix on addition to ATF. Thisgelation may be induced due to change in temperature or pH.

Gelation temperature

It is observed that gelation temperature of the formulations is in the range 34-43.5°C. A range of 37 - 37.5°Cgelation temperature is preferred for the formulations to be installed in to the eye to avoid damage to the eye. The results are shown in Table 2.

Drug content

The drug content was found to be in acceptable range for all the formulations. Table 2 shows the result of percent drug content in all the 13 formulations within the range of 95.13 – 99.88%, indicating uniform distribution of drug.

In vitro drug release kinetics

The release of drug from these in situ gels was characterized by an initial phase of high release that is burst effect.The complete drug release from formulation F1, F2 & F3within 6 hr where as other formulations observed slow, gradual and sustained almost complete drug release until 12 hr. The biphasic pattern of release is a characteristic feature of matrix diffusion kinetics. The results showed that the formed gels had the ability to retain the drug for a period of 12 hr. It was found that the drug release of the formulations were in the range of 65.67 – 100.02%. The*in vitro* release profiles of formulations are shown in Figure 3.

Table 2
3^2full factorial design runs, their respective dependent responses and other parameters

F.Code	Factors		Responses			Other parameters	
	X_1 (Poloxomer, mg)	X_2 (HPMC, mg)	Y_1 (gelling capacity, hr)	Y_2 (gelling temperature, ^{0}C)	Y_3 (Drug releases at 12 hrs, %)	pH	Drug content (%)
1	10	1.00	4.30	43.5	100 (6^{th} hr)	7.14	98.55
2	15	1.00	3.55	42.5	100 (6^{th} hr)	7.29	98.00
3	20	1.00	5.25	41.5	100 (6^{th} hr)	7.34	98.55
4	10	1.75	6.23	37.0	96.63	7.32	99.80
5	15	1.75	7.23	37.0	98.70	7.34	95.13
6	20	1.75	6.27	36.5	92.30	7.12	98.28
7	10	2.50	10.21	34.0	88.57	7.16	95.59
8	15	2.50	10.22	33.5	74.23	7.06	95.56
9	20	2.50	10.00	34.0	65.15	7.18	95.66
10	15	1.75	7.29	37.5	96.00	7.18	95.14
11	15	1.75	7.42	37.0	99.39	7.09	95.14
12	15	1.75	7.30	36.5	98.93	7.32	95.14
13	15	1.75	7.15	37.0	100.02	7.27	95.13

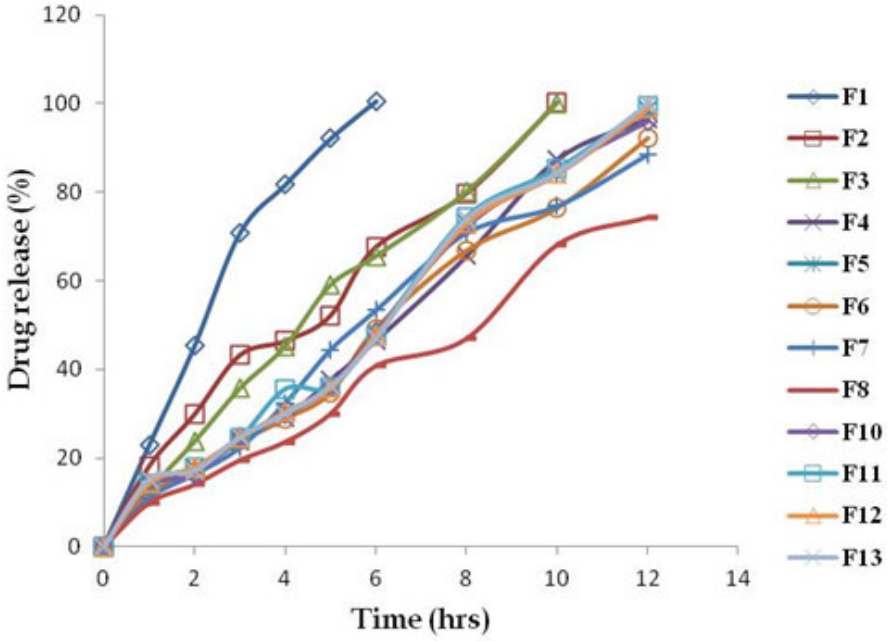

Figure 3
Drug release profiles of all prepared formulations

Rheological studies

The formulations exhibited pseudo plastic behaviour decrease in viscosity is mainly necessary as range of shear experienced during relative movement of eyelids.[37,38] From these studies it was observed that the exhibited pseudoplastic flow as evidence by decrease in viscosity with increasing in angular velocity. The viscosity was directly dependent on the polymeric content of the formulation. The viscosity increased with increasing concentration of poloxamer and HPMC E5. A plot was drawn between shear rate and viscosity shown in Figure 4. These studies confirmed sol to gel transition at physiological eye temperature.

Data analysis

In this 3^2 full factorial design of experiment, two factors ,amount of gelling agent(X_1) and amount of viscosity enhancer (X_2) were studied each, at three levels low, medium and high. By using this data 3 responses gelling capacity (Y_1), gelation temperature (Y_2) and 100 % drug release (Y_3) were selected for statistical optimization and fitted to linear, interactive and quadratic models. The summary of statistics are presented in Table 3, the comparative R^2, adjusted R^2, predicted R^2, PRESS, s.d., F-values and p-values were determined using the Design Expert. A suitable polynomial model for describing the data was selected based on coefficient of determination and PRESS values. Response Y_1 followed linear model, response Y_2 and Y_3 follows quadratic model. Hence these models were selected for further optimization because they showed higher R^2 and F-values and lower PRESS and p-values. These parameters were used to construct the independent variables on the response. The F-value for the responses, Y_1, Y_2 and Y_3 were found to be 71.75, 231.97 and 36.18 respectively, which indicated that the models were significant. The values of Prob> F (less than 0.05) for all the responses indicated the significance of the models.

Table 3
Summary results of regression analysis for selected responses

Model	R^2	Adjusted R^2	Predicted R^2	PRESS	s.d	F-value	p-value	Remarks
Response Y_1 – Gelling capacity (hr)								
Linear	0.9410	0.9279	0.8755	6.76	0.60	71.75	<0.0001	Suggested
Interactive	0.9457	0.9253	0.8063	10.51	0.61	0.69	0.4295	-
Quadratic	0.9549	0.9172	0.6571	18.61	0.64	0.61	0.5743	-
Response Y_2 – Gelation temperature (^{0}C)								
Linear	0.9522	0.9416	0.8925	12.83	0.80	89.64	<0.0001	-
Interactive	0.9606	0.9458	0.8241	21.01	0.71	1.70	0.2287	-
Quadratic	0.9949	0.9906	0.9683	3.79	0.32	19.98	0.0022	Suggested
Response Y_3 – Cumulative percent drug release at 12 hr (%)								
Linear	0.6894	0.6204	0.3444	966.63	7.13	7.13	0.0052	-
Interactive	0.7790	0.6961	0.2737	1070.8	6.38	3.24	0.1095	-
Quadratic	0.9679	0.9411	0.7348	390.96	2.81	17.64	0.0031	Suggested

The models generated were plotted to construct contour (2D) and response surface (3D) plots for Y_1, Y_2 and Y_3 responses of formulations to understand the main and the interaction effects of these two factors (Figure 5).

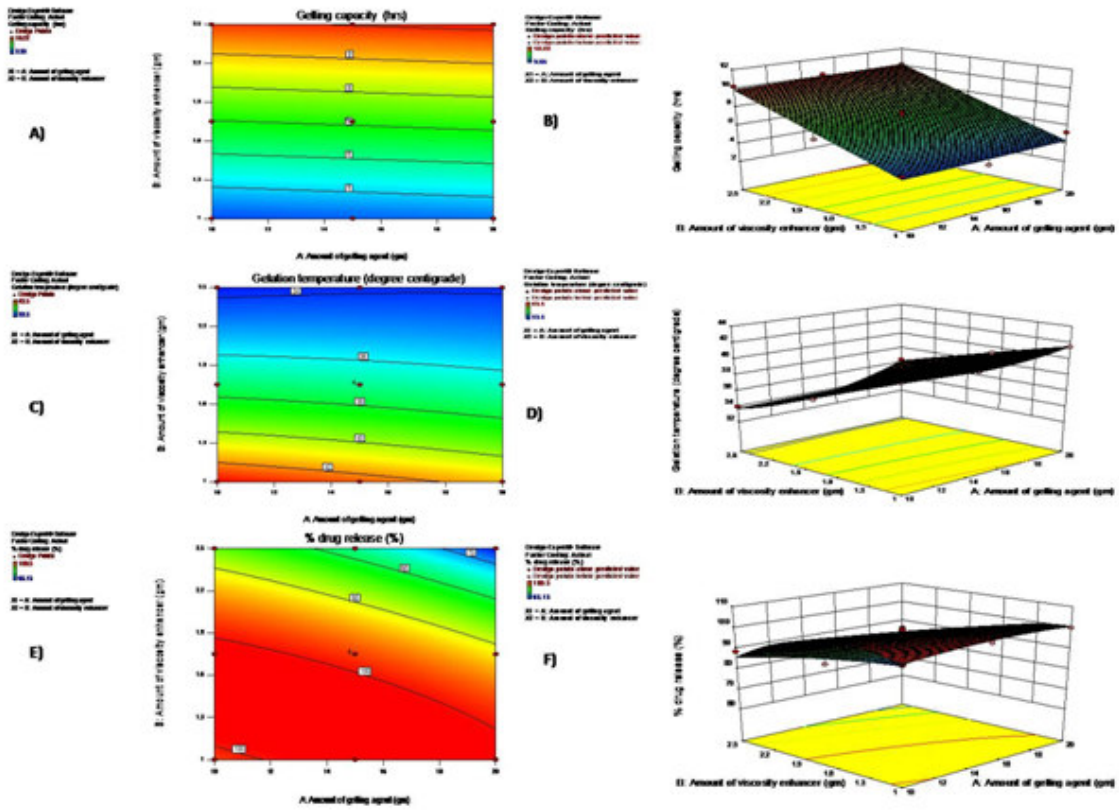

Figure 5
Response surface plots & Contour plots showing influence of X_1& X_2 on responses

The detailed summary of results of multiple regression analysis of dependant variables for all responses is shown in Table 3. The significant parameters in the equations can be selected using a stepwise forward and backward elimination for the calculation of regression analysis. However, in the present study full model having both significant and non-significant p-values were used for obtaining dependent variables. Coefficients with one factor indicate the effect of that particular factor, while the coefficients with more than one factor and those with second order terms represent the interaction between those factors and the quadratic nature of the phenomena, respectively. Positive sign of the term indicates positive (additive) effect, while negative sign indicates negative (antagonistic) effect of the factor on the response.[24,36] Main effects of all the selected independent variables like amount of gelling agent (X_1) and amount of viscosity enhancer (X_2) are highly significant ($p<0.05$).Additive and antagonistic effects were observed for all responses respectively with amount of gelling agent (X_1) and amount of viscosity enhancer (X_2), indicating increased responses with increase in amount of gelling agent and increase in viscosity enhancer. The variance of inflation factor (VIF) as shown Table 4 measures the extent to which the variance of particular model coefficient was inflated by the lack of orthogonality in the design. The VIF values for all the models were found to be nearer to 1, indicating good estimation of coefficient.The contour plots are built to evaluate the relationship between two independent factors, amount of gelling agent(X_1) and amount of viscosity(X_2) enhancer and their effect on dependent factors i.e. responses Y_1, Y_2 and Y_3. Similarly response surface plots were also generated to establish the effect on response factors. For Y_1 responses the independent factor X_1 and X_2 shows an additive effect. For Y_2 responses the independent factor X_1 shows additive effect and X_2 shows antagonistic effect. For Y_3 responses the independent factor X_1 and X_2 shows an antagonistic effect.These results suggest that amount of gelling agent and amount of viscosity enhancer plays a direct role for achieving optimum response.

Table 4
coefficient estimation data for selected responses

Factor	Y_1 – Gelling capacity			Y_2 – Gelation temperature			Y_3 – drug release		
Intercept	***Coefficient of estimation***	***SE***	***VIF***	***Coefficient of estimation***	***SE***	***VIF***	***Coefficient of estimation***	***SE***	***VIF***
Intercept	7.11	0.17	-	37.02	0.15	-	98.19	1.28	-
X_1	0.15	0.24	1.0	-0.42	0.13	1.0	-4.70	1.15	1.0
X_2	2.91	0.24	1.0	-4.33	0.13	1.0	-12.14	1.15	1.0
$X_1 X_2$	-	-	-	0.50	0.16	1.0	-5.74	1.40	1.0
X_1^2	-	-	-	-0.062	0.20	1.13	-1.70	1.72	1.13
X_2^2	-	-	-	1.19	0.20	1.13	-8.94	1.72	1.13

The application of response surface methodology yielded the following regression equations.

$$Y_1 = 7.11 + 0.15\ X_1 + 2.91\ X_2$$
$$Y_2 = 37.02 + 0.42\ X_1 - 4.33\ X_2 + 0.50\ X1X2 - 0.062\ X_1^2 + 1.19\ X_2^2$$
$$Y_3 = 98.19 - 4.70\ X_1 - 12.14\ X_2 - 5.74\ X1X_2 - 1.70\ X_1^2 - 8.94\ X_2^2$$

Where, X_1 and X_2 are the coded values of the test variables of the amount of gelling agent and amount of viscosity enhancer respectively.

Optimization

In this experimental design optimization is done both by numerically and graphically. In numerical optimization the desired character for the response was selected, automatically software will choose the solutions with desired characters and limits. Among different solutions, it also suggests the solution with desirability near to 1. Graphically optimization was done by desirability plot and over lay plot which contains optimal values of independent variables. Higher the desirability the more suitable is the formulation. The desirability plot and overlay plot with optimized formula are given below Figure 6. The model predicts optimized formulation as per the selected criteria such as maximum gelling capacity at body temperature and sustained its drug release until 12 hr. The formulation F5 was optimized by DoE having high desirability factor.

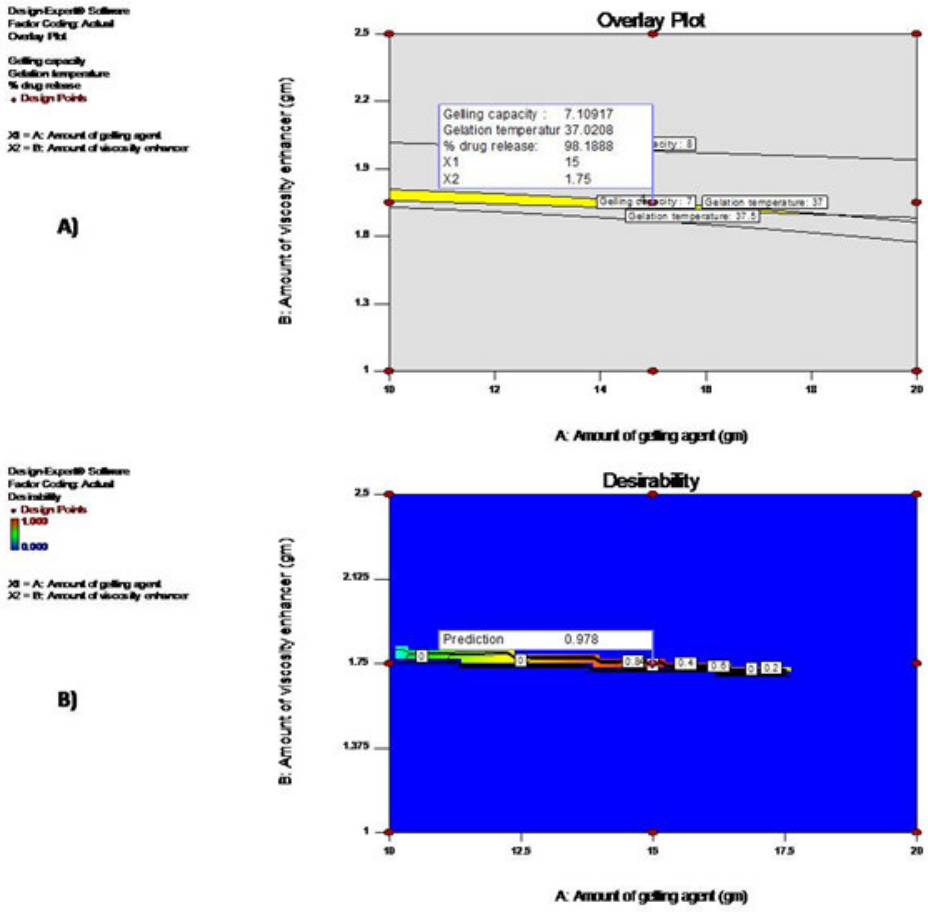

Figure 6
Desirability and overlay plot of optimized formulation

Cross validation of model

Upon comparison of the observed responses with that of the anticipated responses, the prediction error was calculated, and it was lower than 5.0% as shown in Table 5. Lower values of the relative error indicated that there was a close agreement of experimental values with predicted values. This proved the predictability and validity of model and ascertained the effects of amount of gelling agent and amount of viscosity enhancer on responses.

Table 5
Percent prediction error data for optimized formulation (F5)

Response	Predicted value	Experimental value	% Prediction error
Y_1 – Gelling capacity (hr)	7.11	7.23	1.600
(Y_2) Gelation temperature (^{0}C)	37.02	37.00	0.054
(Y_3) Cumulative % drug release	98.18	98.70	0.526

Comparison with marketed formulation

The marketed formulation released the whole drug within 6 hr but the optimised released the complete drug within 12 hr. The comparative dissolution profiles of the optimized and marketed formulation are plotted in Figure7.

Summary

In the present investigation, bromfenac sodium thermo sensitive ocular *in situ* gelwas statistically optimized by 3^2 full factorial designs. In this design the effect of two factors X_1 (amount of gelling agent) and X_2 (amount of viscosity enhancer) were selected and responses Y_1 (gelling capacity), Y_2 (Gelation temperature) and Y_3 (Cumulative percent drug release) were studied. The evaluation parameters for optimized formulation (F5) were found within acceptable limits ofpH (7.34)**,** gelation temperature (37°C), gelling capacity (7.23 hr), rheological behaviour (pseudo plastic), drug content (95.13%) and i*n vitro* drug release of 98.70 % for 12 hr. All the effects of factors and responses of parameters are shown by contour plots and response surface plots.X_1 and X_2, 15mg and 1.75mg respectively, was optimized from the overlay plot with prediction value 0f 0.978.The optimized formula was cross validated using experimental values and less than 5% percent relative error was obtained. This shows the correctness of the values, or the accuracy of the design.Optimized formulation exhibited sustained drug release profile for 12 hrwhen compared to marketed formulation, which sustained the release only for 6 hr.All the evaluated parameters are suitable to the physiological conditions of the eye.

CONCLUSION

Thus twice-a-day thermo sensitive ocular *in situ* gel formulation of bromfenac sodium could be developed using cooling technique sustaining the drug release for a prolonged time. The developed formulation is suitable for easy scale up as the number of steps in the process is less and it can be carried out with the usual equipment available in a pharmaceutical industry. This research also helps in establishing factorial experimentation which may be utilized for optimizing the formulation design.

ACKNOWLEDGEMENTS

The authors would sincerely like to thank Mr. Asimbiswal, FDC Mumbai for providing drug sample. We are grateful to Prof. J VijayaRatna for her suggestions, valuable time and help during work.

CONFLICT OF INTEREST

Conflict of interest declared none

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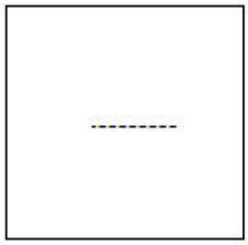

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We sincerely thank the above reviewers for peer reviewing the manuscript