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RESEARCH ARTICLE

FORMULATION DESIGN, OPTIMIZATION AND *IN VIVO* EVALUATION OF A PH AND ION RESPONSIVE OPHTHALMIC *IN SITU* GEL OF FORSKOLIN- BETA CYCLODEXTRIN COMPLEX

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ABSTRACT

The poor bioavailability of ocular solution is caused by dilution and drainage from the eye can be overcome by using *In situ* gel forming ocular drug delivery system prepared from polymers that exhibit *sol* to *gel* transition. The objective of the study was to develop optimized formulation of *In situ* gel of Forskolin (FSK) antiglaucoma agent using pH and Ion activated polymers Carbopol 940 and Sodium alginate respectively as gelling agents, Hydroxy Propyl Methyl Cellulose (HPMC K4M) as viscosity enhancing polymer. The Forskolin (FSK) solid dispersion was prepared by kneeding method in various ratios (1:1 to 1:4) with β- cyclodextrinas solubility enhancing agent. Solid dispersion 1:4 was selected for further formulation of ocular pH and ion activated *In situ* gels. The 2^3 factorial design was employed to optimize the formulation considering Carbopol 940, Sodium alginate and Hydroxy Propyl methyl cellulose (HPMC K4M) as independent variables, *Sol* to *gel* transition time (sec) and *In vitro* percentage drug release as dependent variables. Formulations were prepared successfully and assessed for appearance, gelling capacity, pH, drug content, viscosity, *in vivo* ocular irritation and *in vivo* intra ocular pressure (IOP) reduction studies and results observed in acceptable range. Based on *sol* to *gel* transition time (sec) and *in vitro* percentage drug release F9 formulation was found to be best optimized formulation from the nine formulations developed by 2^3 factorial design. The study revealed that the *in situ* gel system of Forskolin (FSK) sustained the antiglaucoma activity up to 8 h for F9 formulation.

INTRODUCTION

There is a high clinical demand for treating ocular diseases, and priority is in increasing the delivery efficiency of therapeutic drugs to the eye. To date, safe and effective treatments of most ocular diseases rely heavily on topical applications because of ease of use and low-cost manufacturing. Conventional dosage forms, including aqueous solutions, suspensions, and ointments, are administered topically and dominate the global market of ocular drug delivery, accounting for nearly 90% of marketed formulations (**Kaur and Smitha, 2002**). The glaucomas are a group of diseases characterized by gradual visual field loss with excavation and atrophy of the optic nerve head due to the death of retinal ganglion cells. Although intraocular pressure (IOP) is often elevated, which is considered the greatest risk factor for glaucoma, vision loss and progressive neuropathy can occur without elevations in IOP. Other risk factors for the development of glaucoma include advanced age, family history, and black race **(Quigley and Vitale, 1997) (Quigley, 1996) (Sommer, 1996)**. To treat chronic eye diseases such as glaucoma, considerable efforts have been devoted to development of new topical drug carriers and formulations to increase ocular residence time of drugs and increase drug adsorption. Consequently, enhanced drug delivery helps extend the duration of drug activity and reduce dosing frequency for patient compliance improvement. Nonconventional delivery systems and formulations for topical delivery of antiglaucoma drugs are under rapid development. Mucoadhesive polymers such as hyaluronic acid and chitosan are able to enhance retention time and drug penetration through the corneal barriers because of their bioadhesiveness **(Diebold, 2007) (Lele and Hoffman, 2000)**. *In situ*–forming hydrogels are liquid upon instillation and form viscoelastic gels in response to environmental changes such as pH or temperature (**Rozier *et al.*, 1989**). Forskolin (FSK), a labdane diterpene compound

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isolated from the roots of Coleus forskohlii Briq. (**Bhat *et al.*, 1977**), is useful in the treatment of health disorders including cardiovascular diseases, hypertension (**Kansal *et al.*, 1978**) (**Dubey et al., 1997**), asthma, glaucoma (Suryanayanan and Pai, 1998). The pharmacological activities of FSK are mainly due to its role as an activator of adenylate cyclase. FSK increases the amount of cyclic AMP (cAMP) (adenosine monophosphate) in cells by activating adenylate cyclase enzyme. cAMP is an important secondary messengers in the cell, and is considered to be an effective cell regulating compound (**Dubey *et al.*, 1981**). The aim of this work was to formulate ocular *in situ* gelling systems using pH and ion activated polymers containing Forskolin to be appliedtopically, and to evaluate the *in vitro* and *invivo* performance of the prepared *in situ* gelling systems.

MATERIALS AND METHODS

Forskolin (FSK) with purity of > 98 % was obtained from Madvik Labs, Hyderabad, India. Carbopol 940, Hydroxy Propyl methyl cellulose (HPMC K4M), Sodium Alginate with a molecular weight (Mw) of 196 000 g. mol^{-1} was obtained from SD fine chem., Mumbai, India. All the other chemicals were procured from HiMedia Lab, Mumbai, India. All the solvents were of High Performance Liquid Chromatography (HPLC) grade. Triple-distilled water was used throughout the studies.

Preparation of ocular *In Situ* gelling systems

The *In situ* gelling systems were prepared as per the procedure reported by Srividya *et al.* with little modifications (Srividya *et al.,* 2001). Solid dispersions of Forskolin were preparedby kneeding method for improving the aqueous solubility using β-cyclodextrin as complexing agent. Drug with β- cyclodextrin ratios 1:1 to 1:4 were kneeded separately in a mortar and pestle using deionized distilled water as solvent for 30 min. The slurry is allowed to dry for 24 h in vacuum finally obtained granules were pulverized and passed through sieve no. 60 and analyzed for solubility. Accurately weighed quantity of HPMC K4M was dispersed in 50ml of purified water, HPMC K4M was added as viscosity enhancing agent, Carbopol 940 and Sodium alginate were sprinkled over this solution, stirred with an overhead stirrer and allowed to hydrate overnight at room temperature. Forskolin (FSK) solid dispersion (FSK: β- cyclodextrin- 1:4) equivalent to 50 mg of active ingredient was dissolved in small quantity of acidic medium (2% of HCl solution), 0.03% v/v of benzalkonium chloride (BKC) was added and pH was adjusted to 6.0 by using 0.1N sodium hydroxide solution. The drug solution was added to the polymer solution and stirred homogeneously using magnetic stirrer, Isotonicity was adjusted by addition of 0.9% w/v sodium chloride (NaCl) solution. Purified water was then added to make up the volume to 100ml and the solution was filtered through 0.2µm membrane filter and all formulations were sterilized in an autoclave at 121°C for 20 min (**Srividya *et al.*, 2001**).

Optimization by 2^3 factorial design

A three-factor, two-level factorial design (2^3) were employed for optimization procedure with quantity of Carbopol 940 (A), Sodium alginate (B), and HPMC K4M (C) as three prime selected independent variables, which were varied at two levels, low level (−1) and high level (+1). The values of two coded levels of three factors were assumed after preliminary trials. The *In vitro sol* to *gel* transition time (sec) and *In vitro* % drug release were measured as dependent variables. Design-Expert® 9.0.3 Software was used for the generation and evaluation of the statistical experimental design. The factorial designed batches and responses are shown in (Table 1).

Table 1. Composition of different coded values in 2^3 full factorial design

	Factor 1	Factor 2	Factor 3	Response 1	Response 2
Formulations	A:Carbopol 940	B:Sodium Alginate	C:HPMC K4M	*Sol-gel* transition time (sec)	*In vitro* % drug release (%)
F1	-1	-1	-1	29±7	99±1
F2	+1	-1	+1	41±4	87±3
F3	-1	-1	+1	33±8	94±2
F4	+1	-1	-1	35±2	84±4
F5	-1	+1	+1	44±2	82±5
F6	+1	+1	+1	72±5	63±3
F7	+1	+1	-1	64±8	68±4
F8	-1	+1	-1	52±7	78±2

Fourier-transformed infrared (FTIR) spectroscopy

FTIR spectroscopy was carried out to characterize the possible interaction between the drug and excipients, if any. The FTIR spectra of pure drug, FSK solid dispersion and physical mixture of drug with selected polymers were recorded using KBr disc using an FTIR spectrophotometer (Shimadzu, Tokyo, Japan).

pH

pH of the *In-situ* gels after addition of all ingredients was measured using digital pH meter (**Pandey *et al.*, 2010**).

Visual appearance and clarity

Visual appearance and clarity was done under fluorescent light against a white and black back ground for presence of any particulate matter (**Pandey *et al.*, 2010**).

Gelation studies

Gelation studies were carried out in test tube, The studies were carried out using simulated tear fluid (STF) of composition 1 (sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride dihydrate 0.008 g and purified water sufficient to make 100 g) and of composition 2 (bovin serum albumin 0.268 g, lysozyme 0.268 g, γ-globulin 0.134 g, calcium chloride dehydrate 0.008 g, *D*-glucose 0.15 g, sodium chloride 0.65 g and distilled water sufficient to make 100 g), which simulated either the divalent cation content or both the protein and divalent cation content of the tear fluid equilibrated at 37^0 C. The one drop of preparation was carefully placed into the test tube using a micropipette and 2 ml of gelation solution (composition 1 or 2) was added slowly (**Srividya *et al.*, 2001**). Gelation was assessed by visual examination and time taken for *sol* to *gel* formation is recorded (**Nanjawade *et al.*, 2007**).

Viscosity

The viscosity of developed formulation was determined at 20°C by pouring it into the sample adaptor of Brookfield viscometer (DV-II+ Pro, Brookfield Engineering Ltd, Middleboro, MA, USA). Viscosity of sample was measured at different angular velocities. A typical run comprised changing angular velocity from 10 to 100 rpm with equal weight for each rpm. The hierarchy of angular velocity was reversed (100 to 10 rpm) with similar weight. The average of three readings was used to calculate the viscosity. The *sol* was allowed to *gel* and then viscosity was again measured using the same Brookfield viscometer with T spindle in conjunction with heli path stand (**Nanjawade *et al.*, 2007**).

Drug content uniformity

The vials (n = 3) containing the preparation were shaken for 2–3 min manually and 100 µl of the preparation was transferred aseptically to 1ml of ethanol containing 25ml volumetric flasks with a micropipette and the final volume was made up with simulated tear fluid solution pH 7.4 at 35± 1^0c and sonicated for homogeneity. Forskolin concentration was determined at 210 nm by UV spectroscopy (Shimadzu, UV-1601, Japan) (**Srividya *et al.*, 2001**).

Sterility test

It was performed for aerobic, anaerobic and fungi microorganisms using fluid thioglycollate and soya bean casein digest medium as per IP 2007. Formulation took into laminar flow and passed through a membrane filter of 0.45µm with the help of vaccum pump. After filtration the filter paper was removed and cut into two halves. One half was dropped in fluid thioglycollate and other in soya bean casein digest. Both the media kept for incubation at 37°C for 7 days, and observed for any microbial growth (**Singh *et al.*, 2010**).

***In vitro* release study**

Drug released study from prepared formulation was studies using Franz- diffusion cell. Cellophane membrane and artificial tear fluid (ATF) pH 7.4 was used as a diffusion membrane and medium respectively. The cellophane membrane (previously soaked overnight in the receptor medium) was tied at one end of the glass diffusion cell. Accurately weighed 1ml of *gel* was spread uniformly on a cellophane membrane, which was in contact with receptor medium. The receptor medium was stirred continuously at 20rpm to simulate blinking action of eyelids. The whole assembly was adjusted on magnetic stirrer and maintained at 35±1°C. At specify intervals (0.5 h, 1 h, 2 h up to 8 h) 1 ml of sample was withdrawn from receptor compartment, replace with 1ml of freshly ATF and analyzed by UV spectroscopy(Shimadzu, UV-1601, Japan) at 210 nm (**Pandey *et al.*, 2010**) (**Singh *et al.*, 2010**).

***In vivo* Ocular irritancy test**

New Zealand White rabbits, weighing 2.5–3.0 kg, were provided by National Institute of Nutrition, Hyderabad, India. The animals, housed in standard cages in a light controlled room at 19±1°C and 50±5% RH, were given a standard pellet diet and water *ad libitum*. All the studies were conducted in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Draize technique was selected for conducting the Ocular irritancy test on New Zealand white rabbits (**Baeyens *et al.*, 2002**). The experimental protocol dated 05/06/2014. Total sixteen animals are equally divided into four groups. Group I was treated with 0.01 % w/v Sodium lauryl sulfate (SLS) (positive control), Group II animals were treated with blank, Group III with sterile optimized formulation, and Group IV with 0.9%w/v NaCl (negative control) twice a day for a period of 1 week into the rabbit's left eye and observed for redness, swelling, watering. The ocular condition was recorded every day. The eye irritation score was obtained by dividing the total score for all rabbits by the number of rabbits. Irritation was classified according to four grades: practically non-irritating, score 0–3; slightly irritating, score 4–8; moderately irritating, score 9–12; and severely irritating (or corrosive), score 13–16.

Intra ocular pressure (IOP) reduction studies

The Forskolin (FSK) *In situ* gel formulations were tested for their IOP lowering effects on adult normotensive male New Zealand albino rabbits and the obtained results were compared with that of plain *In situ* formulations as well as plain FSK solution. An Increase in IOP was induced by the rapid infusion of a 5% glucose solution through the marginal ear vein. The amounts of injected were 15 ml/kg of body weight and infusion was accomplished in all animals within 30 sec. The IOP was measured by standardized Schiotz tonometer (Zur- Benutzungdes Schioetz, Germany) (**Kaur *et al.*, 2000**) (**Monem *et al.*, 2000**). Before the measurement of the tension, the cornea was anaesthetized with 2 or 3 drops of xylocaine (1% w/v). After 2 min, the eyelids were retracted gently with one hand, without exerting pressure on the eye ball. The lower *cul-de-sac* of right eye of each rabbit of the group (n = 4) received 25 µl of the optimized formulation while the contra lateral eye (left) received no drug and served as a control. The IOP of both eyes of each rabbit was measured immediately before the administration of formulation (zero reading), 30 min after instillation and then at every hour for a period of 8 h. The similar procedure was adapted for the measurement of IOP after instillation of 25 µl of plain FSK solution and plain *In situ* formulations, respectively. The change in IOP (ΔIOP) was determined by following equation:

$$\Delta IOP = IOP_{Dosed\ eye} - IOP_{Control\ eye}$$

All the observations were taken in triplicate and the mean values were reported. All the measurement periods began during the same hour on each day and all the data were recorded with the same tonometer.

RESULTS AND DISCUSSION

The solid dispersions FSK with β- cyclodextrin ratios 1:1, 1:2, 1:3 and 1:4 were prepared successfully by kneeding method and produced good yield. β- cyclodextrin more than four parts formed sticky mass compared to other ratios. The drug content

of formulated solid dispersions was ranging from 94.70 ± 0.26 to 96.90 ± 0.58 %. Prepared Solid dispersions were showed solubility of 2.012±0.012, 2.241±0.018, 2.759±0.016 and 3.495±0.017 mg/ml respectively for ratios 1:1, 1:2, 1:3, and 1:4 in pH 1.2 and 2.020±0.002, 2.255±0.015, 2.768±0.011, 3.499±0.012 mg/ml respectively in pH 7.4 buffer solutions. Solid dispersion 1:4 was selected for further formulation of ocular pH and ion activated *In situ* gels. The purpose of using a full 2^3 factorial experimental design was to conduct a comprehensive study of the effect of polymers and viscosity enhancing agent like Carbopol 940(*A*), Sodium Alginate(*B*), Hydroxy Propyl Methyl Cellulose(*C*) and their interactions using a suitable statistical tool (Design-Expert® 9.0.3 Software) by applying ANOVA at 0.05 levels. The effects of the independent variables (A, B and C) on the *sol–gel* transition time (sec) and *In vitro* % drug release were evaluated, and the following models were obtained:

Final Equation in Terms of Coded Factors

Sol-gel transition time (R1) =+46.25+6.75 A+11.75 B+1.25 C+3.25 AB+2.25 AC-1.25BC+ 1.75ABC

In vitro % drug release (R2)=+81.88-6.38A-9.13B-0.38C-0.87AB-0.12AC+0.13BC-2.13ABC

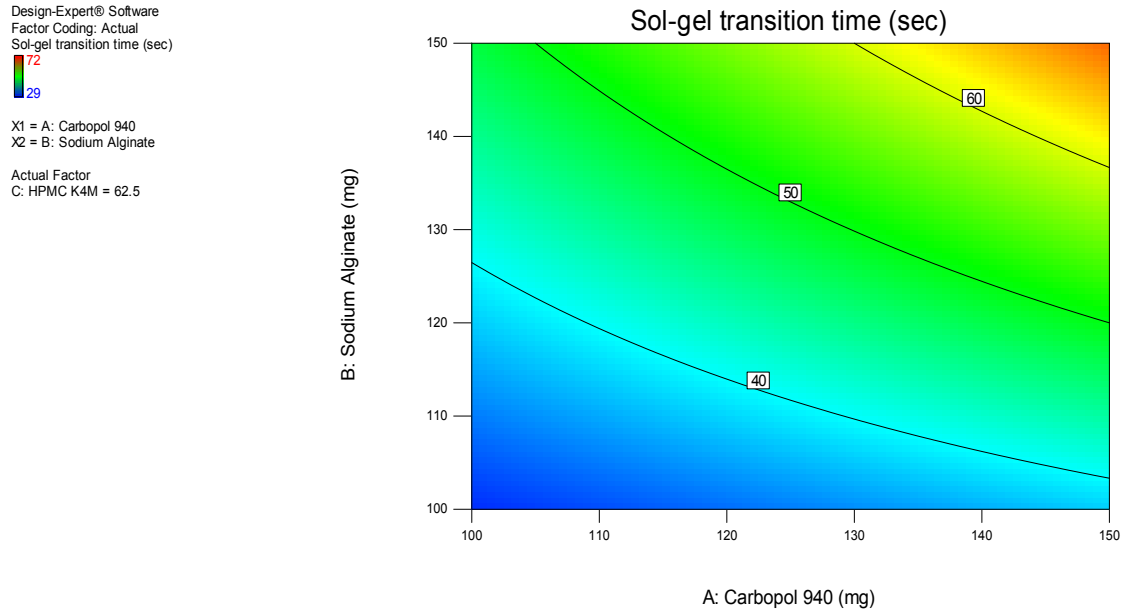
From the ANOVA results (Table 2 and 3) of the model relating *Sol-gel* transition time (sec) and In vitro % drug release as response, it can be noticed that all the coefficients of this model equations had statistical significance ($p < 0.05$) with the R^2 values of 0.9994 and 0.9999 for *Sol-gel* transition time (sec) and *In vitro* % drug release respectively. The three-dimensional response surface graphs (Fig. 1(b), Fig. 2 (b)) and corresponding two-dimensional contour plots (Fig. 1(a), Fig. 2 (a)) were generated by the Design-Expert z® 9.0.3 Software.

Table 2. Response 1: *sol* to *gel* transition time: ANOVA for selected factorial model Analysis of variance table [Partial sum of squares - Type III]

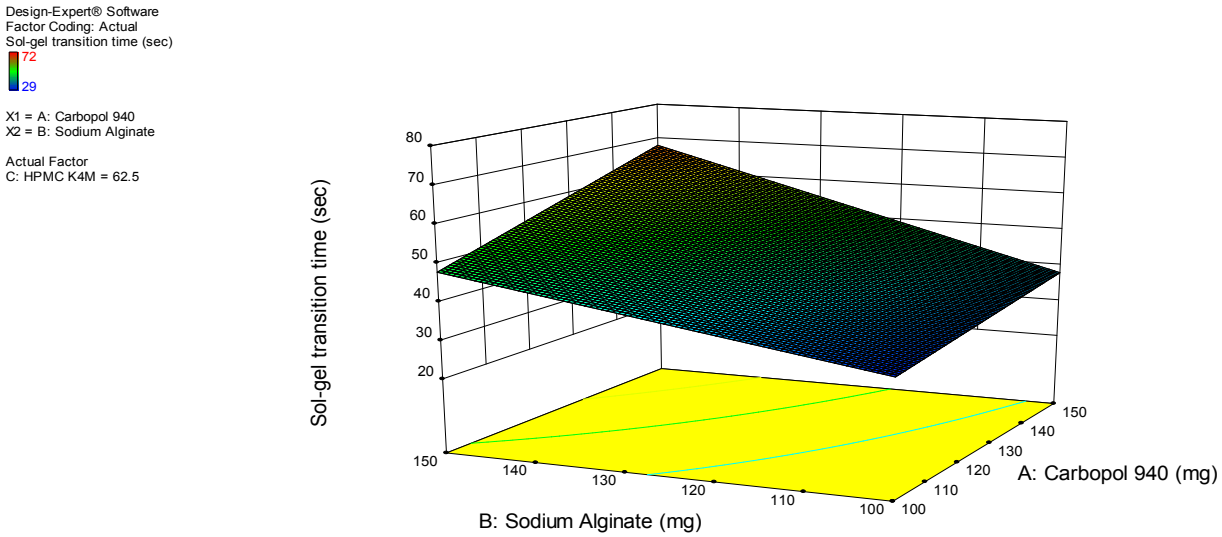
Sources	sum of square	df	mean square	F- value	p-value Prob>F
Model	1643.50	7	234.79	12.19	0.0176
A-Carbopol 940	364.50	1	364.50	9.00	0.0399
B-S0dium Alginate	1104.50	1	1104.50	27.27	0.0064
C-HPMC K4M	12.50	1	12.50	0.31	0.6081
AB	84.50	1	84.50	21.05	0.0039
AC	40.50	1	40.50	10.44	0.0145
BC	12.50	1	12.50	26.84	0.0021
ABC	24.50	1	24.50	84.72	0.0689

Table 3. Response 2: *In vitro* % drug release: ANOVA for selected factorial model Analysis of variance table [Partial sum of squares - Type III]

Sources	sum of square	df	mean square	F value	p-value Prob>F
Model	3.20	7	0.53	75245.80	0.0028
A-Carbopol 940	1.01	1	1.01	1.005	0.0017
B-Sodium Alginate	2.04	1	2.04	2.005	0.0012
C-HPMC K4M	3.885	1	3.885	547.62	0.0272
AB	0.037	1	0.037	5244.30	0.0088
AC	1.635	1	1.635	230.46	0.0414
BC	0.13	1	0.13	15678.78	0.0021
ABC	36.13	1	36.13	84.72	0.0689



(a)



(b)

Fig. 1. Effect of Carbopol 940, Sodium Alginateand HPMC K4Mquantitieson *sol* to *gel* transition time (sec) presented by response surface plot (a), and contour plot (b)

The three-dimensional response surface graph is very useful in learning about the main and interaction effects of the independent variables (factors), whereas two-dimensional contour plot gives a visual representation of values of the response.

The three-dimensional response surface graphs relating *sol* to *gel* transition time (sec) as response (Fig. 1 (b)) depicted the increase in *sol* to *gel* transition time with the increasing of polymer concentration, (Fig. 2 (b)) depicting that *In vitro*% drug release was increase with decreasing of polymer concentration mainly.

The two-dimensional contour plots (Fig. 1 (a) and (Fig. 2 (a)) relating to *sol* to *gel* transition time (sec) and *In vitro* % drug release indicated nonlinear relationships. The optimal value of response was obtained by numerical analysis using Design-Expert® 9.0.3 Software. In order to evaluate the optimization capability of model generated according to the results of the full 2^3 factorial design, optimized *In situ* gel containing FSK was prepared using the optimal process variable settings (Table 4) and the optimized formulation was evaluated to determine the *sol* to *gel* transition time (sec) and *In vitro* % drug release. The *sol* to *gel* transition time (sec) and *In vitro* % drug release for the optimized formulation was measured 37±4 sec and

94±3% respectively with small error value. This reveals that mathematical models obtained from the full 2^3 factorial design was well fitted.

Newtonian behavior in the *sol* state which is shown in the Fig. 5(A).

Table 4. Values of factors for the formulation of optimized *In situ* gel containing FSK (F 9)

Formulation	Carbopol 940 (mg)	Sodium Alginate (mg)	HPMC K4M (mg)	*sol* to *gel* transition time (sec)		*Invitro* % drug release	
				Observed (Mean ± SE, *n* = 3)	Predicted	Observed (Mean ± SE, *n* = 3)	Predicted
F9	100	130	60	37±4	40	94±3	96
				%Error= 7.5		%Error= 2.083	

Table 5. Assessment of FTIR spectra

Functional Group	Standard peak range (cm^{-1})	Observed peak range of Forskolin (cm^{-1})	Observed peak range of Forskolin Solid dispersion (cm^{-1})	Observed peak range of Physical Mixture (cm^{-1})
O-H	3200-3570	3437.49	3385.5	3443.28
C-CH_2	2820-2975	2849.31	2850.27	2164.7
C=O	1500-1995	1702.84	1696.09	1965.11
C- CH_3	1300-1410	1375	1369.21	1405.20

Table 6. Results of evaluation of Ocular *In situ*gel formulations

Formulation	Clarity	pH	Drug Content(%)	Gelling capacity
F1	clear	6.1±0.2	99.2±2	+
F2	clear	5.9±0.1	97±3	++
F3	clear	6±0.4	101±0.5	++
F4	clear	5.8±0.7	99.1±1	++
F5	clear	6.0±0.2	98.3±0.4	++
F6	clear	5.8±0.3	99.7±0.5	+++
F7	clear	6.1±0.1	100±1	++
F8	clear	5.9±0.3	99.1±2	+
F9	clear	6.2±0.1	98.9±1	+++

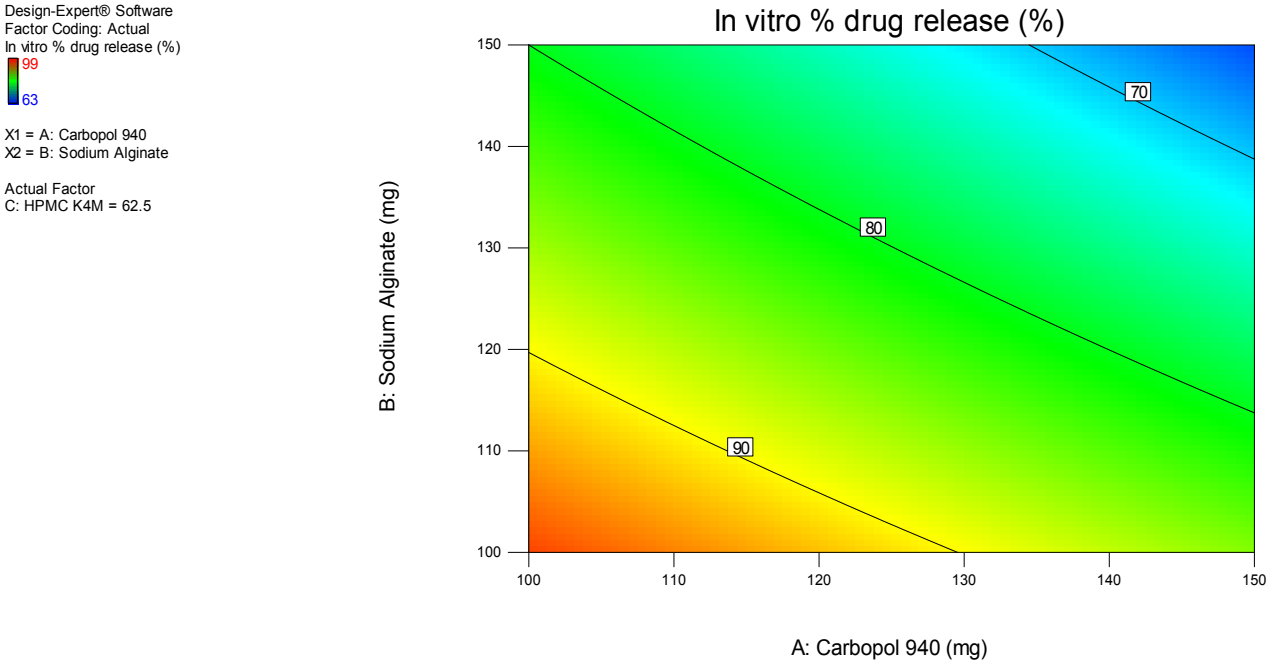
+ Gelation after few minutes, dissolves rapidly; ++ gelation immediate, remains for few hours;
+++ Gelation immediate remains for extended period.

Table 7. Scores for *In Vivo*Ocular irritation evaluation of FSK ocular *In situ* gel in rabbits

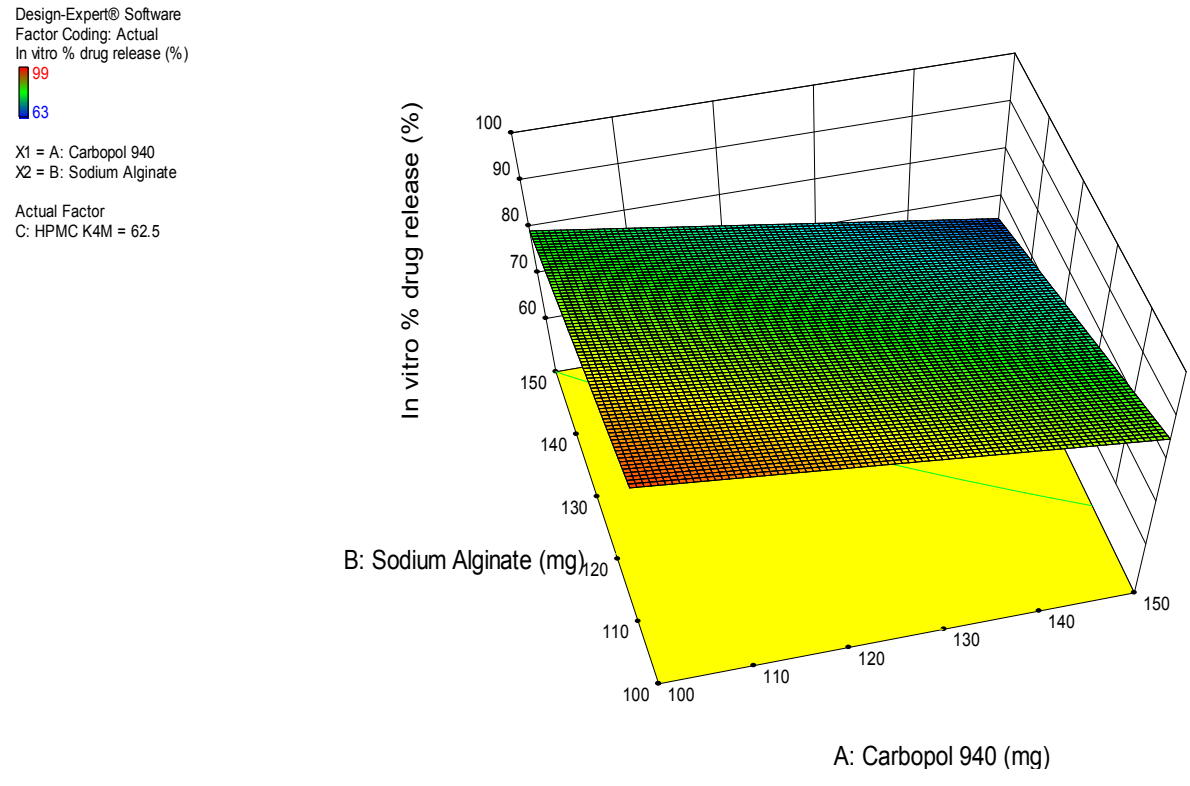
Preparation	Score
Blank Formulation	0
Optimized Formulation	0
0.01% w/v SLS solution (positive control)	8
0.9% w/v Sodium chloride solution (negative control)	0

FTIR spectra analysis revealed no significant interaction between various rational combinations containing physical mixture of drug with polymers (i.e., Carbopol 940, sodium alginate and HPMC K4M) as shown in (Fig. 3, 4, 5, Table 5). The FTIR spectra of drug-polymer mixture confirmed neither any shift in the wave numbers of the peaks nor in the intensity, construed lack of interaction. The formulations exhibited

In the *gel* state the formulations exhibited pseudoplastic rheology shown in Fig. 6(B) as evidenced by shear thinning with an increase in shear stress with increased angular velocity. Viscosity was directly dependent on the polymeric content of the formulation. The viscosity increased with increasing concentration of HPMC K4M. Maximum viscosity was observed with F9 and minimum viscosity with F8.



(a)



(b)

Fig. 2. Effect of Carbopol 940, Sodium Alginate and HPMC K4M quantities on *In vitro* % drug release presented by response surface plot (a), and contour plot (b)

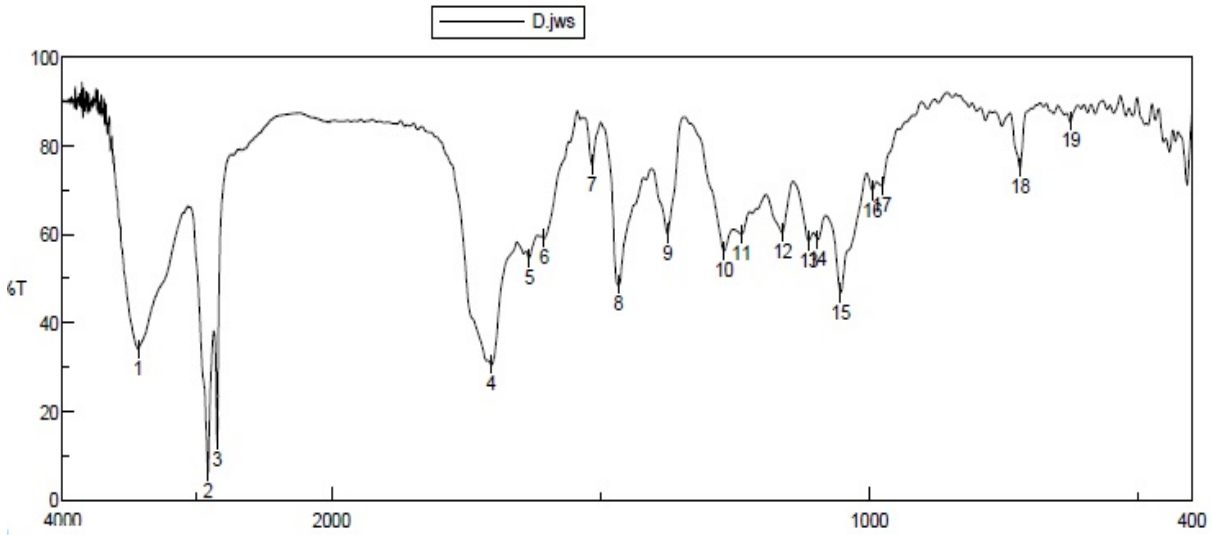


Fig. 3. FTIR spectrum for Forskolin

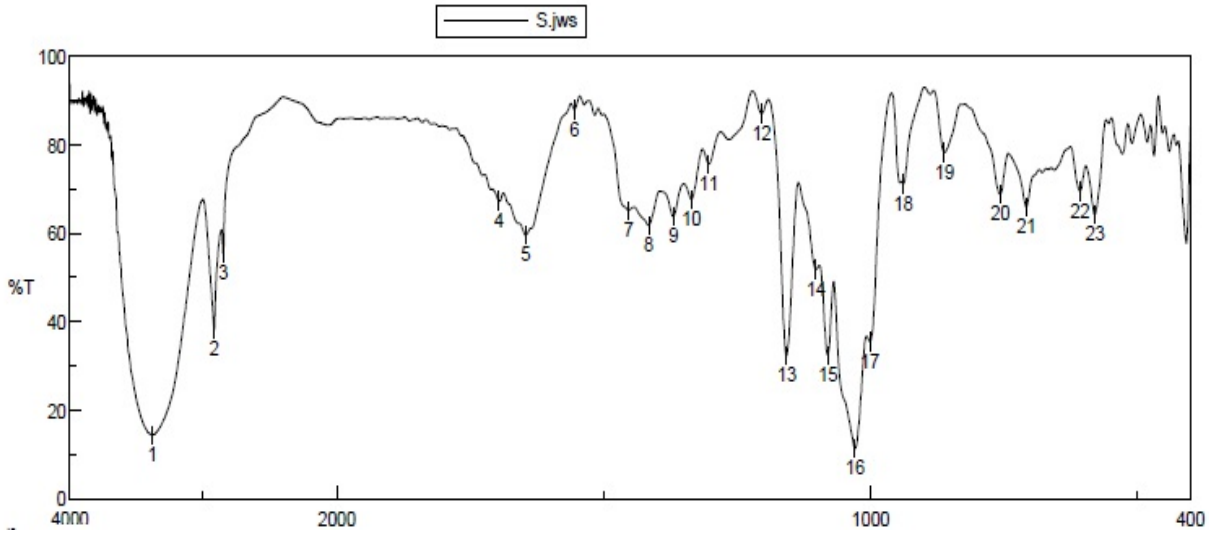


Fig. 4. FTIR spectrum for Forskolin and beta cyclodextrin Solid Dispersion

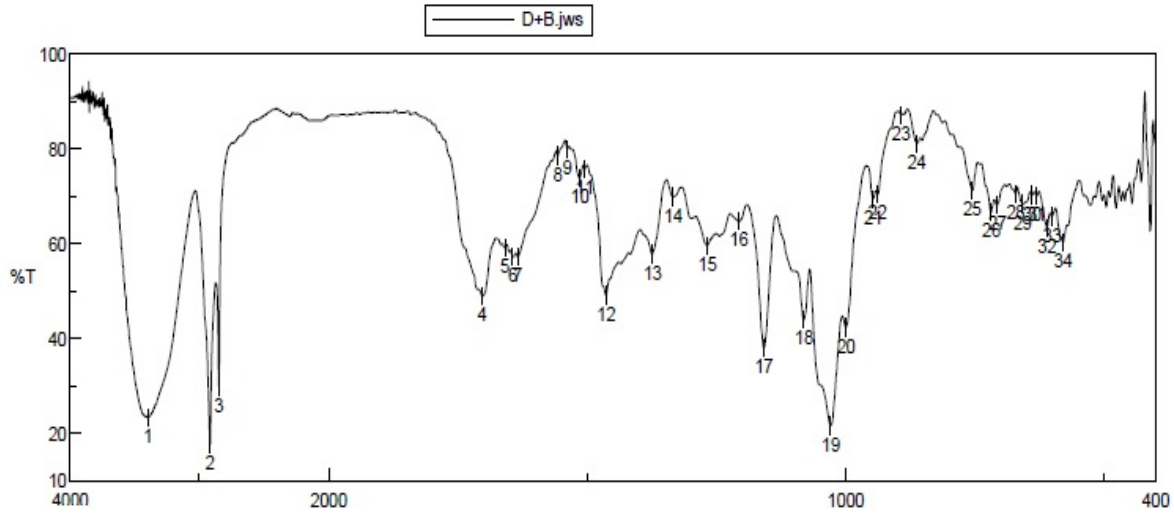
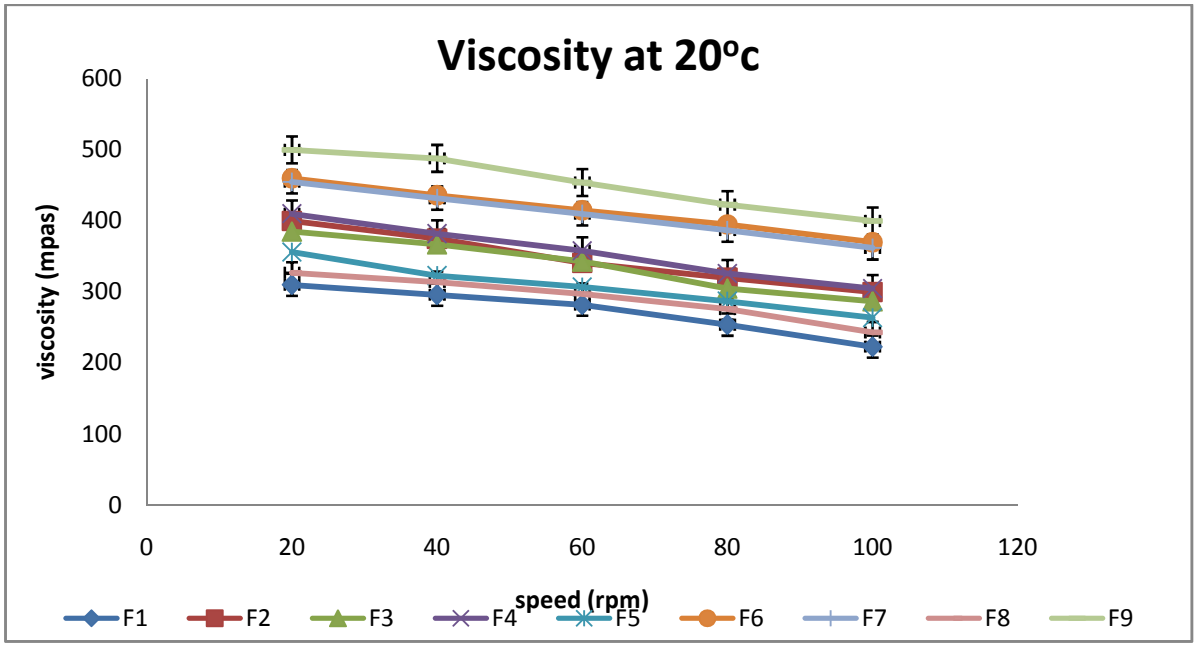
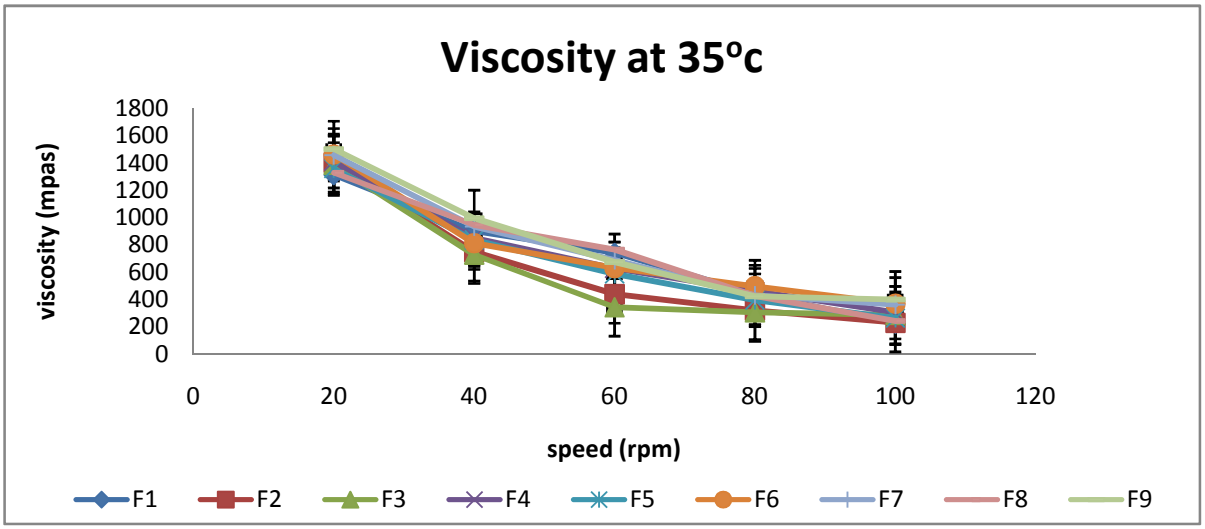


Fig. 5. FTIR spectrum of drug and other ingredients



(A)



(B)

Fig. 6. Graph representing viscosity of formulations (A) at 20°C (B) at 35°C

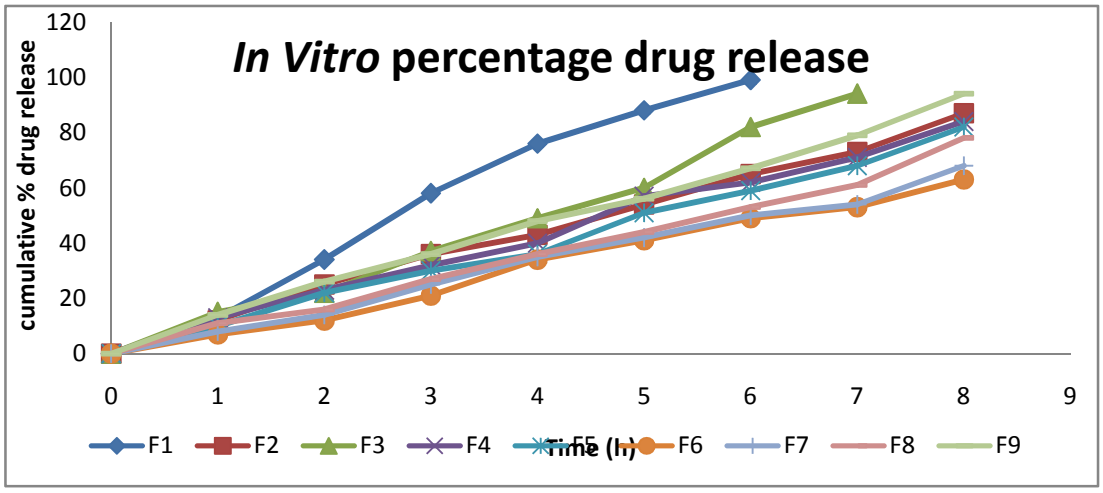
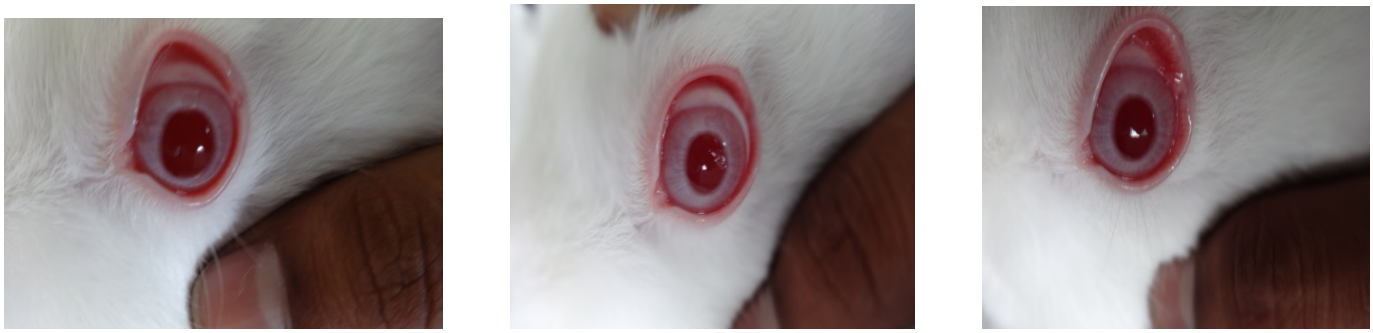


Fig. 7. *In Vitro* percentage drug release of FSK *In situ* gels



(a) (b) (c)

Fig. 8. *In Vivo* ocular irritation studies a) 15 min before installation of *In situ*gel (F9) b) No lesion observed for F9 on 3rd day c) No lesion observed for F9 on 7th day

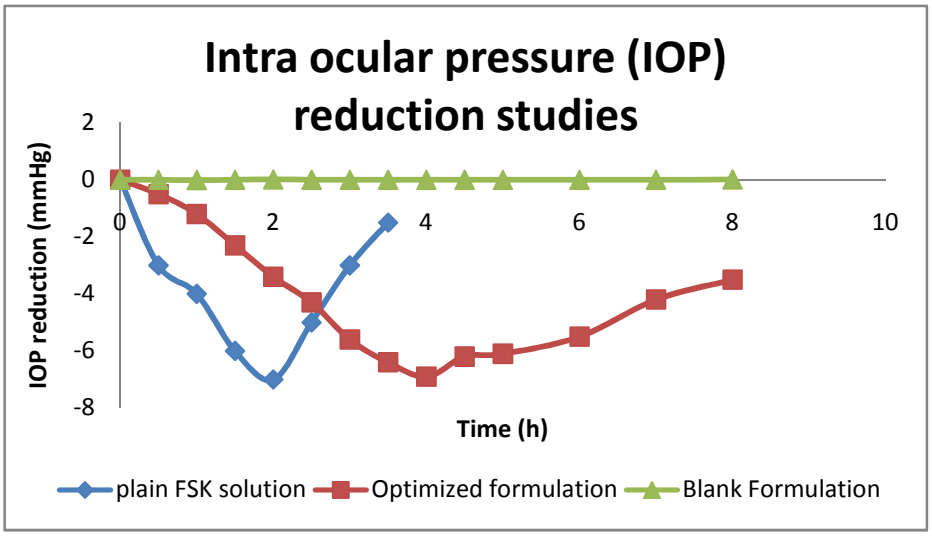


Fig. 9. *In Vivo* IOP reduction studies for F9

This indicates that addition HPMC K4Mlead to increase in viscosity for formulations.

All the formulations were found to be clear in appearance. The pH was within the acceptable range and hence would not cause any irritation when administered into the eye. The drug content was found to be in acceptable range and was in the range of 98.3±0.4 to 101±0.5% indicating uniform distribution of drug. All the formulations gelled instantaneously within 80 sec on contact with 7.4 pH phosphate buffer at 35 ± 1°C, formulations containing optimal quantity of HPMC K4M showed good gelling properties. By visual observations the formulations formed a translucent matrix on gelling. F9 formulation showed good gelling capacity and gelled within 37±4 sec. The pH, drug content and gelling capacity values of all the formulations were shown in Table 6 and *sol* to *gel* transition time (sec) of all formulations were shown in Table 1. There was no microbial growth in all formulations after 7 days of incubation period, showing that the method used for sterilization was reliable.

In vitro release (Fig. 7) through cellophane membrane revealed that with the increase in the concentration of polymers the drug release decreased due to the formation of gel structure.

Comparing to the polymers, more concentration of carbopol 940 contain formulations showed prolonged drug release than sodium alginate with combination of HPMC K4M. These results suggested that FSK was released in a sustained manner from formulation F9 for a period of 8 h with optimal *sol* to *gel* transition time (37±4 sec), hence formulation F9 was selected as optimized formulation for conducting *In vivo* studies. In order to understand the drug release mechanism, the release data was tested assuming common kinetic model. The higher regression coefficient values (Higuchi model) for each formulation suggested that the formulations F1 to F9 showed matrix type of drug release.

The results of the ocular irritation studies (Table 7) indicated that the optimized formulation (F9) was non-irritant with no ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae. As shown in Fig. 8 (a, b and c), no lesion formation was observed during the test. Hence, FSK ocular *In situ* Gel may be considered safe for ophthalmic drug delivery for glaucoma. The Intra ocular pressure (IOP) reduction studies suggested that the hypotensive activity of drug loaded *In situ* gel formulation (F9) was comparable to that of the plain drug solution. In the beginning, IOP decreased sharply for the first

2 h in case of plain FSK solution whereas IOP was observed to decrease slowly in case of drug loaded *In situ* gel formulation (F9). The IOP was immediately and noticeably reduced up to 2 h after instillation of plain FSK solution, but increased slightly over the rest of the period of observation. This type of fluctuation was not observed in case of optimized formulation, where the IOP continued to drop. The results suggested that *In situ* gel formulation (F9) produced a significant and prolonged reduction in IOP throughout 8 h. This overwhelming superiority over plain FSK solution was further magnified when single instillation was considered (Fig. 9). The reduction in IOP was found sustained for a period of approximately 6 h with the *In situ* gel formulation (F9) as compared to the plain drug solution.

Conclusion

The present studies, therefore, shows the successful formulation of pH and Ion activated *In situ* gels of FSK using Carbopol 940, Sodium alginate and HPMC K4M as suitable polymers for glaucoma. The application of experimental design methodology helped to prepare the optimized formulation, which showed good *sol* to *gel* transition time (sec), *In vitro* percentage drug release and *In Vivo* anti glaucoma activity. The drug release was found to be diffusion controlled and followed Higuchi kinetics for all formulations. FTIR studies confirmed absence of any physiochemical interaction between drug and other ingredients. Ocular irritation studies showed that absence of ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae. The Intra ocular pressure (IOP) reduction studies suggested that *In situ* gel formulation (F9) produced a significant and prolonged reduction in IOP throughout 8 h.

Conflicts of interest

The authors report that this article content does not have any conflicts of interest.

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