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FORMULATION AND EVALUATION OF IN SITU GELLING SYSTEM OF KETROLAC TROMETHAMINE FOR OPTHALMIC DRUG DELIVERY

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ABSTRACT

The poor bioavailability and therapeutic responses due to rapid precoreneal elimination by opthalmic solutions of drug maybe overcome by the use of *in situ* gel forming systems that are instilled as drops into eye and undergo a sol-gel transition in the cul-de-sac. The present work describes the formulation and evaluation of an opthalmic delivery system of a NSAID drug, Ketorolac tromethamine based on concept of thermo reversible in situ gelling systems. Poloxamer 407 was used as a gelling agent in combination with hydroxy propyl methyl cellulose (Methocel E50LV), which acted as viscosity enhancing agent. The developed formulations were stable, non-irritant and provided diffusion controlled release over a period of six hours. The developed system is thus a viable alternative to conventional eye drop.

KEY WORDS:

Ketorolac tromethamine, Poloxamer 407, Methocel E50LV, In situ gelling system, controlled release.

INTRODUCTION

Due to the special anatomic structure and efficient protective mechanisms, many challenges are presented in the development of effective ophthalmic dosage forms. Most ophthalmic drugs are administrated topically in the form of eye drops. However,
the rapid turnover of lacrimal fluid and efficient drainage apparatus make the ophthalmic solutions to eliminate rapidly, which causes a short precorneal residence time and a limitation of transcorneal absorption. All these lead to an ocular bioavailability that is commonly less than 10%. Moreover, nasolacrimal drainage is also the major route to enter the circulatory system for drugs that are applied through topical administration. For potent drugs, the systemic exposure through nasolacrimal drainage after topical administration can be sufficiently high to cause systemic toxicity. In order to enhance the ocular bioavailability, many ophthalmic drugs are applied in high concentrations or increased times of administration, but these increase the possibility of causing both ocular and systemic side-effects (Chein Y, 1993).

In order to overcome these drawbacks, many researchers have attempted to retain delivery systems in the front of the eye given the enormous loss of an instilled drug solution that typically occurs. Some retentive systems, such as inserts and ointments were considered. These dosage forms have proven to be of long duration and able to substantially modify drug bioavailability compared to their solution dosage form counterparts (Balasubramaniam J and Pandit J, 2003; Kaur I and Kanwar M, 2002; Peppas A *et al*., 2000).

Physical/chemical change (for example, pH, temperature or a specific ion) induced by the physiological environment has been investigated as a more convenient dosage form of topical application. Poloxamer (trade name Pluronic®), a block copolymer

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that consists of polyethylene oxide (PEO) and polypropylene oxide (PPO) units, is known for exhibiting the phenomenon of reverse thermal gelation under a certain concentration and temperature. At a concentration of 18% (w/w) or higher in aqueous solution, Poloxamer 407 (P407), in which the ratio of PEO and PPO is 7:3, is transformed from a low viscosity solution to a gel under the ambient temperature (Panwar R and Sagar B, 2006; El-Kamel A, 2002; Menqi and Deshpande S, 2005). But this lower concentration solution will lose the gelation ability after diluted by lacrimal fluid. So 25% (w/w) P407 should be used to ensure the completion of the phase transition under physiological condition (Mitan R *et al*., 2007; Nanjawade B *et al*., 2007).

Meanwhile, a retentive dosage form based on a so-called muco/bioadhesive polymer, which is capable of attaching mucosal surfaces, offers the prospects of prolonging the residence time of an ocular drug delivery system at the sites of drug absorption and ensures optimal contact between the formulation the absorbing surface has been investigated to enhance bioavailability of topical administration.

Ketorolac tromethamine a NSAID, 800 times more potent than aspirin, exhibits pronounced analgesic and moderate anti-inflammatory activity. Good water solubility, higher penetration efficacy at tear fluid pH makes it a suitable drug candidate for formulating as in situ gelling system.

MATERIALS AND METHODS

CHEMICALS

The Ketorolac tromethamine was obtained from FDC Ltd. Mumbai. Poloxamer 407 was generously gifted by BASF Corporation. All other chemicals were procured from Research Laboratories Fine Chemical Industries, Mumbai. Evaluation was done on equipments available in laboratory at Appasaheb Birnale college of Pharmacy, Sangli.

ANIMALS

Albino rabbits, weighing 2.5- 3.0 kg were procured from the Krishna institute of medical sciences Karad-Satara. Approval of the Institutional Animal Ethic Committee was obtained prior to commencement of the study.

METHOD

Preparation of thermoreversible PF127 aqueous solutions

The cold method described by Schmolka *et al* (Schmolka I, 2000; Balasubramaniam J and Pandit J, 2003; Kaur I and Kanwar M, 2002; Peppas A *et al*., 2000) was used for the preparation of gel. Poloxamer solutions with concentrations of 16–20% w/v were made by weighing into a cold solvent. The where were kept in to ensure complete dissolution. The solutions were made R. O. water as a solvent. Concentration of the Poloxamer was optimized on the basis of gelation temperature Poloxamer 407. Solutions were also containing Nacl (0.9%) as isotonicity agent. Also they were optimized by the visual inspection method (El-Kamel A, 2002).

The effect of different concentrations of drug on sol-gel temperature was also studied (Nanjawade B *et al*., 2007; Schmolka I, 2000).

Optimization of mucoadhesive polymers with optimum concentration of PF 127

For the optimization of mucoadhesive polymers, various sets of aqueous solution of optimum concentration of PF 127 containing varying concentrations of mucoadhesive polymers were prepared. The prepared sets of formulation were evaluated in terms of gelation temperature. The optimized polymer shows gelation temperature near to the temperature of the *cul-de-sac* temperature. i.e. 34^0C

Mucoadhesion study

In vitro Mucoadhesion study- Modified balance method

The mucoadhesive forces of all the prepared formulae were determined using the mucoadhesive force measuring device (Figure 1), which is a modified balance that was developed in our laboratory according to previously reported methods (Desai & Kumar, 2004; Mikos & Peppas, 1990); was determined by measuring the force required to detach the formulation from a mucin disc using the measuring device. The detachment force (dyne/cm2) was determined using the following equation stated by Ch'ng,Park, Kelly, and Robinson (1985).

$$\text{Detachment Force (dyne/cm}^2) = \frac{m.g}{A}$$

Where *m* is the weight of water in grams;
g is acceleration due to gravity taken as 980 cm/sec^2; and
A is the area of the mucin disc (area of contact) and is equal to πr^2 (r is the radius of the mucin disc).

Formulation studies

Optimized **c**oncentration of mucoadhesive polymers under study selected for further formulation study.

Preparation of Pluronic F127/HPMC formulations

The formulations were prepared with cold method. The drug, Isotonicity adjusting agent were dissolved in distilled deionized water and kept in refrigerator. After cooling Pluronic F127 of required quantity was added and kept at 4^0C with periodical stirring to ensure complete dissolution. Benzalkonium chloride was added as a preservative (Majithiya R *et al*., 2006; Pisal S *et al*., 2004).

In order to identify the composition suitable for use as in situ gelling, aqueous solutions of Pluronic F127 and HPMC with different concentration and grades were prepared and evaluated for gelling capacity and transparency at physiological conditions. The formulations were then subjected to terminal sterilization

by autoclaving at 121^{0}C and 15 p.s.i. for 20 min.The gelling capacity was measured by visual method.

Evaluation of formulation

Content uniformity

Tests for content uniformity were carried out for all the prepared gel formulations (Mansour M *et al*., 2008).

Gelation temperature

Gelation temperature of each formulation was determined by visual inspection method (Majithiya R *et al*., 2006; Pisal S *et al*., 2004).

Determination of mucoadhesive strength

Mucoadhesive strength of each formulation was determined by method described in preformulation study. Measurement was carried in triplicates for each formulation.

Viscosity study

The viscosities of various formulations were determined by using Cone and Plate viscometer (Brookfield viscometer Model Cap 2000+2). Few drops of formulation were applied to lower plate of the viscometer using glass rod. The temperature was increased in steps of 2^{0}C/minute, from 28^{0}C to 36^{0}C. The apparent viscosity was measured as a function of the temperature (^{0}C) (Qi H *et al*., 2007; Li Z *et al*., 2006).

In-vitro drug release study

Keshry Chein diffusion cell was used for evaluation of drug permeation. Cellulose acetate membrane (25mm, 0.45µ pore size) was placed between the receiver compartment and donor compartment. Receiver compartment filled with Simulated Tear fluid and the temperature was maintained at 34± 0.2°C ^{0}C by circulating water bath (Cyber Lab CB 2000) throughout study. Diffusion cells were placed on magnetic stirrer (Mega Whirlmatic SpetcraLab) to provide continuous agitation. Formulation equivalent to 2.265 mg of drug was placed in donor compartment. At regular time intervals, 0.5 ml of sample was withdrawn and replaced by fresh Simulated Tear Fluid in order to maintain sink conditions. The samples were diluted to 10 ml with Simulated Tear Fluid, filtered and the absorbance was measured at 322 nm using UV spectrophotometer (Bain M *et al*., 2009).

Ocular Irritation Studies

In developing a novel ophthalmic delivery system, an injury to the eye was taken into consideration. Since, eye being a sensitive, most delicate and yet most valuable of the sense organs, the injuries to the cornea, conjunctiva, and iris were measured according to Draize test. Approval of the Institutional Animal Ethic Committee was obtained prior to commencement of the study. In vivo ocular irritation studies were performed according to the Draize technique on 3 albino rabbit, each weighing 2.5-3.0 Kg. The left eye was designated as the test eye in which, a single drop (approximately 0.04 ml) of the sterile formulations was instilled twice a day for a period of 3 days, whereas the right eye served as a matched control and was left untreated (figure 11). The observations were made at the time interval of 1h,2h,4h,8h, 24 h, 48 h and 72 h after exposure. Instillation was made into the lower conjunctival cul-de-sac; normal blinking was allowed, although the eyelids can be held together for several seconds after instillation. Rabbits were observed periodically for redness, swelling, and watering of the eye. The evaluation was made according to the Draize test protocol (Earl L *et al*., 1997).

RESULT AND DISCUSSION

Optimization of poloxamer 407

Thermoreversible polymer-based liquid formulations that provide in situ gelling property in ocular cavity were designed to delay clearance of the formulations from the ocular cavity. It was found that gelation temperature decreases as the concentration of Poloxamer increases and sol temperature increases. Table 4 shows different sol-gel temperature obtained from visual inspection method. In the preliminary studies, the minimum concentration of PF127 that formed gel near to the body temperature was found to be 17.5 and 18% w/v. In general, the gelation temperatures have been considered to be suitable if they are in the range of 25^{0}C to 37^{0}C. If the gelation temperature of a thermoreversible formulation is lower than 25^{0}C, a gel might be formed at room temperature leading to difficulty in manufacturing, handling, and administering.

If the gelation temperature is higher than 37^{0}C, a liquid dosage form still exists at the body temperature, resulting in the corneal clearance of the administered drugs at an early stage. As the temperature of the *Cul-de-sac* is 34^{0}C, this study aimed at preparing the liquid formulations of PF127 that shows phase transition temperature below 34^{0}C. Addition of mucoadhesive polymer in the Poloxamer solution leads to decrease in the gelation temperature, when we use 16%w/v of Poloxamer then temperature further goes away from body temperature; hence 14 % w/v Poloxamer concentration was taken for further study.

Sol-gel-sol phase diagram

The gelation of PF127 vehicles is known to result from the change in micellar number with temperature. With increasing temperature, the number of micelles formed increases as a consequence of the negative coefficient of solubility of block copolymer micelles. Eventually the micelles become so tightly packed that the solution becomes immobile and gel is formed. Researchers have attributed gelation to the dehydration of PPO groups in the micelle core, a change in the micellar volume, or a decrease in the critical micelle concentration and an increase in the aggregation number.

Recently, Cabana *et al* suggested a mechanism of gelation based on micelles packing and entanglements. Also, conformational changes in the orientation of the methyl groups in the side chains of poly (oxypropylene) polymer chains, constituting the core of the micelle, with expulsion of the hydrating water from the micelles will contribute to the gelation phenomenon.

Gelation temperature of prepared formulations

From the phase diagram shown in figure no.2 it was found that as the polymers concentration increases there was simultaneous decrease in the gelation temperature and increase in sol temperature. It was clear from the phase diagram that the minimum concentration of polymer has the maximum gelation temperature and minimum sol temperature. The micelle formed from the minimum concentration of polymer was unstable and they required minimum energy to break hydrogen bond formed during aggregation. The energy for breaking the bond supplied in the form of external heat. The binary phase diagrams show critical gel concentration (CGC) and critical gelation temperature (CGT) (a lower transition temperature from sol to gel and an upper transition temperature from gel to sol).

Optimum concentration of the vehicle

- The viscosity of pluronic /HPMC solution was significantly greater than that of individual Pluronic solution.
- The above results and interferences clear in cases that the gel strength of the polymer solution in the physiological condition can be enhanced significantly by combining the two individual solution.
- The results suggest that the combined polymer solution may have more strength to withstand the low shear forces likely to be encountered in the cul de sac of the eye as well as prolong the residence time of the drug in the eye.
- Block copolymer PF 127 gels are thought to formed by hydrogen bonding in aqueous systems, caused by the attraction of the pluronic ether oxygen atom with protons of water. If the hydrogen bonding is supplemented by adding compounds with hydroxyl groups such as the examined cellulose derivatives, the desire gel strength may be achieved with reduced concentration.
- The optimum concentration of combination of HPMC E50 and Pluronic F127 was found to be 1.5% w/v and 14% w/v, respectively.
- Gelation of HPMC solutions is primarily caused by the hydrophobic interaction between molecules containing methoxy substitution.
- At low temperatures, the macromolecules are hydrated, and there is little polymer–polymer interaction other than simple entanglement. As the temperature is raised, the polymers gradually lose their water of hydration, which is reflected by a decline in relative viscosity.

Viscosity of optimized formulations

Viscosity study:

The study of the rheological behavior of liquid and semi-solid pharmaceutical formulations is important in view of their complex nature and their possible influence on manufacturing processes. Poloxamer 407 solution has unusual rheological characteristics including thermoreversible gelling, gel-sol transition temperature and viscosity, which have to be studied. The polymer solution is a highly viscous gel at room temperature, but becomes a liquid at low temperature (5^oC). The gel undergoes thermoreversible gelling and can be cooled and warmed many times without changing its properties. Viscosity studies were performed by changing the temperature and keeping the shear rate constant. The results obtained were given in following table.

Mucoadhesive strength of optimized formulations

The Mucoadhesive force is an important physiochemical parameter for in situ ophthalmic gels because it prevents the formulation from rapid drainage and hence lengthens its precorneal residence time. The results of prepared formulae are tabulated in table no.3

The mucoadhesive force of the prepared Ketorolac tromethamine in situ forming gels formulations significantly increased in formulations containing mucoadhesive polymers such HPMC. Also, increasing the concentration of mucoadhesive polymer in the formulations significantly increased the mucoadhesive force (ANOVA at $p < 0.05$). This could be explained by the fact that secondary bond forming groups (e.g., carboxyl, hydroxyl, ether, oxygen, and amine) principle source of mucoadhesion, and the cellulosic polymers used during the formulation of the in situ forming have an abundance of hydroxyl and ether groups along their length, which are responsible for the mucoadhesive properties.

Increasing the concentration of the cellulosic derivatives in situ forming gels increased the bonds-forming groups, increasing the mucoadhesive force of the formulations.

In-vitro diffusion study

In- vitro diffusion from various formulations was studied by using cellulose membrane having pore size of 45 µm. Percent cumulative drug release obtained from various formulation is given in following table.

Ocular irritation testing

From the above results it is concluded that drug release was decreased for the formulation containing mucoadhesive polymers such as HPMC. The mechanism for such enhanced resistance may be due to reduction in the number and dimension of water channels and due

to the increase in the number and size of micelles within the gel structure. The shorter intermicellar distance leads to greater numbers of cross-links between neighboring micelles leading to higher viscosity and lower rate of drug release. This assumption may be potentiated by the rheology study that indicates direct proportionality between gel concentration and viscosity The retarding effect of the cellulosic mucoadhesive polymers could be attributed to their ability to increase the overall product viscosity as well as their ability to distort or squeeze the extra-micellar aqueous channels of poloxamer micelles through which the drug diffuses, thereby delaying the release process.

Fig.1 In vitro Mucoadhesion study- Modified balance method

Table No.1 Formulation composition and gelling capacity of Pluronic /HPMC formulations.

Batch code	Pluronic 127 F	HPMC E50 LV	Gelling capacity [a]
F1 (Control)	15 %	_	+++ (*)
F2	14 %	0.5%	+
F3	14%	1.0%	++
F4	14%	1.5%	+++
F5	14%	2.0%	+++

(*) : polymer solution form stiff gel at room temperature only.

a : + weak gelation

+ + gelation immediate , remain for few hours

+ + + gelation immediate , remain for extended period

Table No.2 Gel to sol temperature of various concentration of Poloxamer

Sr. No.	Concentration (%w/v)	Gel Temperature (^{0}C)	Sol Temperature (^{0}C)
1	16.0	44.53± 0.057	46.83±0.15
2	16.5	43.433±0.115	47.5±0.32
3	17.0	38.966±0.2516	49.8±0.17
4	17.5	37.733±0.115	51.2±0.17
5	18	31.66±0.642	55.13±0.49
6	18.5	30.4±0.173	58.26±0.40
7	19.0	29.33±0.3055	59.9±0.3
8	19.5	25.4±0.264	61.8±0.346
9	20.0	24.067±0.115	62.53±0.057

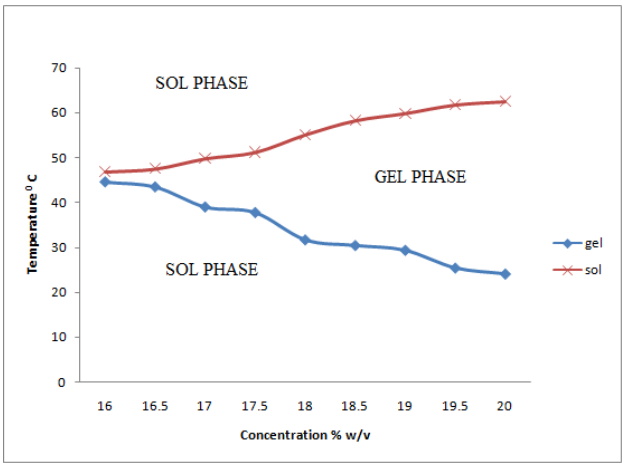

Figure no.2 Binary phase diagrams of Poloxamer 407.

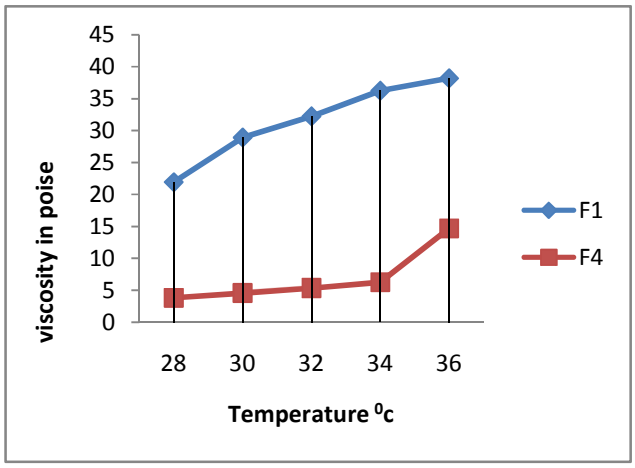

Fig. No. 3 Comparison of F1 with F4 formulation for the apparent Viscosity

Table No. 3 Gelation temperature, $_{p}$H and Mucoadhesion force of prepared formulations-

Formulation	Gelling Temperature	$_{p}$H	Mucoadhesion Force(dyne/cm^2)
F1	23^{0}C±0.5	6.9±0.020	3335.59±78.01
F2	39^{0}C±1.0	6.92±0.030	4818.08±136.30
F3	**36^{0}C±0.5**	**6.85±0.050**	**6437.10±113.7**
F4	34^{0}C±0.5	6.80±0.030	8777.36±97.32
F5	32^{0}C±0.5	6.74±0.030	10494.92±58.27

Table No. 4 Viscosity Study of optimized formulation at different temperatures.

Sr.No.	Temperature^{0}C	ViscosityPoise	
		$F_{1\ (Control)}$	F_4
1	28	21.937	3.812
2	30	28.93	4.56
3	32	32.24	5.34
4	34	36.25	6.23
5	36	38.21	14.687

Table no.5 Diffusion study of Formulation F1-F5

Sr. No.	Time (mins.)	F1		F2		F3		F4		F5	
		% CDR	S.D.	% CDR	S.D.	% CDR	S.D.	% CDR	S.D.	% CDR	S.D.
1	0	0	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0	0.00
2	30	29.571	0.29	20.340	0.27	22.718	0.41	24.536	0.45	23.942	0.12
3	60	37.253	0.24	32.460	0.23	34.419	0.23	36.040	0.33	32.736	0.20
4	120	68.962	1.28	61.450	0.97	63.246	0.45	64.330	0.40	63.602	0.05
5	180	83.067	0.52	69.330	0.54	70.930	0.21	71.515	0.21	70.526	0.11
6	240	95.603	0.27	92.550	0.21	94.737	0.25	84.248	1.40	83.470	0.14
7	300	92.474	0.40	99.450	0.34	100.429	0.36	94.586	0.16	93.568	0.17
8	360	89.216	0.64	94.578	0.63	94.578	0.23	101.858	0.41	99.374	0.13

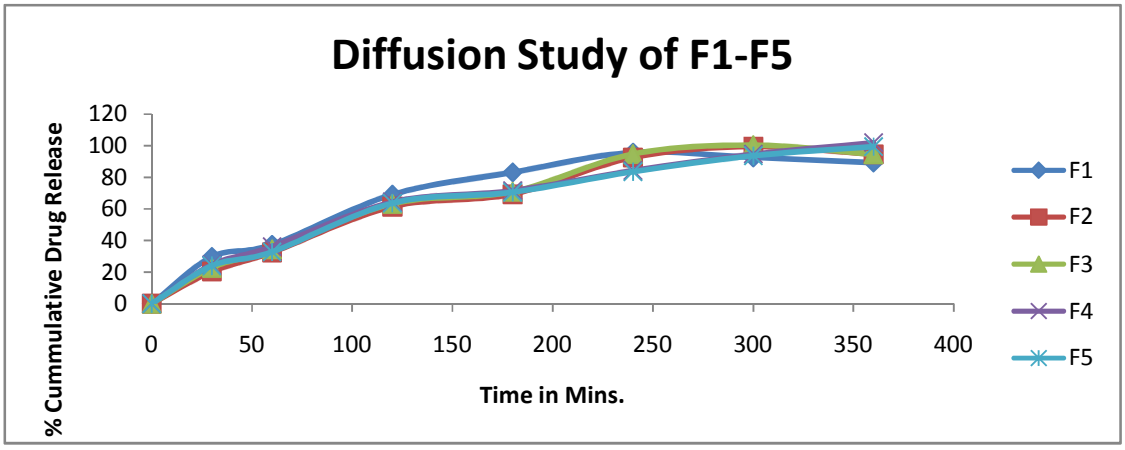

Fig. No. 4 Comparison of F1 with F2, F3, F4, and F5 for Diffusion Study.

Table No.6 Draize eye irritation score

Formulation code	OBSERVATIONS. Time in hrs						
	½ Cj I Co	1 Cj I Co	2 Cj I Co	4 Cj I Co	24 Cj I Co	48 Cj I Co	72 Cj I Co
Blank	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
F4.	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0

Cj – Conjunctiva I – Iris Co – Cornea. Total Score – 0 Irritation Score – 0.00

CONCLUSION

Ketororolac Tromethamine, a NSAID used in the treatment of post-operative ocular infections, was successfully formulated as thermoreversible in situ gel forming ophthalmic solution (0.5%) using Poloxamer 407 as a gelling agent in combination with mucoadhesive polymers as a viscosity-enhancing and release retarding agent. The formulation was subjected to various physicochemical studies, such as visual appearance, pH, drug, content uniformity etc. and was found to be satisfactory.

The gelling temperature & gelling capacity important evaluation parameters showed that the gel formed instantaneously at *cul-de-sac* temperature, which retained its consistency for extended period of time. The rheological profile demonstrated that formulation was liquid at non physiological conditions (pH5.0) and room temperature (25^0C) and underwent rapid gelation upon raising pH to 7.4 i.e. at physiological conditions and temperature 34^0C, a condition of cul-de-sac.

Also, eye irritation studies confirmed that the formulation was non-irritating to eyes. The optimized formulation F4, followed non-fickian diffusion release mechanism indicated by n values found after applying the release data to various models. Statistical analyses of the drug release data exhibited the significant effect of concentration of HPMC on drug release and mucoadhesive strength.

Hence, it can be concluded that in situ gels are a viable alternative to conventional eye drops by providing sustained release of medicament resulting in decreased frequency of administration leading to better patient acceptance.

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