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NOVEL OCULAR SUSTAINED RELEASE FLURBIPROFEN INSITU GELS DEVELOPMENT AND IN-VITRO EVALUATION

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ABSTRACT

Insitu gelation is a process of gel formation at the site of application, like ocular region. Insitu gel phenomenon based upon liquid solution of drug formulation and converted into mucoadhesive semi-solid. So that it can overcome the drawbacks of conventional eye drops like poor therapeutic response, because of high tear fluid flow dynamics. And also the high frequency of eye drop instillation is associated with patient non-compliance. In the present project, insitu gels were prepared by utilising various concentrations of pH responsive gelling agents like carbopol 934, sodium alginate, chitosan, and viscosity modifier agent HPMC K4M is used. From the nine formulation trails F1-F9, formulation F6 containing 0.8% of HPMC K4M and 0.5% of Carbopol 934 was found to be optimised. The developed optimised formulation was tested for various in-vitro quality control tests and was found to sustaining the drug release upto 8 hours, was stable, non-irritant and found to be sterile in the performed tests. The developed system thus could be a viable alternative to the conventional eye drops.

KEYWORDS: Insitu Gelling, Flurbiprofen, Sustained Delivery.

1. INTRODUCTION

The main aim of ocular pharmaco-therapeutics is attainment of effective drug concentration at the intended site of action for a sufficient period of time to elicit the response. Most common conventional drug dosage forms include eye solutions, eye suspensions, eye ointments are used to treat ocular disorders. The use of this dosage system has several drawbacks like poor bioavailability and therapeutic response, because high tear fluid turnover and dynamics cause rapid precorneal elimination of the drug. A high frequency of eye drop instillation is associated with patient non-compliance. Inclusion of excess drug in the formulation is an attempt to overcome bioavailability problem is potentially dangerous if the drug solution drained from the eye is systemically absorbed from the nasolacrimal duct. Various ophthalmic vehicles such as inserts, ointments, Suspensions, and aqueous gels have been developed in order to lengthen the residence time of instilled dose and enhance the ophthalmic bioavailability. These ocular drug delivery systems however have not been used extensively because of some drawbacks such as blurred vision from ointments or low patient compliance from inserts.[1-3]

A significant increase in the precorneal residence time of drugs and consequently bioavailability can be achieved by using delivery systems based on the concept of in situ gel formation. These systems consist of polymers that exhibit sol-to-gel phase transitions due to a change in a specific physico chemical parameter (pH, temperature) in their environment; the cul-de-sac in this case. Dependingon the method employed to cause sol-to-gel phase transition on the eye surface, the following three types of systems are recognized.

- pH triggered systems (e.g. cellulose acetate hydrogen phthalate latex)
- temperature-dependent systems (e.g. pluronics and tetronics) and
- ion-activated systems (e.g. GelriteE and gellan).[4,6,8]

2. MATERIALS AND METHODS

2.1. *Materials*

Flurbiprofen was obtained from SL-drug supplier Hyderabad India, as a gift sample. Carbopol 934 and Sodium alginate were procured from SD-fine chemicals. Ltd. Mumbai, India. HPMC K4M was obtained for Colorcon asia pvt. ltd. Goa. India. Chotosan was obtained form CIFT Cochin, India. And all the other materials used were of analytical grade and double distilled water was used throughout the project.

2.2. *Preparation of formulations*

Buffer salts were dissolved in double distilled water. HPMC K4M was added and Polymer solution was made in phosphate buffer 6.8 pH. Drug is dissolved in buffer solution and pH was adjusted. Benzalkolium chloride was added as preservative. Make up volume upto 100

with phosphate buffer. Filteration of the formulation with 0.2 *u* whattman filter paper Sterilisation was carried out by Autoclave at optimum temperature.[9,10]

2.2.1. *Selection of vehicle*

The solubility of flurbiprofen was tested in phosphste buffer I.P. (pH 4.6, 4.8, 5.0, 5.5 and 6.0), citrophosphatebuffer B.P. (pH 6.0 and 6.2) and phosphate buffer USP (pH 6.0, 6.5 and 7.2). Solutions offlurbipofen (0.03%, w/v) in the buffers in which it was soluble were prepared and these were tested for stability to light, temperature and autoclaving using a stability indicating high-performance thin-layer chromatographic (HPTLC) method.[11,12]

2.2.2. *Preparation of in situ gelling systems*

Aqueous solutions of varying concentrations of Carbopol 934, sodium alginate, chitosan and HPMC K4M of different concentration formulation codes F1, F2. F9 were prepared as shown in the table no.01 and evaluated for gelling capacity and viscosity in order to identify the compositions suitable for using as in situ gelling systems. The gelling capacity was determined by placing a drop of the formulation in a vial containing 2 ml of artificial tear fluid.[10,11]

Table No 1: Compostion of Various Formulations of Ocular Insitu Gels of Flurbiprofen.

Ingredient (wt/v)	F1 %	F2 %	F3 %	F4 %	F5 %	F6 %	F7 %	F8 %	F9 %
Flurbiprofen	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
HPMC K4M	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Sodium Alginate	0.3	0.4	0.5	-	-	-	-	-	-
Carbopol 934	-	-	-	0.3	0.4	0.5	-	-	-
Chitosan	-	-	-	-	-	-	0.3	0.4	0.5
Disodium hydrogen phosphate	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Benzalkolium chloride	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Nacl	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Double Distilled water	q.s 100ml	q.s 100ml	q.s 100ml	q.s 100ml	q.s 100ml	q.s 100ml	q.s 100ml	q.s 100ml	q.s 100ml

2.3. EVALUATION OF pH – TRIGGERED IN-SITU GELLING SYSTEM

1) Determination of visual appearance and clarity.

The appearance and clarity were determined visually against a white and black back ground for presence of any particulate matter.[10-15]

2) pH

pH is one of the most important parameter involved in the ophthalmic formulation. The two areas of critical importance are the effect of pH on solubility and stability. The pH of ophthalmic formulation should be such that the formulation will be stable at that ph and at the same time there would be no irritation to the patient upon administration of the formulation. Ophthalmic formulation should have ph range in between 6.2 to 7.4. The developed formulations were evaluated for pH using control dynamics digital pH meter (equiptronics).[19]

3) % Drug Content:

Uniform distribution of active ingredient is important to achieve dose uniformity. The drug content was determined by diluting 1 ml of formulation to 100 ml with simulated tear fluid pH 7.4. Aliquot of 5 ml was withdrawn and further diluted to 25 ml with STF. flurbiprofen concentration was then determined at 247 nm by using UV-VIS spectrophotometer.[20]

4) Rheological studies

Viscosity of instilled formulation is an important factor in determining residence time of drug in eye. The viscosity determination of prepared formulations was carried out using Brookfield viscometer LVDV-E with spindle 61. Viscosity of sample was measured at different angular velocities between 20-200 rpm.[8]

5) Gelling capacity

All prepared formulations were evaluated for gelling capacity and viscosity in order to identify the compositions suitable for use as in situ gelling systems. The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of artificial tear fluid freshly prepared and equilibrated at 37° C and visually assessing the gel formation and noting the time for gelation and time taken for the gel formed to dissolve.[9]

6) In vitro drug release studies

The in-vitro release of flurbiprofen from the formulation prepared was studied through cellophane synthetic membrane using diffusion cell. The dissolution medium used was artificial tear fluid freshly prepared (pH 7.4) The cellophane membrane previously soaked overnight in the dissolution medium was tied with the help of thread to one end of a specifically designed glass cylinder (opens at both end). A 1 ml volume of formulation was accurately pipette into this assembly. The cylinder was attached to a metallic drive shaft and suspended in 50 ml of

dissolution medium maintained at 37± 1º C so that the membrane just touched the receptor medium surface. The dissolution medium was stirred at 50 rpm using magnetic stirrer. Aliquots each of 1 ml volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with the receptor medium if needed and analysed by a UV-Visible spectrophotometer at 247 nm using receptor medium (STF) as a blank.[10]

7) Sterility test

The sterility test was performed according to Indian Pharmacopoeia. Direct inoculation method was used. 2 ml of liquid from test container was removed with a sterile pipette or with a sterile syringe or a needle. The test liquid was aseptically transferred to fluid thioglycolate medium (20 ml), and soyabean- casein digest medium (20 ml) separately. The liquid was mixed with the media. The inoculated media were incubated for not less than 14 days at 30º C to 35º C in the case of fluid thioglycolate medium and 20º C to 25º C in the case of soyabean- casein digest medium.[11]

8) Ocular irritancy test (HET-CAM)

For the present study, modified hen's egg chorioallantoicmembrane (HET-CAM) test as reported by Velpandian et al. was carried out. Briefly, fertilized hen's eggs were obtained from poultry farm. Three eggs for each formulation weighing between 50 and 60 g were selected and candled in order to discard the defective ones. These eggs were incubated in humidified incubator at a temperature of 37± 0.5°C for 3 days. The trays containing eggs were rotated manually in a gentle manner after every 12 h. On day 3, egg albumin (3 ml) was removed by using sterile techniques from the pointed end of the egg. The hole was sealed by 70% alcohol-sterilized parafilm (American Can Company, USA) with the help of heated spatula. The eggs were kept in the equatorial position for the development of CAM away from the shell. The eggs were candled on the fifth day of incubation and everyday, thereafter, nonviable embryos were removed. On the tenth day, a window (2×2 cm) was made on the equator of the eggs through which formulations (0.5 ml) were instilled. A0.9%NaCl solution was used as a control as it is reported to be practically nonirritant. The scores were recorded according to the scoring schemes as shown in Table2 and score obtained is given in Table 3.[25]

Table 2: Scoring Chart for HET-CAM Test.

S. NO	Effect	Scores	Inference
1	No visible haemorrhage	0	Nonirritant
2	Just visible membrane discoloration	1	Mild irritant
3	Structures are covered partially due to membrane discoloration or haemorrhage	2	Moderately irritant
4	Structures are covered totally due to membrane discoloration or hemorrhages	3	Severe irritant

9) Accelerated stability studies

Optimised formulation F6 was placed in ambient colour vials and sealed with aluminum foil for a short term accelerated stability study at 40ºC±5º and 75±5% RH as per International Conference on Harmonization (ICH) states Guidelines. Samples were analyzed every month for Clarity, pH, gelling capacity, drug content, rheological evaluation, and in vitro diffusion test. The storage conditions and the length of studies chosen was sufficient to cover storage, shipment, and subsequent usage conditions.[12,13]

3. RESULTS AND DISCUSSION

3.1 preformulation studies

Table 3: Melting point.

S NO.	PURE DRUG	REFENCE RANGE	OBSERVED RANGE
1	Fluribprofen	117 °C (243 °F)	117°C.

3.1. *Selection of vehicle*

The studies in various buffer solutions indicated the drug was soluble in acetate buffers of pH 4.6, 4.8 and 5.0 and in citrophosphate buffer of pH 6.0 at the dosage level desired (0.03%, w/v). The solutions were stable to elevated temperatures and autoclaving. However, their instability to light as evidenced by discoloration of the exposed solutions necessitated their packing in amber vials. The marketed eye drop was found to have a pH of 6.2.[14,15,20]

3.2. *Evaluation of formulation*

Table 4: Evaluation of formulations.

Formulation code	*PH-measerment*	*Viscosity*	*Clearity*
F1	6.50 ± 0.01	*560.0 cps*	*Slightly Clear*
F2	6.52± 0.01241	*720.0 cps*	*Moderately Clear*
F3	6.54 ± 0.02549	*880.0cps*	*Clear*
F4	6.50 ± 0.1322	*975.0cps*	*Slightly Clear*
F5	6.57 ± 0.060	*1087.5cps*	*Clear*
F6	6.8± 0.125	*2750.0cps*	*Very clear*
F7	6.62± 0.0565	*524.7cps*	*Slightly Clear*
F8	6.65 ± 0.0132	*859.6cps*	*Clear*
F9	6.68± 0.0605	*986.4cps*	*Moderately Clear*

All values are expressed as mean ± S.D (n=3)

Table 5: Drug content.

Formulation Code	Drug content (%)
F1	97.99± 0.05
F2	99.45± 0.030
F3	99.60± 0.104
F4	98.26± 0.072
F5	99.49± 0.025
F6	100.1± 0.05
F7	99.18± 0.102
F8	97.78± 0.38
F9	96.99± 0.56

Table 6: Gelling capacity.

FORMULATION CODE	GELLING CAPACITY
F1	_
F2	+
F3	++
F4	+
F5	++
F6	+++
F7	_
F8	+
F9	++

+ is gel after few minutes, dissolve rapidly.
++ is gellation immediate, remain for few hours.
+++ is gellation immediate but for few extended periods.

Table 7: In Vitro Release Studies

Formulation /Time in hours	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
0.5	36	25	19	32	36	30	41	34	30
1	50	39	30	40	42	41	62	41	38
2	68	52	43	45	51	52	75	56	50
3	76	63	54	50	59	67	84	68	62
4	89	81	67	56	58.89	71	86	72	69
5	92	89	82	58.23	64.79	84	98	86	76
6	94	90	85	65.6	78.2	100	98	99	84
7	96	93	94	69.8	82	100	98	99	98
8	98	96	98	76.2	92	100	98	99	98

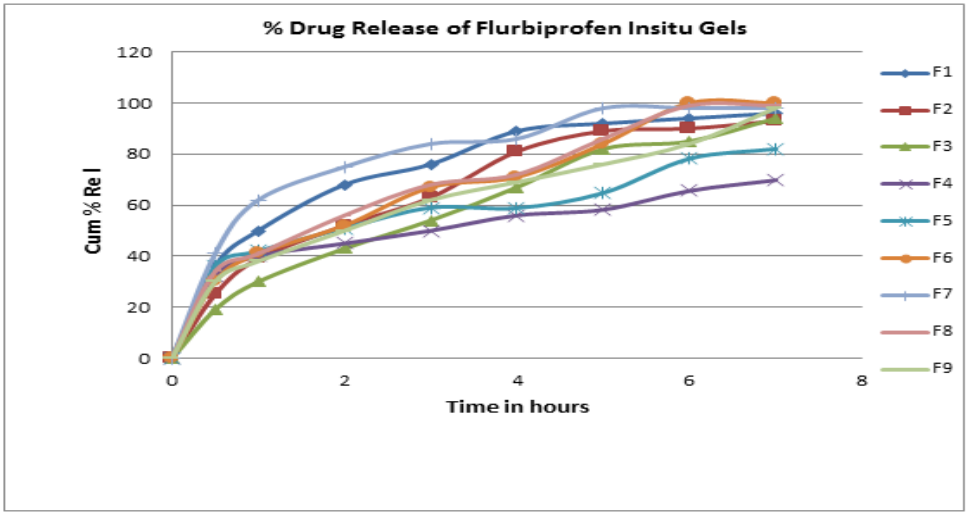

Fig 1: Invitro Drug Release of Formulation.

Test for Sterility

There was no appearance of turbidity and hence no evidence of bacterial growth when optimized formulation was incubated for 7 days at 30ºC to 35ºC in case of fluid thioglycolate medium and at 20ºC to 25ºC in case of soyabean-casein digest medium. The preparations examined, therefore, passed the sterility test.[20-24]

Ocular Irritation Test (HET-CAM Test)

Ocular irritation of the developed formulation was checked by hen's egg chorioallantoic membrane test which is a rapid, sensitive, and inexpensive test. Testing with incubated eggs is a borderline case between in vivo and in vitro systems and does not conflict with the ethical and legal obligations. The chorioallantoic membrane of the chick embryo is a complete tissue

including veins, arteries, and capillaries and is technically very easy to study. It responds to injury with a complete inflammatory process, a process similar to that induced in the conjunctival tissue of the rabbit eyes. Developed formulation was tested by this method and the result was compared with those obtained using normal saline, which was used as control that is supposed to be practically nonirritant. A means score of 0 was obtained for normal saline. carbopol/HPMC-based formulation was nonirritant up to 12 h (mean score 0) while the mean score was found to be 0.33 up to 24 h (Table). The study shows that the formulation is nonirritant to mild irritant and is well tolerated.[25]

Table 8: Scores Obtained in HET-CAM Test.

	Score										
	Time in mins										
S no.	Formulations		0	5	15	20	60	120	240	480	1440
1	Normal saline	Egg 1	0	0	0	0	0	0	0	0	0
2		Egg 2	0	0	0	0	0	0	0	0	0
3		Egg3	0	0	0	0	0	0	0	0	0
4		Mean	0	0	0	0	0	0	0	0	0
5	Optimised formulation	Egg 1	0	0	0	0	0	0	0	0	0
6		Egg 2	0	0	0	0	0	0	0	0	0
7		Egg 3	0	0	0	0	0	0	0	0	1
8		Mean	0	0	0	0	0	0	0	0	0.33

Table 9: Accelerated stability studies.

Stability study of Optimized formulation (F6) Sr.No.	**Observations**	**Before Stability testing**		**After Stability testing**	
Duration		**1 month 2 months**		**3 months**	
1.	Clarity	Clear	Clear	Clear	Clear
2.	Visual Appearance	Transparent	Transparent	Transparent	Transparent
3.	pH	6.8	6.8	6.81	6.82
4.	Drug content	101.5%	101.5%	101.4%	101.3%

CONCLUSION

Flurbiprofen (Anti-inflammatory agent) was successfully formulated as pH-induced in situ gel-forming eye drop. Common drawbacks of eye drops can be solved improving residence time, prolong drug release,thus therapeutic efficacy can be improved. Considering its features, the insitu gel formulation can be seen as an alternative to conventional eye drops with decreased frequency of administration and better patient acceptance. The formulation instead of giving 4 times a day can be given as once in a day as it has sustained drug release. The developed formulation could be a viable alternative to conventional eye drops. Hence, it could be concluded that polymer combination 0.8% HPMC K4M, 0.5% Carbopol 934 showed better potential for flurbiprofen sustained ocular delivery. However, further in-vivo studies needs to be conducted to ensure safety.

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