International Journal of Pharmaceutics & Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

Original Research Article

FORMULATION, DEVELOPMENT AND EVALUATION OF OCULAR IN SITU GEL OF MOXIFLOXACIN AND KETOROLAC TROMETHAMINE

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***Article History:**

Received: 24/04/2023
Revised: 12/05/2023
Accepted: 26/05/2023

ABSTRACT

In situ gelling system of moxifloxacin and ketorolac tromethamine was successfully formulated using polymeric combination of gelling agents Pluronic F127, Carbopol 934 as, temperature sensitive and pH-sensitive respectively along with HPMC 15cps as viscosity enhancing agent. Formulation F6 to F9 show poor gelling capacity in simulated physiological conditions of pH and temperature because of comparatively less concentration of Pluronic F127 in F6 and F9 and due to low concentration of Carbopol in F1, F3, F4 and F5 formulation show better gelling capacity. The comparative study of viscosity was done at 10 rpm. F3, F4, and F5 show comparatively better viscosity and good consistency gel. The *In vitro* drug release data of the optimized formulation was subjected to goodness of fit test by linear regression analysis according to zero order and first order kinetic equation in order to determine the mechanism of drug release. When the regression coefficient values of were compared, it was observed that 'r' values of Higuchi was maximum hence indicating drug release from formulations was found to follow Higuchi release kinetics. In conclusion, evaluation of *in-situ* gel is determined to ensure that the prepared preparation meets the standard and is safe. In the chemical evaluation *in-situ* gel determined the diffusion of the active substance of a compound by measuring its concentration.

Key words: Ophthalmic drug delivery, Moxifloxacin, Ketorolac tromethamine, *In situ* gel, *In vitro* drug release.

INTRODUCTION:

Ocular in situ gels have emerged as a promising drug delivery system for the treatment of ocular diseases. These gels are administered as liquid formulations that undergo gelation upon instillation in the eye, providing prolonged drug release and enhanced therapeutic efficacy. Moxifloxacin and ketorolac tromethamine are commonly used drugs for the treatment of ocular infections and inflammation, respectively (Raval *et al.,* 2012).

The formulation, development, and evaluation of ocular in situ gels of moxifloxacin and Ketorolac tromethamine aim to improve the ocular bioavailability, reduce dosing frequency, and enhance patient compliance. These in situ gels offer advantages such as prolonged contact time with the ocular surface, controlled drug release, and improved therapeutic outcomes (Ghosh *et al.,* 2012).

The development of ocular in situ gels involves selecting suitable gelling agents, viscosity enhancers, and mucoadhesive polymers that can provide a gel-like consistency in the eye. Various techniques such as sol-gel transition, temperature-induced gelation, and ion-activated gelation are employed to achieve gel formation in the ocular environment (Muzaffar *et al.,* 2016; Patel *et al.,* 2012; Tiwari *et al.,* 2008).

The evaluation of ocular in situ gels includes assessing parameters such as gelation time, pH, viscosity, drug content uniformity, drug release profile, and stability. In vitro studies are conducted to evaluate the drug release kinetics, of moxifloxacin and ketorolac tromethamine from the in situ gels.

MATERIALS AND METHODS

Formulation development of *in-situ* gel

- For the preparation of Pluronic F127 based ocular *in-situ* gel all the ingredients were sieved from sieve no 44.
- Then solution of 0.5% and 0.5% of drugs was prepared in acetate buffer 5.0 I.P.
- The solution was cooled in an ice bath and pluronic F127 was added slowly with continuous stirring.
- Then the resulting solution was kept in a refrigerator under 4^0C for 24h. this storage was help in dissolving the Pluronic F 127 completely.
- After 24h carbopol 934 and HPMC 15cps were added slowly along with other exepients with continuous stirring. The stirring should be continued to 2-3 hours for proper mixing and avoid slug formation.

The resulting formulation kept on probe sonicator to remove air bubble. All formulations were stored in LDPE (Low Density Polyethelene) bottles for further use. All the containers stored in refrigerator (Saxena and Kushwaha, 2013).

Evaluations of formulations

Appearance

Clarity is one of the most important characteristic features of ophthalmic preparations. All developed formulations were evaluated for clarity by visual observation against a black and white background (Viram and Lumbhani, 2012).

Drug content

The assay of drug Gentamicin and Dexamethasone was performed by UV method. The calculation was based on calibration curve method using simultaneous equation method (Vodithala *et al.,* 2010).

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 formulations should have pH range in between 5 to 7.4. The developed formulations were evaluated for pH by using calibrated digital pH meter (Shankar and Kalikonda, 2014).

For *In situ* gel pH 5.0 should be optimum because both the drug is stable at pH 3.5-5.0. Lowering the pH from 5.0 can causes irritation to eye and on raise the above 5 will result in gelation of formulation due to presence of carbopol.

In-Situ gelling capacity

In situ gelling capacity determined by visual inspection. The formulation has been exposed to the physiological conditions of temperature and pH. Simulated tear fluid (STF) was prepared and warm up to 37^0C. Formulations were introduce into STF in a ratio of 1:2 Change in consistency of Formulations were visually inspected (Mahesh and Manjula, 2012).

Viscosity study

At pH 5.0 and temperature less than 16^0C the developed formulations were in liquid state and show low viscosity. For viscosity studies the pH of formulations were raised from pH 5.0 to pH 7.4 and the temperature was raised to 37^0C. pH was raised to 7.4 by the addition of 0.5M NaOH [11].

The resulting gel studied for viscosity on Brookfield Synchrolectric Viscometer using Spindle No.7 at 50 RPM for comparative study. The angular viscosity was measured by gradually increase the RPM from 10 to 70.

Sterility testing

The test for sterility is applied to pharmacopoeial articles that are required according to the Pharmacopoeia to be sterile. However, a satisfactory result only indicates that no contaminating viable micro-organisms have been found in the sample examined in the conditions of the test. The test must be carried out under aseptic conditions designed to avoid accidental contamination of the product during testing. For achieving these conditions, a grade A laminar airflow cabinet or an isolator is recommended. The test environment has to be adapted to the way in which the tests are performed. Precautions taken for this purpose should not adversely affect any micro-organisms, which are to be revealed in the tests. The working conditions in which the tests are carried out should be monitored regularly by appropriate sampling of the air and surfaces of the working area and by carrying out control tests (Gratieri *et al.,* 2011).

Method of Test

For aqueous solutions: Remove the liquid from the test containers with a sterile pipette or with a sterile syringe or a needle. Transfer the quantity of the preparation under examination into the culture medium so that the volume of the preparation under examination is not more than 10 per cent of the volume of the medium, unless otherwise prescribed. If the preparation under examination has antimicrobial activity, carry out the test after neutralising this with a suitable neutralizing substance or by dilution in a sufficient quantity of culture medium. When it is necessary to use a large volume of the product it may be preferable to use a concentrated culture medium prepared in such a way that it takes account of the subsequent dilution.

Where appropriate, the concentrated medium may be added directly to the product in its container. Incubate the inoculated media for not less than 14 days. Observe the cultures several times during the incubation period. Observe the containers of media periodically during the 14 days of incubation. If the test specimen is positive before 14 days of incubation, further incubation is not necessary. For products terminally sterilized by a validated moist heat process, incubate the test specimen for not less than 7 days.

In-vitro drug diffusion study

The *in vitro* release of drugs from the formulations was studied through cellophane membrane. The dissolution medium used was Simulated Tear Fluid freshly prepared (pH 7.4). Cellophane membrane, previously soaked overnight in the dissolution medium, was tied to one end of a specifically designed glass cylinder (open at both ends and of 5 cm diameter). A 1-ml volume of the formulation was accurately pipetted into this assembly. The cylinder was attached to the metallic driveshaft and suspended in 50 ml of dissolution medium maintained at 37±1°C so that the membrane just touched the receptor medium surface. The dissolution medium was stirred at 50 rpm using magnetic stirrer. Methodology Aliquots, each of 1-ml volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium (Nayak *et al.,* 2012).

Table 1: Composition of different formulations of *In-situ* gel

S. No.	Ingredient (%)	Formulations								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1.	Moxifloxacin	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
2.	Ketorolac tromethamine	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
2.	Pluronic F127	20	15	10	20	15	10	20	15	10
3.	Carbopol 934	0.2	0.2	0.2	0.3	0.3	0.3	0.4	0.4	0.4
4.	HPMC 15cps	1.0	1.0	1.0	0.75	0.75	0.75	0.5	0.5	0.5
5.	EDTA	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
6.	Benzalkonium Chloride	0.013%	0.013%	0.013%	0.013%	0.013%	0.013%	0.013%	0.013%	0.013%
7.	NaCl	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
8.	Poly ethylene glycol	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
9.	Acetate Buffer (pH 5.0)	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml

Table 2: Clarity test of *in-situ* gel formulations

Formulation code	Clarity
F1	Clear
F2	Clear
F3	Clear
F4	Clear
F5	Clear
F6	Clear
F7	Precipitate observed
F8	Precipitate observed
F9	Turbid

Table 3: Drug content analysis

Formulation	Drug Content (%)*	
	Moxifloxacin	Ketorolac tromethamine
F1	98.22±0.12	96.65±0.65
F2	99.14±0.25	99.89±0.62
F3	97.22±0.32	98.56±0.41
F4	98.65±0.14	97.85±0.32
F5	95.51±0.15	97.65±0.14
F6	95.56±0.32	98.12±0.52
F7	96.69±0.56	95.65±0.14
F8	97.89±0.14	98.85±0.32
F9	98.25±0.54	98.78±0.25

***Average of three determinations (n=3)**

Table 4: pH determination

Formulation	pH	Adjust to
F1	4.5	5.0 ±0.1
F2	4.8	5.0 ±0.1
F3	4.6	5.0 ±0.1
F4	4.7	5.0 ±0.1
F5	4.3	5.0 ±0.1
F6	4.7	5.0 ±0.1
F7	4.6	5.0 ±0.1
F8	4.8	5.0 ±0.1
F9	4.2	5.0 ±0.1

Table 5: *In-situ* gelling capacity of *In-situ* gel formations

Formulation code	*In-situ* gelling capacity
F1	"+++"
F2	"++"
F3	"+++"
F4	"+++"
F5	"+++"
F6	"+"
F7	"+"
F8	"+"
F9	"+"

"+" gelation after five minutes and dissolves rapidly

"++" gelation immediate, remains for few hours

"+++" gelation immediate, remains for extended period 8 hours

Table 6: Comparative viscosity* of *in-situ* formulation

Formulation code	% of Pluronic F 127	Viscosity of solution (in cps)	Viscosity after galation
F1	20	1020	2450
F2	15	945	2254
F3	10	869	2140
F4	20	1120	2250
F5	15	985	2145
F6	10	885	2030
F7	20	985	2145
F8	15	841	2032
F9	10	750	1985

*Spindle no.7, rpm 10

Table 7: Sterility testing of formulations

Formulation code	Observation
F1	No growth observed
F2	No growth observed
F3	No growth observed
F4	No growth observed
F5	No growth observed
F6	No growth observed
F7	-
F8	-
F9	-

Table 8: *In-vitro* drug release profile of Ketorolac tromethamine from *in-situ* Formulation F4

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative*% Drug Released	Log Cumulative % Drug Released	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	21.25	1.327	78.75	1.896
1	1	0	32.65	1.514	67.35	1.828
1.5	1.225	0.176	41.12	1.614	58.88	1.770
2	1.414	0.301	58.85	1.770	41.15	1.614
2.5	1.581	0.398	63.32	1.802	36.68	1.564
3	1.732	0.477	75.65	1.879	24.35	1.386
4	2	0.602	89.98	1.954	10.02	1.001
5	2.236	0.699	98.12	1.992	1.88	0.274

Table 9: Comparative study of regression coefficient for selection of optimize Formulation F4

r^2	Zero order	First order	Higuchi	Peppas
Moxifloxacin	0.960	0.913	0.979	0.692
Ketorolac tromethamine	0.968	0.911	0.988	0.675

RESULTS AND DISCUSSION

Ophthalmic *in situ* gelling system of moxifloxacin and ketorolac tromethamine was successfully formulated using polymeric combination of gelling agents Pluronic F127, Carbopol 934 as, temperature sensitive and pH-sensitive respectively along with HPMC 15cps as viscosity enhancing agent.

The clarity of the prepared formulations was found satisfactory but precipitate observed in formulation during storage. The pH of all formulations was found 5.0. The drug content of the prepared formulations was within the acceptable range, and ensures dose uniformity. The formulation F4 showed maximum drug content.

In-situ gelling capacity determined by visual inspection. The formulation has been exposed to the physiological conditions of temperature and pH. Simulated tear fluid (STF) was prepared and warm up to 37°C. Solution was introduced into STF in a ratio of 1:2 changes in consistency of solution visually inspected. Formulation F6 to F9 show poor gelling capacity in simulated physiological conditions of pH and temperature because of comparatively less concentration of pluronic F127 in F6 and F9 and due to low concentration of carbopol in F1, F3, F4 and F5 formulation show better gelling capacity

The comparative study of viscosity was done at 10 rpm. F3, F4, and F5 show comparatively better viscosity and good consistency gel. For sterility testing formulations were diluted ten times by sterile distilled water. From this

dilution remove quantity and placed in culture media, this quantity should be equivalent to more than 200 mg of the formulation. Petri dishes then placed in incubation chamber for 7 days and observed for microbial growth.

The *In vitro* drug release data of the optimized formulation was subjected to goodness of fit test by linear regression analysis according to zero order and first order kinetic equation in order to determine the mechanism of drug release. When the regression coefficient values of were compared, it was observed that 'r^2' values of Higuchi was maximum hence indicating drug release from formulations was found to follow Higuchi release kinetics.

CONCLUSION

In conclusion, evaluation of *in-situ* gel is determined to ensure that the prepared preparation meets the standard and is safe. In the chemical evaluation *in-situ* gel determined the diffusion of the active substance of a compound by measuring its concentration. In microbiology evaluation determine if the preparations is contaminated or not, also be effective and safe.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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