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Research Article

FORMULATION AND EVALUATION OF ION ACTIVATED OCULAR GELS OF KETOROLAC TROMETHAMINE

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ABSTRACT

In-situ gels are viscous polymer-based liquids that exhibit sol-to-gel phase transition on the ocular surface due to change in a specific physico-chemical parameter like ionic strength, pH or temperature. A major problem in ocular therapeutics is the attainment of optimal drug concentration at the site of action, which is compromised mainly due to pre-corneal loss resulting in only a small fraction of the drug being ocularly absorbed. The effective dose administered can be altered by prolonging the retention time of medication into the eye by using *in situ* gels, thereby preventing the tear drainage. The objective of the present study is to formulation and evaluation of the in situ ocular gelling systems (ion activated gelling systems) of Ketorolac tromethamine. These gelling systems involve the use of Gelrite as polymer. The formulations were evaluated for clarity, pH measurement, gelling capacity, drug content estimation, rheological study, in vitro drug release, ocular irritancy studies (as per draize test) and ex-vivo corneal permeation studies using isolated goats cornea. The developed formulations showed sustained release of drug for upto 6 hrs. The formulations were found to be non-irritating with no ocular damage.

Keywords: Ketorolac tromethamine, *In* situ gels, Draize test, Ex-vivo studies, Gelrite.

INTRODUCTION

Ophthalmic drug delivery is one of the challenging endeavors facing the pharmaceutical scientist today. The structural and functional aspects of the eye render this organ highly impervious to foreign substances. A significant challenge to the formulator is to overcome the protective barriers of the eye without causing permanent tissue damage. The major problem encountered with topical administration is the rapid pre-corneal loss caused by nasolacrimal drainage and high tear fluid turnover which leads to only 10% drug concentrations available at the site of actions. Approaches to enhance the ocular bioavailability aim at increasing the corneal permeability by using penetration enhancers or prodrugs, and prolonging the contact time with the ocular surface by using viscosity-enhancing or *in-situ* gelling polymers. The *in-situ* gelling polymers undergo sol-to-gel phase transition on exposure to the physiological conditions present in the eye. *In-situ* gels are viscous polymer-based liquids that exhibit sol-to-gel phase transition on the ocular surface due to change in a specific physico-chemical parameter (ionic strength, temperature or pH) [1, 2]. In situ gelling systems can be classified as ion activated systems (e.g Gelrite [3,4] and sodium alginate, [5]temperature dependent systems (e.g Pluronics [6,7], Tetronics and polymethacrylates[8]), pH triggered systems (e.g Carbopol [9,10,11] and cellulose acetate phthalate [12] and. The principal advantage of *in-situ* gels is that they can be easily administered with accurate and reproducible dose compared to that of preformed gels and have an advantage over preformed gels that they can be easily instilled in liquid form, and are capable of prolonging the residence time of the formulation on the surface of the eye due to gelling.

Ketorolac tromethamine is a nonsteroidal anti-inflammatory drug, used to treat seasonal allergic conjunctivitis. Ion activated ocular gels of Ketorolac tromethamine were prepared by using the polymer Gelrite. The present investigation deals with development and evaluation of ion activated ocular gels of Ketorolac tromethamine. The prepared dosage regimens provided ease in application and capable to sustained drug release with reduced frequency of administration.

MATERIALS AND METHODS

Ketorolac tromethamine was obtained as a gift sample from Symed labs; Hyderabad. Gelrite was obtained as gift sample from Sigma Labs. Acular 0.5% (Mfg by**:** Allergan) was purchased at local medical stores Glass cylinders were fabricated at Murthy labs works, Hyderabad. A.P. Rabbits used for the Draize eye irritancy studies were from the college animal house and the Goat's cornea for the ex-vivo studies was obtained from the local slaughter house.

Preliminary studies for optimum amount of Gelrite for insitu gelation

Preliminary studies were carried out using different concentrations of Gelrite. Based on the gelation studies, Dummy systems using different amounts of Gelrite were taken for the study as shown in Table 1. To optimize the amount of Gelrite, gelation studies were carried out using simulated tear fluid (pH 7.4) at 34 º ±. 0.5º C. Preliminary studies revealed optimum results with 0.75% w/v gelrite.

Effect of Gelrite on gelation

Gelrite was selected as polymers for ion activated ocular gels due to its gelling property. Gelrite in the concentrations of 0.75% w/v was found to be better carrier system because it shows optimum gelation. As the Gelrite concentration increases the gelation capacity increases because of increase in concentration of Gelrite. Based on the gelation capacity, excipient ratio 0.75% w/v was selected.

Table 1: Gelation studies with Gelrite

Formulation code	Gelrite (%w/v)	Gelation	Gelation Capacity
KT-G1	0.5	YES	+
KT-G2	0.75	YES	++
KT-G3	1	YES	+++

(KT: Ketorolac tromethamine, G-1,G-2, G-3:formulation code with Gelrite). **+** Gels after a few minutes and dissolves rapidly, **++** Gelation immediate and remains for few hours, **+++** Gelation immediate and remains for extended period of time.

Preparation of the formulations

Preparation of in-situ gelling systems

Gelrite of different concentrations of (0.5% w/v, 0.75% w/v and 1% w/v) was dispersed in deionized water, heated to 90°C while stirring then cooled to room temperature [13]. The formulations were shown in Table 2.These prepared gels were evaluated for gelling capacity in simulated tear fluid (pH 7.4). The optimized formula is shown in Table no 3. The ion activated gels with gelrite were characterized for clarity, viscosity, assay by UV, pH, FTIR, ocular irritancy and ex-vivo corneal studies.

Table 2: Formulations with ion activated insitu ocular gels containing Gelrite.

Formulation code	Concentration of drug (KT) (%w/v)	Gelrite (%w/v)
KT-G1		0.5
KT-G2	0.5	0.75
KT-G3		1

(KT: Ketorolac tromethamine, G1,G2,G3: formulation code with Gelrite). (KT: Ketorolac tromethamine)

Table 3: Shows formulation of ion activated insitu ocular gels of kt using Gelrite

Ingredients	Amount (% w/v)
Gelrite	0.75
Drug	0.5
Benzalkonium chloride	0.02
Water	20mL
0.1 M NaOH	q.s for pH adjustment

Characterization for Ion activated ocular gels

a. Clarity

The clarity of the formulations before and after gelling was determined by visual examination of the formulations under light alternatively against white and black backgrounds.

b. pH

Formulation was taken in a beaker and 0.1M NaOH was added dropwise with continuous stirring. pH was checked using pH meter (µ pH Systronics digital pH meter)

c. Assay

Accurately weighed amount gel equivalent to 5mg of drug was taken in a 100ml volumetric flask. Simulated Tear Fluid (STF pH 7.4) was added to it and kept on magnetic stirrer to dissolve the drug. The volume was made to 100ml with STF (pH 7.4).and filtered using Whatmann filter paper (No 42). 10ml aliquot of the above solution was taken and diluted to 100ml with STF (pH 7.4). The absorbance of sample solution was determined at 322nm against STF (pH 7.4)

d. In-vitro Dissolution studies

In-vitro drug release studies of samples were carried out by using modified USP apparatus II paddle method with STF (pH 7.4) as dissolution medium. A glass cylinder of 2.5 cm in diameter open at both ends[14] as shown in Fig 1 was designed for the purpose of the study. Dialysis membrane previously soaked in STF (pH 7.4) was taken, dried, and tied on to one end of the glass cylinder and to this one ml of the formulation was accurately pipetted. The glass cylinder was attached to the shaft of USP apparatus II, in place of basket as shown in Figure 1. The cylinder was then suspended in 50 mL of dissolution medium maintained at 34 ± 0.5°C such that the membrane just touched the dissolution medium. The speed of the metallic device shaft was set at 50 rpm. Aliquots were withdrawn at intervals of 1, 2, 3, 4, 5 and 6 hours and replaced by equal volumes of dissolution medium. Aliquots were suitably diluted with STF (pH 7.4) and analyzed by UV Spectrophotometer at 322 nm. The percent release of the drug was computed as shown on Table 5 and the graph of percent drug release versus time were plotted as shown in Fig 2.

Composition of Simulated Tear Fluid (STF)

Sodium chloride : 0.670 gm

Sodium bicarbonate : 0.2 gm

Calcium chloride dihydrate : 8 mg

Water upto : 1000 ml

Data treatment of dissolution studies

Various models like zero order, first order, Higuchi models, and Korsemeyer & Peppas were tested for explaining the kinetics of drug release based on the release rate data as as shown in Table 7.

e. FTIR spectroscopy

FTIR spectra of drug, and formulation were obtained. Sample is suspended between KBr plates, and examined in 0.1mm KBr sealed cell, and scan for 16 times. The instrument model used for FTIR was Prestige 21, SHIMADZU and the FTIR spectrum was recorded from 3800 cm-1 to 650 cm-1.

f. Rheological Evaluation

Viscosity of formulation was determined before and after gellation by using Brookfield's viscometer (DV II model) in the small volume adaptor and the angular velocity was increased gradually from 10, 20, 50 and 100 rpm. The hierarchy of the angular velocity was reversed. The average of two readings was taken to calculate the viscosity of the gels. Gelation was induced in formulation by adding STF pH 7.4.

Fig. 1: It shows the glass cylinders and dissolution assembly

g. Ocular Irritancy test [16]

The optimized formulation was terminally sterilized and evaluated for in vivo performance in animal model (Albino Rabbits). The protocol is approved by college ethical committee (Ethical committee Registration number is CPCSEA/IAEC/Reg. No. 518/2009).

Three rabbits (Albino rabbits) were used for this study. They were housed and maintained in the animal house at room temperature

(27ºC) during the period of the study. They were fed with standard diet and water. The animals were placed in cages and the eyes were marked as test and control respectively. The control group received no sample and the test eye received the formulation (0.5ml), and the eyes were observed for the ocular irritancy (includes the macroscopic observation of cornea, iris, and conjunctiva) [14,15].

h. Ex vivo corneal permeation studies using goat's cornea

Goat cornea was used for the present investigation to study the permeation across the corneal membrane. Whole eyeballs of goat were procured from a slaughter house and transported to laboratory in cold condition in normal saline maintained at 4ºC. The cornea were carefully removed along with a 5–6 mm of surrounding scleral tissue and washed with cold saline. The washed corneas were kept in cold freshly prepared solution of tear buffer of pH 7.4.The study was carried out by using Franz-diffusion cell in such a way that corneum side is continuously remained in an intimate contact with formulation in the donor compartment. The receptor compartment was filled with STF pH 7.4 at 34 º C ± 0.5 º C. The receptor medium was stirred on a magnetic stirred. The samples were withdrawn at different time intervals and analyzed for drug content. Receptor phase were replenished with an equal volume of STF (pH 7.4) at each time interval. The percent drug released was plotted against time to get dissolution rate curves.

RESULTS AND DISCUSSIONS

Gelrite in the concentrations of 0.75% w/v was found to be better carrier system since it shows optimum gellation. With the increase in the concentration of Gelrite the gellation capacity increases.

Characterization of thermo-reversible insitu ocular gels of ketorolac tromethamine

Assay: The ion activated ocular gels of Ketorolac tromethamine prepared complied with the requirements of assay. The results for assay were tabulated in Table 4.

Table 4: Assay of ion activated gels

Formulation code	Drug content (%)
KT-G1	84.3
KT-G2	99.5

(KT: Ketorolac tromethamine, G-1,G-2, G-3: formulation code with Gelrite)

In vitro dissolution: Simulated tear fluid (basic media at pH 7.4) was selected as media for the dissolution studies. This media is an official dissolution media. Hence, optimized formulation of ion activated ocular gels was subjected to in vitro dissolution testing in STF (pH 7.4). ion activated ocular gel with 0.75% w/v of Gelrite concentrations showed highest dissolution rate as shown in Table 5 and Fig 2

Table 5: Comparative dissolution profile of formulations with gelrite

Time	Formulation code	
	KT-G1	KT-G2
0	0	0
1	42.38	39.10
2	51.17	46.32
3	61.45	56.04
4	70.80	65.82
5	63.27	75.70
6	51.74	86.96

(KT: Ketorolac tromethamine, G2: formulation code with Gelrite)

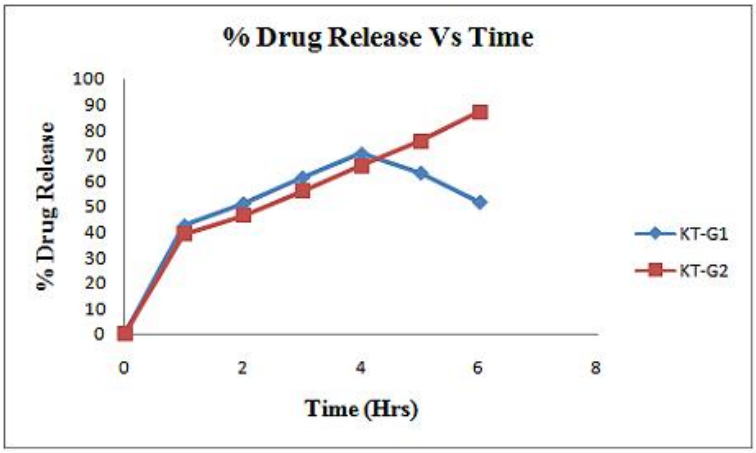

Fig. 2: Effect of excipient ratio on dissolution rate of Ketorolac tromethamine ion activated insitu ocular gels

Table 6: Comparative dissolution profiles of marketed product Vs formulation

Time (Hrs)	Formulation (KT-G2)	Marketed product (Acular 0.5%)
0	0	0
1	39.10	48.27
2	46.32	68.09
3	56.04	87.09
4	65.82	100.32
5	75.70	--
6	86.96	--

(KT: Ketorolac tromethamine, G2: formulation code with Gelrite)

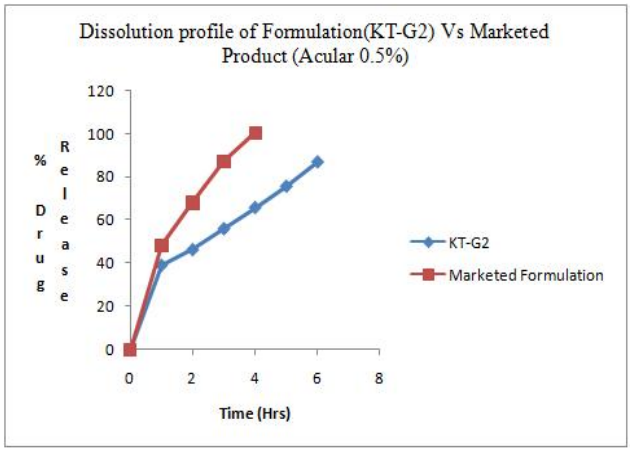

Fig. 3: Comparative dissolution profiles of marketed product and formulation (KT-G2)

Table 7: shows different release rate constants of ion activated ocular gels of Ketorolac tromethamine (formulation code KT-G2)

Formulation	Parameters	Zero order	First order	Higuchi	Korsemeyer & pappas
KT-C9	K	10.16	0.122	27.25	0.436
	r^2	0.991	0.939	0.993	0.979

(KT: Ketorolac tromethamine)

Table 8: Rheological evaluation of ion activate gels

Formulation code	RPM	Viscosity in cps (spindle no. S 34) Before gelation	Viscosity in cps (spindle no. S 34) After gelation
KT-G2	10	1985	4511
	20	1354	2128
	50	746	1563
	100	362	674

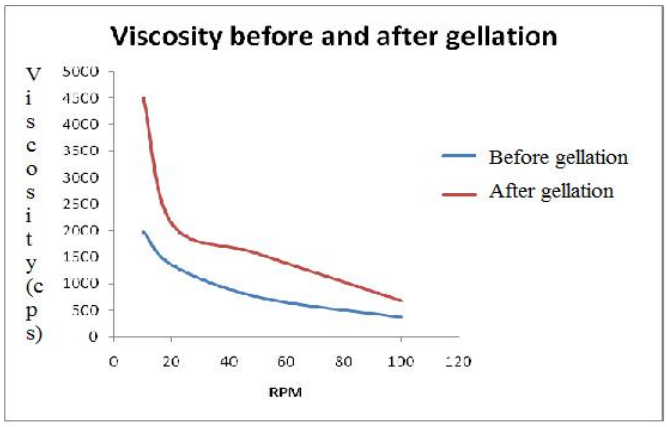

Fig 4: Viscosity before and after gelation (KT-G2)

FTIR spectroscopy

It was performed by KBR pellet method. The principal peaks of Ketorolac tromethamine were observed at 3353, 3082, 2922, 2953cm-1, 1613.The characteristic peaks for formulation were found at 1642 and 3440 cm^{-1} as shown in Fig 5.

Ocular Irritancy studies

Ocular irritation studies indicate that KT-G2 was a non irritant. The formulation was very well tolerated by the eye. No ocular damage or abnormal clinical signs to the cornea, iris, or conjunctivae were visible.

Ex vivo corneal permeation studies

Corneal permeation studies were performed using isolated goat's cornea on Franz-diffusion cell using STF (pH 7.4) at 34 ± 0.5 º C. The samples were withdrawn at regular intervals and analyzed for drug content. The percent drug released was plotted against time to get dissolution rate curves. Table 9 shows the percent drug release and Fig. 6 shows the percent drug release.

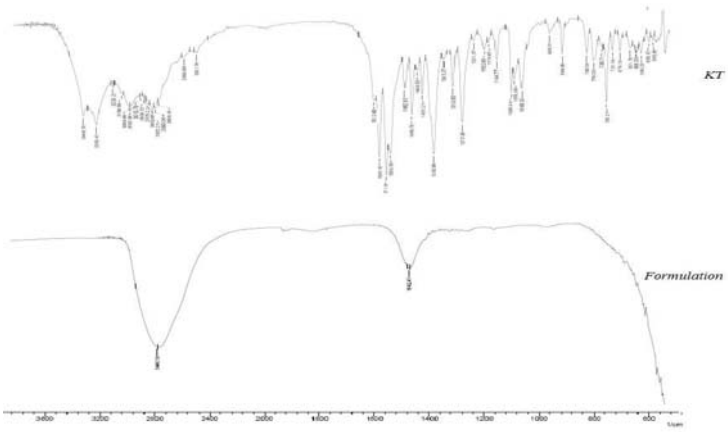

Fig. 5: FTIR of Ketorolac tromethamine and formulation KT-G2

Table 9: Diffusion profile of the formulation (KT-G2) on goat cornea

Time (hrs)	% Drug Release
0	0.00
1	40.93
2	51.50
3	63.09
4	74.06
5	86.79

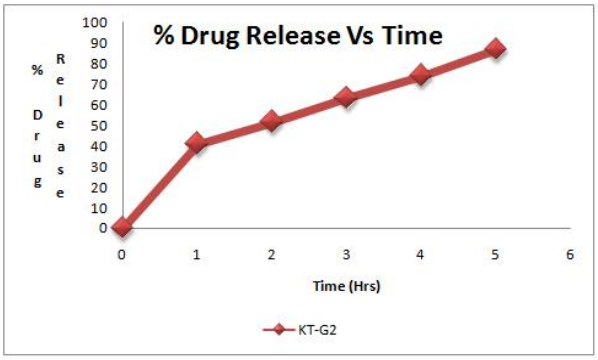

Fig. 6: Diffusion profile of KT-G2 on isolated goats cornea

CONCLUSIONS

Rationale of the present study was to improve the precorneal residence time, and sustain the drug release by utilizing the approach of in situ gelling systems using Gelrite as polymers. It was envisaged that this techniques would prove successful in case of formulations prepared with the drug (KT).Hence, it can be concluded that in situ gelling systems are viable alternative to conventional eye drops by providing sustained release of medicaments to the eye.

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