Ion-activated In-situ Gel Formulation of Gatifloxacin for Sustained Release Ocular Drug Delivery

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Abstract

Presently available conventional ocular dosage forms, viz., solution, suspension, and ointment, etc. possess drawbacks of pre-corneal drug elimination, variability in efficacy, and blurred vision on application and thereby frequently administered to achieve desired ocular drug bioavailability. The *in-situ* gel forming solution (GFS) undergoes a sol-gel transition in cul-de-sac of eye and therefore, overcomes the above drawbacks. In present investigation, an ion-activated *in-situ* gelling topical ocular formulation of gatifloxacin sesquihydrate was developed with an object to provide a long pre-corneal retention time and sustained release drug delivery. Gellan gum as ion-activated *in-sit* gelling agent and HPMC K100M as mucoadhesive release retardant were used in the formulation development. The developed formulations were evaluated for clarity, pH, drug content, isotonicity, *in-vitro* gelation, *in-vitro* drug release and ocular irritancy. The developed products exhibited an instant visco-elastic gelation in contact with simulated lachrymal fluid (SLF) with sustained drug release profile over 8 h period and passed the HET-CAM ocular irritancy test.

Keywords: Gatifloxacin sesquihydrate, In-situ gel, Gellan gum, Simulated lachrymal fluid, HET-CAM.

1. INTRODUCTION

Medication in the form of eye drops is commonly administered in lower cul-de-sac for treatment of ocular diseases(1). Eye drops, in general are however, associated with problem of poor ocular bioavailability due to instant tear formation and drainage of instilled drug, and need to be administered frequently to maintain effective drug concentration(2).

In-situ gel forming solution (GFS) based ocular drug delivery systems are now considered therapeutically more efficacious than eye drops, as they remain in pre-corneal

contact for a longer time and exhibit a sustained drug release profile and therefore, need less frequent drug administration (3). These delivery systems are formulated using some polymers that exhibit sol to gel phase transition under physiological conditions, viz., pH, temperature and ionic content in the eye(4).Depending upon their mechanism, three types of *in-situ* gelling systems have been recognized, i.e., pH triggered systems, like carbopol(5)based formulation; temperature triggered systems, like pluronic(6) and tetronics(7) based formulation; and ion-activated/ triggered systems, like gellan gum(8), and sodium alginate(9) based formulation.

Gatifloxacin sesquihydrate is, a fourth generation flouroquinolone antibiotic, used for treatment of bacterial conjunctivitis and commercially available in the form of eye drops and ointment dosage forms. The objective of present work was to develop an *in-situ* gel forming ocular drug delivery system of gatifloxacin sesquihydrate using an ion-activated/ triggered sol to gel phase transition polymer for effective and sustained release drug delivery into the eye for leading to enhanced ocular bioavailability.

2. MATERIALS AND METHODS

2.1 Materials

Gatifloxacin sesquihydrate was received as a gift sample from M/s. Alkem Laboratories Ltd., Mumbai (India). Gellan gum and HPMC (hydroxypropylmethylcellulose) were procured from M/s. Chempure Pvt. Ltd., Mumbai (India) and M/s. Colorcon Asia Pvt. Ltd. Mumbai (India) respectively. All other chemicals and reagents used in the present study were of analytical grade.

2.2 Selection of gellan gum concentration

Gelation property of gellan gum solution of different concentration (0.2%, 0.4%, 0.6%, 0.8% and 1% w/v) was studied in simulated lachrymal fluid (SLF) having composition of NaCl (0.67 g), $NaHCO_3$ (0.20 g), $CaCl_2.2H_2O$ (0.008 g), and deionized distilled water to 100 g (10). On the basis of in-situ gel forming behavior of gellan gum solution of different concentration (table 1), 0.6% w/v gellan gum solution was selected for further formulation studies.

Table 1: Gel forming behavior of different concentration of gellan gum solution in simulated lachrymal fluid (SLF)

S. No.	Gellan gum Concentration	Observed Gelling Behavior
1	0.2%	-
2	0.4%	+
3	0.6%	++
4	0.8%	++
5	1.0%	+++

- no gelation
+ slow gelation
++ instant gelation persisting for 8 h
+++ instant but very viscous gelation

2.3 Formulation design

In-situ gelling formulations (0.3%w/v ophthalmic solution) of gatifloxacin sesquihydrate were designed using gellan gum (0.6% w/v)as ion-activated *in-sit* gelling agent, HPMC-K100M (0.2%, 0.4%, 0.6% w/v) as mucoadhesive release retardant, phenyl mercuric nitrate as preservative, boric acid as tonicity adjusting agent, and EDTA sodium as stabilizer (table 2).

Table 2: Composition of designed in-situ gelling formulations of gatifloxacin

S. No.	Ingredient	Concentration (w/v)		
		Formulation GTGFS-1	Formulation GTGFS-2	Formulation GTGFS-3
1	Gatifloxacin sesquihydrate (eq. to gatifloxacin base)	0.3 %	0.3 %	0.3 %
2	Gellan gum	0.6 %	0.6 %	0.6 %
3	Boric acid	1.67 %	1.67 %	1.67 %
4	HPMC K100M	0.2 %	0.4 %	0.6 %
5	Phenylmercuric nitrate	0.001 %	0.001 %	0.001 %
6	EDTA sodium	0.04 %	0.04 %	0.04 %
7	Deionized water (q.s.)	100 %	100 %	100 %

Boric acid and EDTA sodium followed by gellan gum and HPMC were dissolved in deionized water under constant stirring to make a homogeneous solution. The drug and phenyl mercuric nitrate were then added into this polymeric solution and mixed thoroughly until a clear and uniform solution is formed. The resultant product was filled and sealed in glass vials and sterilized by autoclaving at 121˚C temperature for 20min.

2.4 Evaluation of formulated products

2.4.1 Physico-chemical characterization

Clarity of formulated products was evaluated by visual observation against white and black back ground and the pH was determined by digital pH meter (Cyberscan 510).

2.4.2 Drug content

One hundred µl of formulated product was diluted to 25 ml and mixed thoroughly with distilled water and then filtered through Whatman filter no. 41 and estimated spectrophotometrically at 286 nm using a double beam UV-visible spectrophotometer (Shimadzu 1700).

2.4.3 Rheological property

Viscosity of formulated products was determined by Brookfield viscometer (LVT model), before and after dilution with simulated lachrymal fluid (SLF) to determine viscosity change after gelation of formulation.

2.4.4 Gelation property

The gelation property of products in simulated lachrymal fluid (SLF) was evaluated by visual observation. Small amount of methylene blue dye solution was uniformly mixed with the formulated product to impart blue color and one drop of this colored solution was added to a test tube containing 5 ml SLF. An instant gelation of developed formulation was clearly observed in the form of colored gelled drop.

2.4.5 Osmolality and isotonicity

The osmolality of formulated product was measured by freezing point determination technique using an Osmometer (Advanced Instruments Inc. USA). Isotonicity of formulated product was studied with red blood cells in plasma fluid. Red blood cells (RBCs) were separated from blood sample using cooling centrifuge (Eppendorf) and the separated RBCs

were mixed with formulated product and kept aside for 1 h. The samples were viewed under polarizing microscope (Leica) and the size and shape of the RBCs was compared with RBCs kept similarly in isotonic saline solution (negative control) and hypertonic and hypotonic solutions (positive control) respectively.

2.4.6 In-vitro drug release

The in-vitro drug release of the formulated product was studied using modified USP dissolution apparatus-1. A 100 µl formulation was placed over a small circular piece of Whatman filter paper No 41 (previously soaked with SLF) and kept inside the USP-1 basket. The basket was placed into 50 ml of simulated lachrymal fluid filled in a beaker and allowed to rotate at 50 rpm. The aliquots of samples (3 ml) were withdrawn at selected time intervals and replaced with an equal volume of simulated lachrymal fluid. The samples were filtered through Whatman filter no. 41and analyzed spectrophotometrically at 286 nm using double beam UV-visible spectrophotometer (Shimadzu 1700).

2.4.7 HET-CAM ocular irritation test

HET-CAM (Hen's Egg Test on Chorio-Allantoic Membrane) ocular irritancy test on formulated product was performed following the reported method (11-12).The ocular irritancy was adjudged on the basis of the effect of test products on chorio-allantoic membrane (CAM) of chick embryo in fertilized hen's eggs. The product was to be considered as non-irritant if it did not induce any adverse change to the CAM, while an irritant product would induce adverse effects, i.e., hemorrhage, vessel lysis or coagulation. The effect of developed product on CAM was compared with a 0.9% sodium chloride isotonic solution, (negative control) and 1% sodium hydroxide solution (positive control).

3. RESULT AND DISCUSSION

The formulated in-situ gelling product was a clear solution with pH 6.0, viscosity 55 cps, drug content and osmolality within acceptable range. The developed formulations also exhibited flow characteristics desired for instillation into the eye (table 3).

Table 3: Evaluation of formulated products of gatifloxacin sesquihydrate

Formulation code	Appearance	pH	% Drug content*	Osmolality (mOsm/kg)	Gelation property	Viscosity# (cps)	
						Sol	Gel
GTGFS-1	Clear	6.1	96.3 ± 0.45	298 ± 6	Good	48	308
GTGFS-2	Clear	6.0	98.2 ± 0.11	318 ± 5	Good	55	325
GTGFS-3	Clear	6.0	96.7 ± 0.75	345 ± 4	Good	61	342

*mean±SD (n=6)

#mean (n=3)

The product underwent a sol-gel phase transition when comes in contact with simulated lachrymal fluid, which was also confirmed by viscosity changes from 55 cps (sol) to 325 cps (gel). Combination of 0.6% w/v gellan gum and 0.4% w/v HPMC provided best gelling attributes and a sustained drug release profile for 8 h (fig.1).

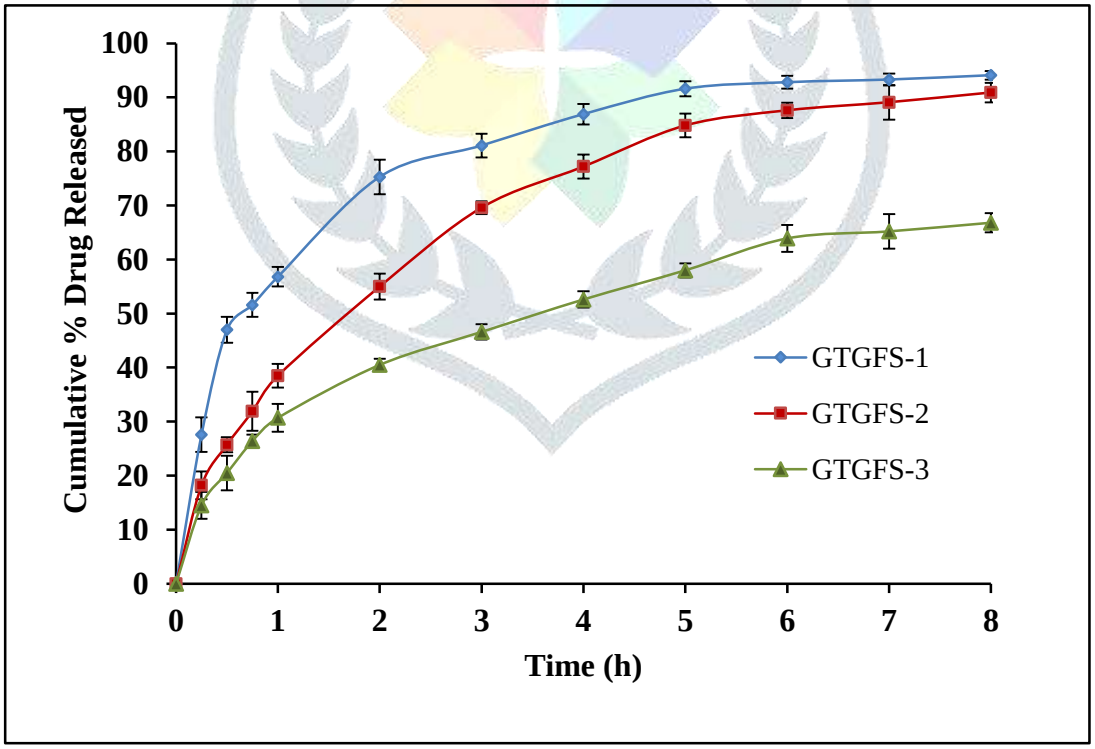

Fig.1: In-vitro drug release of formulated in-situ gel products in simulated lachrymal fluid. Values expressed as mean±SD (n=6)

The osmolality of the developed product was determined to be 318±5 mOsm/kg, which is within acceptable range for ocular products. The developed product was found to be isotonic, as no change was observed in size and shape of RBCs with the formulation and isotonic solution, while the RBCs showed shrinking and bursting with the hypertonic and hypotonic solution respectively (fig.2).

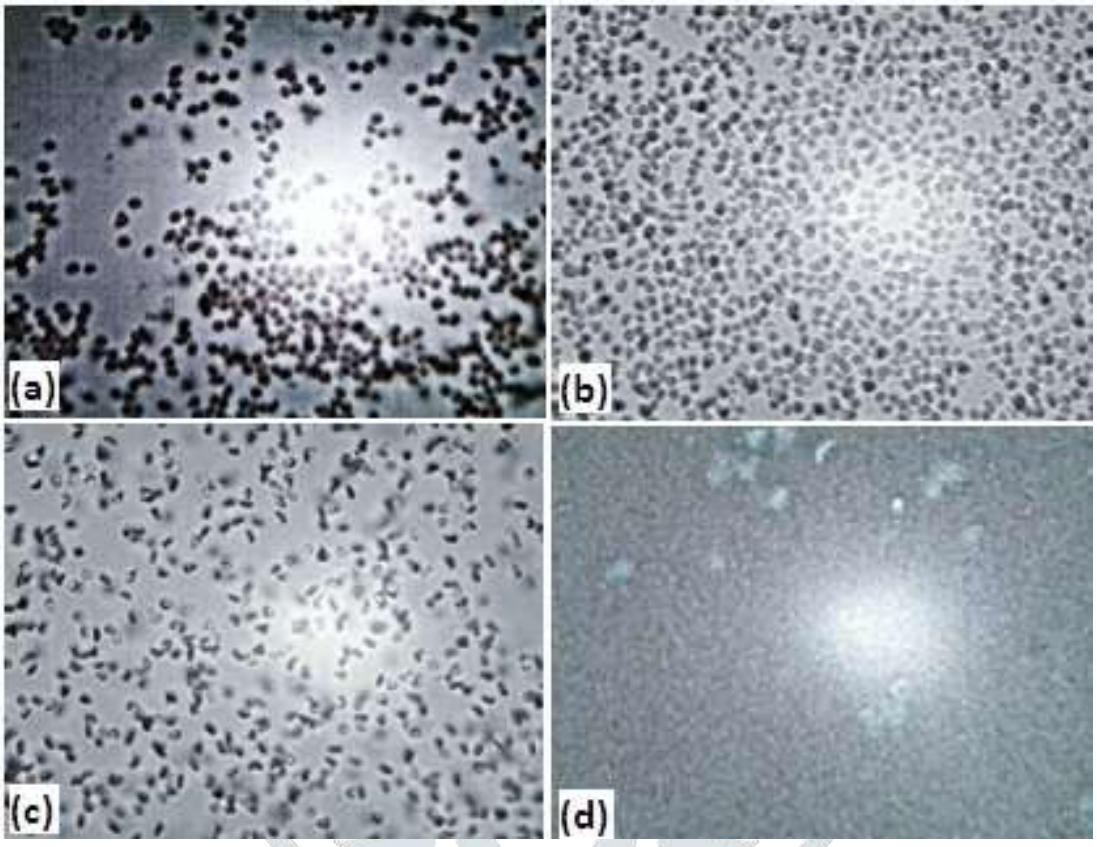

Fig.2: Microscopic view of separated RBCs with developed in-situ gelling product and control samples.

(a) RBCs with developed product showing no change in size and shape

(b) RBCs with isotonic solution (negative control) showing no change in size and shape

(c) RBCs with hypertonic solution (positive control) showing shrinking

(d) RBCs with hypotonic solution (positive control) showing swelling and bursting.

The developed product was also found to be non-irritant in HET-CAM (hen's egg test on chorio-allantoic membrane) ocular irritancy test, as no irritant effect, i.e., hemorrhage, vessel lysis, and coagulation was induced to the CAM of chick embryo in its presence (table 4 and fig.3).

Table 4: Observation of HET-CAM ocular irritancy test

Test product	Observation	Inference
Developed formulation (GTGFS-2)	No adverse change	Non-irritant
0.9% NaCl solution (negative control)	No adverse change	Non-irritant
1% NaOH solution (positive control)	Hemorrhage and vessel lysis	Highly irritant

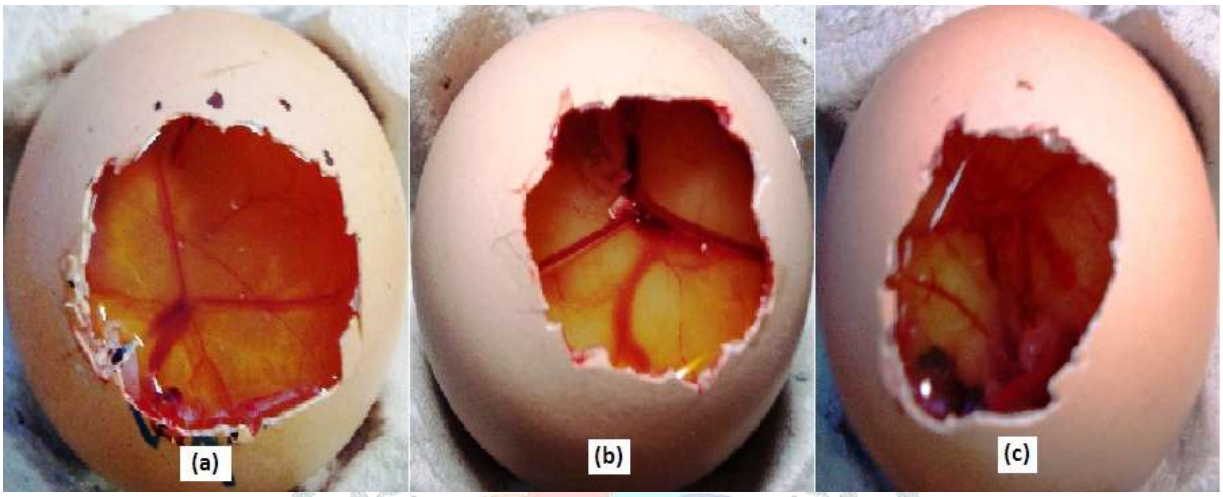

Fig.3: Effect of developed product and control samples on Chorio-Allantoic Membrane (CAM) of chick embryo in fertilized hen's eggs.

(a) Developed product showing no adverse effect
(b) 0.9% NaCl solution (negative control) showing no adverse effect
(c) 1% NaOH solution (positive control) showing hemorrhage and lysis of blood vessels

CONCLUSION

On the basis of above findings, it can be concluded that the developed gatifloxacin *in-situ* gelling formulation provides a sustained ocular drug delivery for 8 h. The gellan gum based ion-activated gelling systems can be explored for designing topical ocular drug delivery.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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