Research Article

Formulation and Evaluation of Acyclovir Occular Ion Activated *In-Situ* Gel by using Sodium Alginate/HPMC E50 LV and Gelrite

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ABSTRACT

The poor bio availability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid pre corneal elimination of the drug may be overcome by the use of in situ gel forming systems that are instilled as drops into the eye and the undergo a sol to gel transition in the cul de sac. The present study was aimed to prepare and characterize ion activated in situ gel based opthalmic drug delivery system of antiviral drug acyclovir. Gelrite and sodium alginate was used as gelling agents in combination with hydroxyl propyl methyl cellulose (HPMC-E50 LV) as a viscosity enhancer. Benzalkonium chloride in suitable concentration used as a preservative. The formulations were sterilized by moist heat sterilization as per IP. The prepared formulations were evaluated for pH, drug content, clarity, gelling capacity, viscosity, sterility study, eye irritation test, gelling capacity and in vitro drug diffusion. Under rheological investigations both solution and gel was found to be in pseudo plastic behavior. The selected formulations showed the sustained release over a period of 8 h with increased resident time. All studies shown favourable results thus acyclovir in situ gelling system is a valuable alternative for the treatment of herpes simplex keratitis with the delivery of the drug in a controlled manner directly to the site of action that is cornea which will reduce the complications and limitations of conventional and oral therapies.

Keywords: Acyclovir, in situ gel, gelrite, sodium alginate and herpes keratitis.

INTRODUCTION

Viruses are the common cause of conjunctivitis in patients of all ages. A variety of viruses can be responsible for conjunctival infection, however adenovirus is the most common cause and herpes simplex is the most problematic[1]. Herpes simplex virus (HSV) is a member of family of herpes viridae, a DNA virus. There are two types of Herpes Simplex Viruses (HSV). Viz HSV type 1 and type 2[2]. Conjunctivitis is an inflammation or redness of lining of the white part of the eye and the underside of the eyelid (conjunctiva) that can be caused by infection, allergic reaction or physical agents like infrared or ultra-violet light. Conjunctivitis is classified as acute and chronic. It can also be classified as bacterial, viral and allergic depending upon the causative factors. Herpes simplex virus (HSV) is the most problematic. Viral conjunctivitis and bacterial conjunctivitis may affect one or both eyes. Viral conjunctivitis usually produces a watery or mucous discharge. Bacterial conjunctivitis often produces a thicker, yellow-green discharge and may be associated with a respiratory infection or with a sore throat[3].

Acyclovir is preferentially taken up by the virus infected cells. Because of selective generation of the active inhibitor in the virus infected cell and its inhibitory effect on viral DNA synthesis, acyclovir has low toxicity for host cells. This is an alternate approach to improve the bioavailability is the use of polymeric solutions, which change to a gel as a result of exposure to the physiological temperature, pH or ionic composition of lacrimal fluid. Phase transition systems are instilled in a liquid

form which gets converted to the gel in the presence of mono or divalent cations[4].

MATERIALS AND METHODS

Acyclovir was received as gift sample from Hetro pharma, Hyderabad; Sodium alginate was purchased from Thomas baker chemicals ltd, Mumbai; HPMC E50 LV was purchased from Colorcon Asia pvt ltd, Goa; Gelrite was purchased from Sigma Aldrich, USA; All other chemicals used were analytical grade.

Preparation of *in situ* gelling system

a) Sodium alginate based formulations

The weighed quantities of Sodium alginate/HPMCE50LV solutions were prepared by dispersing the required amount in de ionized water with continues stirring.0.3 g of drug was accurately weighed and drug solution was prepared to this drug solution 0.02% of benzalkonium chloride was added. The drug solution was added to the polymer solution and the volume was made up to 100 ml by using deionised water. This solution was filtered through membrane filter and subjected to terminal sterilization (autoclave 15lb pressure for 20 min) after sealing into vials.

Table 1: Formulation of Acyclovir *in situ* gel systems using Sodium alginate and HPMC E50LV

Ingredients	Ingredients concentration (percentage w/v)							
	S1	S2	S3	S4	S5	S6	S7	S8
Acyclovir(g)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sodium alginate(g)	0.5	0.75	1.0	1.25	0.5	0.75	1.0	1.25
HPMC E50 LV(g)	0.5	0.5	0.5	0.5	0.75	0.75	0.75	0.75
Benzalkonium chloride(%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Deionised water(q.s)	100	100	100	100	100	100	100	100

b) Gelrite based formulations

The weighed amount of gelrite was dissolved in a beaker containing deionized water and this solution was heated to about 85°C for 15 min, then solution was cooled with stirring. 0.3 g of drug accurately weighed and drug solution was prepared, to this drug solution 0.02% of benzalkonium chloride was added. The drug solution was added to the polymer solution and the volume was made up to 100 ml by using deionised water. This solution was filtered through membrane filter and subjected to terminal sterilization (autoclave 15 lb pressure for 20 min) after sealing into vials.

Table 2: Formulation of Acyclovir *in situ* gel systems using Gelrite

Ingredients	Ingredients concentration (percentage w/v)							
	G1	G2	G3	G4	G5	G6	G7	G8
Acyclovir(g)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Gelrite(g)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
Benzalkonium chloride(%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Deionised water (q.s)	100	100	100	100	100	100	100	100

Evaluation of the formulations

1) Clarity

The developed formulations were evaluated by visual inspection under florescent light against a white and black background in a well lit cabinet for appearance and clarity.

2) pH measurement

The pH of the gel forming ophthalmic solution was measured using pH meter.

3) Gelling capacity

Gelling was determined by mixing the formulation with simulated tear fluid in the proportion 25:7 and the gellation

was assesses by visual examination at 37.5°C.

4) **Drug content estimation**
0.1 ml (≈0.3mg) of 0.3% sample solution was pipette out and was diluted to 10 ml with simulated tear fluid in 10 ml volumetric flask. The absorbance of the resulting sample solution was measured at 252 nm.

5) **In vitro drug diffusion studies**
The in vitro drug diffusion of acyclovir from the formulations was studied through cellophane membrane using franz diffusion apparatus. The diffusion medium used was freshly prepared simulated tear fluid. Cellophane membrane previously soaked overnight in the diffusion medium (STF), was placed in between the donor and receptor compartment. 1 ml volume of the formulation was accurately instilled into donor compartment. 120 ml of STF was placed in the receptor compartment. The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37±0.5°C. The magnetic bead was rotated such that it produced a vortex and touched the cellophane membrane. Aliquots each 1 ml volume were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with STF and analyzed by UV visible spectrophotometer at 252 nm.

6) **Viscosity determination**
The viscosity measurements were done by using Brookfield DV-II+ viscometer using LV-2 spindle. The developed formulations were poured into the adapter of the viscometer and the angular velocity was increased gradually from 10 -100 rpm. The angular velocity was reversed gradually. The average of two readings was used to calculate viscosity. By adding STF the formulations were made into gel form and viscosity was determined as specified above using LV-3 spindle.

7) **Stability studies**
Stability studies were carried out on most satisfactory formulations (S7, G 6) as per ICH guidelines Q1C. Sterile gel forming ophthalmic solution were filled in autoclavable transparent plastic bottles, closed with autoclavable rubber closures and sealed with aluminum foils. The formulations were kept in stability chamber of at 40±2°C & 75±5% RH for 2 months.

8) **Test for sterility**
Test for sterility were performed for aerobic bacteria and fungi by using fluid thioglycollate medium and soyabean casein digest medium.
Preparation of fluid thioglycollate medium:29.3 g of fluid thiogycolate medium was dissolved in 1000 ml distilled water by boiling. Sterilized by autoclaving at15 lbs pressure at 121°C for 20 min.
Preparation of soyabean casein digest medium: 30 g of soyabean casein digest medium was dissolved in1000 ml distilled water. The medium was boiled to dissolve completely. Sterized by autoclaving at15 lbs pressure at 121°C for 20 min.
The media used should comply with the following tests carried out before or in parallel with the test on the preparation being examined.

- **Sterility (negative control) test** fluid thioglycolate media was incubated at 30-35°C and soyabean casein digest medium at 20-25°C for not less than 7 days.
- **Growth promotion (positive control) test**
  The sterile media was inoculated with about 100 viable micro organisms and incubated according to the conditions specified.
  Ophthalmic preparations should be sterile and must be checked for the presence of any bacteria or fungi before it is used.

- **Test for aerobic bacteria**
  20 ml of sterile fluid thioglycolate was transferred to 3 tubes aseptically. The tube labeled as positive control was inoculated with viable aerobic micro organisms bacillus subtilis (ATCC No. 6633) aseptically. 2.5 ml of the ophthalmic preparation was added to the tube labeled as test. Then all three test tubes were incubated at 30-35°C for not less than 7 days.

- **Test for fungi**
  20 ml each of sterile soyabean-casein digest medium was transferred to 3 tubes aseptically. The tube labeled as positive control was inoculated with candida albicans (ATCC No. 10231) aseptically. 2.5 ml of the ophthalmic preparation was added to the tube labeled as test. All the three test tubes were incubated at 20-25°C for not less than 7 days.

9) Eye irritation studies

In the measurement of injury to the eye, a modification of the scoring system of Friedenwald, Hughes and Herrmann (modified Draize Technique) was used. Injuries to the cornea, conjunctiva and the iris were scored separately[13] .

RESULTS AND DISCUSSIONS

Table 3: Evaluation of ophthalmic *in situ* gel

Formulation Code	Drug Content (%)	Clarity	Gelling capacity	pH
S1	98.59 ±2.97	Translucent	-	6.3
S2	98.87±1.87	Translucent	+	6.29
S3	99.85±2.49	Translucent	++	6.4
S4	98.87±4.05	Translucent	O.V	6.6
S5	99.71±2.95	Translucent	-	6.42
S6	97.97±3.82	Translucent	+	6.23
S7	97.96±1.95	Translucent	+++	6.34
S8	98.87±2.95	Translucent	O.V	6.5
G1	98.31±2.06	Transparent	-	6.43
G2	99.43±1.96	Transparent	+	6.34
G3	99.15±2.65	Transparent	+	6.4
G4	99.71±1.87	Transparent	++	6.45
G5	98.63±0.97	Transparent	++	6.30
G6	99.43±1.78	Transparent	+++	6.25
G7	98.43±1.04	Transparent	+++	6.4
G8	97.40±1.87	Transparent	O.V	6.7

*Average of three readings
+: Gels after few min, remains for upto 2-3 h.
++: Gelation immediate remains for upto 4-6 h.
+++: Gelation immediate remains for upto 7-9 h.
O.V: Outside viscous

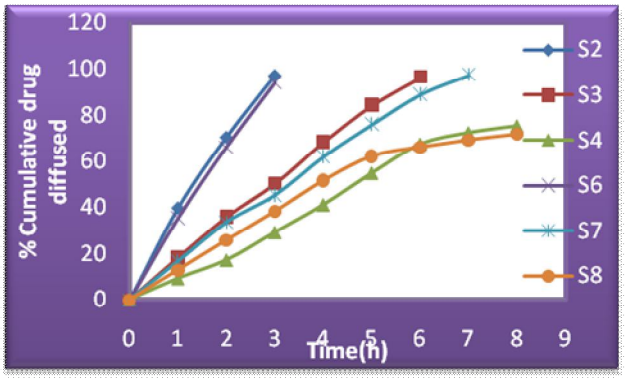

Fig. 1: %Cumulative drug diffusion of S1-S8

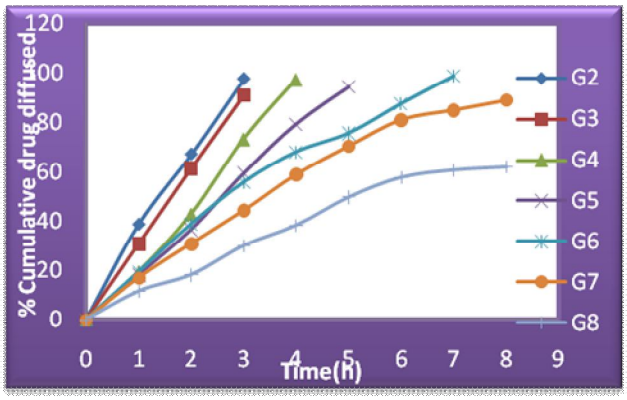

Fig. 2: %Cumulative drug diffusion of G1-G8

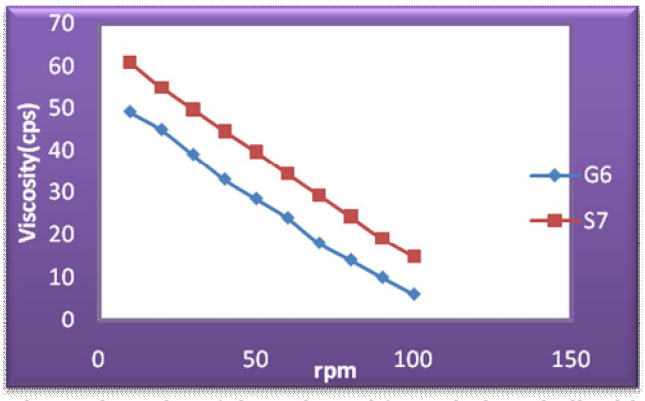

Fig 3: Viscosity of the selected formulations in liquid

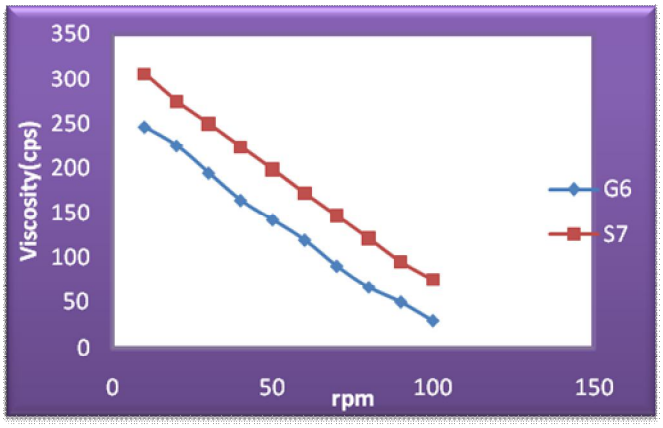

Fig 4: Viscosity of the selected formulations in gel

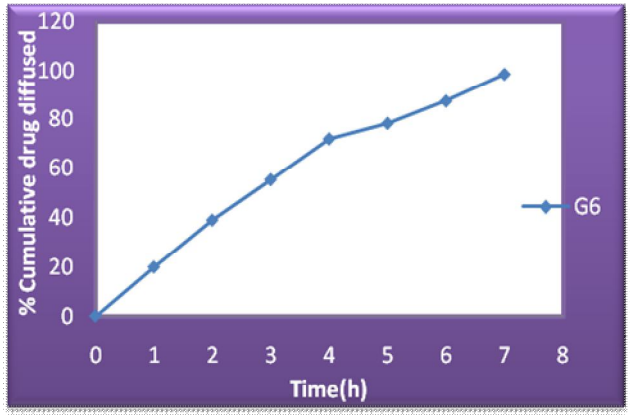

Fig 5: % Cumulative drug diffusion of G6 after two months at 40 ± 2°C& 75 ± 5% RH

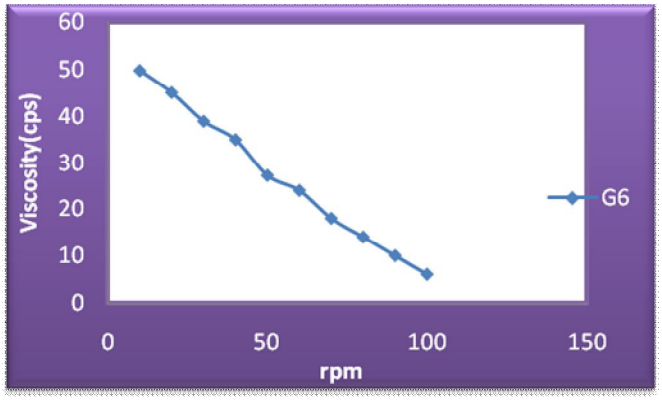

Fig 6: Viscosity of G6 in liquid after two months at 40 ± 2°C& 75 ± 5% RH

Table 4: Observations of sterility testing

Sterility Tests	Results Obtained																			
	Negative control							Test							Positive control						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Test for aerobic bacteria	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
Test for Fungi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+

* (-) sign suggests negative results (No growth of microorganisms)
** (+) sign suggests positive results (Formation of colonies of microorganisms)

Acyclovir in situ gel systems were prepared by ion activated methods. The formulations from S1 to S8 were light yellow in color and the clarity was found to be translucent and the formulations from G1 to G8 were transparent. The pH of all the formulations was within the acceptable range. The drug content of all the formulations were in range of 93.97% to 99.81%. Except the formulations S1, S5 and G1 all the formulations gelled instantaneously with a translucent matrix on addition to STF, which may be due to ion cross linking of the alginate chains by the divalent cations and extended for few hours. The evaluation results are mentioned in table -3.

The in vitro release studies indicated that amongst all the formulations S7 and G6 showed sustained drug release for 7 h, which may be due to optimum concentration of sodium alginate, HPMC E50LV and Gelrite. The evaluation results are mentioned in fig 1 and 2. The viscosity of the formulations G6 and S7 in liquid form ranged from 5-60cps and in the gel

form ranged from 30-300 cps. All the formulations exhibited pseudo-plastic rheology, as shown by shear thinning and a decrease in the viscosity wit increase in angular velocity. The evaluation results are mentioned in fig 3 and 4. Stability studies of the formulations were carried out as per the ICH guidelines. The clarity of gelrite based G6 formulation did not show any significant change as compared to the sodium alginate based S7 formulation which develop haziness when stored for 30 days at 40±2°C & 75±5% RH. So S7 batch was discarded. The results showed that there were no significant changes in the invitro drug diffusion studies of G6 batch. The results are mentioned in fig 5 and 6.

The formulation passed the sterility test as there was no appearance of turbidity and hence no evidence of microbial growth when incubated for not less than 7 days. The results of the sterility test showed that the prepared ophthalmic formulation passed the sterility test, the evaluation results are mentioned in table-4.

The results of ocular irritation studied indicated that the formulations were non irritant. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae were observed.

CONCLUSION

Acyclovir is a antiviral drug used in the treatment of herpes infections of the eye was successfully formulated as an ion activated in situ gel forming ophthalmic solution using sodium alginate in combination with HPMC as a viscosity enhancer and Gelrite. Acyclovir entrapped in an in situ gel forming systems was formulated in a solution form such that the acyclovir drops when instilled into the eye undergo a solution-gel transistion in cul de sac.the loss of drug is overcome due to the immediate gel formation. Buy considering the results of all the evaluation parameters G6 is considered as ideal formulation.

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