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Research Article

FORMULATION AND EVALUATION OF OCULAR *INSITU* HYDROGELS OF ACYCLOVIR

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ABSTRACT

The present research work deals with the formulation and evaluation of *in-situ* gelling system based on sol-to-gel transition for ophthalmic delivery of an antiviral agent acyclovir , to overcome the problems of poor bioavailability and therapeutic response exhibited by conventional formulations. Aciclovir an antiviral agent preferentially used in the treatment of infections caused by herpes simplex virus, which is the main cause of viral conjunctivitis.so in the present study ocular insitu hydogels of acyclovir were prepared to treat viral conjunctivitis.Carbopol 940 was used as the gelling agent in combination with HPMCK100M which acted as a viscosity enhancing agent.Poly vinyl alcohol was used as crosslinker. The prepared formulations were evaluated for pH, viscosity, clarity, drug content, gelling capacity, *Invitro* drug release, *Exvivo* diffusion studies, antimicrobial efficacy ,ocular irritancy, The clarity, pH, and drug content of the developed formulations were found in range 6.0-6.8, , 82-95% respectively. The gel provided sustained drug release over an 6 hour period. The developed formulation can be used as an *in-situ* gelling vehicle to enhance ocular bioavailability and helped in the reduction in the frequency of instillation thereby resulting in better patient compliance.

Keywords: *In-situ* gelation; acyclovir; Carbopol 940; HPMC K100M, HPMCK4M ,HPMC E50LV.

INTRODUCTION

Ophthalmic drug delivery is one of the most attractive and challenging field facing the pharmaceutical scientist. A significant challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage [8]. Most of the ocular treatments call for the topi-cal administration of ophthalmically active drugs to the tissues around the ocular cavity [5] .The most conventional ocular dosage forms for the delivery of drugs are eye drops (solution, suspension) and ophthalmic ointments. Short residence time, pulsed dosing of drug, frequent in-siltation, and large drainage factor are the limitation associated with conventional ocular do-sage form. Newer ocular drug delivery systems are being explored to develop extended duration and controlled release strategy.

Formulation of *in-situ* ocular gel of acyclovir's an antiviral drug was to treat viral conjunctivitis of the eye, using biodegradable polymers is the approach to overcome the drawbacks of conventional eye preparations. Acyclovir is preferentially taken up by virus infected cells because of selective generation of active inhibitor in the virus infected cell and its inhibitory effect on viral DNA synthesis[4]. Carbopols are mainly used in liquid or semisolid pharmaceutical formulations as suspending or viscosity increasing agents. Formulations containing carbopol include creams, gels and ointments for use in ophthalmic, rectal and topical preparations. HPMC is widely used in oral and top-ical pharmaceutical preparations as coating agent, film formers, rate controlling polymers for sustained release, stabilizing agents, viscosifier

The aim of present study is to formulate and evaluate ph triggered *in situ* ophthalmic gel forming solution of acyclovir by using combination of hydroxyl propyl methyl cellulose HPMC K4M K100M , E50LV[1]. HPMC was used as viscosifying agent and

carbopol940 as gelling agent. To achieve the objective, independent formulation variables such as polymer to polymer ratio and different viscosity grades of HPMC (K4M K100M E50LV) were examined to identify the best formulation were using three square full factorial design.

MATERIALS AND METHODS

Acyclovir was obtained as a gift sample from hetero pharmaceuticals pvt. ltd.,. hydroxypropylmethyl cellulose (HPMC) and HPMC k15m were obtained from colorocon asia pvt. ltd, Mumbai, and CARBOPOL 940 was obtained from sd fine pvt. ltd., Mumbai, India. all other chemicals/reagents used were of analytical grade, available commercially and used as such without further processing. a uv/vis spectrophotometer (systronics, double beam uv-vis spectrophotometer) was used for drug analysis.

PROCEDURE FOR THE PREPARATION OF *IN SITU* GELLING SYSTEMS

A 3^2 full factorial design was adopted to optimize the variables and 9 experiments were conducted in total. In this design, two factors were evaluated each at 4 levels [7]. The polymer-to-polymer (HPMC, HPMC K15M) (X1) and the amount of bioadhesive polymer (Carbopol 940) (X) were chosen as independent variables and Y as de-pendent variables (viscosity, drug content, and *in-vitro* drug release). The levels of independent variables are shown in Table 1.

The dispersions of HPMC and carbopol were prepared .Carbopol940 was sprinkled and allowed to hydrate over night the solution was stirred under an overhead stirrer and buffer salts were added to the above solution. Polyvinyl alcohol was added to the above polymer solution . Acyclovir was added to the polymer solution .Purified water was then added to make up the volume to 100ml and the prepared formulations was sterilized 120°C for 15 min.

Table 1: Factorial design of insitu gel forming systems

CARBOPLO 940	HPMCK100M	HPMCE50LV
1	1	1
1	2	1
1	3	1

Table 2: Formulation table of *insitu* hydrogels

FORMULATIONS	DRUG (g)	CARBOPOL 940(g)	HPMC K4M(g)	HPMC K100M(g)	HPMCE50LV (g)	POLYVINLY ALCOHOL (PVA)
F1	0.5	0.1	0.1	-	-	1ml
F2	0.5	0.1	0.2	-	-	1ml
F3	0.5	0.1	0.3	0.2	-	1ml
F4	0.5	0.3	-	0.1	-	3ml
F5	0.5	0.3	-	0.2	0.2	3ml
F6	0.5	0.3	-	0.3	0.3	3ml
F7	0.5	0.5	-	0.1	0.2	5ml
F8	0.5	0.5	-	0.2	0.4	5ml
F9	0.5	0.5	-	0.3	0.6	5ml

EVALUATION STUDIES

PHYSICAL APPEARANCE

The physical appearance of formulations was observed visually under clariy test apparatus. The clarity of formulations was observed against white and black back ground respectively .

pH DETERMINATION

0.3g of gel was dissolved in 100ml of distilled water and ph was measured using pHmeter

DETERMINATION OF VISCOSITY

Viscosity of instilled formulation is an important factor in determining residence time of drug in the eye. Viscosity of samples was determines using Brookfield digital viscometer with spindle3 at an angular velocity run from 10 to 100 rpm per minute.

GELLING CAPACITY

Determination of *invitro* gelling capacity was done by visual method. Colored solutions (1%amaranth solution in water) of *in situ* gel forming drug delivery systems were prepared. The *in vitro* gelling capacity of prepared formulation was measured by placing 5ml of artificial tear fluid in a glass test tube maintained at 37±1 °c temperature .1ml of colored solution was added with the help pipette as the solution comes in contact with gelation solution, it was immediately converted in to a stiff gel like structure the *invitro* gelling capacity was graded in three categories on the basis of gelation time and time period for which the formed gel as remained as such.

\+ gelation slowly and dissolve .

++ gelation immediate and remains for few hours

+++ gelation immediate and remained for extended period of time

***IN VITRO* RELEASE STUDIES**

The *in-vitro* release of aciclovir from the prepared formulations was studied through cellophane membrane using a modified USP XXIII dissolution testing apparatus. The dissolution medium used was pH 7.4 buffer. Cellophane membrane was previously soaked overnight in the dissolution medium and was tied to one end of a specifically designed glass cylinder (open at both ends of 5 cm diameter). A 2ml volume of the formulation was accurately pipetted into this assembly. The cylinder was attached to the metallic drive shaft and suspended in 100ml of dissolution medium maintained at 37±1°C so that the membrane just touched the receptor medium surface. The shafts was rotated at 50 rpm/min. Aliquots each of 1ml volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with receptor medium and absorbance was measured at 275 nm % cumulative release was calculated for all the formulations.

STABILITY STUDIES

The 30 days stability studies were carried out for optimized formulations. sterile gel forming ophthalmic solution were filed in glass vials closed with gray butyl rubber closures and sealed with aluminum caps the formulation vials kept in stability chamber maintained at 40±2°C and relative humidity 75±5% RH for one month samples are with drawn at 0,7,15,30 days interval and evaluated for drug content, pH, appearance, clarity .

***EX VIVO* DIFFUSION STUDY**

Ex vivo permeation study was carried out using franz diffusion chamber and goat corneal membrane used to separate donor and receptor compartment the whole eyeball of goat were procured from a slaughter house and carried to laboratory in cold condition in normal saline maintained at 4° c. The corneal membrane was washed and placed in pH 7.4 phosphate buffer and then it was mounted on by sand-witching between the clamped donor and receptor compartment prior to application of formulations the membrane was allowed to equilibrate for 30 minutes. Accurately weighed 1ml of gel was spread uniformly on corneal membrane which was in contact receptor medium. The receptor compartment was filled with phosphate buffer at ph 7.4 at 37±0.5°c and stirred it continuously at 20 rpm to simulate blinking action of eye lids whole assembly adjusted to magnetic stirrer and pre determined intervals (30min,1hr, 2hrs up to 6hrs). 1ml sample was with drawn from receptor compartment replacing the sampled volume with phosphate buffer solution pH7.4 after each sampling for a period of 6hrs the samples withdrawn were analyzed by UV-visible spectrophotometer at 275nm

DRUG CONTENT

Drug content was determined by taking 1ml of formulation and diluting it to 100ml distilled water aliquot of 5ml was with drawn and diluted to 25ml with distilled water .Acyclovir concentration was determined at 275nm by uv visible spectrophotometer .

STERILITY STUDIES

ANTI MICROBIAL ACTIVITY

Antimicrobial efficiency studies were carried out to ascertain the biological activity of sol-to-gel systems against micro organisms .This was determined in agar diffusion medium

employing cup plate technique. Sterile solutions of marketed acyclovir eye ointment was used as standard. The standard and developed test solutions were taken into separate cups bored into sterile nutrient agar medium previously seeded with organisms pseudomonas aeruginosa. After allowing the diffusion of solutions for 2hrs, plates were incubated for 24hrs at 37°c .The zone of inhibition was compared with that of standard .each samples were tested .

OCULAR IRRITATION STUDIES(DRAIZE TEST)

Albino rabbit are used as test species one eye is designated the test eye was done under the guidance of institutional animal ethics commitee.The contra lateral eye severs as a matched control and is usually left untreated .Single drop approximately 0.04ml is instilled in to lower conjunctival cul-de-sac; normal blinking is allowed ,although the eyelids can be held together for several seconds after instillation .observations was done at 1,24,48,72 hours one week after exposure. Ocular changes was graded by a scoring system that includes rating any alterations to the eyelids, conjunctiva, cornea, and iris.

RESULTS AND DISCUSSION

Physical appearance of the formulations were light white in color and clear. The pH value of all the prepared formulations ranged from 6.0 to 6.8, which is considered acceptable to avoid the risk of irritation upon application to the eye. The two main fundamentals of gelling system are viscosity and gelling capacity. The viscosity of the different formulations was compared as shown in Table 4. The viscosity was directly dependent on indicated that the viscosity increased with increase in concentration of HPMC K15M and carbopol 940 (1 to 4%). F7, showed the maximum viscosity whereas the minimum viscosity at 100 rpm was shown by F1. Except for the formulations F1, F2, F5, F6, All the formulations gelled instantaneously on addition to the simulated tear fluid and extended for few hours. The *in-situ* formed gel should preserve its integrity without dissolving or eroding for prolonged period to facilitate sustained release of drugs locally.

On the basis of physicochemical properties (viscosity and gelation capacity) nine formulations (F1,F2,F3, F4, F5, F6, F7, F8 and F9) were selected and evaluated for drug content, and *in-vitro* dissolution . The drug content of all the formulations was in range (82-95%).

The *Exvivo* diffussion studies were carried out for optimizing the in situ gelling systems, namely F4, F5,F6, F1, F2, F3,F4 ,F5,F6,F7,F8 and F9. The release profiles are shown in the figure 7.8 . FormulationF3, F4 and F5 containing only Carbopol940 released around 80% of the drug in the first 3-4 hrs. Hence it was necessary to use additional release retardant to obtain the desired release profile. So further studies were carried out using Methocel (HPMC K 4M , K 100M , E50 LV as a release retarding polymers.

Anti microbial studies revealed that zone of inhibition(ZOI) was not observed in all formulations .Hence formulations were treated as sterile preparations.The results of the ocular irritation studies indicate that the developed formulation was non-irritant. Excellent ocular tolerance was noted. No ocular or abnormal clinical signs to the cornea, iris or conjunctiva were visible.The formulated insitu gelling system F1,F2,F3, F4,F5, F6,F6,F7,F8, F9 batch were subjected to stability study which indicates No significant change in physical parameters and drug content of the final formulation stored at stressed condition (40ºC with 75% relative humidity) after one month. Therefore it can be concluded that drug is stable in final formulation at room temperature and at 40ºC for one month.

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 . 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 transition 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 F4,F5,F6 were considered as ideal formulations.

Table 3: Clarity Testing Of All Formulations

FORMULATION	CLARITY
F1	Clear
F2	Clear
F3	Clear
F4	Clear
F5	Clear
F6	Clear
F7	Clear
F8	Clear
F9	Clear

Table 4: Gelling Capacity of All Formulations

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

Table 5: Viscosity of Different Formulation

Formulation code	VISCOSITY @ pH 6.1 At different shear rates				
	2	4	6	8	10
F 1	5389.2	4592	3832	3265	2721
F 2	7322	6392	5449	4539	3679
F 3	9481	8298	7090	5741	4621
F 4	832	729	620	519	420
F 5	953	830	714	582	479
F 6	1167	1031	897	752	591
F 7	2778	2489	2142	1825	1420
F 8	3554	3107	2821	2341	1788
F 9	4121	3581	3121	2621	2061

Table 6: Invitro Drug Release of All Formulations

Time (hr)	Cumulative % release of drug								
	F1	F2	F3	F4	F 5	F 6	F 7	F 8	F 9
0	0	0	0	0	0	0	0	0	0
1	9.21	8.39	11.89	17.35	15.22	22.60	17.95	18.40	12.67
2	14.73	15.24	15.22	29.89	38.72	39.32	29.02	33.88	29.23
3	21.32	24.27	19.29	38.81	55.31	61.01	44.83	45.12	42.59
4	28.19	29.18	27.44	53.22	67.27	76.43	52.93	54.24	53.82
5	35.34	32.09	34.53	67.53	79.01	84.32	59.87	59.82	60.05
6	47.76	41.35	40.21	79.87	86.32	91.36	67.50	62.85	69.78
7	55.79	52.54	51.67	85.34			75.43	69.72	75.73
8	63.62	61.53	59.32				79.04	77.03	80.06
9	72.13	72.37	68.01				82.71	82.14	81.12
10	80.33	76.89	75.98					85.92	83.14

Table 7: Cumulative% Release of All Formulations

Cumulative % release of drug			
Time (hr)	Cumulative % release of drug		
	F4	F5	F6
0	0	0	0
1	29	29	24
2	38	41	42
3	67	63	59
4	91	86	71
5	--	--	88

Table 8: Zone Of Inhibition Observed For Different Formulations

Formulation code	ZOI (cm)	Test organism
Marketed formulation	Nill	*staphylococcus aureus*
F1	Nill	*staphylococcus aureus*
F 2	Nill	*staphylococcus aureus*
F 3	Nill	*staphylococcus aureus*
F 4	Nill	*staphylococcus aureus*
F 5	Nill	*staphylococcus aureus*
F 6	1.2	*staphylococcus aureus*
F7	1.4	*staphylococcus aureus*
F 8	Nill	*staphylococcus aureus*
F 9	Nill	*staphylococcus aureus*

Table 9: Ocular Irritation Testing

S.NO	PREPARATION	RESULTS
1	F 1	NIL
2	F 2	NIL
3	F 3	SIGN OF LACHRYMATION
4	F 4	NIL
5	F 5	NIL
6	F 6	NIL
7	F 7	NIL
8	F 8	NIL
9	F 9	NIL
10	MARKETED FORMULATION	NIL
11	0.9% NACL NEGATIVE CONTROL	NIL

Table 10: Stability Studies of All Formulations

TIME	PHYSICAL APPERANCE	pH	DRUG CONTENT
0	+	6.7	85.33 ±0.41
2	+	6.7	84.95 ±0.06
3	+	6.7	84.95 ±0.06
7	+	6.7	83.65 ±0.19
10	+	6.7	83.50 ±0.53
15	+	6.7	82.39 ±0.62
30	+	6.7	82.39 ±0.62

Fig. 1: Clarity Testing of All Formulations

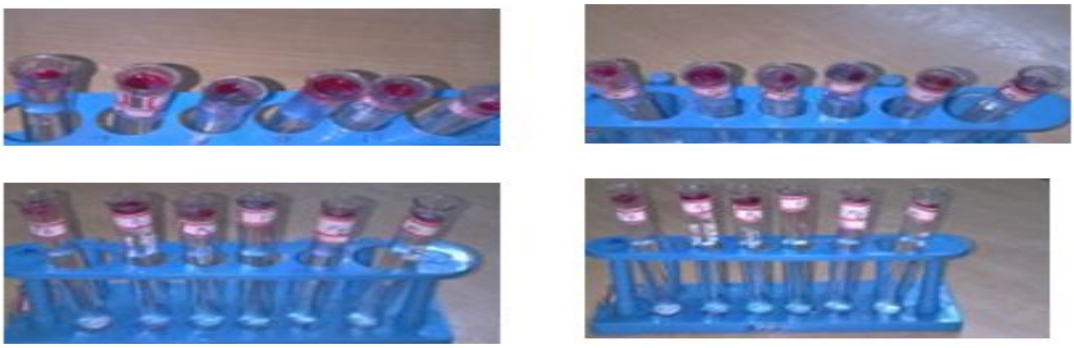

Fig. 2: Gelling Capacity of All Formulations

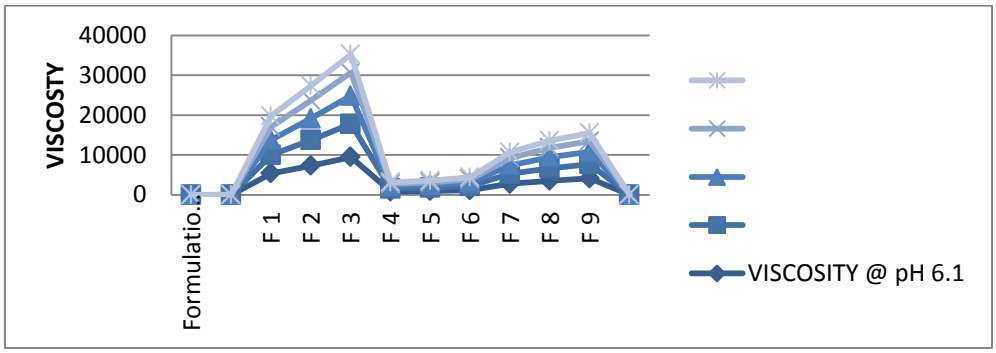

Fig. 3: Viscosity of All Formulations

Fig. 4: *Exvivo* Diffusion Studies

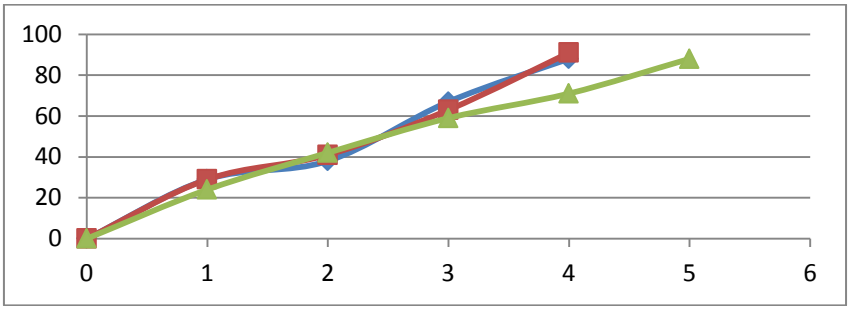

Fig. 5: Cumulative %Release of All Formulations

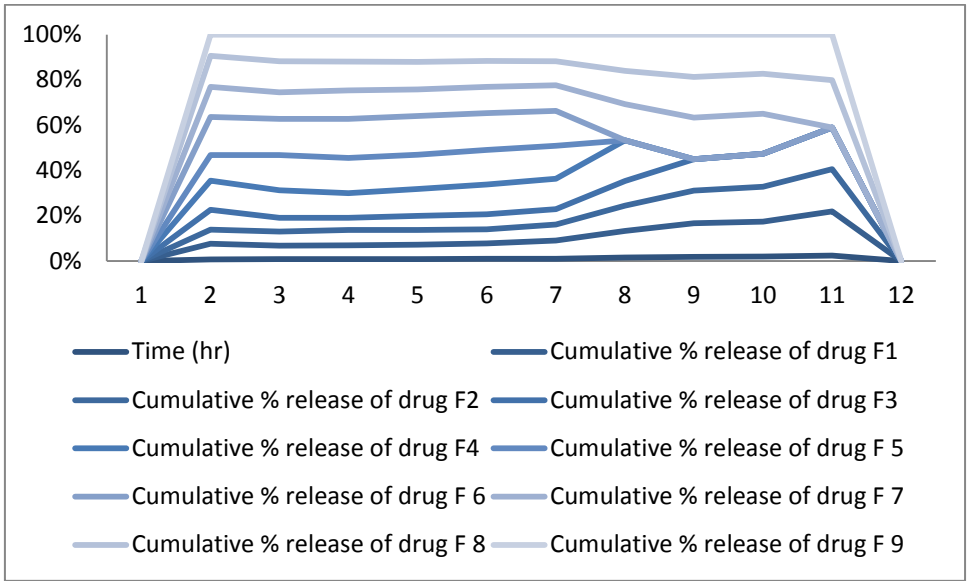

Fig. 6: Invitro Release of All Formulations

Fig. 7: Zone Of Inhibition of All Formulations

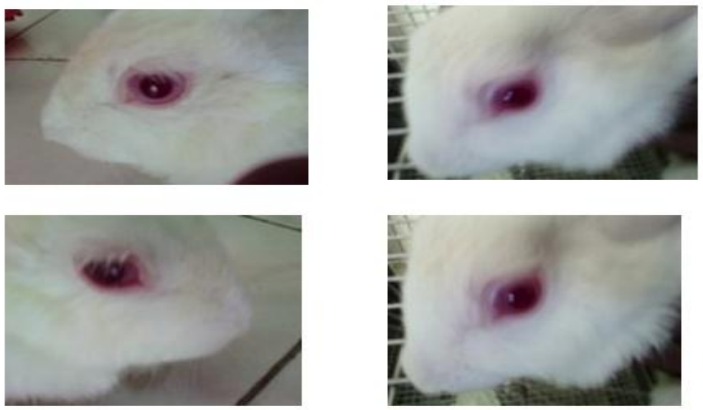

Fig. 8: ocular irritancy of all formulations

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