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Formulation and *in vitro* Characterization of Ocular *in situ* Gels of Valcyclovir

V.Vijaya Kumar[1*], C. Madhusudhana Chetty[1], Y. Dastagiri Reddy[1], R. E.Ugandar[1] & B. Deekshi Gladiola[1]
Department of Pharmaceutics, Santhiram College of Pharmacy, Nandyal.

Abstract:

Rapid precorneal elimination of drug results in poor availability and exhibits poor therapeutic response. This can be overcome by the use of novel drug delivery systems. The present work mainly aims to develop and evaluate Valacyclovir ophthalmic in-situ gels based on the concept of a p^H-triggered system for the prolonged corneal residence time. Formulations were prepared using Carbopol 940 as a gelling agent and HPMC K100 M as a viscosity enhancer. The prepared formulations were evaluated for various parameters like viscosity, gelling capacity, and drug content, sterility test, and in-vitro drug release profile for selection of an optimized formulation. The developed formulations with satisfactory visual appearance, clarity, drug content, viscosity, gelling capacity were selected for in-vitro release studies. An optimized formulation that shows sustained and prolonged release for an extended period of time for 10 hours was finalized for further evaluations like; antimicrobial efficacy and irritation test. Formulations were found to be a nonirritant, effective against the test and exhibited Zero-order release kinetics. The developed formulation with Carbopol 940 as a gelling agent and HPMC K100M as a viscosity enhancer can be a viable alternative to conventional eye drops.

Keywords: Carbopol 940, HPMC K100M, Insitu gels, Ocular drug delivery, Sustained delivery, Valacyclovir**.**

INTRODUCTION:

Ocular drug delivery has remained as one of the most demanding tasks for pharmaceutical scientists. The unique structure of the eye does not allow the drug molecules at the required site of action [1]. Conventional systems like solutions, suspensions and ointment have not been used extensively due various drawbacks like increased precorneal elimination, reduced drug concentration and blurred vision [2] .Low absorption results are shorter duration of action due to this high frequency of eye drop installation required. An alternative approach that significantly increases the precorneal residence time and bioavailability of the drug can be achieved by using the novel delivery system based on the concept of in situ gel formation [3]. In situ gel forming system can be described as liquids dosage form that can be delivered in a drop form and they undergo a phase transition in the ocular cul-de-sac to form a visco-elastic gel. In situ activated gelling systems on contact to physiological conditions will change to a gel phase [4] Gelation occurs via the cross-linking of polymer chains that can be achieved by covalent bond formation (chemical cross-linking) or non-covalent bond formation (physical cross-linking). In situ gel-forming systems on installation in the conjunctival cul-de-sac form visco-elastic gels due to conformational changes of polymers in response to the physiological environment. In the present study, Ophthalmic in-situ gels of Valacyclovir were prepared based on the concept of P^H triggered system of the formulation. Valacyclovir is a nucleoside analog that mimics one of the building blocks of DNA, which is active against the herpes viruses [5]. It is used to treat infections with Shingles (herpes zoster), Genital herpes (herpes simplex genitals), cold sores (herpes labialis). The high penetration into the aqueous humor and low toxicity of Valacyclovir make it a good candidate for consideration as a topical ocular antiviral agent.[6]

MATERIALS:

Val acyclovir was received as a gift sample from Hetero Pharma Ltd, Hyderabad. Carbopol 940, HPMC K 100M , Tween 60 were purchased from S.D. Fine Chem, Mumbai, Sodium Chloride was purchased from Karnataka Fine Chemicals, Bangalore and Benzalkonium chloride was procured from Ozone chemicals, Mumbai

EXREPIMENTAL METHODS:

Drug-excipients compatibility studies:

Fourier Transform-Infrared spectroscopic studies:

Interaction between drug and polymer should be observed to check the compatibility between various ingredients of the formulation by Fourier Transform Infra Red (FTIR) spectroscopy analysis of their physical mixture by employing Potassium bromide pellet method (2 mg sample in 200 mg KBr). The spectrum of each sample was recorded over the range of 400-4000 cm^{-1} and the resolution was 1 cm-1.Compatibility studies were performed for the pure drug of Valacyclovir and Valacyclovir along with physical mixture (Carbopol 940 and HPMC K100 M).

Formulation Development:

Total nine formulations have been prepared by employing pH-triggered in-situ gelation method using different concentrations of gelling agents i.e., Carbopol 940 as p^H-sensitive polymer and HPMC K100 M. The composition of each and every formulation was shown in the formulation Table 1. All the nine formulations contain Carbopol 940 as p^H-sensitive polymer, HPMC K 100 M as viscosity enhancer, sodium chloride as an Isotonicity ad justifier, Tween 60 as a surfactant and Benzalkonium chloride as preservative. The method of preparation Valacyclovir ophthalmic in situ gels formulations are as follows:

pH triggered in situ gelation method: All the Formulations have been prepared by this method in which carbopol 940 has been used as pH triggered polymer.

Dissolve the 0.9g of sodium chloride in 50ml of water and to that HPMC K30 was added with continuous stirring by using magnetic stirrer for one hour until there is no lumps of HPMC should be observed. Carbopol 940 was sprinkled over the above mixture and allowed for overnight. The weighed quantity of drug was dissolved in Tween 60 and benzalkonium chloride was added to this drug solution. Finally drug solution was added to the above polymer solution with continuous stirring.

Evaluation of Ophthalmic gels:

Physical appearance and clarity:

The formulations of Valacyclovir ocular *in situ* gels were observed for general appearance and for the presence of suspended particulate matter by naked eye for their characterization. The clarity of the formulated solution is to be determined under black & white background by visual inspection [7].

Determination of pH:

The pH of Valacyclovir ocular *in situ* gels must be checked using pH meter immediately after preparation. The pH range of the gel formulation should be between 6 to7.4 and preferably be near to ocular pH to avoid ocular irritation and enhance patient compatibility and tolerance [8].

Gelling capacity:

The gelling capacity is determined by placing a drop of the formulation in a vial containing 2 ml of freshly prepared simulated tear fluid and formation of gel is observed visually [9]. The time taken for its gelling is noted. The composition of simulated tear fluid contains sodium chloride 0.670g, sodium bicarbonate 0.200g, calcium chloride 0.008g and purified water quantity sufficient to 100ml.

Rheological studies of Valacyclovir ocular *in situ* gels:

Rheological properties of *in situ* forming gel is an important parameter in determining the residence time of the drug in the eye after its instillation.[10].The viscosity of solution and gels was measured by Nunes viscometer.

Drug Content:

It is determined by taking 1ml of the formulation and diluting it to 100ml with distilled water. 1 ml was withdrawn and further diluted to 10 ml with distilled water. Concentration was determined at 255 nm by using UV visible spectroscopy [11].

Sterility testing:

Sterility is the important parameter for ocular gels, hence sterility test should be performed for the all nine Valacyclovir ocular *in situ* gels. Sterility testing must be performed for aerobic and anaerobic bacteria and fungi by using fluid thioglycolate medium and soya bean-casein digest media under aseptic conditions. The 1ml sterile optimized formulation was taken and diluted with 100 ml sterile water for injection. From this, 5mlsolution was added in each medium and incubated for not less than 14 days at 20-25^0 C in the fluid thioglycolate medium and at 20-25^0 C in soybean casein digest medium to find outgrowth of any microorganisms in the formulation.

In-vitro Drug Release Studies:

The drug release studies are carried out by using bi-chambered donor and receptor compartment model (Franz diffusion cell.) in which the formulation is placed in donor compartment and freshly prepared simulated tear fluid in the receptor compartment. Between donor and receptor compartment cellophane membrane is placed. Samples are withdrawn at regular intervals of time and these are diluted with a respective solvent to a specific volume and analyzed by UV spectrophotometer at respective nm using reagent blank. The percentage cumulative drug release (% CDR) calculated and the obtained data is subjected to drug release kinetics [12].

RESULTS AND DISCUSSION:

Drug - Polymer Compatibility Studies:

In the physical mixture of Valacyclovir with HPMC K 100 M and carbopol 940 the major peaks belonging to drug functional groups were obtained almost at the same wave numbers. However, additional peaks were obtained in physical mixtures which could be due to presence of impurities but there is no influence in the drug peaks, which indicates that there is lack of significant interaction between the drug and polymers and the entrapment of drug is only by physical process.

Evaluation of In-Situ Ophthalmic Gels: All the in situ gel formulations were evaluated for physical appearance, clarity, pH, drug content, gelling capacity, rheological study, sterility testing and in vitro diffusion study.

Physical appearance and clarity: All Formulations were checked for its clarity under dark background and also for visual appearance. All the prepared Formulations of Valacyclovir ocular *In situ* gels F1-F9 were found to be clear and transparent and the results were shown in the Table No.2

Evaluation of gelling capacity: All the prepared formulations of Valacyclovir ocular *In situ* gels were evaluated for its gelling capacity in stimulated tear fluid having pH 7.4 and results were shown in the Table No.2. Formulations containing carbopol 940 as gelling agent shown good gelling capacity which remains its gelling strength for extended period as shown in Fig. No.1

Rheological Studies: Viscosity of the formulations governs the behavior of the formulations in the cul-de-sac resisting against the hydrodynamism and the blinking of the eye. In order to evaluate the rheological behavior, viscosity of the formulation before and after addition of simulated lachrymal fluid was evaluated using Nunes viscometer. All the formulations were found to be shear thinning exhibiting pseudoplastic behavior. All the formulations were liquid at room temperature and underwent rapid gelation upon raising the pH to 7.4. The samples were analyzed both at room temperature at 25°C and also at 37 ± 0.5°C. Results of viscosity studies before and after gelation were presented in the Table No.4. Viscosity of pH triggered formulations was increased due to pH change after instillation of the formulation into the tear film leads to an almost instantaneous transformation of the highly fluid latex into a viscous gel.

Table No.1 Formulation table of Valacyclovir ophthalmic in situ gels

Ingredients (gm)	Formulation Code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Valacyclovir	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Carbopol 940	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8
HPMCK100M	1.8	1.6	1.4	1.2	1.0	0.8	0.6	0.4	0.2
Benzalkonium Chloride	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Sodium chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Tween 60 (ml)	1	1	1	1	1	1	1	1	1
Distilled water(ml)	100	100	100	100	100	100	100	100	100

Table No. 2. Visual appearance and clarity of formulations F1-F9

Formulation Code	Visual appearance Clarity
F1	Transparent Clear
F2	Transparent Clear
F3	Transparent Clear
F4	Transparent Clear
F5	Transparent Clear
F6	Transparent Clear
F7	Transparent Clear
F8	Transparent Clear
F9	Transparent Clear

Table No. 3. Gelling capacity of formulations F1-F9

Formulation Code	Gelling Capacity
F1	++
F2	+++
F3	+++
F4	+++
F5	+++
F6	+++
F7	+++
F8	+++
F9	+++

Table No. 4.Viscosity of all Formulations before and after Gelation

Formulation Code	The viscosity of solution at 37^0 C (in cps) (mean ±SD)
F1	1725±1.58
F2	1135.27±2.59
F3	4057±3.46
F4	2518±2.28
F5	2518±2.28
F6	2604.36±3.21
F7	292.7±2.02
F8	1090±3.54
F9	474 ±2.42

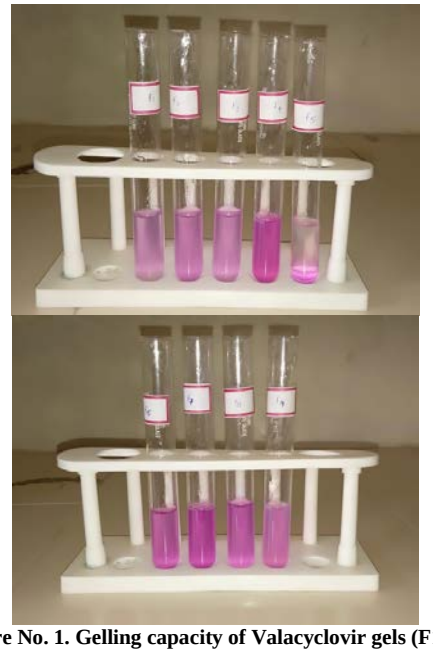

Figure No. 1. Gelling capacity of Valacyclovir gels (F1-F9)

Figure No 2. Sterility testing of Valcyclovir Ocular *In situ* gels (F1-F9)

Table No. 5.Zone Of Inhibition Observed For Different Formulations F1-F9

Formulation Code	Zone of inhibition(cm)	Test organism
F1	Nil	staphylococcus aureus
F2	Nil	staphylococcus aureus
F3	Nil	staphylococcus aureus
F4	Nil	staphylococcus aureus
F5	Nil	staphylococcus aureus
F6	Nil	staphylococcus aureus
F7	Nil	staphylococcusaureus
F8	Nil	staphylococcus aureus
F9	Nil	staphylococcus aureus

Table No. 6 In-vitro drug release Data of Formulations F1-F9

	Cumulative drug release								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
0	0	0	0	0	0	0	0	0	0
1	21.40	17.12	17.35	17.94	12.67	17.95	8.39	8.89	9.21
2	37.12	39.14	29.89	34.82	29.23	29.02	15.24	13.13	15.13
3	63.14	56.44	48.81	46.32	42.59	44.83	24.27	19.22	21.32
4	78.13	66.36	53.22	56.51	53.82	52.93	29.18	26.44	29.29
5	85.12	78.61	67.53	61.22	60.05	59.87	32.09	33.53	36.14
6	93.61	85.24	79.87	64.44	69.78	67.50	41.35	40.21	48.36
7	96.61	92.43	85.34	70.72	75.73	75.43	52.54	51.67	56.29
8	75.03	80.06	79.04	61.53	58.32	61.72	84.14	81.12	82.71
9	72.37	67.01	72.13	72.10	86.92	83.14	76.89	74.98	79.33

Table No.7. In-vitro drug release kinetics F1-F9

Formulation Code	Zero order	First order	Korsemeyer Peppas plot	Higuchi plot
	R2	R2	R2	R2
F1	0.917	0.947	0.973	0.945
F2	0.941	0.962	0.980	0.951
F3	0.975	0.920	0.978	0.925
F4	0.841	0.979	0.980	0.984
F5	0.876	0.981	0.936	0.962
F6	0.906	0.996	0.989	0.971
F7	0.986	0.937	0.678	0.853
F8	**0.992**	**0.913**	**0.729**	**0.936**
F9	0.985	0.916	0.616	0.843

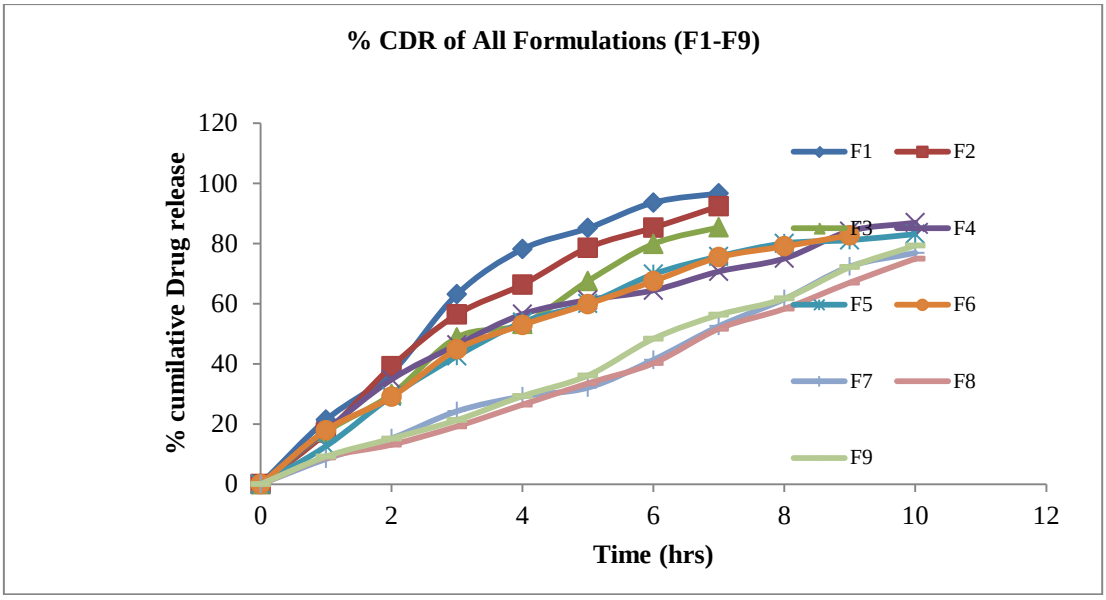

Figure No.3 .Cumulative drug release of F1-F9

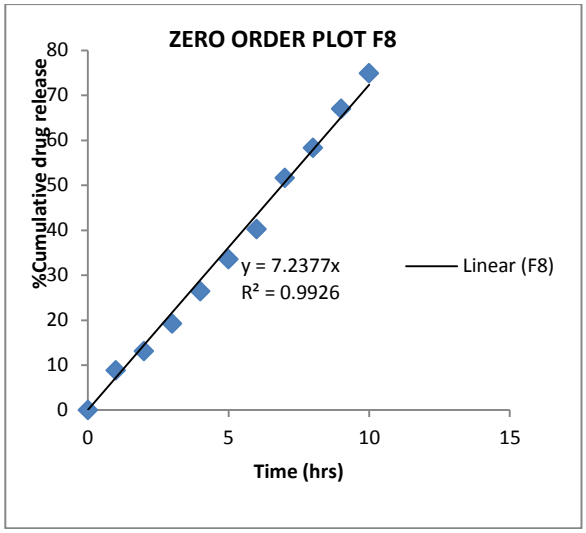

Figure No 4 . Zero order profile of F8

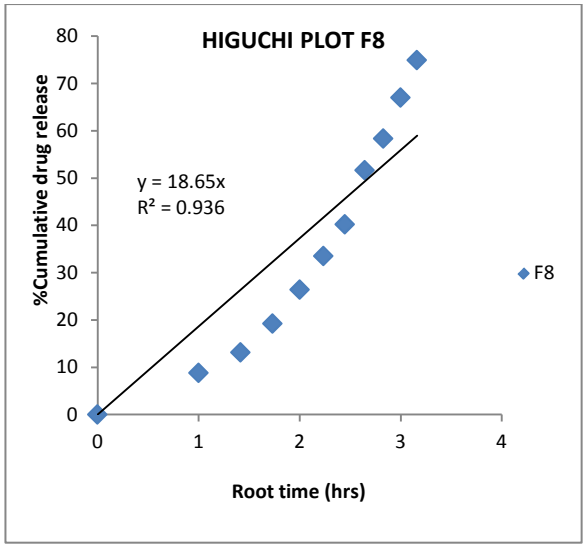

Figure No 5.Higuchi profile of F8

Sterility testing: All the prepared formulations of Valacyclovir ocular *In situ* gels were evaluated for its sterility test by using fluid thioglycolate medium ., All the formulations were checked for the growth of any microorganism in the respective mediums after its incubation period. It was found that all the formulations shown that there is no growth of any bacteria or fungi. Hence all the formulations are sterile and found to be free from microorganisms and the results were shown in the Table No.5.

***In Vitro* drug Release Studies:** The in vitro diffusion of Valacyclovir ocular *In situ* gels was studied using Franz diffusion cell using STF of pH 7.4 as diffusion medium. Egg membrane placed between receptor and donor compartment was used as diffusion membrane. The diffusion studies of prepared in situ gelling systems were carried up to 10 hours. Samples are withdrawn at regular time intervals and the same have been evaluated by using UV Visible spectrophotometer at 255 nm.. Results of drug release of all formulations were shown in the Table No.6 and 7. Comparative in vitro drug release plots of all formulations were shown in Figure No.3, 4 and 5. Formulation F8 containing carbopol 940 and higher amount HPMC K 100 M shows drug release up to 10 hrs with controlled release. Increased concentration of Carbopol 940 shows rapid dissolution and this can be controlled by the addition of HPMC K 100 M as release retardant.

CONCLUSION:

From this study, we can conclude that Valacyclovir ophthalmic gels enhance ocular bioavailability by extending precorneal residence time and ability to maintain sustained drug release, also important is the ease of administration afforded and decreased frequency of this system of administration resulting in better patient compliance. In order to maximize the potential of this system for ocular delivery further in vivo studies and stability, studies are needed to examine its feasibility for prolonged drug delivery. Ophthalmic *in situ* gels will be promising approach to overcome the drawbacks of conventional ophthalmic solutions. Hence further work is recommended to support its efficacy claims by in vivo studies and stability studies.

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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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