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RESEARCH ARTICLE

Acyclovir Loaded Ophthalmic Lyophilisate Carrier System (OLCS): Development, Characterization and Ocular Irritation Study

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ABSTRACT

The eye is a complex organ with a unique anatomy and physiology. A major problem in ocular drug delivery is the availability of optimal drug concentration at the site of action, which is compromised mainly due to precorneal loss resulting in only a small fraction of the drug being occularly absorbed. The present study was aimed to design Ophthalmic Lyophilisate Carrier System (OLCS) for Acyclovir (ACV) to defeat the cons of conventional ophthalmic formulations. Various hydrophilic polymers were tried to select best polymer for giving acceptable strength to the system. Methocel® E5 was selected as an optimal polymer for making drug-polymer solution having desirable strength. Poly propylene was optimized as a most effective carrier. Exhaustive preformulation study and drug excipients compatibility study was performed. The supportive media was sterilized by steam sterilizer. OLCS was finally lyophilized by conventional laboratory freeze dryers. The developed formulation was characterized for various physicochemical parameters. Ocular irritation study of OLCS was performed using a modified het-cam test and assessed by irritation score. The OLCs were charged for the accelerated stability studies as per ICH guidelines (25±°C/60% RH, 40°C/75% RH) for a period of 6 months. The results of drug excipients compatibility study and FTIR spectra revealed compatibility of drug with proposed excipients. The IS score of the developed OLCS was 0.60 indicating non ocular irritancy of OLCS. The results of all physicochemical parameters were within the acceptable limit for eye application. The results of short term stability study showed desirable stability of developed formulation. Thus, OLCs can be a promising approach for ophthalmic delivery of drug with desirable bioavailability and minimal loss.

KEYWORDS

Ophthalmic Lyophilisate Carrier System, Sterility, Het-Cam Test, Stability Study

INTRODUCTION

The eye is a complex organ with a unique anatomy and physiology which is easily accessible but a difficult organ as a drug delivery site.

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The difficulty arises from the effective protective mechanisms the eye poses. The protective mechanisms include lacrimal secretion/drainage and blinking reflex, both of which cause rapid loss of drug after topical administration[9].

Eye-drops are the conventional dosage forms that account for 90% of currently accessible ophthalmic formulations[8]. Despite the excellent acceptance by patients, one of the major problems encountered is rapid precorneal drug

loss. However, most of the topically administered dose is lost due to reflux blinking and only 20% (~7 µl) of instilled dose is retained in the precorneal pocket[11]. To improve ocular drug bioavailability, there is a significant effort directed towards development of novel ocular drug delivery system. Elderly patients are suffered as they are unable to recline their head more than particular angle without feeling dizzy. Several eye drops may flood the eye with medication, leading to overtreatment resulting in topical and systemic side effects due to the rapid absorption of the active ingredient via the nasopharyngeal mucosa. The extensive precorneal fluid loss caused by the drainage and high tear fluid turnover during the application of conventional ophthalmic water solutions and suspensions with ocular tissues is short (1 minute). This is one of the disadvantages of high concentrations being administered in conventional ocular therapy. Another important disadvantage of conventional eye drops is the use of preservatives. Preservatives are meant to destroy micro-organisms and protect the eye against possible infections, but their action is non-specific and they have been proved to damage ocular tissues[13].

So, it is justifiable to formulated novel ocular drug delivery system which prevails the drawbacks related with conventional ophthalmic dosage forms. An ideal form of ocular DDS should be easy in use, have neutral pH, absence of preservatives, cause a more linear drug inflow into the eye, sterile true single dosage with minimal discomfort and minimal influence on visual acuity.

To overcome the ocular drug delivery barriers and improve ocular bioavailability, various conventional and novel drug delivery systems have been developed and are not limited to nanomicelles, nanoparticles, liposomes, dendrimers, implants, contact lenses, nanosuspensions, microneedles, and *in situ* thermosensitive gels and many of them were introduced unsuccessfully[12, 17].

Acyclovir, (Figure 1) chemically known as 9-[(2hydroxyethoxy) methyl] guanine is a purine nucleoside analogue, active against herpes simplex virus type 1 and 2 and against viricella zoster virus. The inhibitory activity of acyclovir is highly selective due to its affinity for the enzyme thymidine kinase (TK) encoded by HSV and VZV[14].

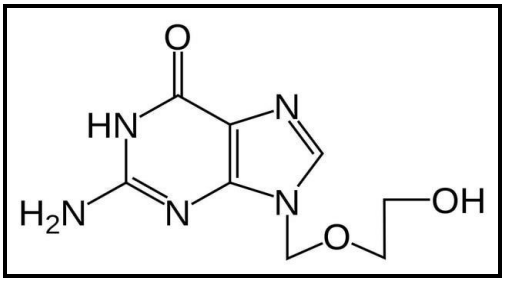

Figure 1: Chemical structure of Acyclovir

A new preservative free, freeze dried ophthalmic drug delivery system (lyophilisate) (Figure 2) demonstrated a very good tolerability and excellent safety. Generally OLCS in which a single dose of active ingredient is dissolved or dispersed in a drop of aqueous solution of a hydrophilic polymer, which is freeze dried on a soft hydrophobic carrier membrane attached to a paper handle. Upon administration, the lyophilisate is stripped off its carrier by a wiping motion over the lower eyelid, adheres to the conjunctiva and dissolves in the tear fluid[15].

Figure 2: Design and way of application of OLCs

So, in the present work, ACV loaded OLCS was prepared and characterized it substantially along with ocular irritancy test using eggs.

MATERIAL AND METHODS

Materials and Method

Acyclovir was received as a gift sample from Xcelris Labs, Ahmedabad, India. Different grades of HPMC and Carbopol 931P were

procured from Suvik Pharmaceutical Pvt. Ltd, Gandhinagar, India. All other reagents were of analytical grade.

Preformulation Study

The ACV powder was tested for various physicochemical properties like solubility in water and organic solvents, organoleptic properties, melting point, pH and derived properties. Pharmacokinetics parameters of drug were collected from literature[4].

Drug Excipients Compatibility Study

The physical mixture of drug and excipients was kept in petri dishes for 1 month at different storage conditions and it was evaluated for various physicochemical characteristics[1].

Analysis of ACV

UV-visible double beam spectrophotometer, Shimadzu UV-160AUV with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells were used. The quantification of ACV in phosphate buffer solution (pH 7.4) was done by UV spectrophotometric method at 252 nm[6].

Formulation of OLCs

Small batches of 5 to 50 OLCS were prepared in conventional laboratory freeze dryers. A drop containing the drug and a hydrophilic polymer was placed on each of the pre-sterilized strips under aseptic conditions in a cylindrical stainless steel container. The container was closed with a lid containing a large opening covered with a 0.2 mm filter and the solution was frozen and lyophilized after transfer into the freeze drier.

Selection of Appropriate Polymer for OLCs

The selection of hydrophilic polymer should be judicious to provide stable architect to dosage form. In the primary selection of hydrophilic polymer, different polymers including HPMC (5 cps), Hydroxyethylcellulose (HEC), Carbomer (Viscopol 931P), Chitosan and Poloxamer (Pluronic® F-127) were tried. From primary study the best polymer was further used in different concentration for optimization[19].

Selection of Appropriate Carrier Surface for OLCs

The selection of carrier significantly plays an important role in formulation of OLCs. The selection of material of carrier greatly affected by choice of method of sterilization and the condition of later. In the present study, only two carriers PVC film (Polyvinyl Chloride) and non-PVC film (Poly Propylene) were tried for its suitability of carrier of OLCS.

Selection of Appropriate Sterilization Process

Sterility is mandatory for ophthalmic preparation. Aseptically sterilization is more suitable process due to lyophilize nature of drug product. For sterilization of supporting media feasible method are Gamma sterilization, ETO sterilization and Steam sterilization.

Characterization of OLCs[15, 18, 19]

Density

The mean mass of a lyophilisate was from the average of mass of 3 Lyophilizates determined. From the mass and volume of the individual Lyophilisates, the density was calculated. In this case, the volume of the solution used (30 µl) is approximately the Lyophilisate volume equated. (n=3).

Reconstitution

The time period in which the OLCS was dissolved without leaving a clearly visible residue was noted. (In 2 mL distilled water at room temperature). (n=3)

Uniformity of Mass

Twenty OLCS were detached individually from the support with tweezers and weighed using weighing balance (Analytical balance, Entris®). (n=3)

Clarity and Particulate Matter

One unit of OLCs was reconstituted with 1.0 mL of purified water and its clarity was compared with equal volume of purified water in a glass test tube. The clarity of both the solutions must be significantly comparable and there must not be any visible residue as undissolved matter.

Table 1: Formulation of ACV OLCS

Formulations of ACV OLCS (%)									
Ingredients	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**
ACV	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
HPMC	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
Mannitol	0.0	0.0	0.0	5.0	5.0	5.0	10.0	10.0	10.0
Sodium Hydroxide	q.s. to pH adj.								
WFI	q.s. to 100%								

(n=3)

pH

One unit of OLCS was reconstituted with 1.0 mL of purified water. The pH of a reconstituted solution was measured using a calibrated pH meter. (n=3)

Water Content

Methanol dried (35.0-40.0 mL) was taken in titration vessel of Karl-Fischer instrument and was neutralized with Karl-Fischer reagent. 0.15 g of Disodium Tartrate was weighed and transferred in to the titration vessel using the butter paper. The system was titrated to the electrometric end-point with Karl-Fischer reagent. Ten OLCs were placed in a glass vial, and 10.0 mL methanol dried was added into it with the help of dry syringe and needle. The contents were mixed well. Blank reading for 10.0 mL of methanol dried was recorded. The content of the vial was transferred into the titration vessel, which contained pre-neutralized methanol. The system was titrated to the end-point and burette reading was recorded. (n=3)

$$Water\ content\ (mg\ per\ container) = V * F$$

Where,

V = Volume of KF reagent consumed for the sample (Burette reading – Blank reading)

F = Karl- Fischer reagent factor in mg/mL

Dimension of OLCs

The diameter of each dry drop was measured by vernier caliper. The average of six drops was considered in result.

Wetting Time

A piece of paper tissue was folded twice and placed in a small culture dish (i.d. = 6.5 cm) containing 8 ml of amaranth solution. One unit of OLCs was placed on the paper. The time required to completely remove lyophilisate was record as wetting time.

Osmolality of a Reconstituted Solution

Two OLCS were taken and reconstituted with 1.0 mL purified water. 250 µL of reconstituted solution was pipetted out and transferred into clean and dry sample tube using micropipette. The sample tube was placed into sample well of the Osmometer and the Osmolality of the reconstituted solution was found out.

In Vitro Corneal Permeation Study[16]

A modified Franz diffusion cell was used for study. A modification was made in the donor and receptor compartments to hold corneas and maintain normal corneal curvature without wrinkling. The temperature was kept at 34°C. Receptor chambers were filled with a modified ringer's solution to preserve the integrity of the excised cornea. The permeation medium was

constantly stirred using small magnetic bars. A sample of each formulation was introduced into the donor compartment. Samples of 0.4 mL was withdrawn at pre-determined time points and replaced with fresh receptor medium.

In Vitro Drug Release Study

The release of drug from OLCs (n = 3) was performed using glass vials in an oscillating water bath. An OLCs was accurately weighed and transferred to a glass vial containing 1.00 mL simulated lacrimal fluid (pH 7.4) [Simulated Lacrimal Fluid (SLF) prepared with 8.3 g/l NaCl, 1.4 g/l KCl and 84 mg/l $CaCl_2$ in MilliQ water]. To avoid water evaporation, the vials was covered with rubber caps and placed in an oscillating (25 rpm) water bath at 32 ± 1°C. Throughout the experiment, aliquots were withdrawn after 5 min. The concentration of drug was determined spectrophotometrically.

Ocular Irritation Study Using a Modified Hetcam Test

The chorioallantoic membrane (CAM) is the vascularized respiratory membrane that surrounds a chick developing inside an egg. This assay involves isolating an area of this membrane. OLCs were applied to the prepared surface. After incubation, the membrane was inspected visually and changes in its morphology were scored. There are several variations in CAM assay protocols. One of the scores determines the irritation potential from a formula which includes the time in seconds at which haemorrhage, vasoconstriction and coagulation appears. The irritation score ranges from 0 to 21. Substances are categorized according to these values[2, 10].

$$\text{Irritation score (IS)} = \left(\left(\frac{(301-\text{Hemorrhage time})}{300}\right)\times 5\right)+\left(\left(\frac{(301-\text{Lysis time})}{300}\right)\times 7\right)+\left(\left(\frac{(301-\text{Coagulation time})}{300}\right)\times 9\right)$$

Where,

Hemorrhage time= Time (sec) of the first appearance of blood hemorrhages

Lysis time= Time (sec) of the first appearance of vessel lysis

Coagulation time = Time (sec) of the first appearance of protein coagulation

Short Term Stability Study of OLCs

The OLCs were charged for the accelerated stability studies as per ICH guidelines (25±°C/60% RH, 40°C/75% RH) for a period of 3 months in stability chambers (Model-TH 90 S, Thermolab, India). They were placed in flint vials and hermetically sealed with rubber plugs and aluminum caps. The samples were taken out at 1, 3 and 6 months and evaluated for the various parameters[7].

RESULTS AND DISCUSSION

Preformulation Study

The results of preformulation studies are depicted in Table 2.

Table 2: Results of Preformulation Studies

Parameter	Observation[3, 5, 14]
Organoleptic Properties	
Color	White crystalline powder
Odor	Odorless
Solubility (mg/mL)	
Solubility	Slightly soluble in water, freely soluble in DMSO (45 mg/mL), very slightly soluble in ethanol.
pKa	pKa: 2.16, pKb:9.04
Log P	-1.59
Partition Coefficient (n-octanol/water)	-1.56
Physical Properties	
Melting point (°c)	256-260
Molecular weight	225.2
pH (10% w/v)	4-5
Pharmacokinetics parameters	
Half-life (hrs)	2.5-3.3
BCS class	IV
Oral B.A.	10% to 20%,
Protein binding	9%-33%

Table 3: Drug-Excipients Compatibility Study

Parameter	Before storage	Observation after 1 month storage at		
		Room temperature	Controlled room temperature (25^0C and 60 ± 5%RH)	Higher temperature (45^0C and 75 ± 5%RH)
Colour	White	No change	No change	No change
Odour	Characteristic	No change	No change	No change
Degradation products (visual)	No	No	No	No
Physical state	Solid	Solid	Solid	Solid
Drug content	100.54%	98.26.%	99.06.%	99.86%
Any other sign of instability	Not any	Not any	Not any	Not any

Drug Excipients Compatibility Study

The results of drug excipients compatibility study are depicted in Table 3. The results indicated that drug was found compatible with proposed excipients.

Formulation of OLCs

Selection of Appropriate Polymer for OLCs

OLCs prepared with HPMC (5 cps) have more elegant look and good disintegration pattern than OLCs prepared with other polymers. The success of the formulation is based on formation of a continuous layer in the cornea upon application of OLCs. To achieve patient compliant smooth layer, polymer should be hydrated immediately, which is better possible with HPMC than other polymer tried.

Selection of Appropriate Carrier Surface for OLCs

Non-PVC film (Poly Propylene) was more suitable over the PVC film.

Selection of Appropriate Sterilization Process

Risk associated with ETO sterilization is minute quantity of residue present on media after sterilization. In case of Gamma sterilization, its change the polymorphic form of supporting media.

The clarity of media converts to pale yellow from clear colorless. Therefore, steam sterilization method was selected to sterilize the supporting media.

Advantage associate with this method is that it also depyrogenate the carrier system by washing with WFI. Samples taken after steam sterilization were tested for sterility test and results proved that at 121°C for 15 min it produced sterile supporting media.

Characterization of OLCS

The results of physicochemical study are given in Table 4. The results indicated the Batches B4-B9 was passed and the values were in the limit.

Table 4: Results of Physicochemical Study (Batches B1-B9)

Parameter	B1	B2	B3	B4	B5	B6	B7	B8	B9
Assay	Defined particles, mechanically unstable			99.94%	98.13%	100.07%	96.98%	94.57%	94.01%
Residual amt. of API on the carrier film				0.64%	1.98%	0.41%	3.26%	5.02%	5.39%
Determination of density (g/cm^3)				0.073	0.078	0.081	0.135	0.140	0.146
Determination of the moisture content				4.03%	3.12%	4.58%	4.95%	5.57%	6.11%
Reconstitution (sec)				<4	<5	<4	<8	<8	<10
Uniformity of mass				Passes	Passes	Passes	Passes	Passes	Passes
Osmolality				267	310	319	300	298	299
Wetting time				<1 sec	<2 sec	<3 sec	<3 sec	<3 sec	<3 sec
Dimension of OLCs				Uniform	Uniform	Uniform	Uniform	Uniform	Uniform
pH				6.3	6.5	6.2	6.5	6.4	6.5
Clarity and particulate matter				Complies	Complies	Complies	Complies	Complies	Complies

Table 5: Results of stability study

Parameter	Initial	40°C/75%RH			25°C/75%RH		
		1M	3M	6M	1M	3M	6M
Assay	100.45%	98.36%	99.05%	100.25%	99.68%	100.74%	99.37%
Total Related Compounds	0.53%	0.89%	1.24%	1.47%	1.22%	1.37%	0.67%
pH	4.7	4.7	4.3	4.5	4.3	4.5	4.5
Osmolality	307	316	300	311	297	305	324

Ocular Irritation Study using a Modified Het-Cam Test

The results of ocular irritation study are depicted in Figure 3. Figure 3B indicates occurrence of haemorrhage in the egg treated with positive control. There were no any signs of haemorrhage, vasoconstriction and coagulation in egg treated with ACV OLCs (Figure 3A). The morphology of the vein in the egg was similar to the negative control used in the study. The IS score calculated in case of ACV OLCs is 0.60 which testifies the non-ocular irritancy from developed formulation.

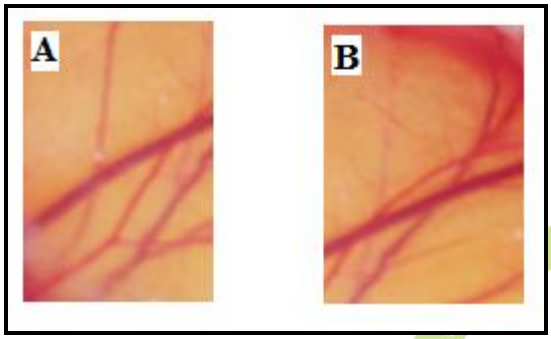

Figure 3: Ocular Irritation Study of ACV OLCs

Stability Study

The results of stability study of OLCS at two different conditions are depicted in Table 5. The results of stability study indicate that OLCS was stable at both the conditions for tested period.

CONCLUSION

ACV was found compatible with auxiliary excipients used in the proposed study. Judicious selection of polymer and carrier for development of OLCs was found prominent due to sterility issue. Stem sterilization method yielded optimal sterilization without compromising the quality of product. Het cam test confirms the non-irritancy of OLCs to eye. Short term stability study data revealed stable characteristics of proposed formulation.

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