Twice a Day Ocular Inserts of Acyclovir by Melt Extrusion Technique

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Acyclovir, an antiviral is used in the treatment of ocular infections. Acyclovir is effective against human herpes viruses including *Herpes simplex* virus type 1 and 2, Varicella Zoster virus, Epstein-Barr virus and Cytomegalovirus. Acyclovir is available as a 3%w/w eye ointment to be applied 5 times a day in the eye. The present investigation was aimed at designing a twice a day ocular inserts of acyclovir by melt extrusion technique to improve patient compliance, using hydroxypropylcellulose as a thermoplastic polymer. Also, the developed formulation would overcome the greasy nature of the eye ointment. The developed inserts were stable, non–irritant and provided release of the drug over a period of 10 hours *in vitro*.

Key words: Ocular insert, hot-melt extrusion, hydroxypropylcellulose, Klucel®

Topical ophthalmic application is considered the preferred way to achieve therapeutic levels of drugs used to treat ocular diseases[1]. The conventional preparations for this route are solutions, suspensions, semisolids like ointments, etc. Bioavailability, particularly for ocular solutions ranges from 1-10% of the total administered dose. This is due in part to the rapid precorneal clearance kinetics resulting from reflex tearing and blinking, where half-life times of instilled isotonic solutions or suspensions approximate only 15 s in humans. The problem associated with the use of ophthalmic ointment is poor patient acceptance[2]. To overcome these problems, various novel ophthalmic delivery systems such as inserts, *in situ* gels, etc have been investigated in an attempt to extend the ocular residence time of medication for topical application to the eye.

Melt extrusion is a technique in which during extrusion, a polymer melt is pumped through a shaping die and formed into a profile. This profile can be a plate, a film, a tube, or have any shape of its cross section[3]. The process often is referred to as profile or line extrusion in which the shape of the extrudate like a tube is determined by the die. The extruded profile proceeds horizontally to the cutoff equipment, which controls its length. Profiles may be further processed, for example, as in film extrusion, blow molding, or injection molding. In film extrusion, the polymer melt is extruded through a long slit die onto highly polished cooled rolls which form and wind the finished sheet. This is known as cast film[4].

Melt extrusion technology has been exploited in polymer industries since 1930's[5]. Since then it has been extensively used in polymer[6], food[7,8], chemical[9], rubber[10] and metal industries[11]. In pharmaceutical industries this technology is also exploited in preparation of pellets[12,13], solid dispersion[14-16], topical dosage forms[17], powder coating[18], gastroretentive dosage forms[19], tablets[20] and sustained release oral dosage forms[21-23]. However this technique has not yet been exploited in preparation of sustained release ophthalmic formulations.

Acyclovir has an *in vitro* and *in vivo* inhibitory activity against human herpes viruses including Herpes Simplex virus (HSV) type 1 and 2, Varicella Zoster virus, Epstein–Barr virus and Cytomegalovirus[24]. Acyclovir is available as a 3% w/w eye ointment to be applied 5 times a day in the eye[25]. The objective of the present investigation was to prepare long acting ocular inserts of acyclovir to be placed in the eye twice a day, by melt extrusion technique.

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MATERIALS AND METHODS

Acyclovir was obtained as a gift sample from Cipla Limited. Methocel® and Starch 1500 were obtained as a gift sample from Colorcon Asia Pvt. Ltd. Klucel® was gifted by Signet Chemical Corporation. All other chemicals and solvents used were of analytical grade.

Formulation considerations:
Extrusion of each plain polymer Methocel® A (methylcellulose), Methocel® E and Methocel® K (hydroxypropylmethylcellulose), Klucel® (hydroxypropylcellulose, HPC), Natrosol® (hydroxyethylcellulose) and Starch 1500 were carried out on Melt Flow Rate Apparatus, Model 3/80, Davenport. Extrusion dies of dimensions 1 mm, 1.2 mm and 1.5 mm were tried to afford the product suitable for instillation in the eye. The polymer used for the study was medium viscosity grade Klucel® GFF. The dose of Acyclovir was calculated so that an ocular insert for twice a day use could be fabricated using the technique of melt extrusion. Plasticizers such as propylene glycol, glycerine and polyethylene glycol 400 were tried as they are non-irritant for ocular use and their concentrations were optimised.

Method of preparation of ocular insert:
Acyclovir and the polymer were sieved through 60#, weighed and blended geometrically. The plasticizer was added and blended. The blend was then charged to the barrel of Melt Flow Rate apparatus and extruded. The extrudate was cut into appropriate size of 4.5 mm × 1 mm and packed in polyethylene lined aluminium foil (thickness 100 µ), heat sealed and sterilized by gamma radiation (2.5 Mrad for 4 h).

Evaluation of insert:
The developed inserts were evaluated for several parameters viz. appearance, uniformity of weight, dimensions, drug content, uniformity of content, Differential Scanning Calorimetric (DSC) analysis, eye irritation test and *in vitro* release studies. The inserts were observed for appearance/elegance, colour, surface irregularities, air bubbles, tackiness and suitability for ocular use. Twenty inserts were weighed and the average weight was determined. Deviation of individual insert's weight with respect to average weight was determined. Three inserts from a batch were powdered and dissolved in 50 ml of purified water by stirring on a magnetic stirrer for 2 h. The absorbance of each of these solution was then measured on a Jasco V530 UV/Vis Spectrophotometer at 251 nm. The concentration was extrapolated from the standard curve. Six inserts from a batch were individually crushed and dissolved in 50 ml of purified water. The absorbance of this solution was then measured spectrophotometrically at 251 nm. The concentration was extrapolated from the standard curve.

Differential scanning calorimetric (DSC) analysis:
DSC of the selected samples was carried out to study the thermal behaviour under specified conditions. Each sample was heated over the temperature range from ambient to 425° at a heating rate of 10°/min under nitrogen environment (20 ml/min). The instrument used was Perkin Elmer Differential Scanning Calorimeter. Thermograms were integrated using Pyris 6 software.

Ocular irritation test:
Ocular irritation studies were performed according to the Draize technique. Assessment of ocular irritation potential of ophthalmic formulations is an extremely important step in the development of ophthalmic formulations. The test has been standardized at the international level, e.g. using the OECD guideline No.405[26]. Acute eye irritation/corrosion and is the most widely used test for classification and labelling of chemicals according to their ocular safety. Six female rabbits each weighing 2-3 kg were used for the study of the formulations. The sterile formulations were placed twice a day for a period of 21 d and the rabbits were observed periodically for redness, swelling and watering of the eyes.

***In vitro* release studies:**
The *in vitro* release studies were performed in a modified dissolution apparatus as per USP specification[27]. The dissolution conditions were: temperature was kept at 37±1°, horizontal amplitude was 3.8 cm and frequency was set at 32 cycles/min. Each insert was tied in muslin cloth and was placed in the test tube containing 10 ml dissolution medium with the help of the hanger, in triplicates. Aliquots were withdrawn at 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 h. The aliquots were suitably diluted and analysed by spectrophotometrically at 252 nm. The % cumulative release of the drug was computed and graph of % cumulative release vs. time was plotted.

Sterilisation studies:
The inserts were packed in polyethylene lined aluminium foil (thickness 100 μ), heat sealed and sterilized by gamma radiation (2.5 Mrad for 4 h). Radio sterilised inserts were evaluated for appearance, uniformity of weight, dimensions, content and uniformity of content, *in vitro* release profile, DSC characterisation and sterility testing

Accelerated stability studies:
The optimized formulation in its final pack was stored at ambient conditions, 30±2°/65±5% RH and 40±2°/75±5% RH. Sampling was done at 0, 1, 2 and 3 mo and the formulations were evaluated for physical parameters, *in vitro* release, sterility and drug content.

RESULTS AND DISCUSSION

Melt extrusion offers the advantages of being a single step, simple, continuous process with relatively high throughput rates. It provides the facility of mixing inside the extruder body thus bypassing problem of segregation during premixing. It obviates the need for organic solvents in processing and circumvents associated hazards. Also, it obviates the need for water and hence can work for water sensitive drugs. Also, there is no time consuming drying step involved. The bioavailability of the drug substance could be improved when it is dispersed at the molecular level in hot-melt extruded dosage forms.

Of the various polymers evaluated for melt extrusion, only all grades of Klucel® could be melt extruded. Methocel® A, Methocel® E, Methocel® K, Natrosol® and Starch 1500 could not be melt extruded. Polymers were extruded using different die of diameters 1, 1.2 and 1.5 mm at 122-128°. The compression force required to extrude the polymer was found to be 15 kg for 1.5 mm die diameter and was 21.5 kg for 1 mm and 1.2 mm die diameter, respectively. However, smallest diameter (1 mm) die was chosen for further studies after considering the size of the marketed formulation i.e. Lacrisert® (dimension: 5 mm×1.16 mm). The same die was used for further studies. Acyclovir is available as a 3%w/w ointment to be placed in the eye five times a day as a 1cm ribbon each[25]. The weight of such 5 ribbons approximates 66 mg of the ointment that contains around 2 mg of acyclovir daily. Hence, it was decided to formulate ocular inserts containing 1 mg of acyclovir for twice-daily use.

Plasticizers are normally used with polymers in melt extrusion to ensure smooth, uniform melt flow and flexible, homogeneous end products. The advantages of plasticizers in melt extrusion are lower processing temperatures and ease of manufacturing[3]. The effect of plasticizers at a concentration of 5% w/w viz. propylene glycol, polyethylene glycol and glycerine on processing conditions using Klucel® GFF was as shown in Table 1. The incorporation of the plasticizer was found to reduce the processing temperature as well as the compression force for melt extrusion. Propylene glycol was chosen as a plasticizer for further studies since it afforded a lowest processing temperature and therefore its concentration was optimised using Klucel® GFF as a melt extrudable polymer. The results are as depicted in Table 2. Propylene glycol was optimised as a plasticizer at a concentration of 5% w/w.

Klucel® GFF, Klucel® MFF and Klucel® HF (Table 3) were tried as a matrix for the insert along with propylene glycol (5%w/w) as the plasticizer and the polymer that afforded a desired release profile of more than 90% at the end of 10 h, in an *in vitro* dissolution study was selected as the optimum formulation. Klucel® HF gave the desired release profile. The results are as depicted in fig. 1.

TABLE 1: SELECTION OF PLASTICIZER FOR EXTRUSION

Plasticizer	Concentration % w/w	Polymer	Extrusion temperature (°)	Compression force (kg)	Appearance
Propylene glycol	5	Klucel® GFF	104	5	Good
Glycerine	5	Klucel® GFF	116	5	Good
Polyethylene glycol 400	5	Klucel® GFF	120.8	5	Good

TABLE 2: OPTIMIZATION OF CONCENTRATION OF PROPYLENE GLYCOL AS A PLASTICIZER

Plasticizer concentration	Polymer	Extrusion temperature (°)	Compression force (kg)	Appearance
2.5% w/w	Klucel® GFF	105.3	5	Brittle
5% w/w	Klucel® GFF	104	5	Good
7.5% w/w	Klucel® GFF	102.5	5	Tacky

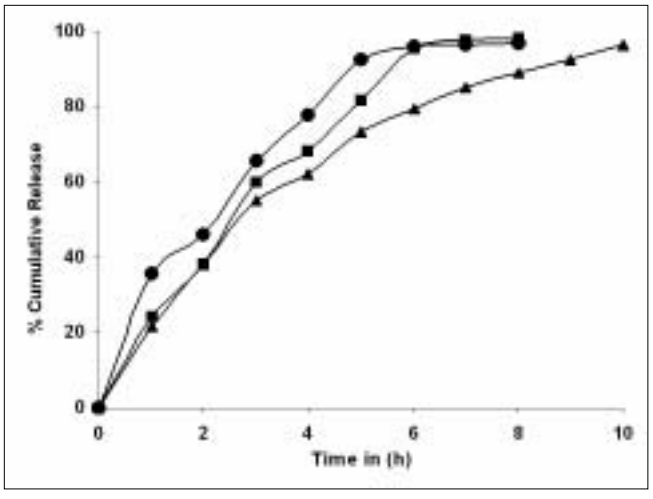

Fig. 1: Selection of Klucel® as a matrix for the insert Klucel® GFF (●); Klucel® MFF (■); Klucel® HF (▲)

The drug release from the Klucel® matrices was found to exhibit first order kinetics. This can be exhibited by the value of R and the values for T25, T50, T75 and T90% for the drug release from the matrix as shown in Table 3

The results of the evaluation of the ocular inserts of acyclovir are as depicted in Table 4. There were no changes in the quality control parameters of the insert before and after sterilization. Similarly, no change was observed in the release kinetics before and after sterilization as depicted in Table 4. The DSC studies confirm that there is no degradation of the drug as evident by the absence of any additional peaks in the DSC Thermograms. As illustrated in fig. 2 the reduction in the peak area of DSC thermogram of

TABLE 3: OPTIMIZATION OF GRADE OF KLUCEL®

Ingredients	Quantity per insert		
Formulation	**GFF**	**MFF**	**HF**
Acyclovir	1.00 mg	1.00 mg	1.00 mg
Propylene glycol	0.30 mg (5% w/w)	0.30 mg (5% w/w)	0.30 mg (5% w/w)
Klucel® GFF q.s.	6.00 mg	-	-
Klucel® MFF q.s.	-	6.00 mg	-
Klucel® HF q.s.	-	-	6.00 mg
Extrusion Temperature (°C)	104.0	114.0	115.8
Compression Force (Kg).	5	5	5
Observations	Good	Good	Good
Release kinetics			
R-value First	-0.978	-0.9748	-0.98978
Zero	0.9753	0.9732	0.9752
Higuchi	0.9744	0.974	0.9744
Time in hours			
T25	0.9	1.377	1.383
T50	1.72	2.12	2.69
T75	3.12	3.38	4.95
T90	4.97	5.04	7.9

TABLE 4: EVALUATION OF THE INSERTS

Parameter	Results	
	Before Sterilization	**After Sterilization**
Appearance	White and smooth devoid of air bubbles	White and smooth devoid of air bubbles
Uniformity of weight (mg) ±SD	5.97±0.17	5.892±0.21
Diameter (mm) ±SD	1.23 ± 0.0295	1.22±0.0286
Length (mm) ±SD	4.56±0.22	4.52±0.235
Content %	99.56	99.21
Content uniformity ±SD	99.38±1.4	99.83±1.94
Ocular irritation test	-	Non irritant
Differential Scanning Calorimetric (DSC) analysis	As shown in the fig. 3	As shown in the fig. 3
In vitro release	More than 90% release at the end of 10 hours (fig. 4)	More than 90% release at the end of 10 hours (fig. 4)
Sterility testing	-	Sterile
Release kinetics		
R First	-0.98978	-0.99892
Zero	0.9752	0.9792
Higuchi	0.9744	0.9700
Time in hours		
T25	1.383	1.03
T50	2.69	2.35
T75	4.95	4.62
T90	7.9	7.61

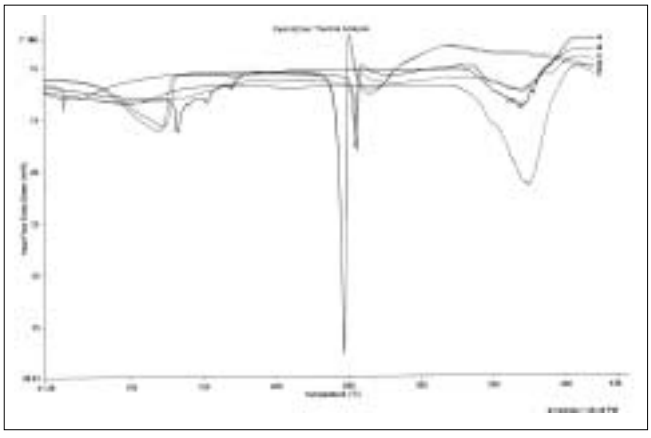

Fig. 2: DSC thermogram of sterilised and unsterilised drug, polymer and formulation.
Unsterilized Insert (A); Sterilized Polymer (B); Sterilized Insert (C); Unsterilized Polymer (D); Unsterilized Drug (E); Sterilized Drug (F)

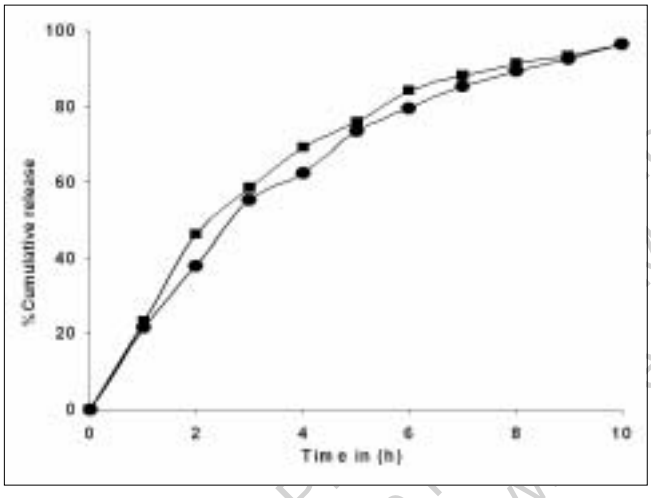

Fig. 3: Comparison of release profile of ocular insert of acyclovir
Comparison of release profile of ocular inserts before and after sterilization: before sterilization (●); after sterilization (■)

insert confirms the partial solubilisation of the drug in the polymer.

No irritation was observed in the rabbit eye during ocular irritation test. The overall irritation was found to be 4 out of 110 on the scale of scores for reading the severity of ocular lesions given by OECD guidelines no. 405[26]. It was also observed that after 12 h, the inserts completely dissolved in eye indicating biodegradable nature of the inserts.

Stability studies were carried out at ambient conditions, 30°±2°/65% RH±5%, 40°±2°/75% RH±5% for a period of 3 mo. The formulation was found to be stable, sterile and the drug content was found to be within limits.

The technique of melt extrusion was applied to the fabrication of acyclovir ocular inserts as solid polymeric rods to be placed in the cul-de-sac of the eyes. These inserts were retained in the eye for required period of time and sustained the release of the drug for 10 h. The polymer slowly released the drug via swelling and dissolved slowly in the tear fluid, thus avoiding the need to remove insert after drug administration. Further, the polymer is also non-greasy, thus potentially increasing patient acceptability.

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