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Short communication

CONTROLLED RELEASE POLYMERIC OCULAR INSERTS FOR DELIVERY OF ACYCLOVIR

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Abstract

The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid precorneal elimination of drug may be overcome by the use of ocular inserts, which achieve increased bioavailability by controlled release of drug. The purpose of the present research is to fabricate reservoir-type ocular inserts, by sandwiching the hydroxypropyl methylcellulose (HPMC) matrix containing acyclovir (ACV) in between two rate controlling membranes of cellulose acetate phthalate (CAP). ACV is a poorly aqueous soluble drug, and to improve its solubility it was complexed with β-cyclodextrin (BCD) and then incorporated into HPMC matrix. Polyethylene glycol (PEG-400) (10 % w/w) was incorporated as a plasticizer as well as permeation enhancer. Nine inserts (AO-1 to AO-9) with varying ratio of HPMC (drug matrix) and CAP (rate controlling membrane) were developed. The formulations were subjected to physicochemical evaluations, drug content uniformity studies, in vitro and in vivo drug release studies, interaction and stability studies. The in vitro release studies revealed that the formulations with 5% CAP was found to be optimum as rate controlling membrane. Based on the results of in vitro release studies the optimized formulations were selected for in vivo evaluation in rabbits. A high correlation coefficient was found between in vitro/in vivo release rate studies. Also the study confirmed the improved solubility of ACV when complexed with β-cyclodextrin. It can be concluded that the ocular inserts was remained stable and a shelf life of 1.8 years was assigned.

Key words: *Acyclovir, β-cyclodextrin, Ocular Insert, Binary systems.*

Asiklovir İçeren Kontrollü Salım Polimerik Oküler İnsertler

Konvansiyonel oftalmik çözeltilerle, ilacın hızlı prekorneal eliminasyonu nedeniyle ortaya çıkan düşük biyoyararlanım ve terapötik yanıt sorunları, ilacın kontrollü olarak salımı yoluyla biyoyararlanımını artırabilen oküler insertlerin kullanılmasıyla önlenebilir. Bu çalışmanın amacı, asiklovir (ACV) içeren hidroksipropil selüloz (HPMC) matriksi, hız control edici olarak 2 selüloz asetat ftalat (CAP) membrane arasına yerleştirerek, depo tipi oküler insert elde etmektir. ACV nin suda az çözünen bir ilaç olması nedeniyle, çözünürlüğünü artırmak için, β-siklodekstrin (βCD) ile kompleks hale getirilmiş, daha sonra HPMC matrikse eklenmiştir. Polietilen glikol (PEG-400) (% 10 a/a) plastik özellik kazandırıcı ve absorpsiyon artırıcı olarak formülasyona ilave edilmiştir. Farklı HPMC (ilaç matriksi) ve CAP (hız kontrol edici membrane) oranları kullanılarak 9 (AO-1 – AO-9) insert geliştirilmiştir. Formülasyonlarda, fizikokimyasal değerlendirmeler, içerik tekdüzeliği çalışmaları, in vitro ve in vivo ilaç salım çalışmaları gerçekleştirilmiştir. İn vitro salım çalışmaları sonucunda, % 5 CAP içeren formülasyonlar, hız kontrol edici membran özelliği açısından en uygun bulunmuştur. İn vitro ile in vivo salım çalışmaları bulguları arasında yüksek bir korelasyon katsayısı bulunmuştur. Bu çalışma ile, ACV nin β-siklodekstrin ile kompleks oluşturması sonucunda çözünürlüğünün arttığı doğrulanmıştır. Son olarak, hazırlanan oküler insertlerin 1.8 yıllık raf ömrüyle dayanıklı kaldığı belirlenmiştir.

Anahtar kelimeler: *Asiklovir, β-siklodekstrin, Oküler insert, İkili sistem.*

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INTRODUCTION

The vast majority of existing ocular delivery systems are still fairly primitive and inefficient; these dosage forms bear the limitations like quick elimination from the pre-corneal area, solution drainage by gravity, induced lacrymation and normal tear turnover resulting in poor bioavailability and increased severity in the systemic adverse effects from the topically applied drugs. Also, the traditional or primitive ophthalmic solutions, suspensions and ointment dosage forms are clearly no longer sufficient to combat some present virulent diseases (1-5).

In order to remove the constraints placed by traditional ocular therapies, newer delivery systems are being explored to develop extended duration and controlled release strategies. Some of the newer, sensitive and successful ocular delivery systems like inserts, biodegradable polymeric systems and collagen shields are being developed in order to attain better ocular bioavailability and sustained action of ocular drugs (6).

Currently, in the management of herpes simplex infections, ACV is used as a 3% ointment preparation. The drug bears 10-30% of bioavailability with mean 2.5 h plasma half-life. These conventional preparations are ill-accepted on account of their short pre-corneal retention time, greasiness, vision-blurring effects etc (7).

Considering the drawbacks associated with the current formulation, the present investigation has been dedicated to prolonging the retention time of medication on the eye surface and to the improvement of transcorneal penetration as well as solubility of poorly soluble drug ACV by co-administration with β-cyclodextrin.

EXPERIMENTAL

Materials

ACV was a gift sample from Glaxosmithkline Pharmaceuticals Mumbai, India. Pharmaceutical grade βCD was donated by Biocon India Ltd., Bangalore, India. Hydroxypropyl methylcellulose (HPMC-K_4M) was kindly supplied from Colorcon India Pvt. Ltd., Goa, India. Cellulose acetate phthalate (CAP) was purchased from Spectrochem Pvt.Ltd., Mumbai India. PEG-400 was purchased from Loba Chemie, Mumbai, India.

Preparation of binary system by co-evaporation method

Binary systems containing ACV (MW 225.21) and β-Cyclodextrin (MW 1135.0) were prepared in the molar ratio of 1:1 on the basis of the previous results obtained from the preliminary phase solubility studies (8).

Co-evaporated product was obtained by addition 10 ml of aqueous solution of β-cyclodextrin to 10 ml of 25 % ammoniacal drug solution. The mixture was stirred with heating at temperature not greater than 50 °C. The clear solution obtained was further heated with stirring, till a pasty mass was obtained. The residual solvent was removed under vacuum at room temperature. The obtained solid was ground, sieved through a 250 μm sieve and placed in an oven at 45 °C for 48 h (8-10).

Table 1. Composition of Various Polymers and Plasticizers in Different Formulations.

Formulation Code	Drug Reservoir (HPMC-K_4M) %w/v	Rate Controlling Membrane (CAP) %w/v.	Plasticizer*/ Penetration enhancer %v/w.	Complexed ACV (mg)
AO – 1	1.0%	4.0%	10.0%	0.690
AO – 2	1.0%	5.0%	10.0%	0.690
AO – 3	1.0%	6.0%	10.0%	0.690
AO – 4	1.5%	4.0%	10.0%	0.690
AO – 5	1.5%	5.0%	10.0%	0.690
AO – 6	1.5%	6.0%	10.0%	0.690
AO – 7	2.0%	4.0%	10.0%	0.690
AO – 8	2.0%	5.0%	10.0%	0.690
AO – 9	2.0%	6.0%	10.0%	0.690

12 ml of the cast solution was poured into petridish to prepare uniform circular cast film.
* Based on polymer weight.

Characterization of ACV Binary Systems (ACV-β-CD)

Analysis of inclusion efficiency in the binary mixtures

Solid inclusion complex (50 mg) was taken in a 50ml volumetric flask containing 30 ml of dimethyl sulphoxide and this mixture was kept in an ultrasonicator. After centrifugation, the supernatant was filtered through a 0.45 μm filter and the filtrate was suitably diluted. The absorbance of the resulting solution was measured at 254 nm (11). The inclusion efficiency was calculated usingequation below.

$$\text{Inclusion efficiency} = \frac{\text{Estimated \% drug content}}{\text{Theoretical \% drug content}} \times 100$$

Differential scanning calorimetry (DSC)

Differential scanning calorimetric analysis was performed using Mettler TA 4000 system. Samples of drug, β-Cyclodextrin and binary mixture containing 5 - 7 mg of ACV, were placed in a sealed aluminium pan and heated at a rate of 10 °C/min in 50-300 °C range, using an empty sealed pan as a reference.

Dissolution rate studies for complex

The USP XXIII-8 station (Electro Lab, India) dissolution test apparatus with a paddle stirrer and a stirring speed of 50 rpm was employed for the studies. pH 7.4 phosphate buffer saline was used as a dissolution medium (thermostated at 37 ± 2 °C). Powdered samples (Granulometric

fraction < 250 μm) of ACV and complex (ACV-β-CD), each containing 100 mg ACV, were added over a surface of 900 ml dissolution medium. Samples of 5ml were withdrawn, through a filter of 0.45 μm at 5 minutes time interval and same amount of fresh medium was replaced. The progress of the dissolution was followed by circulating the dissolution medium through the cell of the spectrophotometer for continuous recording over 60 min, at 254 nm (8-10).

Preparation of Ocular Inserts

The preparation of ocular inserts involved three different steps (1,12,13):

Drug reservoir film

The polymeric drug reservoir (DR) cast films were prepared by dissolving 1.0, 1.5 and 2.0 % of HPMC-K_4M in 15 ml of doubly distilled water. Along with this 26.95 mg of binary mixture containing ACV-β-CD was separately dissolved in dilute alkali hydroxide solution, and then it was poured to the polymeric solution. The solution was stirred using magnetic stirrer at 100 rpm and PEG-400 (10 % w/w), which was previously optimized for its concentration, was incorporated as a plasticizer as well as permeation enhancer to above solution under same stirring conditions.

After complete mixing, the uniform solution was caste on mercury surface using a ring of 5.0 cm diameter. The cast solution was allowed to evaporate by placing it inside an oven maintained at 37 ± 2 ° C, 30 ± 0.5 % RH for 24 hours. After drying, the medicated films of 8 mm diameter, each containing 0.69 mg of dose were cut using a sterile stainless steel borer.

Rate controlling membrane

For the preparation of rate-controlling membrane (RCM), weighed quantities of cellulose acetate phthalate was dissolved in 10 ml of acetone to obtain 4.0, 5.0 and 6.0 % polymeric solutions. Stirring was continuously maintained until the clear solution was obtained. These solutions were poured on a mercury surface using a ring of 5.0 cm diameter.The solution was evaporated slowly by inverting a glass funnel on a petridish at room temperature for 12 h. The dried films were cut into 9 mm diameter using a stainless steel borer.

Sealing Step

For fabricating the insert system, a medicated reservoir disc was sandwiched between two rate controlling membranes. Then this whole unit was placed in a desiccators and saturated with ethanol / acetone (60:40) for 4 - 5 minutes, over a wire mesh inside. This procedure resulted into successful sealing of the medicated reservoir film between two-rate controlling membranes. The sealed ocular inserts were stored in an airtight container under ambient conditions.

The amount of plasticizer weight was based on weight of the polymer. All the above experimentation was carried out under laminar airflow to maintain the sterility conditions of ophthalmic product. Nine batches of ocular inserts were formulated by the above mentioned method and labeled as AO-1 to AO-9 (Table 1).

Drug content uniformity in ocular inserts

For drug content uniformity, the ocular inserts were placed in 5 ml of dimethyl sulphoxide and were shaken in orbital shaker incubator at 50 rpm to extract the drug from inserts. After incubation for 24 h, the solution was filtered through a 0.45 μm filter and the filtrate was suitably diluted. The absorbance of the resulting solution was measured at 254 nm.

Thickness of film

Films were evaluated for the thickness using a Dial Caliper (Mitutoyo, Japan). The average of 10 readings was taken at different points and the mean thickness was calculated. The standard deviations (SD) in thickness were computed from the mean value (14).

Uniformity of weight

The weight variation test was carried out using digital balance, by weighing three patches from each formulation. The mean value was calculated and the standard deviations of weight variation were computed from the mean value (14).

Folding endurance

A small strip of film was cut evenly and separately folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gives the folding endurance.

Percentage moisture absorption

The percentage moisture absorption test was carried out to check physical stability or integrity of ocular films. Ocular films were weighed and placed in a dessicator containing 100 ml of saturated solution of aluminium chloride and 79.5 % humidity was maintained. After three days the ocular films were taken out and reweighed. The percentage moisture absorption was calculated using the formula below:

$$\text{Percentage moisture absorption} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Percentage Moisture Loss

The percentage moisture loss was carried out to check integrity of the film at dry conditions. Ocular films were weighed and kept in a desiccator containing anhydrous calcium chloride. After 3 days, the ocuserts were taken out and reweighed; the percentage moisture loss was calculated using the formula below:

$$\text{Percentage moisture loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Determination of water uptake and swelling behavior

Water uptake was determined gravimetrically. Drug reservoir films were placed on a filter. The lower side of the filter was immersed in a beaker containing simulated lachrymal fluid (SLF), and incubated at 32 °C which is the eye surface temperature. To prevent evaporation during the experiment the beaker was closed with parafilm. The weight of each insert was determined with the digital balance at predetermined time points. The size changes of the inserts due to swelling was investigated macroscopically (5).

In vitro drug release studies

The inserts from each batch were taken and placed in a 15 ml vials containing 10 ml of pH 7.4 phosphate buffered saline. The vials were placed in an oscillating water bath at 32 ± 1°C with 25 oscillations per minute. One ml of the drug releasing media was withdrawn at

various time intervals of 1, 2, 4, 8, 12, 16 and 20 h and replaced by the same volume of phosphate buffer saline pH 7.4. These samples were filtered through 0.45 μm membrane filter. The filtrate was diluted suitably with the buffer and the drug was estimated in each batch by UV-vis spectrophotometer at 254 nm (5, 15).

In vivo Drug Release Study

Based on *in vitro* drug release studies, formulations showing promising release behavior (out of nine formulations, AO-5 and AO-8 were selected) were selected for *in vivo* study. The inserts were sterilized by using gamma radiation before *in vivo* study (4,16).

The New-Zealand strain albino rabbits of either sex, weighing between 2.5-3.0 kg, free of any sign of ocular or gross abnormality were used for the experiment. Animal procedures are followed as per CPCSEA guidelines.

The ocular inserts containing ACV were taken for *in vivo* study, which were previously sterilized on the day of the experiment and were placed into the lower conjunctival cul-de-sac. The inserts were placed into one eye each of the seven rabbits and another eye served as control. Ocular inserts were removed carefully at 1, 2, 4, 8, 12, 16 and 20 h and analyzed for drug content by suitable dilutions. The drug remaining was subtracted from the initial drug content of inserts that will give the amount of drug released in the rabbit eye. Observation for any fall out of the inserts was also recorded throughout the experiment. After one week of washed period the experiment was repeated for two times as before.

Stability studies

Based on the *in vitro* and *in vivo* performance, the formulations AO-5 and AO-8 selected for the short-term stability studies and inserts were packed in amber-colored bottles with induction sealing. They were exposed to varying temperatures (60°, 40°, 20°, 10°, and 0 °C) for 90 days. At regular time intervals, the inserts were taken in 10 ml of pH 7.4 buffer and shaken for 12 hour in an orbital shaker. The resultant solutions are filtered, suitably diluted and estimated spectrophotometrically using pH 7.4 buffer as blank. The logarithmic percent of undecomposed drug was plotted against time and decomposition rate constants (K) were obtained at each temperature. The logarithm of decomposition rate constants were plotted against reciprocal of absolute temperature and the resulting line was extrapolated to K at 25° C (17,18).

Shelf life can be obtained by using formula below:

T_{90} = 0.104/K at 25 °C.

RESULTS AND DISCUSSION

Inclusion Efficiency in the Binary Mixtures

The inclusion efficiency of the co-evaporated product found to be 97.2 %, which suggests good relation between theoretical and actual drug content.

The DSC thermogram for drug as well as β-cyclodextrin and binary mixture are represented in Figure 1. DSC analysis of ACV shows the endothermic peak at its melting point i.e. at 248.5 °C (ΔH = 143.82 J/g).DSC curves of the binary systems were observed at 118.2 °C and they showed complete disappearance of the melting endotherm of ACV, which could indicate the complete amorphization of the drug as well as loss of drug crystallinity, interpreting that the drug has been engulfed in the cyclodextrin cavity.

Dissolution Rate Studies for Binary System

The dissolution rate of the binary compound was evidently higher (61.18 %) than that of the drug itself (34.27 %) after 60 min. This clearly indicates the enhancement in dissolution rate of poorly soluble drug acyclovir when it is complexed with β-cyclodextrin. These results can be attributed to the possible evidence of amorphization of the drug as well as reduction in the size of the drug particles. (Figure 2). For the spectrophotometric assay of ACV, the standard calibration curve was obtained with a good reproducibility, having a slope 0.0549 and with regression value of 0.9997.

Differential scanning calorimetry (DSC)

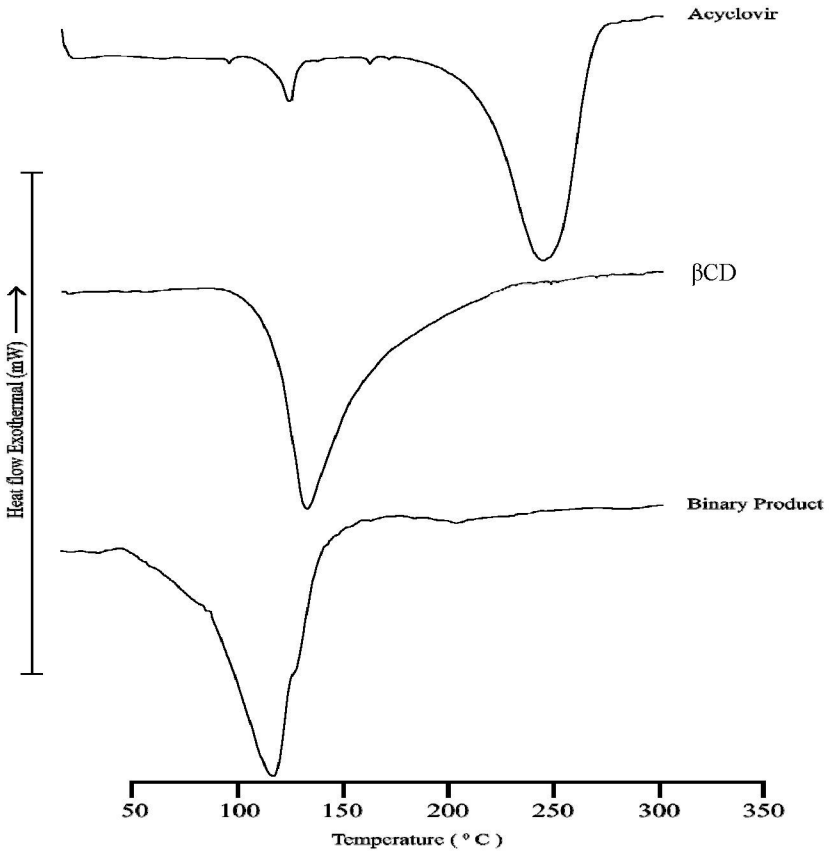

Figure 1. DSC of ACV, βCD and binary complex showing the endothermic peak at its melting point.

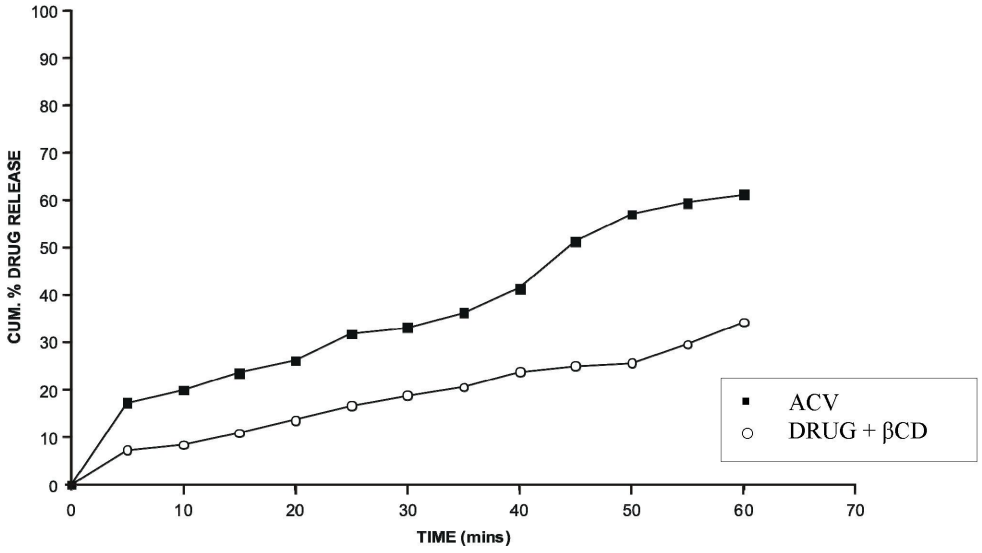

Figure 2. *In vitro* dissolution profiles of ACV and binary mixture.

Drug content uniformity in films

Table 2 shows the results of drug content uniformity of each formulation. Three replicates of each batch were analyzed; mean and standard deviations were calculated. The drug content uniformity found between 0.675 ± 0.012 to 0.689 ± 0.006 mg. Formulations AO-5 & AO-8 showed the maximum and uniform percent drug content of 99.85 and 99.49 %, respectively.

Table 2. Physicochemical Evaluation of Formulations.

Formulation Code	Drug Content (Mean ± SD) mg (n=3)	Thickness (Mean ± SD) mm (n=3)	Weight (Mean ± SD) mg (n=3)	Percent Moisture Loss (Mean±SD) (n=3)	Percent Moisture Absorption (Mean ±SD) (n=3)
AO – 1	0.675 ± 0.148	0.152 ± 0.003	14.55 ± 0.124	6.61 ± 0.241	7.78 ± 0.124
AO – 2	0.685 ± 0.019	0.151 ± 0.000	13.3 ± 0.141	9.34 ± 0.057	5.43 ± 0.090
AO – 3	0.686 ± 0.036	0.158 ± 0.006	16.33 ± 0.169	10.12± 0.0075	5.56 ± 0.182
AO – 4	0.679 ± 0.027	0.151 ± 0.008	14.65 ± 0.314	10.49 ± 0.116	8.24 ± 0.229
AO – 5	0.689 ± 0.018	0.157 ± 0.006	15.77 ± 0.122	7.33 ± 0.263	9.49 ± 0.228
AO – 6	0.684 ± 0.087	0.164 ± 0.008	18.59 ± 0.224	13.49 ± 0.199	6.08 ± 0.150
AO – 7	0.682 ± 0.193	0.151 ± 0.008	15.15 ± 0.156	9.17 ± 0.026	11.83 ± 0.346
AO – 8	0.683 ± 0.013	0.152 ± 0.008	15.21 ± 0.319	8.44 ± 0.240	8.75 ± 0.095
AO – 9	0.680± 0.017	0.171 ± 0.006	17.61 ± 0.113	11.61 ± 0.092	7.28 ± 0.151

Film Thickness

The mean film thickness (n = 3) was uniform and consistent with all the nine formulations, it was found to vary between 0.151 ± 0.001 to 0.171 ± 006. The little variation observed with formulations AO-5 and AO-9 may be due to the presence of higher concentrations of rate controlling membrane (Table 2).

Uniformity of weight

The uniformity of weight was calculated for three different films of each formulation, mean and standard deviation were noted. Uniformity of weight of the films found to vary from 13.33 ± 0.141 to 18.59 ± 0.224. Formulations from AO-1 to AO-9 showed good uniformity in weight (Table 2).

Folding endurance

The films were folded manually on an average of more than 90 times. The values for folding endurance varied from 87.9 to 103.6. And no cracks were observed. Formulations AO-1 and AO-6 showed maximum folding endurance.

Percentage moisture absorption

Each formulation was analyzed in triplicate for percentage moisture absorption and the highest moisture absorption was marked from formulation AO-7 (11.83 ± 0.346). This may be due to the presence of larger concentration of hydrophilic polymer HPMC-K_4M (Table 2).

Percentage moisture loss

There was no change in integrity of all the formulations. The moisture loss for all the formulations varied between 6.61 ± 0.241 to 11.61 ± 0.092. Formulation AO-1 showed the minimum amount of moisture loss in dry conditions, and this might be due to presence of less concentration of hydrophobic polymer cellulose acetate phthalate (Table 2).

Water uptake and swelling behavior

Maximum water uptake was found with 2 % HPMC-K_4M inserts due to the presence of more concentration of swellable polymer. After complete hydration, moderate gelling and swelling of the polymer was observed which may be responsible for the drug release from the matrix and had a diameter of approximately 10.2 mm.

In vitro drug release studies

All the nine formulations were subjected to *in vitro* drug release studies. The overall cumulative percentage drug release for formulations, AO-1 to AO-9 was found to be 89.97, 94.64, 80.14, 91.00, 98.31, 76.11, 83.61, 98.03 & 72.50% respectively at the endof 20 h as shown in Table 3. The release data obtained were grouped in five mathematical models of data treatment. Based on the highest regression value (r), which is nearing to unity, formulations AO-4, AO-5, AO-6, AO-8 and AO-9 followed Higuchi-Matrix kinetics (Table 4). This suggests that the drug releases by swellable polymer matrix through the diffusion of simulated tear fluids. The drug release from such systems is found to control by the dissolution fluid permeating through the membrane until a sufficient internal pressure is reached to drive the drug away from the reservoir membrane. Formulations AO-1, AO-2, AO-3 and AO-7 were best fitted into Peppa's model with 'n' values 0.5111, 0.5383, 0.7704 & 0.6275 respectively. This indicates that the drug release is by non-Fickian-diffusion mechanism. Cumulative percentage drug release for AO-5 and AO-8 found to be 98.31%, 98.03% respectively, and is depicted as linear, complete and more controlled pattern of drug release of all the other formulations and were selected as better formulations (Figure 3). From the release pattern, it was also ascertained that the drug release could be more controlled by using 5 % concentration of cellulose acetate phthalate as a rate controlling membrane, because above which the polymer concentration will restrict the drug release with therapeutic point of view.

Table 3. *In Vitro* Drug Release Data for Different Formulations.

Time (T) h	CUMULATIVE PERCENT DRUG RELEASE								
	AO-1	**AO-2**	**AO-3**	**AO-4**	**AO-5**	**AO-6**	**AO-7**	**AO-8**	**AO-9**
1	16.12	17.88	14.00	18.04	9.08	9.12	17.20	14.20	6.22
2	29.83	28.61	23.31	26.98	21.02	12.03	23.84	26.68	10.34
4	47.70	42.27	35.68	42.64	37.79	27.28	36.25	43.25	24.31
8	68.68	59.64	55.88	61.31	60.91	44.23	45.34	58.66	39.47
12	81.24	68.69	69.76	70.20	69.51	61.08	61.01	70.15	58.66
16	89.65	81.66	73.52	84.69	86.91	69.39	71.31	84.59	67.21
20	89.97	94.64	80.14	91.00	98.31	76.11	83.61	98.03	72.50

Table 4. Kinetic Values Obtained From Mathematical Model Treatment and Best Fit Models.

Formulation code	Slope	Regression coefficient (r)	n-value	Best Fit Model
AO – 1	0.199	0.9838	0.5111	Peppas
AO – 2	0.184	0.9951	0.5383	Peppas
AO – 3	0.209	0.9952	0.7704	Peppas
AO – 4	0.195	0.9972	0.5473	Higuchi-Matrix
AO – 5	0.268	0.9974	0.7536	Higuchi-Matrix
AO – 6	0.270	0.9962	0.6228	Higuchi-Matrix
AO – 7	0.188	0.9967	0.6275	Peppas
AO – 8	0.216	0.9970	0.5820	Higuchi-Matrix
AO – 9	0.306	0.9956	0.7793	Higuchi-Matrix

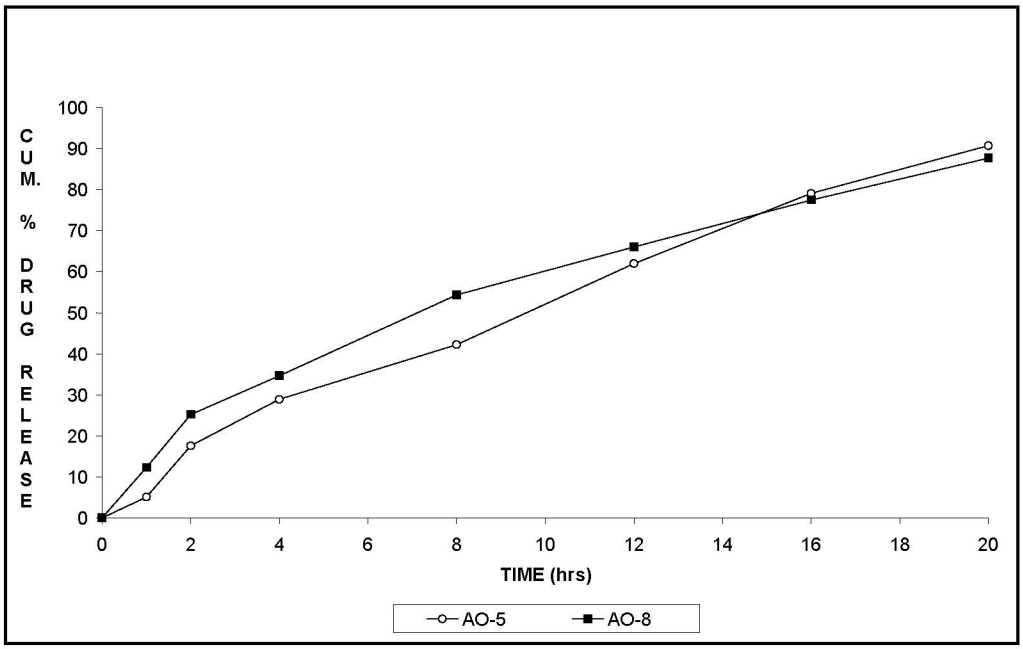

Figure 3. *In vitro* drug release profiles of the formulations AO-5 and AO-8.

In vivo release studies

In vivo drug release studies were performed on New Zealand strain albino rabbits. Out of nine formulations AO-5 and AO-8 were subjected to *in vivo* drug release studies, because these two formulations shown good linearity, maximum in vitro release rate and fulfilled many requirements of the optimized formulations. Hence they were considered as the formulations of choice for *in vivo* studies.

The study was carried out in triplicate for different time intervals, samples were withdrawn and cumulative amount of drug release were calculated by subtracting drug remaining from the main content of respective insert and on the basis of the amount of drug release percentage of ACV was calculated. The results obtained for the formulations AO-5 and AO–8 were calculated the *in vivo* cumulative percentage drug released as a function of time (Figure 4).

Scattered graph based on correlation between *in vitro/in vivo* cum. Percentage drug release for both the formulations were shown in Figures 5 and 6.

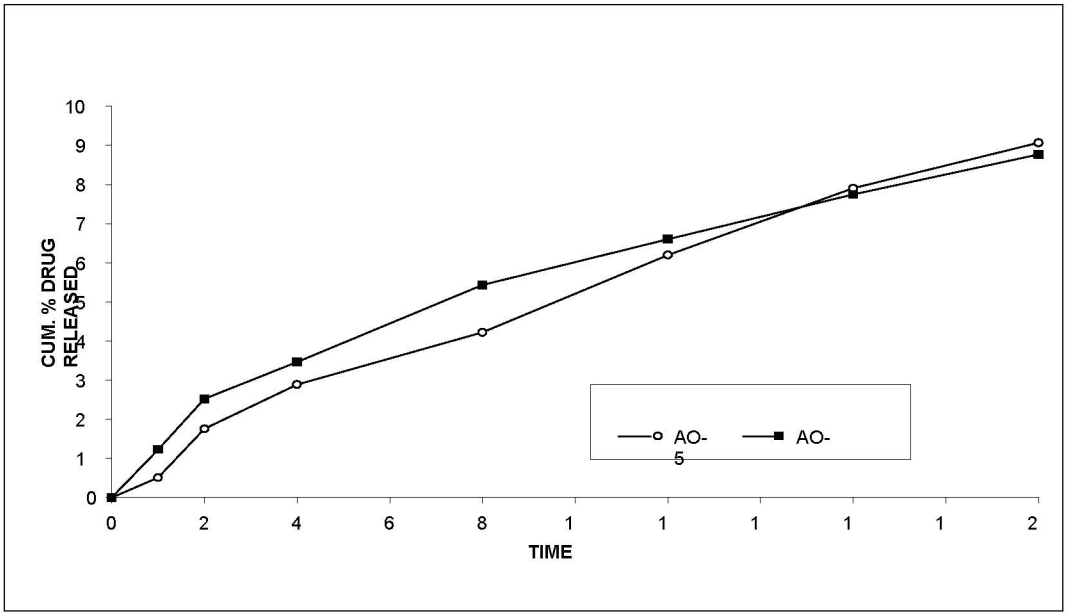

Figure 4. Plot of *in vivo* cumulative % drug release vs time for formulations AO-5 and AO-8.

The cumulative percentage of drug release for AO-5 and AO-8 were found to be 90.71 % and 87.70 % respectively after 20th h as shown in Figure 4. Also, there was complete absence of expulsion of films from the rabbit eye during the entire study. For *in vitro – in vivo* correlation, best possible correlation graph was plotted using least square technique for percentage of *in vivo* drug release v/s percentage of *in vitro* drug release. From the scattered graph represented in Figure 5 and 6 it is stated that the correlation between *in vitro* and *in vivo* was strong and positive. *In vitro/In vivo* correlation i.e. r = 0.9986 (AO-5) and r = 0.9979 (AO-8) substantiates the reproducibility and reliability of *in vitro* method of choice in present study.

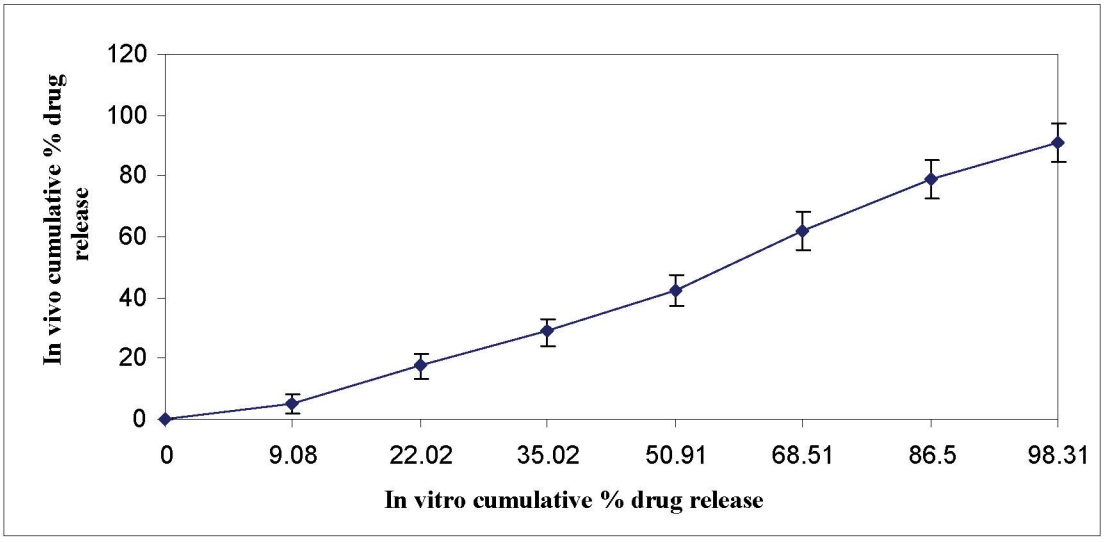

Figure 5. *In vitro – In vivo* correlation for the formulation AO-5.

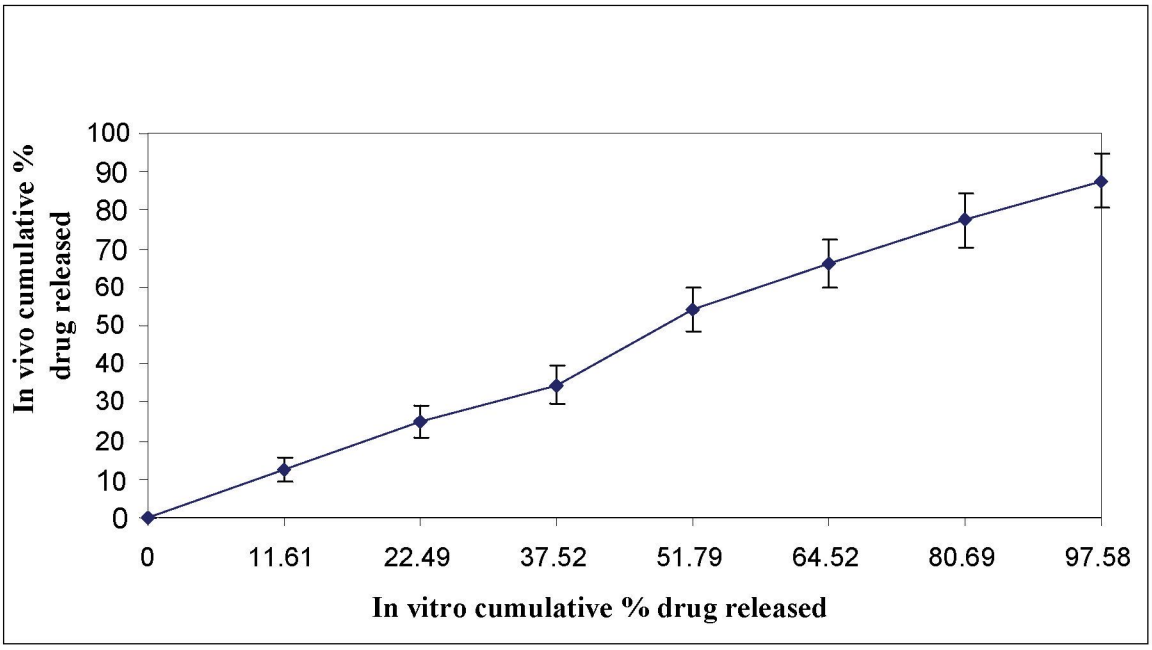

Figure 6. *In vitro – In vivo* correlation for the formulation AO-8.

Stability studies

Stability studies were carried out to predict the degradation that may occur over prolonged periods of storage at normal shelf condition for the formulation AO–5. The results of the stability studies, which were conducted for short term stability studies as per ICH guidelines for 30 days, gives the parameters obtained from the stability studies data. Stability data derived from Free and Blythe method, the shelf life of the fabricated device was calculated based on these parameters.

Stability data at different temperatures and humidity conditions revealed no significant changes in uniformity in drug content, weight, thickness, and folding endurance. The drug remained intact and stable in the ocular inserts on storage and a shelf life of 1.8 years was assigned.

CONCLUSION

From the above findings it is evident that, polymeric system of ACV has achieved the objectives of increased contact time, prolonged release and decreased frequency of administration. Drug release studies indicate that the hydrophobic nature of therate- controlling membrane plays a key role in releasing the drug from drug reservoir membrane. The results obtained for binary systems demonstrate that the solubility and dissolution rate of ACV can be significantly improved by using β-cyclodextrin as an inclusion carrier.

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