Introduction

Anatomy and physiology of the eye makes it a highly confined organ. Ocular drug delivery is an extremely challenging area due to its restrictive barrier functionalities. Design of stable and effective dosage form for ocular diseases has been considered as a formidable task. Numerous efforts have been made to enhance the bioavailability and to prolong the residence time of the drugs, for treatment of ocular diseases. The drug transport via corneal or non-corneal routes involves several intricate biological processes, such as drug penetration across the ocular barriers and transfer to the anterior or posterior chambers of the eye. The majority of existing ocular delivery systems are still fairly primitive, inefficient and have limitations such as quick elimination from the pre-corneal area, solution drainage by gravity, induced lachrymation and normal tear turnover resulting in poor bioavailability and increased severity in the systemic adverse effects from the topically applied drugs.[1–6] Controlled delivery systems, such as ocular inserts, mini-tablets and disposable lenses, can be applied topically for the treatment of diseases affecting the anterior segment of the eye.[6] The increase in residence time following topical application has the potential to improve bioavailability of the administered drug. The development of therapeutic agents that require repeated, long-term administration is a driver for the development of sustained-release drug delivery systems to result in less frequent dosing and non-invasive techniques. Novel
drug delivery systems are being explored to control the drug release for prolonged duration. The successful and susceptible ocular delivery systems such as inserts, biodegradable polymeric systems and collagen shields are being developed in order to attain better ocular bioavailability and sustained action of ocular drugs. The potential use of polymers as drug carriers offers several favorable biological properties, such as biodegradability, non-toxicity and biocompatibility. Additionally a range of strategies has been tested to improve penetration including cyclodextrins, HPMC etc. The ocular inserts, are solid or semi-solid devices which are usually composed of polymeric vehicle and are placed in the cul-de-sac. The potential advantages offered by the inserts are increased ocular residence, accurate dosing, reduction in systemic side effects and better patient compliance due to reduced frequency of administration, and increased stability and shelf life.

Some of the available technologies like Ocusert®, Ocufit® SR, Minidisc, etc., have shown capable of diminishing the systemic absorption of ocularly absorbed drug. Ocusert® developed by Alza Corporation, first marketed in the US in 1974. It is a flat, flexible and elliptical insoluble device consisting two layers of drug reservoir membrane used to deliver pilocarpine for seven days. The Ocufit® SR is a sustained release rod-shaped device made of silicone elastomer, developed by Escalon Ophthalmics Inc. (Skillman, NJ, USA) allows controlled release of active ingredients over a period of at least two weeks. Minidisc devices consisting of a contoured disc with a convex front and a concave back surface described by Bausch & Lomb (Rochester, New York, USA), reported to require less time and less manual dexterity for insertion.

In spite of the several advantages demonstrated by the extensive investigations, a capital disadvantage resides in their solidity, initial discomfort, movement around the eye and loss during sleep. Several investigators have studied impending ocular polymeric systems as promising new solid devices for ocular drug delivery systems. Charoo et al. fabricated the reservoir-type ocular inserts using sodium alginate containing ciprofloxacin hydrochloride as the drug reservoir that was sandwiched between Eudragit and polyvinyl acetate films showed zero-order release kinetics of the drug. On the other hand, Hornof et al. developed thiolated poly (acrylic acid) based ocular inserts of diclofenac sodium and fluorescein for controlled release. These films were evaluated for water uptake, swelling behavior and drug release studies. The in vivo study showed that films provided a drug concentration on the eye surface for more than 8h. Furthermore, Rao et al. prepared norfloxacin-β-cyclodextrin complex to improve the solubility of norfloxacin, which is then incorporated into the films. These films were formulated using hydroxypropyl methylcellulose (HPMC) as matrix and ethyl cellulose (EC) as the rate controlling membrane along with small concentrations of polyvinylpyrrolidone (PVP K30) and evaluated for drug release kinetics. Vijaya et al. have prepared and evaluated chloramphenical ocluserts using HPMC, EC and Eudragit RL-100 at different concentrations. The in vivo/in vitro correlation was found to be affirmed the adaptability of the delivery system to the biological environment where it can release the drug in zero order pattern. The traditional ophthalmic solutions, suspensions and ointment dosage forms are clearly no longer sufficient to combat some present virulent diseases. Currently acyclovir is used as a 3% ointment preparation in the management of herpes simplex infections five times daily because of its low bioavailability to the ophthalmic epithelium. The drawbacks associated with the available formulation are short pre-conveal retention time, greasiness, vision-blurring effects etc. Hence the present study was aimed in developing controlled release ocular systems of acyclovir with improved solubility by complexing with β-cyclodextrin. In addition to that the present research is dedicated to increase the retention time of medication on the eye surface, reduce frequency of dosing, to prolong the drug release and improvement of transcorneal penetration. The ocular polymeric system consists of hydrophilic drug reservoir core (HPMC-K_M) and hydrophobic rate controlling membrane (Cellulose acetate phthalate) in purview to achieve the controlled ocular drug delivery. The formulated ocular inserts were evaluated for film thickness, folding endurance, water uptake studies, drug content, in vitro and in vivo drug release studies, transcorneal penetration evaluation, antimicrobial efficacy studies and stability studies.

Materials and methods

Materials

Acyclovir was a gift sample from Glaxosmithkline Pharmaceuticals Mumbai, India. Pharmaceutical grade β-cyclodextrin was donated by Biocon India Ltd, Bangalore, India. Hydroxypropyl methylcellulose (HPMC-K_M) was kindly supplied by 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. All other reagents used were of analytical grade.

Methods

Preparation of binary system by co-evaporation method

Binary systems containing acyclovir (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.\textsuperscript{[11]}

The methodology in preparation of co-evaporated product goes as follows: Acyclovir (2.49 mg) was dissolved in 10 mL of distilled water containing 2.5 mL of ammonia solution. β-cyclodextrin slurry was separately prepared by adding 12.60 mg of β-cyclodextrin in 10 mL of distilled water. This β-cyclodextrin slurry was then added to the acyclovir drug solution. The mixture was heated under stirring at temperature not more than 50°C. The clear solution obtained was further heated with stirring, until a pasty mass was formed. The residual solvent was removed under vacuum at room temperature. The obtained solid mass was ground, sieved through a 250 μm sieve and dried in oven at 45°C for 48 h.\textsuperscript{[11–13]}

**Characterization of acyclovir binary systems (ACV-β-CD)**

**Analysis of inclusion efficiency in the binary mixtures**

Solid inclusion complex (50 mg) was taken in a 50 mL volumetric flask containing 30 mL of dimethyl sulfoxide and this mixture was sonicated in an ultrasonicator for 30 min. After sonication, volume was made up to 50 mL and the sample solution was centrifuged at 5000 rpm for 10 min. The supernatant was filtered through a 0.45 μm filter and the filtrate was suitably diluted. Further, absorbance of the resulting solution was measured at a wavelength of 254 nm\textsuperscript{[14]} using UV spectrophotometer (Shimadzu, Japan) and percentage (%) inclusion efficiency is calculated using the formula:

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

**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 drug, 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 II dissolution test apparatus with a paddle stirrer and a stirring speed of 50 rpm using pH 7.4 phosphate buffer saline as a dissolution medium (thermostated at 37 ± 0.5°C) was employed for the studies. Powdered samples (Granulometric fraction < 250 μm) of pure drug acyclovir and complex (ACV-β-CD), each containing 100 mg drug, were added to 900 mL dissolution medium.\textsuperscript{[11,13]} A sample of 5 mL were withdrawn, through a filter of 0.45 μm at 5 min time interval and was replace with same amount of fresh medium. 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.

**Preparation of ocular inserts**

The polymeric drug reservoir (DR) cast films were prepared by dissolving 1.0, 1.5 and 2.0 % of HPMC-K₄M in 15 mL of double distilled water. Along with this 26.95 mg of binary mixture containing ACV-β-CD was separately dissolved in 10 mL 0.1N NaOH, and then it was poured to the polymeric solution.\textsuperscript{[1,9,10]} The solution was stirred using magnetic stirrer at 100 rpm. For optimization of plasticizer concentration different levels of PEG-400 (5, 10 and 15% w/w) were screened to study the effect on physical strength of the film. For screening of suitable plasticizer concentration (10% w/w) other experimental parameters like stirring speed, concentration of drug and polymer were kept constant.

The uniform solution was spread 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 40 ± 2°C, 30 ± 5% RH for 24 h. After drying,

**Table 1. Composition of various polymers and plasticizers in different formulations.**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug reservoir(HPMC-K₄M) % w/v</th>
<th>Rate controlling membrane (CAP) % w/v.</th>
<th>Plasticizer*/ penetration enhancer % v/w.</th>
<th>Complexed ACV (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB-1</td>
<td>1.0%</td>
<td>4.0%</td>
<td>10.0%</td>
<td>0.690</td>
</tr>
<tr>
<td>AB-2</td>
<td>1.0%</td>
<td>5.0%</td>
<td>10.0%</td>
<td>0.690</td>
</tr>
<tr>
<td>AB-3</td>
<td>1.0%</td>
<td>6.0%</td>
<td>10.0%</td>
<td>0.690</td>
</tr>
<tr>
<td>AB-4</td>
<td>1.5%</td>
<td>4.0%</td>
<td>10.0%</td>
<td>0.690</td>
</tr>
<tr>
<td>AB-5</td>
<td>1.5%</td>
<td>5.0%</td>
<td>10.0%</td>
<td>0.690</td>
</tr>
<tr>
<td>AB-6</td>
<td>1.5%</td>
<td>6.0%</td>
<td>10.0%</td>
<td>0.690</td>
</tr>
<tr>
<td>AB-7</td>
<td>2.0%</td>
<td>4.0%</td>
<td>10.0%</td>
<td>0.690</td>
</tr>
<tr>
<td>AB-8</td>
<td>2.0%</td>
<td>5.0%</td>
<td>10.0%</td>
<td>0.690</td>
</tr>
<tr>
<td>AB-9</td>
<td>2.0%</td>
<td>6.0%</td>
<td>10.0%</td>
<td>0.690</td>
</tr>
</tbody>
</table>

12 mL of the cast solution was poured into a Petri dish to prepare uniform circular cast film. *Based on polymer weight.
the medicated films of 8 mm diameter were cut using a sterile stainless steel borer, each film containing 0.69 mg of drug.

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 Petri dish at room temperature for 12 h. The dried films were cut into 9 mm diameter using a stainless steel borer.

For fabricating the insert system, a medicated reservoir disc was sandwiched between two rate controlling membranes. This intact unit is placed in a desiccator saturated with ethanol/acetone (60:40) for 4–5 min, 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.

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

Evaluation of polymeric ocular inserts

Drug content uniformity

For drug content uniformity, the ocular inserts were placed in 5 mL of dimethyl sulphoxide and were agitated in orbital shaker incubator at 50 rpm to extract the drug from inserts. After incubation for 24 h, solution was filtered through a 0.45 μm filter and the filtrate was suitably diluted. The absorbance of the resulting solution was measured at a wave length of 254 nm using UV spectrophotometer (Shimadzu, Japan).

Thickness of film

Fils were evaluated for the thickness using a Dial Caliper (Mitutoyo, Japan). Ten readings were taken at different points and the mean thickness was calculated. The standard deviations (SD) in thickness were calculated from individual data value.[10]

Uniformity of weight

The weight variation test was carried out using digital balance (Mettler Toledo), by weighing three inserts from each formulation. The standard deviations (SD) were calculated from individual weight of the inserts.[10]

Folding endurance

A small strip of film was cut evenly and separately folded at the same place until 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 ensure physical stability or integrity of ocular films. Ocular films were weighed and placed in a desiccator containing 100 mL of saturated solution of aluminium chloride and 75±5% RH was maintained. After three days the ocular films were taken out and reweighed; the percentage moisture absorption was calculated using the formula,

\[
\% \text{ Moisture absorption} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100
\]

Percentage moisture loss

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

\[
\% \text{ Moisture loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100
\]

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, the eye surface temperature.[7] The beaker was wrapped with parafilm to prevent evaporation of SLF during the study. The weight of each insert was determined with the digital balance at predetermined time points. The size changes of the inserts due to swelling investigated microscopically.

In vitro drug release studies

Since there was no official method prescribed for in vitro drug release studies of ocular inserts, a simple in-house laboratory assembly was utilized simulating the conditions of ocular cavity. 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
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32 ± 0.5°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 the buffer. 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 (Shimadzu) at 254 nm.[7,15]

In vivo drug release study

Based on in vitro drug release studies, formulations showing promising release behavior (out of nine formulations, AB-5 and AB-8) were selected for in vivo study. The inserts were sterilized by using gamma radiation before in vivo study.[6,15]

Seven healthy male New-Zealand strain albino rabbits, weighing between 2.5–3.0 kg were used for the experiment. The animals housed in an individual cage and acclimatized to laboratory conditions for three days with free access to food and water.

The sterilized ocular inserts AB-5 and AB-8 containing Acyclovir were selected for in vivo study. On the day of the experiment, inserts were placed into the cul-de-sac of one eye of the rabbit and other eye served as a control.

Ocular inserts were removed carefully at 1, 2, 4, 8, 12, 16 and 20 h post insertion and the amount of drug remaining in each ocular insert was determined by placing the sample in 5 mL dimethyl sulfoxide and agitated in an orbital shaker at 50 rpm. After incubation for 24 h the solution was filtered through 0.45 µm filter. The absorbance of the resulting solution was measured at 254 nm using UV spectrophotometer. 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.

Estimation of acyclovir concentration in the aqueous humor of the rabbit’s eye

Fourteen healthy male New-Zealand strain rabbits weighing between 2.5–3.0 kg, free of ocular inflammation were selected for the study. The rabbits were divided into two groups of seven each. Based on in vitro drug release studies, optimized formulations AB-5 and AB-8 were selected for the present study. AB-5 ocular inserts were placed in the right eye and Acivir® (Marketed Acyclovir ointment by Cipla Ltd, India) placed in the left eye of each rabbit. After 1, 2, 4, 8, 12, 16 and 20 h time interval 150 µL aqueous humor was withdrawn with 26G syringe. The sample solution was centrifuged at 2000 rpm for 15 min, the supernatant was filtered through 0.2 µm membrane filter and filtrate was analyzed using high performance liquid chromatographic analysis. Chromatography was performed with a Hewlett Packard model 1100 equipped with a diode array detection (DAD) operating at 254 nm. A bonded C18 reversed phase column (4.6 x 250 mm internal diameter, 5 µm analytical column, Waters, USA) thermostated at 25°C was used. The mobile phase consists of 0.01M trifluoroacetic acid/methanol (93:7) with a flow rate of 1 mL min⁻¹. The calibration curve was obtained using the concentration range of 1.0 – 50.0 µg/mL and regression value (r²) was found to be 0.9977. Aqueous humor Acyclovir concentration was estimated using the standard graph and relating peak area concentration (Figure 6). A similar experiment was carried out on the second group of rabbits with AB-8 formulation.[6,16]

In vitro antimicrobial efficacy test

Antimicrobial activity efficacy test was performed using agar diffusion method to find out the biological activity of the optimized formulation and marketed eye ointment (Acivir®). Staphylococcus aureus and Pseudomonas aeruginosa representing gram-positive and gram-negative bacteria respectively were used as the test microorganisms. A layer of nutrient agar (20 mL) was seeded with the 100 µL of test microorganism and allowed to solidify in the petri dish. Optimized ocular discs (AB-5 and AB-8) and Acivir® marketed eye ointment containing equivalent amount of drug were placed at a suitable distance on the Petri dish which were then incubated at 37 ± 0.5°C for 24 h. The plates were visually examined for zones of inhibition around the ocular disc, and the size of the zone diameter was measured at two cross-sectional points and the average was taken as the zone of inhibition.[6]

Stability studies

Based on the in vitro and in vivo performance, the formulations AB-5 and AB-8 selected for the short-term stability studies. 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 phosphate buffer and agitated for 12 h in an orbital shaker. The resultant solution was filtered, suitably diluted and estimated spectrophotometrically using pH 7.4 phosphate 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 (K) were plotted against reciprocal of absolute temperature and the resulting line was extrapolated to K at 25°C.[17,18] Shelf life was obtained by using formula:

\[ T_{90} = 0.104/K \text{ at } 25°C \]
Results and discussion

Inclusion efficiency in the binary mixtures

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

Differential scanning calorimetry (DSC)

The DSC thermogram for drug as well as β-cyclodextrin and binary mixture are represented in Figure 1. The results are in good correlation with those previously reported by Rossel et al. The DSC analysis of acyclovir shows the sharp endothermic peak at its melting point, i.e. 248.5°C (ΔH = 143.82 J/g) whereas the binary system showed a sharp endothermic peak at 118.2°C and which implies complete disappearance of the melting endotherm of acyclovir. These results show the complete amorphization of the drug as well as loss of drug crystallinity, indicating that the drug has been engulfed in the cyclodextrin cavity.

Dissolution rate studies for binary system

The solubility of acyclovir is very low, 1.3 mg mL⁻¹ as previously reported by Rossel et al. The results of in vitro drug release profile of pure drug and complexed binary system are shown in Figure 2. Dissolution rate of the binary compound was evidently higher (61.18%) than that of the pure drug (34.27%) after 60 min. These results are well correlated with those data obtained by Govindarajan and Nagarsenker. It can be attributed to the increase in solubility of the drug by complexing with β-cyclodextrin, indicating evidence for amorphization of the drug as well as reduction in the size of the drug particles.

Drug content uniformity

The amount of drug present in the ocular films was found to be in the range of 0.675 ± 0.148 to 0.689 ± 0.018 mg per film, as shown in Table 2. Formulations AB-5 and AB-8 showed drug content of 0.689 ± 0.018 and 0.683 ± 0.013, respectively.

Film thickness

The mean film thickness (n = 3) was uniform and consistent with all the nine formulations and it was found to vary between 0.151 ± 0.001 to 0.171 ± 0.006 mm as shown in Table 2. The little variation observed with formulations, AB-6 and AB-9 may be due to the presence of higher concentrations of rate controlling membrane. The formulation were not thick enough, therefore they did not produce any discomfort when placed in cul-de-sac.

Uniformity of weight

Uniformity of weight for films was found to vary from 13.73 ± 0.141 to 18.59 ± 0.224 mg. Formulations AB-1 and AB-9 showed good uniformity in weight of 14.55 ± 0.124 and 17.61 ± 0.113, respectively.
Folding endurance
The values for folding endurance varied from 88 ± 2.01 to 104 ± 3.87. Formulations AB-8 and AB-1 exhibited minimum and maximum folding endurances respectively. The values depended on hydrophilic polymer concentrations used. Folding endurance test results imply that the patches would not break and would maintain their integrity with general skin folding when used. Shinde et al.[24] correlated folding endurance with the composition of polymer used and type of plasticizer incorporated in the film.

Percentage moisture absorption and loss
The highest moisture absorption was marked for formulation AB-7 (11.83 ± 0.346) as tabulated in Table 2. This may be due to the presence of higher concentration of hydrophilic polymer HPMC-K4M. As the moisture content increases the films become soft and may affect the integrity of the formulation. Similar findings were reported by Shinde et al.[24] The moisture loss for all the formulations varied between 6.61 ± 0.241 to 13.49 ± 0.199 (Table 2). Formulation AB-1 showed the maximum amount of moisture loss in dry conditions might be due to presence of less concentration of hydrophobic polymer cellulose acetate phthalate. There was no change in integrity in all the formulations.

Water uptake and swelling behavior
Maximum water uptake was found with 2% HPMC-K4M discs due to the presence of more concentration of swellable hydrophilic polymer. The swelling behavior of these polymers in dry dosage forms has a great impact on their adhesive properties, drug release and stability.[19] After complete hydration, moderate gelling pressure is reached to drive the drug away from the reservoir membrane.[6,10,20] Formulations AB-1, AB-2, AB-3 and AB-7 were best fitted into Peppa’s model with ‘n’ values of 0.5111, 0.5383, 0.7704 and 0.6275, respectively. This indicates that the drug release is by non-Fickian-diffusion mechanism. Cumulative percentage drug release for AB-5 and AB-8 found to be 98.31% and 98.03%, respectively, and is depicted as linear, complete and more controlled patterns of drug release of all the other formulations and were selected as optimized formulations. 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. Charoo et al.[6] obtained almost constant and controlled drug release with the similar kind of ocular insert systems prepared with sodium alginate as a drug reservoir and Eudragit RL 100 and RS 100 as a rate controlling membrane.

### Table 2. Physical parameters evaluation of formulations.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug content (Mean ± SD)</th>
<th>Thickness (Mean ± SD)</th>
<th>Weight (Mean ± SD)</th>
<th>Percent moisture loss (Mean ± SD)</th>
<th>Percent moisture absorption (Mean ± SD)</th>
<th>Folding endurance (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg (n = 3)</td>
<td>mm (n = 3)</td>
<td>mg (n = 3)</td>
<td>(n = 3)</td>
<td>(n = 3)</td>
<td>(Mean ± SD)</td>
</tr>
<tr>
<td>AB-1</td>
<td>0.675 ± 0.148</td>
<td>0.152 ± 0.003</td>
<td>14.55 ± 0.124</td>
<td>6.61 ± 0.241</td>
<td>7.78 ± 0.124</td>
<td>104 ± 3.87</td>
</tr>
<tr>
<td>AB-2</td>
<td>0.685 ± 0.019</td>
<td>0.151 ± 0.001</td>
<td>13.73 ± 0.141</td>
<td>9.34 ± 0.057</td>
<td>5.43 ± 0.090</td>
<td>93 ± 2.83</td>
</tr>
<tr>
<td>AB-3</td>
<td>0.686 ± 0.036</td>
<td>0.158 ± 0.006</td>
<td>16.33 ± 0.169</td>
<td>10.12 ± 0.075</td>
<td>5.56 ± 0.182</td>
<td>103 ± 3.58</td>
</tr>
<tr>
<td>AB-4</td>
<td>0.679 ± 0.027</td>
<td>0.151 ± 0.008</td>
<td>14.65 ± 0.314</td>
<td>10.49 ± 0.116</td>
<td>8.24 ± 0.229</td>
<td>91 ± 4.27</td>
</tr>
<tr>
<td>AB-5</td>
<td>0.689 ± 0.018</td>
<td>0.157 ± 0.006</td>
<td>15.77 ± 0.132</td>
<td>7.33 ± 0.263</td>
<td>9.49 ± 0.228</td>
<td>89 ± 4.12</td>
</tr>
<tr>
<td>AB-6</td>
<td>0.684 ± 0.087</td>
<td>0.164 ± 0.008</td>
<td>18.59 ± 0.224</td>
<td>13.49 ± 0.199</td>
<td>6.08 ± 0.150</td>
<td>94 ± 2.88</td>
</tr>
<tr>
<td>AB-7</td>
<td>0.682 ± 0.193</td>
<td>0.151 ± 0.008</td>
<td>15.15 ± 0.156</td>
<td>9.17 ± 0.026</td>
<td>11.83 ± 0.346</td>
<td>93 ± 5.12</td>
</tr>
<tr>
<td>AB-8</td>
<td>0.683 ± 0.013</td>
<td>0.152 ± 0.008</td>
<td>15.21 ± 0.319</td>
<td>8.44 ± 0.240</td>
<td>8.75 ± 0.095</td>
<td>88 ± 2.01</td>
</tr>
<tr>
<td>AB-9</td>
<td>0.680 ± 0.017</td>
<td>0.171 ± 0.006</td>
<td>17.61 ± 0.113</td>
<td>11.61 ± 0.092</td>
<td>7.28 ± 0.151</td>
<td>90 ± 4.02</td>
</tr>
</tbody>
</table>

occular insert based on thiolated poly (acrylic acid) and correlated the water uptake and swelling behavior with the adhesion properties of the polymers.

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, AB-1 to AB-9 was found to be 89.97, 94.64, 80.14, 91.00, 98.31, 76.11, 83.61, 98.03 and 72.50%, respectively, at the end of 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 ($r^2$), which is nearing to unity, formulations AB-4, AB-5, AB-6, AB-8 and AB-9 followed Higuchi-matrix kinetics as shown in 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.[6,10,20] Formulations AB-1, AB-2, AB-3 and AB-7 were best fitted into Peppa’s model with ‘n’ values of 0.5111, 0.5383, 0.7704 and 0.6275, respectively. This indicates that the drug release is by non-Fickian-diffusion mechanism. Cumulative percentage drug release for AB-5 and AB-8 found to be 98.31% and 98.03%, respectively, and is depicted as linear, complete and more controlled patterns of drug release of all the other formulations and were selected as optimized formulations.
In vivo drug release studies

For in vivo drug release, optimized formulations AB-5 and AB-8 were selected. The cumulative percent drug release for AB-5 and AB-8 were found to be 90.71% and 87.70% at 20 h post insertion, respectively, as shown in Figure 3. There was complete absence of expulsion of films from the rabbit eye during the entire study. For in vitro-in vivo correlation, the best possible correlation graph was plotted using least square technique for percentage in vivo drug release vs. percentage in vitro drug release. From the scattered graph represented in Figures 4 and 5 it is stated that the correlation between in vitro and in vivo was strong and positive. In vitro/in vivo correlation, i.e. $r^2 = 0.9987$ (AB-5) and $r^2 = 0.9979$ (AB-8) substantiates the reproducibility and reliability of in vitro method used in present study. Mundada and Shrikhande\cite{25} formulated gelatin-based ciprofloxacin hydrochloride ocular inserts and described strong in vitro and in vivo correlation, revealing efficacy of the formulation.

Estimation of acyclovir concentration in the aqueous humor of the rabbit’s eye

The concentrations of drug in the aqueous humor were plotted as a function of time after an instillation of marketed ointment and ocular inserts as shown in Figure 6. Ocular inserts showed a higher potential of making the drug permeate into aqueous humor compared with marketed Acivir\textsuperscript{®} preparation. Following topical application of the acyclovir ointment, it was detectable up to 80 min with a maximum drug concentration of 0.34 µg/mL. Furthermore both AB-5 and AB-8 drug concentrations detectable still 20 h with maximum drug concentration of 3.7 µg/mL. The inserts provided a sustained drug release in the aqueous humor compared to free acyclovir ointment from which acyclovir levels in the aqueous humor drastically decreased, may be because of lachrymal drainage. The ability of formulated inserts to improve and prolong the corneal penetration and also to show higher acyclovir concentrations compared to marketed preparation can be attributed to a longer residence time with the corneal epithelium. It is believed that the water soluble polymers interact with the drug/cyclodextrin (CD) complexes in the same way as polymers interact with micelles forming drug CD polymer aggregates. Such macromolecular clusters

Table 3. In vitro drug release profile for different formulations.

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

Table 4. Kinetic values obtained from mathematical model treatment and Best fit models.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Slope</th>
<th>Regression coefficient ($r^2$)</th>
<th>n-value</th>
<th>Best fit model</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB-1</td>
<td>0.199</td>
<td>0.9838</td>
<td>0.5111</td>
<td>Peppas</td>
</tr>
<tr>
<td>AB-2</td>
<td>0.184</td>
<td>0.9951</td>
<td>0.5383</td>
<td>Peppas</td>
</tr>
<tr>
<td>AB-3</td>
<td>0.209</td>
<td>0.9952</td>
<td>0.7704</td>
<td>Peppas</td>
</tr>
<tr>
<td>AB-4</td>
<td>0.195</td>
<td>0.9972</td>
<td>0.5473</td>
<td>Higuchi-Matrix</td>
</tr>
<tr>
<td>AB-5</td>
<td>0.268</td>
<td>0.9974</td>
<td>0.7536</td>
<td>Higuchi-Matrix</td>
</tr>
<tr>
<td>AB-6</td>
<td>0.270</td>
<td>0.9962</td>
<td>0.6228</td>
<td>Higuchi-Matrix</td>
</tr>
<tr>
<td>AB-7</td>
<td>0.188</td>
<td>0.9967</td>
<td>0.6275</td>
<td>Peppas</td>
</tr>
<tr>
<td>AB-8</td>
<td>0.216</td>
<td>0.9970</td>
<td>0.5820</td>
<td>Higuchi-Matrix</td>
</tr>
<tr>
<td>AB-9</td>
<td>0.306</td>
<td>0.9956</td>
<td>0.7793</td>
<td>Higuchi-Matrix</td>
</tr>
</tbody>
</table>
Controlled release polymeric ocular delivery of acyclovir

would be more readily absorbed to biological membranes than the individual drug/CD complexes, and the polymers adhering to the outer surface of the cornea may promote the release of drug molecules from the cyclodextrin inclusion complex into the solution leading to a high concentration of drug molecules at the corneal surface, resulting in enhanced permeation.\cite{21-23}

The studies conducted by Fresta et al.\cite{16} have reported that the polyethylene glycol (PEG) coated polyethylencyanacrylate (PECA) nanospheres are able to increase acyclovir bioavailability compared with the pure drug.\cite{16}

**In vitro antimicrobial efficacy test**

Clear zones of inhibition were observed in case of ocular discs and marketed ointment Acivir®. The zone of inhibition diameter produced by ocular discs AB-5 and AB-8 against both test organisms were greater than those produced by marketed ointment as shown in Table 5. These clear findings can be endorsed to an antimicrobial effect of the ACV in formulation is probably due to a fairly constant release of drug from the drug reservoir. Similar studies were carried out by Charoo et al.\cite{6} in which they have showed a clear zone of inhibition with ciprofloxacin hydrochlorothiazide over a period of five days and attributed to its constant drug release.

**Stability studies**

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 shelf life of 1.8 years may be assigned. Ocular inserts could be stored safely at study storage conditions. However, storage temperature not exceeding 40°C and moisture-proof packing are recommended to ensure stability of formulations.\cite{6}

**Conclusion**

In summary, polymeric system of acyclovir has achieved the objectives of increased contact time, prolonged drug release, improved transcorneal penetration and decreased frequency of administration. Drug release studies indicate that the hydrophobic nature of the rate-controlling membrane plays a key role in releasing the drug from drug reservoir membrane. The

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**Table 5.** Area of the zone of inhibition (mm²) after 24 h of incubation at 37 ± 0.5°C.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Ocular inserts AB-5</th>
<th>Ocular inserts AB-8</th>
<th>Marketed Acivir® ointment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>472 ± 3.2</td>
<td>511 ± 1.6</td>
<td>214 ± 2.7</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>514 ± 1.8</td>
<td>589 ± 1.9</td>
<td>253 ± 1.4</td>
</tr>
</tbody>
</table>
results obtained for binary systems demonstrate that the solubility and dissolution rate of acyclovir can be significantly improved by using β-cyclodextrin as an inclusion carrier. This new device would be beneficial, as placement of one ocular insert in the eye might be feasible to maintain a constant concentration of antiviral drug for several hours.

Acknowledgments

The authors wish to thank GlaxoSmithKline Pharmaceuticals Ltd, Mumbai, India, for providing gift sample of Acyclovir and Principal, K.L.E.'s College of Pharmacy, Belgaum, Karnataka, India, for providing the necessary facilities.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References