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Azithromycin β –cyclodextrin ocular films: Preparation and evaluation

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Abstract

This research work was targeted to prepare Azithromycin ocular films by complexation with β-CD (Beta cyclodextrin) and evaluate the same. Release retardants viz., HPMC K_4M and Ethyl Cellulose were used and Glycerin was adopted as permeability enhancer. Interactions of Azithromycin with excipients used were studied by Differential Scanning Calorimetry and Fourier Transform infrared spectroscopic studies. The prepared ocular films were evaluated for physicochemical parameters, *in vitro* release studies. The *in vitro* drug dissolution data was best fitted to zero order release modeling. The optimized formulation (F-5) was subjected for accelerated stability studies. The results revealed that Azithromycin has no negative incompatibility with the excipients used. The prepared ocular films gave satisfactory physicochemical characteristics, *in vitro* release characteristics. The optimized formulation reservedits physiognomies even after stability studies.

Keywords: Azithromycin, β-Cyclodextrin, Ocular films, evaluation

Contents

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1. Introduction

Conventionally available ocular dosage forms viz., eye drops/ointments have more frequent administration, poor availability of drug, random doses and drainage of drug by tears or nasolacrimal fluid as drawbacks. Ocular films are sterile preparationsexclusively for eye [1-2]. Azithromycin has broad-spectrum antimicrobial action and used in the treatment of trachoma, conjunctivitis and keratitis [3]. Topical applications of Azithromycin is hydrophobic in nature and sparingly soluble in water at neutral pH. Therefore, there is a need to formulate a novel ocular dosage form which deliver the drug at the site of application and at the site of action for the prolonged period of time. Cyclodextrins (CDs) are characterized by cyclic torus-shaped molecules with a hydrophilic outer surface and a lipophilic central cavity. β-CD is popularly included to increase water solubility of drugs [4].

2. Materials and Methods

Materials

Azithromycin was a gift sample obtained from IPCA Laboratories, Hyderabad, India. B-Cyclodextrin (β-CD), Acetic acid and Glycerin were purchased from Merck chemicals, Goa, India. All other reagents and solvents used in the study were of analytical reagent grade. Double distilled water was used throughout the experiment.

Preparation of ocular films

Preparation of Drug reservoir film

HPMC K_4M polymeric films were prepared by preparing various proportions of HPMCK_4M in double distilled water. Azithromycinβ-CD mixture dissolved in dil. Na OH solution and mixed with above solution. Later Glycerin was fusedwith stirring [5].

The above mixture was poured in Glycerin lubricated petri dishes and allowed to dry placing it in a hot air oven (37±2^{o}C, 30±0.5% of RH) for 24 h. After drying the medicated films of 10 mm diameter each containing 2.5 mg of drug were cut using a sterile stainless steel borer.

Preparation of Rate controlling membrane (RCM)

Different concentrations of Ethyl Cellulose solutions were prepared in Acetone with stirring. Later the solutions were poured in Glycerin lubricated petri dish using a glass ring (5cm diameter). The solution was kept aside for evaporation at room temperature for 12 h. The dried films were cut into 10 mm diameter using a sterile stainless steel borer [5].

Sealing

Azithromycin rich reservoir disc was sandwiched between two RCM. Later the preparation was placed in desiccator for 5 min (desiccator was formerly saturated with Ethyl Alcohol: Acetone (60:40). The so formed films were stored in an airtight container under ambient conditions [5-7].

All the above work was carried out under laminar airflow to maintain the sterility conditions of ophthalmic products. The composition of ocular films was shown in Table 1.

Evaluation of Polymeric Ocular Films

Compatibility studies

Differential Scanning Calorimetry studies

Differential Scanning Calorimetry (DSC) thermo grams were obtained by a differential scanning calorimeter (Schimadzu DSC-50, Tokyo, Japan).

Fourier Transform infrared (FTIR) spectroscopy

The FTIR spectrums of Azithromycin and F-5 were studied by FTIR spectrophotometer (Perkin Elmer, spectrum-100, Japan).

Physical Characterization

Uniformity of Thickness: Thicknesses of five Films were measured using a vernier caliper (For-bro Engineers, Mumbai, India) and mean was calculated [8].

Uniformity of drug

It was resolute by assaying the individual films. Each film was placedin5 ml of simulated tear fluid (STF) (NaCl: 0.670 g, $NaHCO_3$: 0.200 g, $CaCl_2$: 0.008 g, Purified water q. s. 100 g)of pH 7.4 and were shaken in orbital shaker incubator (SLM-TPS-60, Bangalore) at 50 rpm. After 24 h of incubation, the solution was filtered through a 0.45 μm membrane filter and the filtrate was suitably diluted with SLF [9]. The resulting solution's absorbance was measured at 215 nm for drug content in UV–Vis Spectrophotometer (CT-50 Ltd., India).

Uniformity of weight: 10 films from each batch were weighed individually and the average was calculated [10] by using Electronic balance (Sartorius GmbH, Gottingen, Germany).

Folding endurance

Ocular film was folded at the same place till it breaks. The number of times the filmcan be folded at the same place till it breaks gives the value of folding endurance [8].

Percentage moisture absorption

Ocular films were weighed and placed in a dessicator containing 100 ml of saturated solution of $AlCl_3$ and 80±5% RH was maintained. After 3 days the ocular films were taken out and reweighed. The percentage moisture absorption was calculated using the following equation [8].

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

Percentage Moisture Loss

Ocular films were weighed and kept in a desiccator containing anhydrous $CaCl_2$. After 3 days, the ocular films were taken out and reweighed; the percentage moisture loss was calculated using the following equation [9].

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

Determination of the Swelling Index

The ocular films were coated on the lower side with ethyl cellulose (to avoid sticking to the dish) then weighed (W_1) and placed separately in petri dishes containing 25 ml of distilled water. The dishes were stored at room temperature. After 5, 10, 15, 20, 30, 45 and 60 min, the ocular films were removed and the excess water on their surface was removed with the aid of a filter paper. The swollen discs were weighed (W_2) and the percentage of swelling was calculated by the following formula [11].

$$\text{Swelling index} = W_2 - W_1 / W_1 \text{ X100}$$

Determination of the Surface pH: Five prepared films were separately permitted to swell in petri dish at room temperature (27±2^{o}C) for 30 min in 0.1ml double distilled water. The swollen patches were placed on universal pH paper. After 1 min the colour developed on pH paper was compared with standard pH colour scale [12].

***In vitro* drug release studies:** The ocular films from each formulation were taken and placed in a 15 ml vials containing 10 mlof STF of pH 7.4. The vials were placed in an oscillating water bath at 32±1^{o}C with 20 rpm. 1ml of the drug releasing media was withdrawn at regular time intervals till 24 h and replaced by the same volume with STF of pH 7.4.These samples were filtered through 0.45 µm membrane filter [13]. The filtrate was diluted suitably with the buffer. The drug was estimated in each batch by double beam UV-Vis spectrophotometer at 215 nm.

Microbiological studies: The selected ocular films were evaluated for antimicrobial activity. A Layer of nutrient agar seeded with the test organism (*Staphylococcus aureus* and *Escherichia coli*) was allowed to solidify in Petri dish. The formulated ocular films were carefully placed over the agar layer at a suitable distance. The plates were then incubated at 37±0.5^{o}C for 24 h. After incubation the zone of inhibition was measured around the ocular film.

Ocular irritation: The prepared patches were tested on 5 rabbits by placing the films in the cul-de-sac of the left eye of the rabbit. Both eyes of the rabbits under test were examined for any signs of irritation before treatment and were observed up to 12 h. The potential ocular irritation or damaging effects of the ocular films under test were evaluated by observing the eyes for redness, increased tear or inflammation [15].

Stability Studies: The optimized formulation (F-5) of ocular films was packed in amber-colored bottles tightly plugged with cotton and capped. They were exposed to various temperatures (60^o, 40^o, 20^o, 10^o, 0^{o}and 25^{o}C) for 30 days. At regular intervals, the films were taken in 5 ml of SLF of pH 7.4 and were shaken in orbital shaker incubator at 50 rpm to extract the drug from ocular films. After incubation for 24 h, the solution was filtered through a 0.45 µmmembrane filter and the filtrate was suitably diluted with SLF. The resulting solution's absorbance was measured at 215 nm. The shelf life can be obtained by using formula [16].

$$T_{90} = 0.104/K \text{ at } 25^oC.$$

3. Results and Discussion

The DSC thermo gram of Azithromycin showed a short endothermic peak at 113.11^{o}C and F-5 blend showed peak at 114.18^{o}C (Fig.1)indicating a slight change in terms of shifting towards the lower temperature. This minor change in the melting endotherm in the drug could be due to the mixing of the drug and excipients which lower the purity of each component in the mixture and may not necessarily indicate potential incompatibility. The compatibility of Azithromycin with the polymers used was confirmed by FTIR spectrums (Fig.2). The Thickness, weight, drug content were found to be uniform. After the moisture loss the ocular films showed no change in integrity and the moisture absorption was within the limits. The Folding endurance reveals that no cracks were observed. The surface pH values of all ocular films were in the range of 5.0-6.7. All these values were represented in Table 2. Water uptake values were within the limits and were shown in Table 3. *In vitro* drug release profile is shown in fig.3. F-5 was tested bacteria. Clear zone of inhibition were obtained against both test organisms, which was shown in Table 4.Stability data indicates the formulations were stable and no major degradation was found and a shelf life of 0.91 years was assigned to the ocular films (F-5).

Table 1: Composition of various polymers in different formulations per ring

Formulation	HPMC-K_4M (%w/v)	EC (%w/v)	Glycerin (%w/w)	Azithromycin and β-CD (mg)
F-1	1.0	1.0	10.0	30
F-2	1.0	2.0	10.0	30
F-3	1.0	3.0	10.0	30
F-4	2.0	1.0	10.0	30
F-5	2.0	2.0	10.0	30
F-6	2.0	3.0	10.0	30
F-7	3.0	1.0	10.0	30
F-8	3.0	2.0	10.0	30
F-9	3.0	3.0	10.0	30

*12 ml of the cast solution was poured into petri dish to prepare circular cast film.

Table 2: Physicochemical Evaluation of different formulations

Formulation	Thickness (mm)	Weight Uniformity (mg)	Moisture Loss (%)	Moisture Absorption (%)	Folding Endurance	Drug content (%)
F-1	0.11±0.001	18.09±0.01	2.94±0.02	2.40±0.05	59±1.0	84.06±3.26
F-2	0.10±0.001	18.95±0.11	6.50±0.01	2.45±0.08	56±1.5	86.20±5.51
F-3	0.13±0.001	18.54±0.15	4.64±0.05	3.99±0.06	66±1.0	89.12±4.64
F-4	0.09±0.001	18.02±0.02	6.32±0.03	3.44±0.08	69±2.5	89.55±5.94
F-5	0.12±0.006	17.99±1.85	5.31±0.06	4.94±0.06	58±1.5	96.40±4.52
F-6	0.10±0.005	18.65±0.12	4.95±0.02	3.44±0.04	73±3.5	95.21±2.12
F-7	0.09±0.003	17.85±0.14	3.84±0.06	4.64±0.61	69±5.0	95.52±8.59
F-8	0.11±0.006	18.51±0.25	5.16±0.06	3.61±0.02	59±2.5	93.25±6.48
F-9	0.13±0.007	18.94±0.58	6.84±0.02	6.84±0.89	71±5.0	94.51±6.21

*All values expressed as mean ± S.D; Number of trials (n)=5

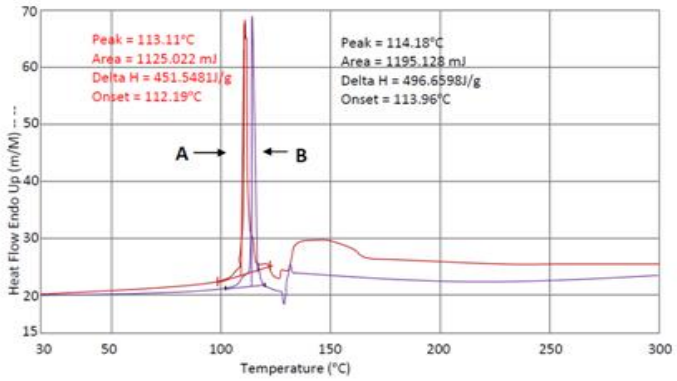

Figure1: DSC of A: Azithromycin, B: F-5 blend

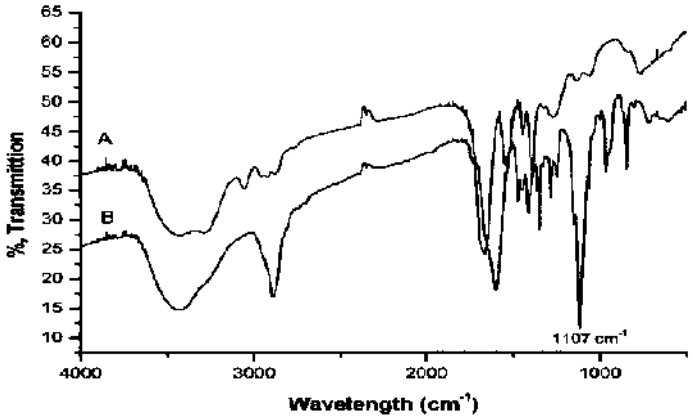

Figure 2: A. FTIR spectrum of Azithromycin pure drug, B: FTIR of F-5 blend

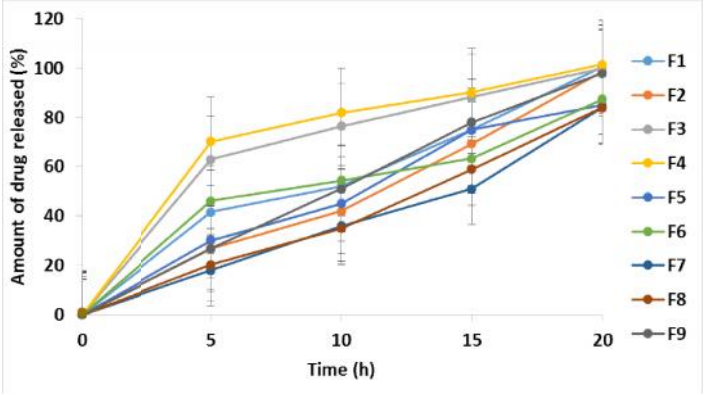

Figure 3: *In vitro* drug release Zero order plots

Table 3: Water uptake and swelling behavior of F-5

Time (h)	Water uptake for F-5 (mg)
0	3.51± 0.02
1	2.25± 0.01
2	1.29± 0.02
3	3.29± 0.02
4	2.21±0.01
5	3.21±0.01

All values expressed as mean ± S.D
Number of trials (n)=5

Table 4: *In vitro* inhibition of the growth of microorganisms by selected ocular insert

Formulation	Zone of inhibition	
	Staphylococcus aureus	***Escherichia coli***
F-5	31.48±0.64	35.84±1.58
All values expressed as mean ±S.D; Number of trials (n)=5		

4. Conclusion

An approach has been made to formulate ocular films of Azithromycin with improved bioavailability, avoidance of repeated administration and dose reduction. From the experimental finding, it can be concluded that Hydroxy Propyl methyl cellulose is a good film forming hydrophilic polymer and is a promising agent for ocular delivery. Ethyl Cellulose was a satisfactory polymeric ingredient to fabricate the rate-controlling membrane of the ocular film. Incorporation of Glycerin enhances the permeability of Azithromycin and thus therapeutic levels of the drug could be achieved. Complexation of Azithromycin with β-cyclodextrin suggested, enhancing the solubility profile of poorly soluble drug Azithromycin and also permeability of the drug through cornea.

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