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Development and Evaluation of Chitosan Ocuserts Containing Ciprofloxacin - β CD Complex

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Abstract: The objective of the study was to develop an ocular insert of Ciprofloxacin -β CD Complex and evaluate for sustained ocular delivery of drug. Such developed ocuserts were subjected to invitro characterization and evaluation. The conventional method of ophthalmic drug administration often resulted in poor availability & therapeutic response due to rapid precorneal elimination of the drug. The conventional/traditional method of ophthalmic administration also requires frequent instillation of medication into the eye, which resulted in poor patient compliance. With an aim to overcome the above drawbacks, a novel drug delivery system of β CD complexed ocular insert was developed and evaluated for its physical characters, thickness and diameter, weight variation, folding endurance, percentage moisture absorption, stability studies, microbiological studies and in vitro release studies. The study clearly showed that an effective drug concentration at the intended site of action for a sufficient period of time could be obtained to elicit the desired response.

Keywords: Ciprofloxacin HCl, Ocuserts, β CD, Invitro characterization and evaluation

Introduction and Experimental

Ophthalmic inserts are defined as sterile preparations, with a solid or a semi solid consistency, whose size & shape are especially designed for ophthalmic application. They are essentially composed of a polymeric support containing drug(s), the latter being incorporated as dispersion or a solution in the polymeric support. The inserts can be used for topical or systemic therapy[1,2].

Different types of drug molecules especially antibiotics are tried in various ophthalmic symptoms. Ciprofloxacin HCl is a very commonly used antibiotic in ophthalmic infections. Ciprofloxacin HCl, a broad spectrum Fluroquinoline antimicrobial with a half-life of 3.3 to 4.9 h has frequently been used in ocular superficial bacterial infections, e.g. corneal ulcers, conjunctivitis[3]. The current literature indicates that none of the Ciprofloxacin ocular inserts are made of biodegradable systems. Hence this investigation was taken up to study the drug release kinetics of Ciprofloxacin HCl from biodegradable Chitosan ocuserts. Chitosan is a deacetylated product or partially Chitin derivative. Chitin is a major polysaccharide of the shells of crustaceans & exoskeletons of insects. It is (1-4)2-amino-2-deoxy-β-D-glucan $[C_6H_{11}O_5N]_n$ and possesses various biological activities like wound healing, antacid-antiulcer, bacteriostatic, fungistatic, haemostatic & spermicidal activity. Sawayange et. al reported that the drug dispersed in Chitosan was released at a constant rate, so Chitosan could be a useful matrix for sustained release of drugs.

Cyclodextrins (CD) are cyclic torus-shaped molecules with a hydrophilic outer surface and a lipophilic central cavity that can accommodate a variety of lipophilic drugs. CD are available in their

α,β and γ forms. In most of the cases we use β-CD as inclusion complexes to improve the solubility properties[4]. It is also observed from earlier studies that formulations such as implants, strips, patches etc had improved their stability properties on the incorporation of β CD.

Acetic acid has been selected as the standard solvent for solution property measurement. Chitosan readily dissolves in glycerol-water (3:1), when the mixture contained 1%acetic acid, resulting in clear, odourless & viscous solution. Because it is insoluble at pH above 5.5, Chitosan functions only in acid system to show possible utility as a thickener, stabilizer, suspending agent or film former.

It was aimed to prepare ocular films containing Ciprofloxacin HCl- β CD complex, Ciprofloxacin and evaluate them to identify which one has better totality as ocusert.

Materials and Methods

Chitosan and Ciprofloxacin HCL were gift samples from CFTIR, Cochin and Ipca, Mumbai respectively. βCD, Acetic acid, Propylene glycol were procured from Nice chemicals, Cochin. All other reagents and solvents were of analytical grade.

Preparation of Ciprofloxacin-β CD inclusion complex (Kneading method)[5]:

β CD was taken into a glass mortar. Distilled water was added to obtain a homogenous paste. The drug was then incorporated slowly with grinding. The mixture was ground for 1hr. During this process, an appropriate quantity of distilled water was added to maintain suitable consistency. The paste was dried in a hot air oven at 40˚C for 48hrs. The dried complex was taken for further study.

Preparation of ocular insert by glass substrate technique[6,7]:

Drug reservoir film: 1% $^{w}/_{w}$ Chitosan was soaked in 1%$^{v}/_{v}$ Acetic acid solution for 24hrs, to get a clear solution of Chitosan in Acetic acid solution. The solution was filtered through a muslin cloth to remove undissolved portion of the polymer (Chitin). Required quantity of drug-β CD complex was added and vortexed for 15minutes to dissolve the complex in Chitosan solution. 1%$^{w}/_{v}$ propylene glycol (plasticizer)f was added to it and mixed well with stirrer. The viscous solution was kept aside for 30 minutes for complete expulsion of air bubbles.

The rate controlling filims for Ciprofloxacin HCl as well as for control (Placebo) prepared using same method.

The films were casted by pouring solution into the center of leveled glass mould and allowing it to dry at room temperature for 24hrs. After drying, films were cut into ocuserts of desired size (13mm diameter) so that each contains equal quantity of the drug. Then, the matrix was sandwiched between the rate controlling membranes using non-toxic, non-irritating, water insoluble gum. They were wrapped in aluminium foil separately and stored in a desiccator until further use[6,8].

The ocusert with Ciprofloxacin HCl as well as control (Placebo) were prepared using the same method

Invitro Characterisation and Evaluation:

a) Physical Characterization[9]:

The ocular inserts were evaluated for their physical characters such as shape, colour, texture, appearance, etc. Various other physical parameters were also evaluated as follows.

b) Thickness and Diameter[9]:

The thickness & diameter of 20 randomly selected ocuserts were measured using micrometer screw gauge and the average was calculated.

c) Weight Variation Test[9]:

20 randomly selected ocuserts were taken and weighed individually using caliberarted digital balance .The average weight of the prepared ocusert was calculated.

d) Folding Endurance[9]:

Folding endurance for ocular inserts were calculated by folding the ocuserts repeatedly in the same position till a crack appeared. Number of folds required to produce the crack were counted. Folding endurance test was repeated using more sets of ocular inserts.

e) Percentage Moisture Absorption[9]:

The Percentage of moisture absorption was measured by keeping the ocuserts at 37±0.5°C and 80%±5% RH for 2-3 days. Initial weights and final weights of the ocuserts were taken.

Percentage moisture absorption was calculated using the formula:

$$\% \text{ Moisture absorption} = \frac{(\text{Final weight} - \text{Initial weight})}{(\text{Initial weight})} \times 100$$

f) Stability Studies[10]:

Stability studies were conducted by storing the prepared ocuserts under different conditions of temperature like: room temperature, elevated temperature (incubator), and refrigeration temperature. Ocuserts were evaluated for weight variation, colour change, change in texture and change in appearance for a period of 7 days. The ocuserts evaluated for 7 days for stability studies were then tested for microbiological sensitivity.

g) Microbiological Studies[11,12]:

For microbilogical studies, standard pour plate method has been used. During the study a strict aseptic condition was maintained.Staphylococcus aureus has been taken as test organism for the study.Ocular inserts (Control, Drug, Drug-βCD) were evaluated

microbiologically for controlled drug release for a period of 7 days. Melted nutrient agar medium was taken in a test tube and inoculated with 4-5 loops full of test microorganism (Staphylococcus aureus) from the provided culture. The inoculated medium was then poured into a sterile Petri dish and allowed to solidify. A sterile ocular insert was placed carefully on the surface of solidified nutrient agar medium using a sterile forcep. The Petri dish was incubated in inverted position for 24 hours at 37±0.5°C. The Petri dish was observed for the zone of inhibition around the ocusert. The zone of inhibition was measured and recorded. The ocusert was then transferred to a fresh, similarly prepared plate. The same procedure was repeated for 7 days, i.e., by transferring the same insert to a fresh Petri dish at an interval of 24 hours to observe the microbiological activity.

h) In Vitro Drug Release Studies[13,14]:

From standard stock solution, a series of dilutions were made with 1% Acetic acid. The absorbance of these solutions were measured against blank of 1% Acetic acid in UV/visible spectrophotometer at 274nm.

Static dissolution studies[15]:

An ocusert was taken and placed in a test tube containing 10ml of phosphate buffer with pH 7.4. The tubes were sealed and kept at 37˚C for 24hrs. The buffer was drained off and replaced with fresh buffer. Using a UV/ visible spectrophotometer, the concentration of drug in buffer was measured at 274nm.

Results and Discussion

The solvent casting technique was used to prepare the ocuserts containing drug & drug-βCD complex. The optimum concentration of Chitosan [1%] was used for the preparation of the ocusert. At this concentration the ocuserts prepared were flexible & easily removable from the die. When higher concentrations of Chitosan were used, the polymer solution was highly viscous & very difficult to filter by laboratory methods; also ocuserts were brittle & hard, and difficult to remove from the die.

The prepared ocuserts were observed for their physical characteristics such as colour, shape, texture and edge. They were circular in shape, smooth & uniform, and pale yellow in colour. The average thicknesses of the ocuserts were 0.25mm & 0.21mm for Drug & Drug-βCD ocuserts respectively (Table-1). However, difference in thickness between ocuserts was negligeble. The βCD complex enabled the proper dispersion of drug in the polymeric solution, which manifested in the reduced thickness of Drug-βCD ocuserts. The diameter & area of ocuserts were 13mm & 132.67 mm^2 respectively.

The average volume of Drug-βCD ocusert was lesser compared to Drug ocusert. The reason for lower volume of Drug-βCD ocusert may be the breakage of the linear polymer structure of chitosan. The average weight of Drug ocusert was slightly higher than Drug-βCD ocusert, which may be due to the crystallization in the formulated ocusert[14]. βCD probably prevents the occurrence of crystallization in the formulation.

The folding endurance of ocusert for Drug & Drug-β CD ocusert was 45.65 &60.65respectively (Table -1). Folding endurance was better for Drug-β CD ocusert. This improved folding endurance may be due to the presence of β CD.

All these evaluations on physical characterization clearly show that, the presence of βCD doesn't interfere with physical characteristics to a great extent but helps to improve some of its physical attributes

The percentage moisture absorbed by Drug-β CD ocusert was lesser than the Drug ocusert.

The ocuserts were subjected to stability evaluation by exposing them to different conditions viz room, refrigeration and oven temperatures. Ocuserts with β CD complex were found to be stable under all the above conditions. The drug-β CD ocuserts did not show any significant weight variation even after 7 days of storage (Table-2).

Microbiological efficacy was evaluated on the basis of zone of inhibition formed around the ocusert. As per the observation (Fig 1,2&3), in the first 3 days Drug-β CD ocusert showed excellent microbiological activity with large zone of inhibition. However in the next 4 days, the microbiological activity dropped to some extent.This improved microbiological activity of Drug-β CD may be due to the presence of β CD complexation, which provides an initial burst release of Ciprofloxacin HCL for first 3 days and then sustains the release there after (Table 3).

Invitro release studies were carried out in phosphate buffer(pH 7.4). The release of Ciprofloxacin HCl from Drug ocusert was 20.12µg on first day, followed by 15.67 µg and 12.24µg on day 2 and 3 respectively. The release on 4^{th} day was reduced to less than 10µg and still poorer thereafter. For Drug-β CD ocusert, ciprofloxacin release on day 1 was 26.21µg, followed by 18.53µg and 14.11µg on day 2 and 3 respectively. The release dropped to less than 10µg only after 7^{th} day (Table-4).

From the release pattern of Drug and Drug-β CD ocusert (Fig 4) it is evident that there was initial high burst release from Drug – β CD ocusert. This initial burst release was observed for more number of days in Drug-β CD ocusert. The incorporation of β CD with drug in the Chitosan polymer may be the reason for the high initial burst as well as the extended release pattern observed for more number of days. According to review of literature, the β CD complexes help not only in improving the solubility of drug but also in the sustained release of drugs. But here the

release from ocusert was believed to be controlled because of the incorporation of these complexes in Chitosan polymer. The drug-β CD ocusert remained intact over 14 days during dissolution study period, and was found to degrade after a month.

The main objective of this study was to over come the major draw backs of the conventional ophthalmic formulations such as drops, ointments etc. The results of this study clearly showed a positive sign in the field of development in controlled drug delivery system such as ocuserts.The attainment of an effective drug concentration at the intended site of action for a sufficient period of time to elicit the response may be possible with such ocusert formulations in presence of β CD .It has been shown that, β CD at an optimum concentration helped in sudden and greater initial burst release of drug from ocusert, and later in controlling the release pattern of drug along with Chitosan polymer. β CD also helped to improve the stability of the prepared formulation. All these improvements came with out causing the damage to physical characteristics of the formulation.The advantages of having β CD in this formulation has been clearly proved in the obtained evaluation datas.Apart from β CD,the inherent properties of Chitosan as a good biodegradable polymer has been fully utilized in this formulation.Invitro results of this formulation is defenitly encouraging hence there is a huge scope for further invivo evaluation so that the designed Ocusert can be converted to a promising marketable product in the category of ophthalmic products.

Table -1 Average Thickness& Folding endurance of Preapared Ocuserts

Type of Ocusert	Average Thickness	Average Folding Endurance
Drug- Only	0.25mm	45.65
Drug-βCD	0.21mm	60.65

Table -2 Stability evaluation data for Ocuserts under different Temperature conditions

No. of Days	Wt. at Room Temp (30°C) (mg)		Wt. at Refrigeration Temp (4-6°C) (mg)		Wt. at Oven Temp (45°C) (mg)	
	Drug	D-βCD	Drug	D-βCD	Drug	D-βCD
1	26	23	26	21	25	22
2	25	23	28	22	25	22
3	20	22	28	25	24	21
4	20	22	24	25	24	21
5	20	22	24	25	23	21
6	20	22	24	25	21	21
7	20	22	24	25	21	21

Table -3 Antibiotic sensitivity for prepared Ocuserts against control

No of Days	Antibiotic Sensitivity		
	Control	Drug	Drug-βCD
01	+	++	+++
02	-	+++	+++
03	-	++	+++
04	-	+	++
05	-	+	++
06	-	+	++
07	-	-	++

- : No release ++ : Good release
+ : Moderate release +++: Excellent release

Table -4 Invitro dissolution study data for prepared Ocuserts

No. of Days	Concentration [µg/ml]	
	Drug	Drug-βCD
1	20.21	26.21
2	15.67	18.53
3	12.24	14.11
4	8.75	15.67
5	6.94	12.31
6	3.78	15.61
7	3.61	8.56
8	2.75	8.68
9	2.01	8.65
10	0.98	8.42
11	NIL	8.40
12	NIL	7.89
13	NIL	7.80
14	NIL	7.52

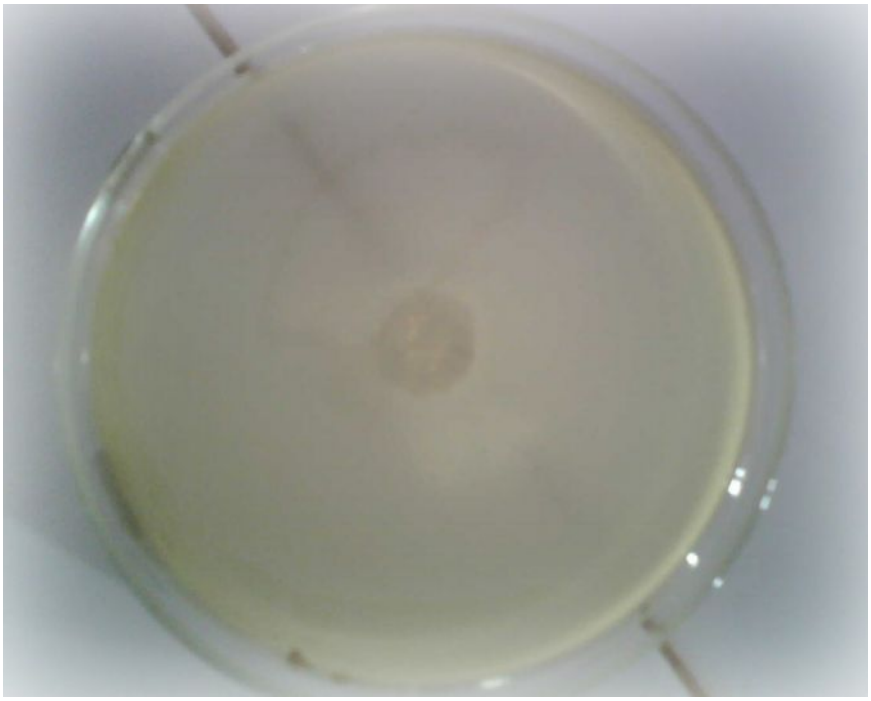

Fig 1: Zone of Inhibition for Control

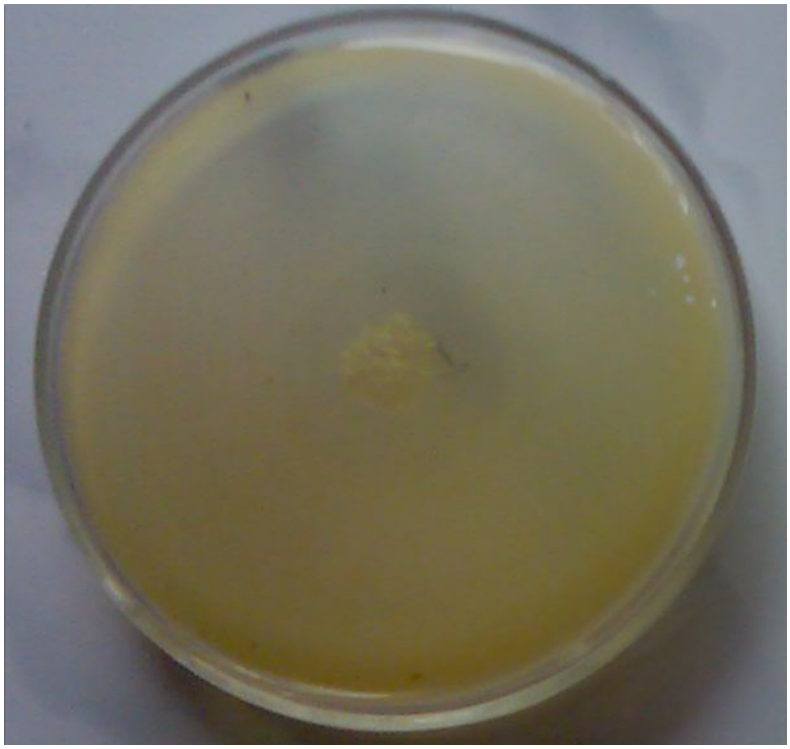

Fig 2: Zone of Inhibition for Drug-Ocusert

Fig 3: Zone of Inhibition for Drug- β CD Ocusert

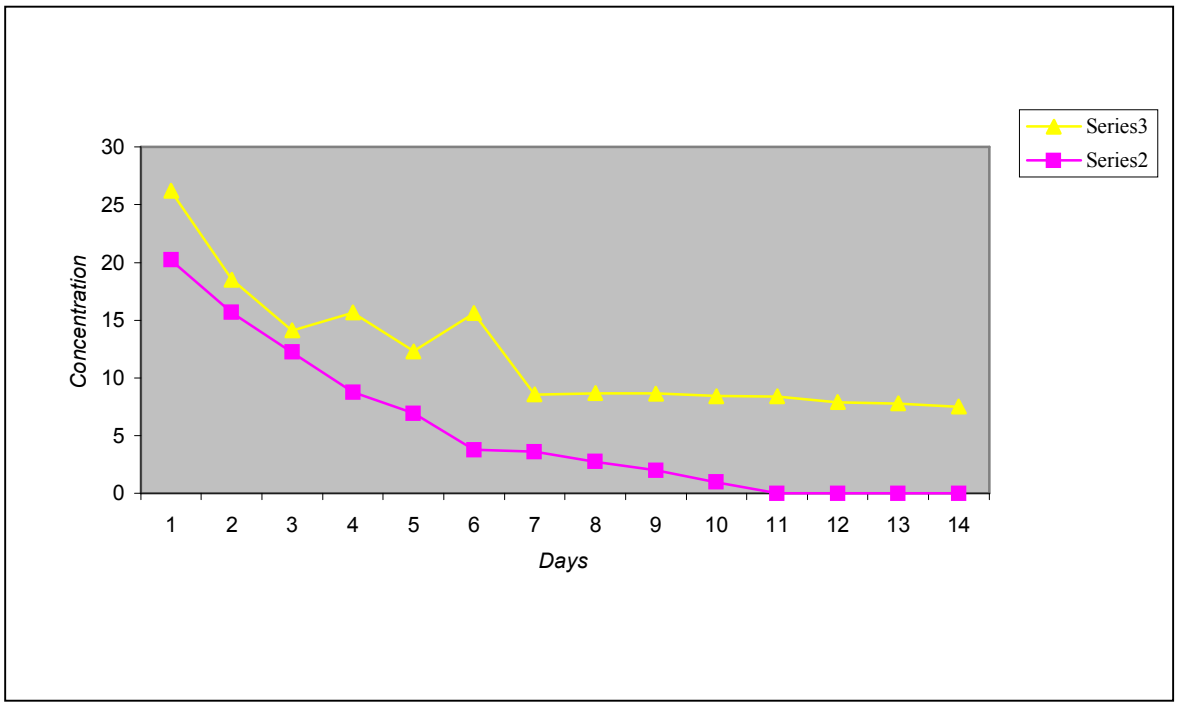

Fig 4 Comparison of release pattern between Drug & Drug-β CD Ocuserts

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