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Development and evaluation of chitosan ocuserts containing Ciprofloxacin - βCD complex

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ABSTRACT

The objective of the study was to develop an ocular insert of ciprofloxacin hydrochloride & evaluate for sustained ocular delivery of drug. Physical characterization, stability studies, microbiological and invitro release studies were done. The ocuserts were made which were of matrix diffusion control system. Kneading and Solvent casting technique and was used to prepare the ocuserts. Two formulations were prepared, a plain formulation consisting of the polymer chitosan and ciprofloxacin hydrochloride and the second one consisting of the polymer, drug-beta cyclodextrin complex in specific ratios. The ocuserts were physically examined for colour and various other physical parameters were evaluated. Based on the physical parameters further studies were carried out like stability, microbiological and invitro release studies. The ocuserts were evaluated for thickness and diameter, weight variation test, folding endurance, moisture absorption, stability studies, microbiological studies and invitro release studies. This was done for the two formulations and the formulation with beta cyclodextrin showed a better result on basis of all the tests that were performed.

Key words : Ciprofloxacin, ß CD complex, chitosan ocuserts.

INTRODUCTION

Millions of people suffer from a wide variety of ocular diseases, many of which lead to irreversible blindness. The leading causes of irreversible blindness in the elderly--age-related macular degeneration and glaucoma--will continue to affect more individuals as the worldwide population continues to age. Although there are therapies for treating glaucoma, as well as ongoing clinical trials of treatments for age-related macular degeneration, there still is a great need for more efficacious treatments that halt or even reverse ocular diseases. The eye has special attributes that allow local drug delivery and non-invasive clinical assessment of disease, but it is also a highly complex and unique organ, which makes understanding disease pathogenesis and ocular drug discovery challenging. As we learn more about the cellular

mechanisms involved in age-related macular degeneration and glaucoma, potentially, new drug targets will emerge. This study provides insight into some of the new approaches to therapy. [3]

“Ophthalmic disorder" refers to physiologic abnormalities of the eye. They may involve the retina, the vitreous humor, lens, cornea, sclera or other portions of the eye, or physiologic abnormalities which adversely affect the eye, such as inadequate tear production. Major ophthalmic disorders affect the posterior segment, including the retina and lens, as well as the anterior segment which includes the cornea, conjunctiva and sclera. Among the most important posterior segment disorders are macular holes and degeneration, retinal tears, diabetic retinopathy, vitreoretinopathy and miscellaneous disorders. The most important disorder of the lens is cataract. The most important disorders of the cornea are refractive disorders such as the sequelae of radial keratotomy, dry eye, viral conjunctivitis, ulcerative conjunctivitis and wound healing (such as corneal epithelial wounds) and the consequences of Sjogren's syndrome. [2,6]

Most ocular preparations like eye drops and suspensions call for the topical administration of active drugs to the tissues around the ocular cavity. These dosage forms are easy to instill but suffer from the inherent drawback that the majority of the medication they contain is immediately diluted in the tear film as soon as the eye drop solution is instilled into the cul-de-sac and is rapidly drained away from the precorneal cavity by constant tear flow and lacrimo-nasal drainage. Therefore the target tissue will absorb a very small fraction of the instilled dose also concentrated solutions and frequent dosing is required for to achieve an adequate level of therapeutic effect. Apart from this, the eye as a portal for drug delivery is generally used for local therapy against systemic therapy in order to avoid the risk of eye damage from high blood concentrations of the drug, which is not intended. The unique anatomy, physiology and biochemistry of the eye render this organ impervious to foreign substances, thus presenting a constant challenge to the formulator to circumvent the protective barriers of the eye without causing permanent tissue damage.

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 or not 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. This invention is a medical treatment for ophthalmic conditions which are associated CDDS .[7,8.]

Different types of drug molecules especially antibiotics are tried in various ophthalmic symptoms. Ciprofloxacin Hydrochloride is a very commonly used antibiotic in ophthalmic infections. Ciprofloxacin Hydrochloride is a broad spectrum Fluroquinoline antimicrobial with a half-life of 3.3 to 4.9 h frequently used in ocular superficial bacterial infections, e.g. corneal ulcers, conjunctivitis. The current literature indicate that none ocular inserts are made of biodegradable systems containing ciprofloxacin Hydrochloride. Hence this investigation was taken up to study the drug release kinetics of Ciprofloxacin from biodegradable chitosan. It was aimed to prepare ocular films containing Ciprofloxacin Hydrochloride with better solubility and longer duration of action delivering the drug in zero order kinetics.[9.10.]

Cyclodextrins (CD) is cyclic torus-shaped molecules with a hydrophilic outer surface and a lipophilic central cavity that can accommodate a variety of lipophilic drugs. The number of applications of CDs in pharmaceutical formulations has been increasing in recent years because of their approval by various regulatory agencies. The formation of inclusion complexes by β-CD with various lipophilic drugs has increased their solubility and absorption. However, the use of

CDs in solid oral dosage forms is limited to low-dose drugs with large stability constants because of the mass limitations of oral dosage units. CD are available in their α,β and γ forms. In most of the cases we use β-CD as inclusion complexes and improve the solubility properties. It is also observed from earlier studies that a formulation such as implants, strips, patches etc has improved their stability properties by the incorporation β-CD.[11,13.]

CD's are not well-known solublizers, but constitute very powerful tool as permeation enhancers. CD may increase the ophthalmic drug delivery of drugs through multiple routes. -By increasing the solubility of poorly soluble drugs; by altering corneal permeability by damaging the corneal membrane. CD's act as anti-irritants –i.e. CD's may decrease the irritation of drugs by formation of inclusion complexes, thereby masking the irritating nature of the drug. Since ophthalmic drug irritation is mostly due to drug concentration on the corneal surface tissues, CD's may be able to eliminate the ocular irritation by changing the rate of drug absorption.

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 $[C_6H_{11}O_5N\ ]_n$. It is insoluble in water but soluble in dilute acids forming the corresponding salts. It is tough, biodegradable, non-toxic, and biocompatible. Is a cationic polyamine; posses various biological activities like wound healing, antacid-antiulcer, bacteriostatic, fungistatic, haemostatic & spermicidal activity.

MATERIALS AND METHODS

I. Preparation of Ocuserts:

The solvent casting technique was used to prepare the ocuserts containing drug(Ciprofloxacin) & drug-βCD complex. The optimum concentration of chitosan [1%] was used for the preparation of the ocusert; with this concentration the ocusert prepared were flexible & easily removable from the dye. When higher concentrations of chitosan were used, the polymer solution was highly viscous & very difficult to filter by laboratory methods. As per earlier works it was observed that such ocuserts were brittle & hard, also difficult to remove from the dye.

A. Preparation of Ciprofloxacin-βCD inclusion complex (Kneading method):

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.

B. Preparation of ocular insert by glass substrate technique:

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, thus formed solution was filtered through a muslin cloth to remove undissolved portion of the polymer (chitin). Propylene glycol (1% $^{v}/_{v}$) was used as plasticizer. Required amount of drug-βCD [DβCD] complex was added and vortexed for 15minutes to dissolve the complex in Chitosan solution. Plasticizer (propylene glycol) of 1%$^{w}/_{v}$ was added to it and mixed well with stirrer. The viscous solution is kept aside for 30 minutes for complete expulsion of air bubbles

The rate controlling films were prepared using the same method [1% $^{w}/_{w}$ Chitosan in 1%$^{v}/_{v}$ acetic acid. Propylene glycol (1% $^{v}/_{v}$) was used as plasticizer.], but without addition of the drug.

The films were casted by pouring solution into center of leveled glass mould and allowing it to dry at room temperature for 24hrs. After drying, films were cut into ocusert of desired size (13mm diameter) so that each contains equal quantity of drug.. Then, the matrix was sandwiched between the rate controlling membranes using non-toxic, non-irritating, water insoluble gum. They were wrapped in aluminum foil separately and stored in a dessicator until further use. Placebo ocuserts were also prepared by same method. [4,5]

Table – I Formulation details

TYPE	POLYMER	DRUG	βCD
Plain	Chitosan	-	-
Drug only	Chitosan	Ciprofloxacin	-
Drug-βCD	Chitosan	Ciprofloxacin	βCD

II. Evaluation of Ocuserts

1. **Physical characterization**

The ocular inserts were evaluated for their physical characters such as shape, colour, texture, appearance, etc and reported.[14.] Various other physical parameters were also evaluated as follows:

a) **Thickness and diameter**
b) **Weight variation test**
c) **Folding endurance**
d) **Percentage moisture absorption**
2. Stability studies
3. **Microbiological studies**
4. **Invitro release studies.**

I Physical characterization

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

Shape : Circular
Colour : Pale yellow
Texture : Smooth & Uniform
Edge : Smooth & Uniform

a. Thickness and diameter

Among the prepared ocular inserts, 20 were selected. The thickness & diameter was measured using screw gauge and average was determined.

Table - II

TYPE OF OCUSERT	THICKNESS	DIAMETER	AREA	VOLUME	DENSITY
PLAIN	0.25mm	13mm	132.67mm^2	33.17mm^3	0.754mg/mm^3
DβCD	0.21mm	13mm	132.67mm^2	27.86mm^3	0.754mg/mm^3

b. Weight variation test

Among the prepared ocular inserts, 20 were selected and weighed. Average weights of ocular inserts were determined.

Table -III

WEIGHT OF OCUSERTS	PLAIN [mg]	Drug-βCD[mg]
Av. weight	25	21

c. Folding endurance

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 was counted. Folding endurance test was repeated using more sets of ocular inserts.

Table – IV

TYPE OF OCUSERT	AV. FOLDING ENDURANCE
PLAIN	45.65
DβCD	60.65

d. Percentage moisture absorption

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:

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

Table –V

DAY	%MOISTURE CONTENT	
	DRUG OCUSERT	DRUG-βCD OCUSERT
1	3.2%	3.7%
2	9.5%	7.4%

II. Stability studies

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

Table -VI

DAY	WT. AT ROOM TEMP MG		WT. IN REFRIGERATOR MG		WT. IN OVEN MG	
	PLAIN	DβCD	PLAIN	DβCD	PLAIN	DβCD
1	26	23	26	21	25	22
2	25	23	88	102	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

III. Microbiological studies

Ocular inserts were evaluated microbiologically for controlled drug release for a period of 7 days. Melted nutrient agar medium was taken and inoculated with 4-5 loops full of test microorganism from the provided culture. The inoculated medium was poured into a sterile petridish and allowed to solidify. A sterile ocular insert was placed carefully on the semisolid medium. The petridish was incubated in inverted position for 24 hours at 37±0.5°C. After incubation, observed for the zone of inhibition. The zone of inhibition was 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 petridish at an interval of 24 hours.

Table –VII

NO: OF DAYS	**ANTIBIOTIC SENSITIVITY**		
	CONTROL	PLAIN	DRUG-βCD
01	+	++	+++
02	-	+++	+++
03	-	++	+++
04	-	+	++
05	-	+	++
06	-	+	++
07	-	-	++

- : No release *++ : Good release*

+ : Moderate release *+++: Excellent release*

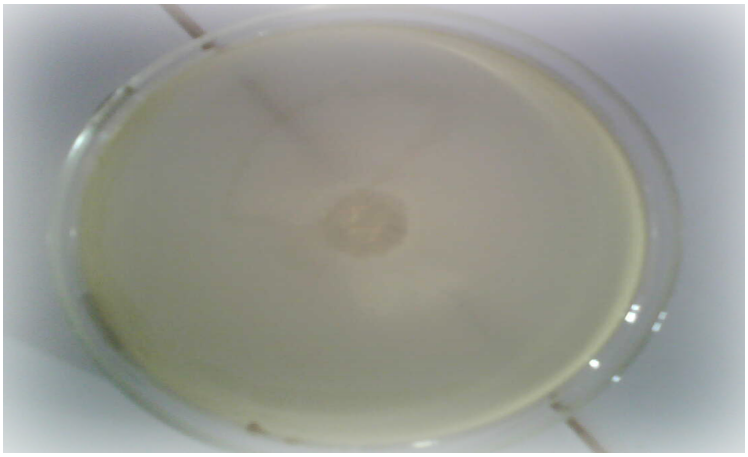


Fig no: I Microbiological study- Control

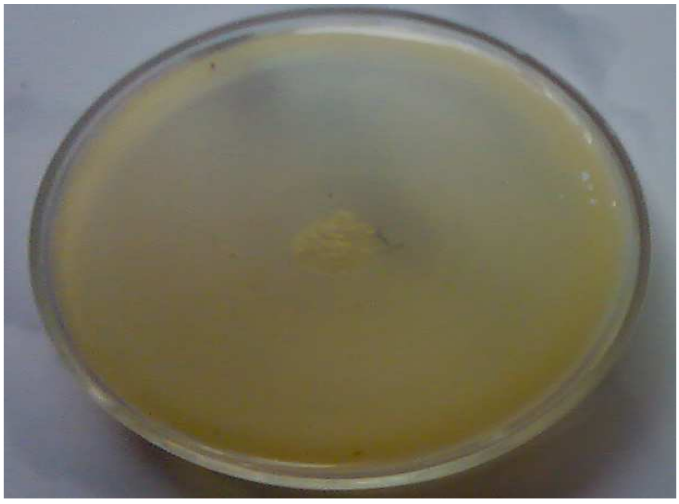


Fig no: II Microbiological study of drug

Fig no: III Microbiological study of drug- βCD

IV. *In vitro* release studies

A. Calibration plot:

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

Table -VIII

DAY	CONCENTRATION [µg/ml]	
	PLAIN	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 no: IV

STANDARD PLOT OF CIPROFLOXACIN-bCD COMPLEX

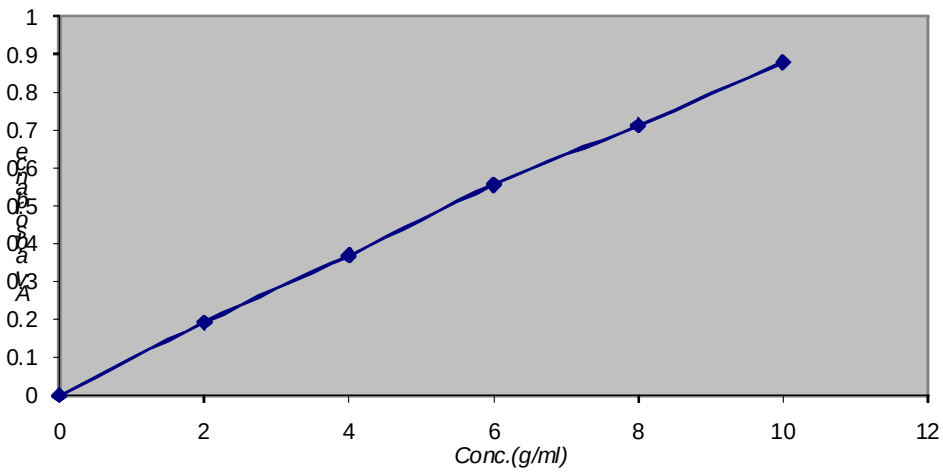


Fig no: V

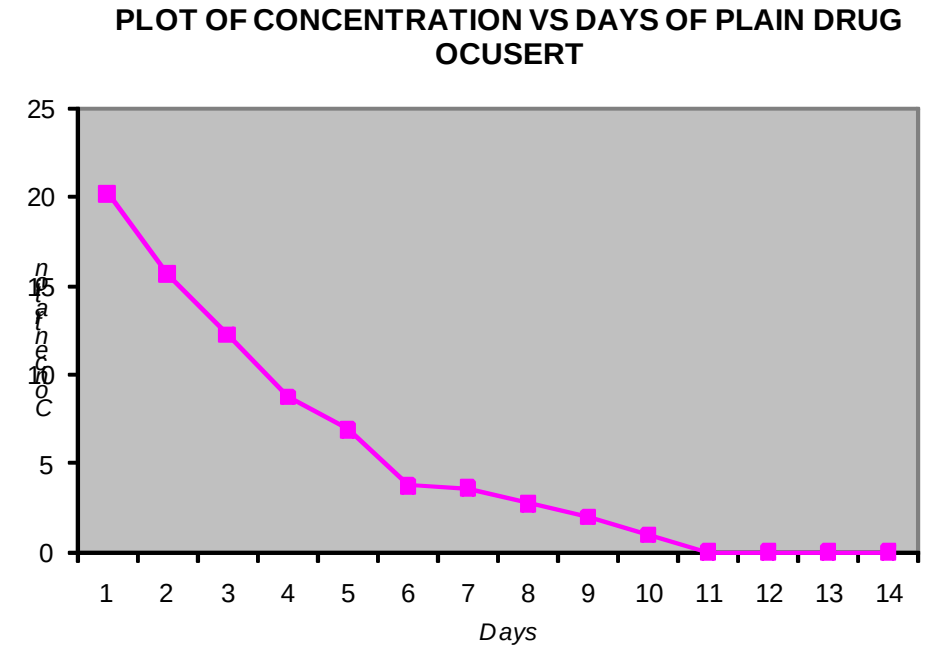


Fig no: VI

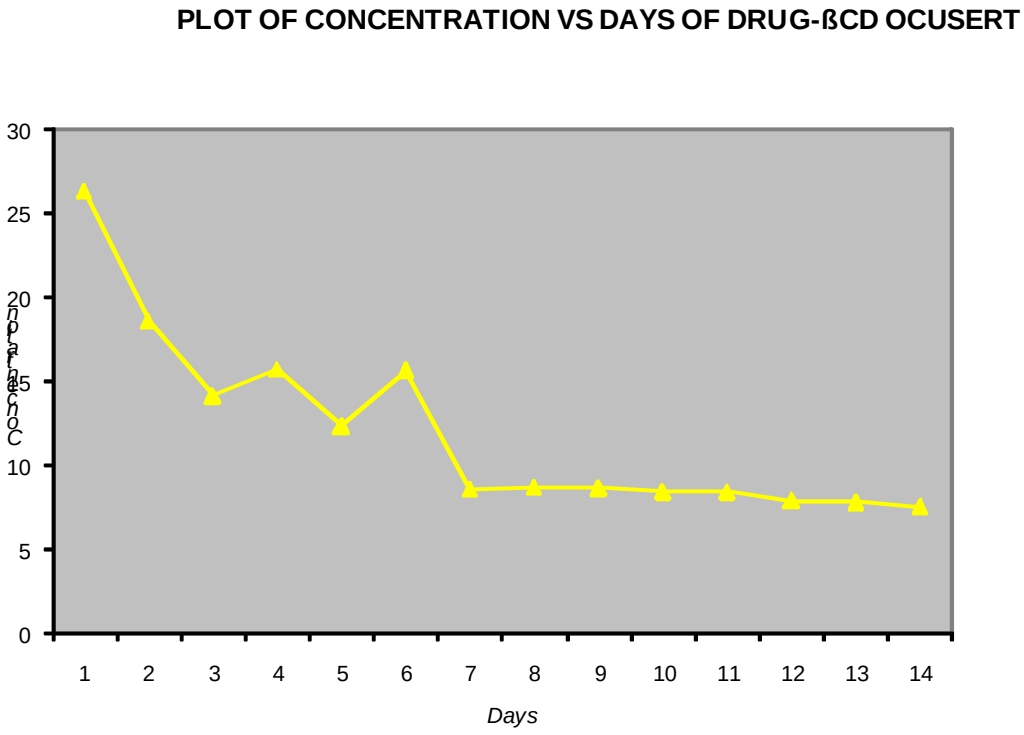
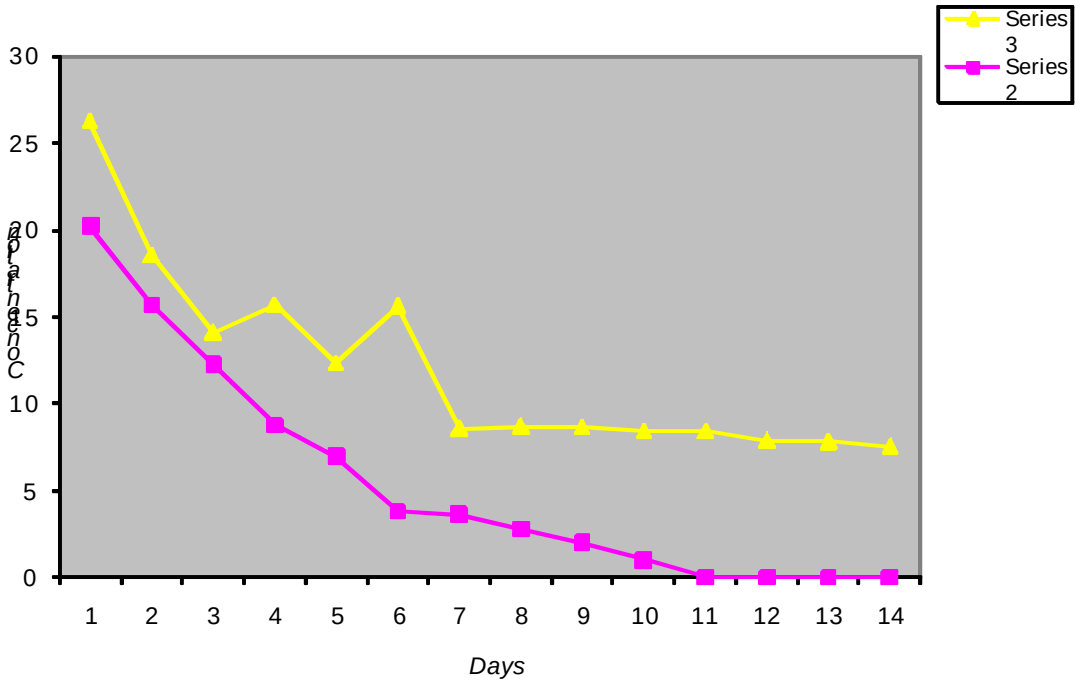


Fig no: VII

COMPARITIVE PLOT OF PLAIN & COMPLEXED DRUG OCUSERTS



RESULTS AND DISCUSSION

The ocuserts were observed for their physical characteristics such as colour, shape, texture and edge. They were circular in shape, smooth & uniform, with a pale yellow colour. Diameter & area of both ocuserts were same, i.e. 13mm & 132.67 mm^2. It clearly indicates the accuracy of the prepared formulation. It also proved that, the presence of βCD doesn't interfere with physical characteristics to a great extent.

The volume of both ocuserts was ranging between 25-35 mm^3, where the average volume of Drug-βCD ocusert was lesser when compared with plain ocusert. Similarly density was 0.754mg/mm^3 for both ocuserts. The reason for lesser volume of Drug-βCD ocusert was due to the breakage of the linear polymer structure of chitosan. The average weight of both, plain & Drug-βCD ocuserts was almost similar to each other. The plain ocusert showed slight higher weight, may be because of the crystallization in the formulated ocusert.

The folding endurance of ocusert was 45.65 for plain ocusert and 60.65 for drug-βCD ocusert. Folding endurance was better for drug-βCD ocusert since the crack was observed in these strips after more number of foldings. This improved folding endurance of drug-βCD ocusert may be due to the presence of βCD in the formulation.

The percentage moisture absorption was calculated for both ocusert and may be due to the presence of βCD complex, the percentage moisture absorbed by drug-βCD ocusert was lesser than the plain ocusert after 2 days evaluation. The percentage moisture absorption was not so significant and the integrity of the ocuserts was stable throughout the study duration.

The ocusert were observed for physical changes in colour, appearance, flexibility and texture. Only a slight change in colour of ocusert was observed when exposed to different conditions of room temperature, refrigeration temperature and oven temperature.

The weight variation data obtained after 7 days showed that weight variation was in lesser extent with drug-βCD ocusert than plain ocusert. It suggests that presence of βCD improves the stability of ocusert.

7 Days microbiological studies were conducted with a control, plain & drug-βCD ocusert, where the microbiological activity was evaluated on the basis of release characteristics. As per the observation, in the first 3 days drug-βCD ocusert showed excellent microbiological activity with large zone of inhibition, were as in the next 4 days the microbiological activity were good in drug-βCD ocusert when compared with moderate activity of the plain ocusert.

This improved microbiological activity of drug-βCD may be due to the presence of βCD complexation, which in turn increases the release of the drug from the formulation with the sustain release properties.

The release of ciprofloxacin from plain ocusert was 20.12µg/ml on first day, followed by 15.67 µg/ml and 12.24µg/ml on day 2 and 3 respectively. The release after 4th day was reduced to less than 10µg and was found to be poorer for subsequent days.

In case of drug-βCD ocusert, ciprofloxacin release for day 1 was 26.21µg/ml, followed by 18.53µg/ml and 14.11µg/ml on day 2 and 3 respectively. There was high release maintained for more number of days than the plain ocusert. The release was reduced to less than 10µg only after 7th day and was still maintained at good rate for subsequent days.
From the release pattern of plain and drug-βCD ocusert it is evident that there was initial high burst release in drug – βCD ocusert than the plain ocusert. This initial burst release was present for more number of days in drug-βCD ocusert.

The incorporation of β-CD with drug in the chitosan polymer may be the reason behind 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 for increasing the solubility of drug but also increases the release for more number of days. But here the release from ocusert was believed to be controlled because of the incorporation of these complexes in chitosan polymer.

The drug-βCD ocusert remained intact over 14 days during dissolution period, and was found to be getting degraded after a month or so.

CONCLUSION

Ocuserts of ciprofloxacin were prepared by using the drug ciprofloxacin hydrochloride, chitosan and ß cyclodextrin and the ocuserts were smooth, flexible and pale yellow in colour. The diameter and area of the ocuserts were same and clearly indicates the accuracy of the preparation.

The percentage moisture absorption was not so significant and the integrity of the ocuserts was stable throughout the study duration. Folding endurance was better for drug-βCD ocusert since the crack was observed in these strips after more number of foldings. This improved folding endurance of drug-βCD ocusert may be due to the presence of βCD in the formulation. The

percentage of moisture absorption was not very significant and the integrity of the ocuserts were stable during the duration of study. Only a slight change in colour of ocusert was observed when exposed to different conditions of room temperature, refrigeration temperature and oven temperature.

The weight variation studies was performed for seven days and weight variation was in lesser extent with drug-βCD ocusert than plain ocusert. It suggests that presence of βCD improves the stability of ocusert. The microbiological activity were good in drug-βCD ocusert when compared with moderate activity of the plain ocusert. Results of the invitro permeation studies shows that the drug-βCD ocusert showed a better release and this was due to incorporation of these complexes in chitosan polymer. Comparative plot of plain drug ocuserts and complex drug ocuserts showed a good release.

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