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Formulation, Optimization and Evaluation of Ocular Inserts Containing Anti-Fungal Drug.

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Anti-Fungal, Infection, Keratitis, ocular insert

Abstract
The goal of the current study was to create Clotrimazole betacyclodextrin ocuserts and assess the physicochemical aspects of in vitro release and . For improved ocular bioavailability and retention of the drug, several polymeric methods are employed to create ocular inserts. Gelling systems have shown benefits of simple administration and prolonged contact time. Utilizing gelatin as film-forming polymers and glycerin as a plasticizer, Clotrimazole beta-cyclodextrin ocular inserts were prepared. Beta-Cyclodextrin was complexes with Clotrimazole so that the solubility of the Clotrimazole can be increased. Solid dispersion of Clotrimazole was done to increase its solubility. Thirteen formulations in all were made using the solvent casting technique and evaluated for their surface pH, thickness, weight fluctuation, drug content, moisture loss, and in vitro and in vivo release tests. Excised goat cornea was placed between the donor and receptor compartments of Franz diffusion cells for the in vitro release tests. At the conclusion of two hours, Formulation F9 exhibits a maximum cumulative percentage drug release via excised goat cornea of 72.62%.

1. Introduction

Opthalmic drug delivery system is the most challenging and crucial delivery of drug to the eye. Due to its ease and safety for ocular chemotherapy, topical administration of medications is typically the method of choice. It is a huge task for the formulator to get around (bypass) the eye's defences without enduring long-term tissue damage. Ocular delivery systems with high treatment efficacy continue to be made possible by the development of better, more sensitive diagnostic procedures and innovative therapeutic substances. .To get the best medication concentration at the active site for the right amount of time is the specific goal while creating a therapeutic system.(1) The eye is a precise, important organ that is impenetrable to outside substances due to its anatomy, physiology, and biochemistry. As a result, it is challenging for formulators to get through the eye's protective barriers without causing any long-term tissue harm. Numerous eye conditions may harm the eye and result in vision loss. The most common and well approved route of administration for treating a variety of ophthalmic problems is applying topical medications to the eye. However, due to the eye's effective defence mechanisms, the bioavailability of ophthalmic medications is extremely low.(2)

The major concern associated with ocular medication is maintaining and obtaining a desired therapeutic level at the site of action and that too for the desired period. The anatomy and physiological conditions of the eye make it an organ exquisitely impervious to foreign substances (3). Ocular inserts, which are solid objects inserted into the eye's cul-de-sac, have many advantages over liquid versions. The effective drug concentration in the eye can be guaranteed over an extended length of time due to the prolonged retention of the devices and a regulated release. Additionally, the medicine is dosed more precisely, lowering the possibility of systemic side effects. (4)

Advantages:-

•prolonged ocular contact, which enhances drug bioavailability

•enhanced ocular permeability in comparison to normal formulation, enabling longer drug action and consequently enhanced ocular bioavailability of medication

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•Capability to deliver a steady rate of medication release in some instances

•Better patient compliance as a result of reduced drug frequency and a lower incidence of ocular and systemic side effects. (5)

Cyclodextrins(CDs) are cyclic torus-shaped molecules with a lipophilic core chamber and a hydrophilic outer surface. There are many forms of CDs available . Among these Beta-CD, which significantly alters the physical and chemical properties of the drug molecule, particularly in terms of water solubility.Cyclodextrin inclusion compounds containing hydrophobic molecules can permeate bodily tissues and can be employed to release biologically active compounds under certain conditions.(6)

2. Methodology

The preparation of Clotrimazole Beta-cyclodextrin complex ocuserts involves three steps.(7)

2.1 . Preparation of Drug polymeric film

The polymeric drug reservoir film were prepared by dissolving 1.5, 2.0, 2.2 % of gelatin in 15ml of hot water. Clotrimazole betacyclodextrin binary mixture was separetly dissolved in water. Then this binary mixture solution was added to the polymeric solution . The solution was stirred under magnetic stirrer at 100rpm and glycerine was added to this solution.After complete mixing, the solution was poured on to the petriplate dish(previously lubricated with glycerine). The cash solution was allowed to evaporate at room temperature. After drying, the medicated film were cut with the help of stainless steel borer which is previously sterilized.

2.2. Preparation of Rate controlling membrane.

The required quantity of Ethyl cellulose was dissolved in ethanol and dibutyl phthalate was added to the solution and poured into the petri plate(previously lubricated with glycerine) and allowed to dry at room temperature. After drying of the film, the films were cut with the help of stainless steel borer.(8)

2.3. Sealing.

The drug polymeric film is sandwiched between the two rate controlling membrane. After that this whole unit is kept on a wire gauge and kept for 5-7 min in desiccator which is previously equilibrium with ethanol . This results in sealing of the layers.

2.4 Formulation table

Ocuserts of clotrimazole betacyclodextrin complex were prepared by solvent casting technique

Sr.no	Batches	Gelatin (gm)	Glycerin (ml)	Water (ml)
1	F1	1.8	0.72	15
2	F4	1.8	0.336	15
3	F5	2.1	0.45	15
4	F6	1.5	0.45	15
5	F7	1.5	1	15
6	F9	2.22	0.72	15
7	F11	2.1	1	15
8	F12	1.37	0.72	15
9	F13	1.8	1.11	15

Where, F =formulation

Table 1: Formulation table for drug film

Solid dispersion of drug.

Despite having strong therapeutic efficacy, the majority of recently discovered chemical entities have low water solubility and poor bioavailability, which results in inadequate absorption in the gastrointestinal tracts. It is essential for medications that are poorly water-soluble to be present in solution state in GI fluid in order for the drug to be absorbed from the gastrointestinal tracts (GIT). Particle-size reduction techniques, such as micro- and nanosizing, salt production, solubilization, and complexation with cyclodextrins, are frequently utilised to address this issue.

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Method adopted: -

By kneading, solid clotrimazole in cyclodextrin dispersions in three different ratios (1:1, 1:2, and 1:3) were created. Here, betacyclodextrin was placed in a mortar, to which a small amount of ethanol was added, then triturated to create the consistency of a homogenous slurry. The drug was added to the slurry gradually, and after an hour of trituration, the mixture was dried at 25° C for 24 hours before being ground and sieved through mesh no. 100.

Figure 1: Solid dispersion of clotrimazole with beta-cyclodextrin

3. Evaluation Parameter:-

The prepared ocuserts are need to be evaluated.

3.1 Physical Characterization

The ocuserts' physical characteristics, such as shape, colour, texture, and appearance, were assessed.

3.2. Thickness of the film.

Using a vernier calliper, films' thickness was measured. The mean thickness was estimated using the average from the three readings made at various film spots. The thickness's standard deviations (SDs) were calculated using the mean value.(9)

3.3. Average Drug content:-

Three ocuserts that had been precisely weighed were dissolved in phosphate buffer solution with a pH of 7.4 and agitated for up to 15 minutes.After stirring, Whatman filters were used to remove the medication solution.The average drug content of occuserts was computed using the devised UV spectrophotometric method of drug quantification, and the drug content of filter paper No. 40 was estimated by measuring absorbance using a UV spectrophotometer. The entire procedure was performed three times, with the average recorded.(10)

3.4. Uniformity of weight.

Three of each batch formulation's ocuserts were weighed for the weight variation test using an electronic balance. The weight variation's standard deviations were computed from the mean value after the mean value had been calculated.

3.5. Folding endurance:-

The number of folds (the number of times the insert is folded at the same location) necessary to break the specimen or cause visible cracks to appear is how the folding endurance is stated.This test is crucial to determine whether the sample can withstand folding.This may also show signs of brittleness. Between the fingers and thumbs, the specimen was folded in the middle and then opened.This was known as a single folding. The insert was broken and cracked in the centre after this technique was performed several times. The folding endurance value was used to describe the entire folding process.(11)

3.6. Moisture content

Each of the manufactured ocuserts was precisely weighed before being stored at room temperature in a desiccator containing calcium chloride.At 3, 6, 12, 24, and 48 hour time period Ocuserts were weighed repeatedly until their weight remained consistent, and the average percentage of moisture in the ocuserts was calculated.(12)

$$\% \text{ moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} * 100$$

3.7. Moisture absorption

The % moisture absorption test was used to evaluate the ocular films' physical stability or integrity. Ocular Films were weighed and put in a desiccator which contain 100mL of saturated aluminum chloride solution, where 79.5% humidity was kept. The ocular films were removed and reweighed after three days. The following calculation was used to compute the percentage of moisture absorption.(13)

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% moisture absorption= Final weight – Initial weight *100

Final weight

3.8. Swelling index

Weighed separately, three occuserts were added to beakers with 4ml of Stimulated tear fluid. After five minutes have passed, Ocuserts were taken out, and the extra water on their enlarged surface was wiped off and weighed.(14)

The % swelling index was calculated as:

(Weight of swollen insert after time – original weight of insert at zero time) x 100

Original weight of insert at zero time

3.9. Surface PH

By keeping the occuserts in contact with 5 ml of distilled water for an hour in a petridish, the ocuserts were first given time to swell. By placing the glass electrode close to the formulation's surface and letting it eqillibriate for a minute, the pH was measured.

3.10. In vitro drug release study

Using a self-assembled modified diffusion cell, in-vitro drug release of prepared ocuserts was performed in phosphate buffer 7.4 pH (ophthalmic simulated media) . In the donor compartment of the diffusion cell, the ocuserts was put with 0.2 ml of phosphate buffer 7.4 pH ocular saline media (OSM). The glass tube was complete assembly and then put on a magnetic stirrer and subjected to steady agitation at a speed of 50 rpm while being heated to a temperature of 37 °. By adding new receptor fluid, the receptor fluid that had been withdrawn for drug analysis was replaced. The developed U.V spectrophotometric method of drug analysis was used to determine the drug concentration.(15)

3.11. In vitro transcorneal permeation study.

The entire goat eyeball was delivered from the neighbourhood butcher shop, and the cornea, along with 2 to 4 mm of surrounding tissue, was carefully separated and cleaned with cold, protein-free normal saline. BetweAt intervals of 15, 30, 45, 60, 90, 120, and 150 minutes, 0.5 ml of the test sample was withdrawn from the receptor and replaced with new buffer. The test samples were filtered, and using a UV spectrophotometer and reagent blank (pH 7.4 STF), the absorbance of each sample was determined at 260 nm. The standard curve was used to translate the absorbance into concentration. It was computed the release rates.en clamped donor and receptor compartments of Franz diffusion, an isolated cornea was attached . The temperature was kept at 37 0 C while a strip of film (1 cm) placed in the donor compartment and the solution (pH 7.4 STF) in the receptor compartment were swirled at 100 rpm using a magnetic stirrer. At intervals of 15, 30, 45, 60, 90, 120, and 150 minutes, 0.5 ml of the test sample was withdrawn from the receptor and replaced with new buffer. The test samples were filtered, and using a UV spectrophotometer and reagent blank (pH 7.4 STF), the absorbance of each sample was determined at 260 nm. The standard curve was used to translate the absorbance into concentration. It was computed the release rates.

3.12. Sterility test.

Since all ophthalmic preparations should be sterile, the sterility test is a crucial evaluation factor. The Indian Pharmacopoeia was followed when conducting the sterility test. Using a sterile pipette, sterile syringe, or sterile needle, 2 ml of the produced Sulfacetamide sodium ocusert solution was withdrawn. The test liquid was aseptically transferred to separate soyabean-casein digest and fluid thioglycolate media. The medium and the liquid were combined. The infected media were incubated for a minimum of 14 days at 20°C to 25°C for the soyabean-casein digest medium and 30°C to 35°C for the fluid thioglycolate medium.(16)

4. Result:

3.1. Physical Evaluation.

The physical characteristics of all the developed ocular inserts, including their size, shape, colour, and smoothness, were assessed.

Test	Specification	Observation	Remarks
Description	White powder	White powder	compile

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Texture	Smooth	smooth	compile
Solubility	In methanol	In methanol	compile
M.P	147°c-149°c	148°c	compile
Odor	Odorless	odorless	compile

Table 2: Physical evaluation of prepared ocuserts.

3.2. FTIR

Infrared spectrum of Clotrimazole was recorded on Fourier Transform Infrared spectrophotometer.The sample was scanned over wavelength region of 400 to 600cm- and compared with literature data.

The Figure 2 and 3 shows that there was no change in the position or disappearance of any characteristic peak of Clotrimazole, indicating the compatibility between the drug Clotrimazole and Beta-cyclodextrin.

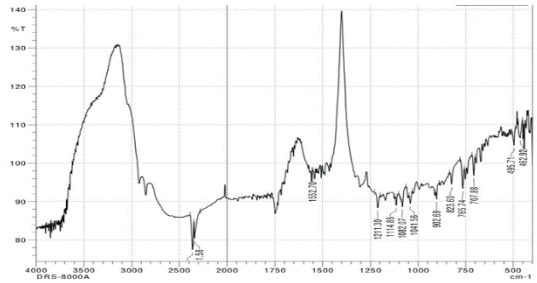

Figure 2: FTIR of Clotrimazole

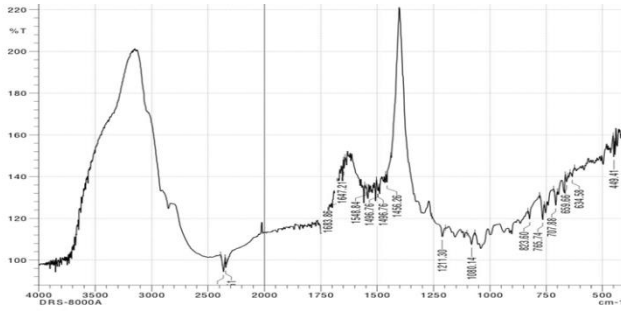

Figure 3: FTIR of Clotrimazole and Betacyclodextrin

3.3. Solubility Study:

The solubility of Clotrimazole was found to be very soluble in methanol. It is soluble in Ethanol, Phosphate buffer and slightly soluble in water.

3.4. Calibration curve of Clotrimazole:-

Development and standard curve of drug using UV-spectrometer clotrimazole dilutions were scanned in the range of 400 nm to 200 nm showed maximum absorption λ 270 nm. Absorbance of prepared solution was measured at 270nm using UV spectrophotometer. Drug followed Beers and Lamberts law in the range of 0 to 10 µg/ml.

Table3 :- Absorbance value of clotrimazole in phosphate buffer

Sr no.	Concentration(ug/ml)	Absorbance(nm)
1.	2	0.052
2.	4	0.121
3.	6	0.186
4.	8	0.253
5.	10	0.327

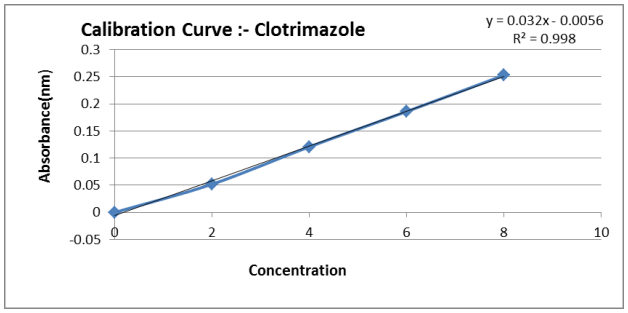

Figure 4: Calibration curve of Clotrimazole

3.5 Uniformity of thickness:

Ocuserts ranged in thickness from 0.22 mm to 0.38 mm on average. There were no obvious differences in the Ocuserts' thickness within any formulation, showing that the film behaved consistently throughout the whole process.

3.6. Uniformity of weight:

Ocuserts' average weights were found to be between 21.3 mg and 30.8 mg. Ocusert's weight was uniform, indicating that the medication, plasticizer, and polymer were distributed well.

3.7. Folding endurance.

All formulas' folding endurance was personally measured. It was discovered in the 52 to 85 range. This test illustrates how flexible ocuserts are. Through this test, it is confirmed that the created ocuserts were

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fit for mass production in order to create lengthy, continuous film without tearing or breaking.

3.8. Surface PH.

Digital pH metres were used to measure the surface pH of each formulation, and the results are shown in table . Since none of the formulation's surfaces were acidic, all formulations were in neutral range so, no eye irritation was expected.

F1	F4	F5	F6	F7	F9	F11	F12	F13
7.1	7.2	7	7.2	7.5	7.6	7.4	6.8	7

Table 4: Surface PH of the ocuserts

3.9. Percentage moisture absorption and loss:-

Percentage moisture absorption and loss of the formulation was found to be in range .

3.10. In vitro drug release study

In vitro drug release of the Clotrimazole betacyclodextrin ocusert was formulated by making 9 batches. The best formulation, F9, demonstrated better controlled release of drug content in-vitro (84.37%) out of all formulations that were observed using zero order release kinetic.

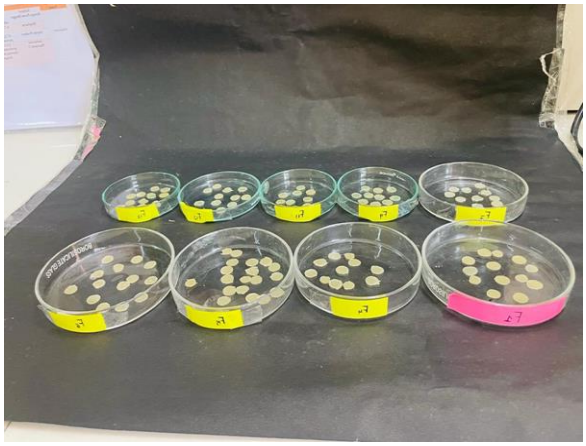

Figure 5: Prepared Clotrimazole beta-cyclodextrin ocuserts

Formulation batch	% Drug release
F1	75.30±0.54
F4	59.36±0.24
F5	83.21±0.38
F6	68.23±0.83
F7	62.21±0.52
F9	84.37±0.34
F11	68.74±0.52
F12	69.51±0.13
F13	78.12±0.39

Table5: Drug release of the prepared ocuserts

3.10.In-vitro transcorneal permeation study.

Table 6: Invitro transcorneal permeation study

Time(min)	% Drug release
15	14.18±0.32
30	34.17±0.77
45	42.82±0.58
60	57.14±0.33
90	63.53±0.04
120	72.49±0.69

Figure 6: Extraction of cornea for drug release study.

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3.11. Sterility test.

When the formulation was incubated for at least 14 days at 20°C to 25°C in the case of soybean casein digest medium (Figure7), and at 30°C to 35°C in the case of fluid thioglycolate medium (Figure8), there was no sign of turbidity and no indication of microbial growth. The formulation under examination therefore passed the test for sterility.

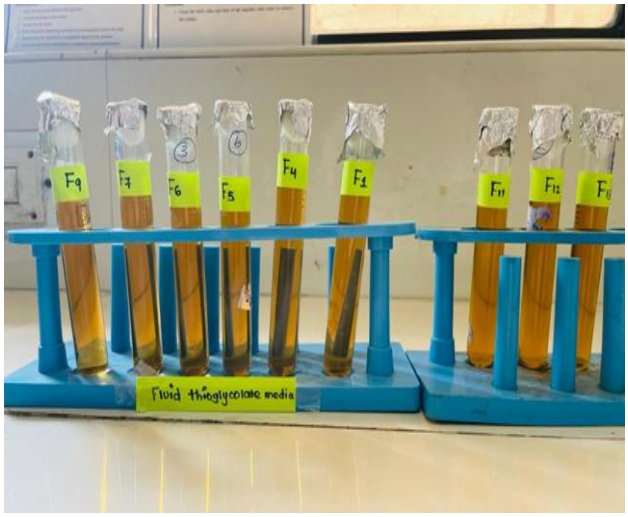

Figure 6: Fluid thioglycolate media

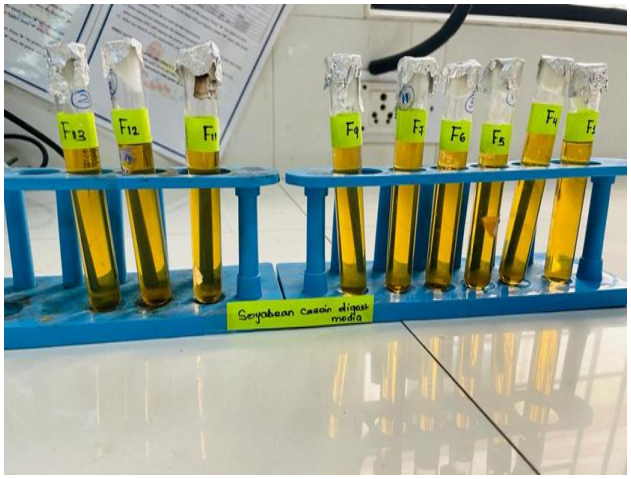

Figure 7: Soyabean casein digest media

5. Discussion

Numerous disorders are treated using the medication clotrimazole. It is recommended for eye conditions. In the current work, many batches of clotrimazole ocuserts were developed utilising the solvent casting process, and they were assessed. Gelatin, polymers were effective at forming films. The amount of ethyl cellulose allowed for a slow release of the medication from the polymeric reservoir. According to the findings of the drug compatibility investigations, there was no chemical interaction between the excipients and the pure medication. The weight of all the films were found to be in between 20.01 and 28.02 mg, indicating that there is uniform distribution of the drug and polymer, folding endurance of all the formulations were to be in the range of 42-85 , and the surface pH of all the formulations were found to be in range 6.60-7.5. All of these findings demonstrated that eye-friendly ocusert preparations do not cause ocular inflammation or redness.

6. Conclusion:

By employing polymers (gelatin) in various ratios and proportions, the solvent casting method was successfully used to create ocular inserts of Clotrimazole betacyclodextrin complex. As a plasticizer, glycerin was employed. The optimum Clotrimazole betacyclodextrin complex in vitro release was demonstrated by the final formulation F9. We discovered that glycerin were effective hydrophilic film-forming polymers and a viable ocular delivery agent. The rate-controlling membrane of the ocusert system was made using EC, a suitable polymeric component. The solubility of Clotrimazole was increased by solid dispersion of the pure drug and then incorporating this solid dispersion in the ouserts.

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