DESIGN AND OPTIMIZATION OF OCULAR INSERTS FOR PROLONGED DELIVERY OF CIPROFLOXACIN WITH CORTICOSTEROID

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ABSTRACT

Ocular drug delivery is very challenging and fascinating for pharmaceutical researchers, especially in the treatment of ocular conjunctivitis. The challenges include delivery of the drug to the eye without causing permanent tissue damage while maintaining a stable therapeutic level at the site of action for a prolonged period of time. The present work focuses on the treatment of ocular conjunctivitis by using combined mechanisms of fluroquinolone antibiotics, providing sustained release of drug from ocular inserts, and using corticosteroid for anti-inflammatory effect and providing comfort to the patient. Quinolone antibiotics, anti-inflammatory agents and polymers with excipients were procured from pharmaceutical companies as gift samples. Different combinations of antibiotics and anti-inflammatory agents were prepared by solvent cast method and the dried films were cut into defined size containing drug and evaluated for different parameters and sustained drug release. Based on *in vitro*, *in vivo* correlation stability studies, the formulation (antibiotic + anti-inflammatory agent) promising the best once a day sustained release of the drug has been chosen.

Keywords: Quinolone antibiotics, anti-inflammatory agents, conjunctivitis, occuserts, *in vitro* release pattern, physico-chemical evaluation

INTRODUCTION

Sustained release or prolonged release of drugs to the eye offers major advantages over the available conventional therapies which involves instillation of drops of eye solutions or suspensions. These conventional dosage forms suffer from the drawback of small fraction (typically 5 %) getting absorbed and reaching the intraocular tissues. Frequent instillation to maintain a continuous sustained therapeutic level is required, which often results in local or systemic side effects[1].

Ophthalmic inserts offer a lot many advantages when compared to conventional dosage forms in terms of improved bioavailability, residence time and possibility of releasing drugs at slow and constant rate, accurate dosing and exclusion of preservatives. These ocuserts also ensure better compliance by the patients as the frequency of administration of drug is reduced, as seen in case of drops and suspensions[2-6].

Bio adhesive and soluble ocular inserts were hence developed for the treatment of external ocular diseases like conjunctivitis using broad spectrum fluoroquinolones in combination with corticosteroid aiming anti-inflammatory activity[7]. The aim of the present work was to formulate ocuserts with a definite concentration of ciprofloxacin and dexamethasone for the treatment of ocular conjunctivitis and evaluate for the sustained release of the active ingredient. The formulation was developed with the objective of increasing the residence time of the drug, reducing the dosing frequency by combining with Carbopol® 974 NF, 980, 981 NF, PEG 400 LR, polyvinyl alcohol and glycerine[8].

MATERIALS AND METHODS

Chemicals and reagents

Pure sample of ciprofloxacin was obtained as a gift sample from Indoco Remedies, Verna, Goa and dexamethasone was from Symbiotic Pharma Private Limited. Carbopol ® 974 NF, 980, 981 NF were gifted by Lubrizol Pvt. Ltd. Mumbai. PVA, PEG 400 and beta cyclodextrins were procured from Hi-Media Laboratories

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Table I: Composition of Ocuserts

Ingredient	Quantity		
	CD 74	CD 80	CD 81
Ciprofloxacin	11.5 mg equivalent to 0.18 mg ciprofloxacin		
Dexamethasone : beta cyclodextrin	34 mg equivalent to 0.06 mg dexamethasone		
Carbopol®974 NF	60 mg	--	--
Carbopol®980	--	60 mg	--
Carbopol®981 NF	--	--	60 mg
Poly vinyl alcohol	540 mg	540 mg	540 mg
Polyethylene glycol 400	0.5 mL	0.5 mL	0.5 mL
Glycerine	25 mg	25 mg	25 mg
Distilled water	20 mL	20 mL	20 mL

Private Ltd., Mumbai. All chemicals used were of analytical grade.

Instrumentation

Preformulation studies were performed on the procured drug samples and excipients with respect to description, melting point, solubility, IR spectra, UV-vis (UV) spectroscopic studies and Differential Scanning Calorimetry (DSC)[9].

UV-VIS SPECTROSCOPY STUDY

Determination of wavelength of maximum absorbance

Pure ciprofloxacin and dexamethasone were weighed and diluted with distilled water. The prepared solutions were scanned in the wavelength region of 200 – 400 nm. This procedure was conducted using UV-visible spectrophotometer (UV - 1800 240V, Made in Japan, Shimadzu Corporation) to estimate the wavelength.

Determination of linearity and range

25 mg of ciprofloxacin was weighed and transferred to 25 mL volumetric flask, dissolved and diluted up to mark with methanol to give a stock solution having strength of 1 mg mL^{-1}. Similarly, accurately weighed 50 mg of pure dexamethasone was transferred into 50 mL volumetric flask, dissolved and diluted up to mark with methanol to give a stock solution having strength of 1 mg mL^{-1}. It was further diluted to get a stock solution of strength 100 µg mL^{-1}.

Table II: Preformulation studies on drugs

Observed parameter	Ciprofloxacin	Dexamethasone
Description	Ciprofloxacin is white in colour, powder form	Dexamethasone is off-white, powder form
Melting point	254.5 ^{0}C	267 ^{0}C
Solubility	Slightly soluble in distilled water, freely soluble in DMSO, DMF and methanol	Poorly soluble in water, freely soluble in DMSO, DMF and methanol and sparingly soluble in ethanol

Aliquots of 0.3 mL, 0.6 mL, 0.9 mL, 1.2 mL and 1.5 mL of working standard solution of individual drugs were transferred to a series of 10 mL standard volumetric flasks and diluted with phosphate buffer pH 6.8 to get 3, 6, 9, 12 and 15 µg mL^{-1} each of ciprofloxacin and dexamethasone respectively.

Respective working λmax of 273.2 nm and 242 nm were used to check absorbance against the blank solution prepared using methanol and phosphate buffer pH 6.8 without drug. The Beer-Lambert law was verified from the calibration curve by plotting the graph of concentration against absorbance[10].

DSC

The thermal property of dexamethasone with beta cyclodextrin, individually and in combination, were studied using DSC (DSC-60 Shimadzu, TA-60 WS collection software). Endothermic and exothermic parameters of the dexamethasone with beta cyclodextrin were subsequently obtained.

IR

The FT-IR spectrum of the obtained sample was compared with the reference standards FT-IR spectra of ciprofloxacin and dexamethasone by potassium bromide method.

PREPARATION OF OCUSERT

Preparation of beta cyclodextrin and dexamethasone complex

Dexamethasone being a poorly soluble drug in water, its solubility was enhanced by forming complexes with β-cyclodextrin. Six different molar ratios were prepared and

evaluated. The solubility profile of the drug was checked and the ratio of 1:1.5 to be used (drug: β cyclodextrins) was finalized based on % cumulative drug release[11].

Preparation of ocuserts of ciprofloxacin and dexamethasone

Area based on 9 cm dimension petri dish was chosen and calculated. Based on area value, drug to be incorporated was calculated. 1:9 proportion of Carbopol® : PVA was dissolved in 20 mL of distilled water the previous night, followed by incorporation of drug and PEG 400 and glycerine with stirring on magnetic stirrer for 6 h. At the end of 6th h, the stirred preparation was poured in the mentioned perti dishes and dried at 50 ^{0}C for 4 h. For the evaluation parameters, 1cm x 1cm areas of the prepared films were employed[12]. Composition of the ocuserts is as shown in Table I.

Table III: IR interpretation

Functional group	Ciprofloxacin (Drug A)	Dexamethasone (Drug B)	Drugs + Carbopol®974	Drugs + Carbopol®980	Drugs + Carbopol® 981
O-H	3377.36	3419.79	3523.95	3523.95	3523.95
N-H	3203.76	--	3419.79	2012.81	2412.08
Aromatic C-H	3099.61	2941.44	2935.66	2937.59	2939.52
Aliphatic C-H	2929.87	2875.86	2873.94	2873.94	2873.94
C = O (Ketone)	1708.93	1714.72	1712.79	1712.79	1714.72
C-F	1273.02	1107.14	1273.02	1273.02	1273.02

Table IV: Evaluated parameters

Formulation code	Surface texture	Thickness (mm)*	Weight (mg)*	Tensile strength (g cm^{-2})*	% Drug content (± SD*)	
					DRUG A	DRUG B
CD 74	Smooth	0.109 ± 0.04	185 ± 0.05	415 ± 0.05	100	83.33
CD 80	Smooth	0.112 ± 0.02	198 ± 0.03	430 ± 0.08	108.33	94.44
CD 81	Smooth	0.117 ± 0.01	192 ± 0.08	425 ± 0.03	100	77.77

Table V: % Cumulative drug diffusion profile

Time (h)	CD74		CD 80		CD 81	
	% Cumulative drug release					
	273.2 nm	242 nm	273.2 nm	242 nm	273.2 nm	242 nm
01	11.84	21.26	7.51	6.78	16.14	19.65
02	19.61	26.94	12.49	18.03	25.01	34.97
03	36.09	57.49	18.86	21.38	39.26	56.44
04	43.32	64.36	23.30	30.60	45.08	64.67
05	50.89	67.48	27.07	34.83	50.37	70.89
06	59.04	77.80	29.80	35.63	53.88	74.18
07	62.31	79.19	31.02	37.63	56.11	76.16
08	65.23	82.65	33.23	40.08	58.80	78.80
09	69.96	95.53	45.55	79.91	75.15	75.70
10	98.38	91.88	55.43	74.95	81.35	95.65
11	108.49	100.36	71.43	95.59	92.33	95.40
12	115.53	105.64	91.36	106.83	101.06	95.85

Table VI: Zone of inhibition value

Formula	Zone of inhibition	
Negative control	--	--
Positive control	--	--
Ciprofloxacin	Present	3.9 cm
Dexamethasone	Absent	0 cm
Ciprofloxacin + Dexamethasone	Present	4.0 cm
CD 74	Present	4.2 cm
CD 80	Present	4.3 cm
CD 81	Present	4.0 cm

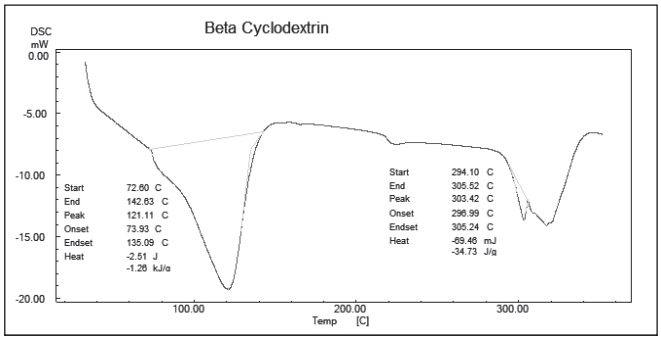


Fig. 1: DSC – β cyclodextrin

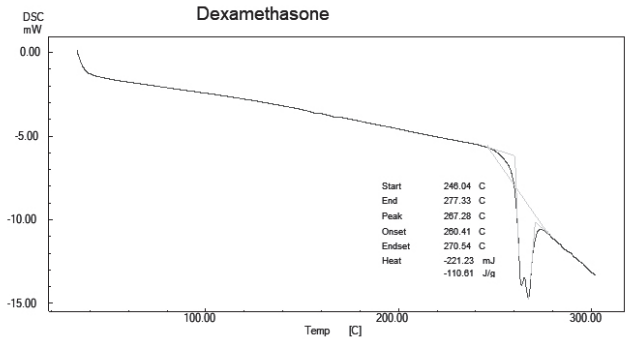


Fig. 2: DSC - Dexamethasone

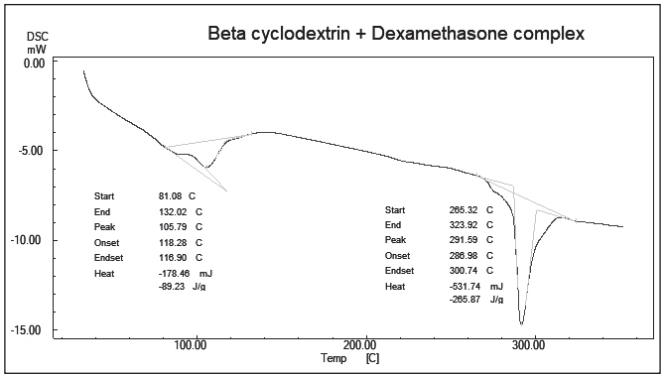
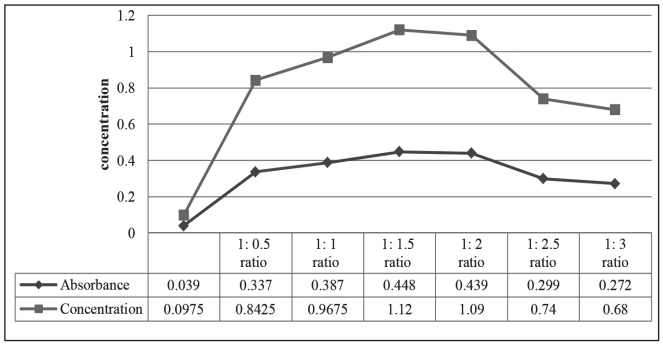


Fig. 3: DSC - β cyclodextrin + dexamethasone



		1: 0.5 ratio	1: 1 ratio	1: 1.5 ratio	1: 2 ratio	1: 2.5 ratio	1: 3 ratio
Absorbance	0.039	0.337	0.387	0.448	0.439	0.299	0.272
Concentration	0.0975	0.8425	0.9675	1.12	1.09	0.74	0.68

Fig. 4: Graph of absorbance v/s concentration

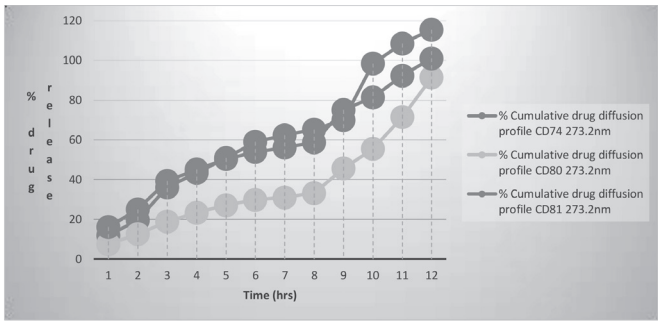


Fig. 5: % Cumulative release of ciprofloxacin

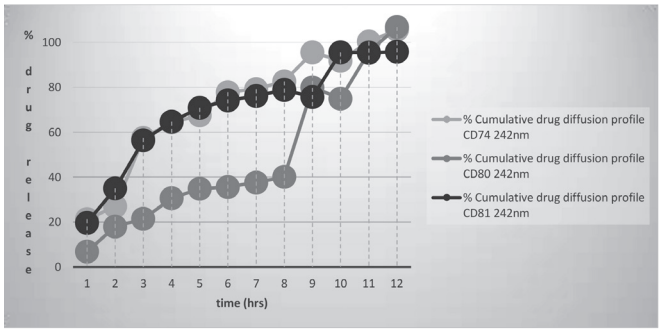


Fig. 6: % Cumulative release of dexamethasone

Surface pH

Prepared ocular insert should be non-irritating to eye and should always be compatible with the lachrymal fluid. The surface pH was checked by dissolving prepared films in 0.1 mL of double distilled water at room temperature. The swollen films were placed in a digital pH meter and the surface pH was recorded[13, 14].

Drug content

Formulated films were cut into 1 cm x 1 cm and dissolved in 10 mL phosphate buffer pH 6.8. 1mL from above solution was further diluted to 10 mL, and analysed using UV-visible spectrophotometer at the absorbance value of 273.2 nm and 242 nm, respectively[15, 16].

Table VII: MIC determination of film CD 80 and pure drug

Concentration µg mL^{-1}	128	64	32	16	8	4	2	1	0.5	0.25	0.125	NC	MC	PC
Turbidity in CD 80	-	-	-	-	-	-	-	-	-	-	+	-	-	+
Turbidity in pure drug	-	-	-	-	-	-	+	+	+	+	+	-	-	+

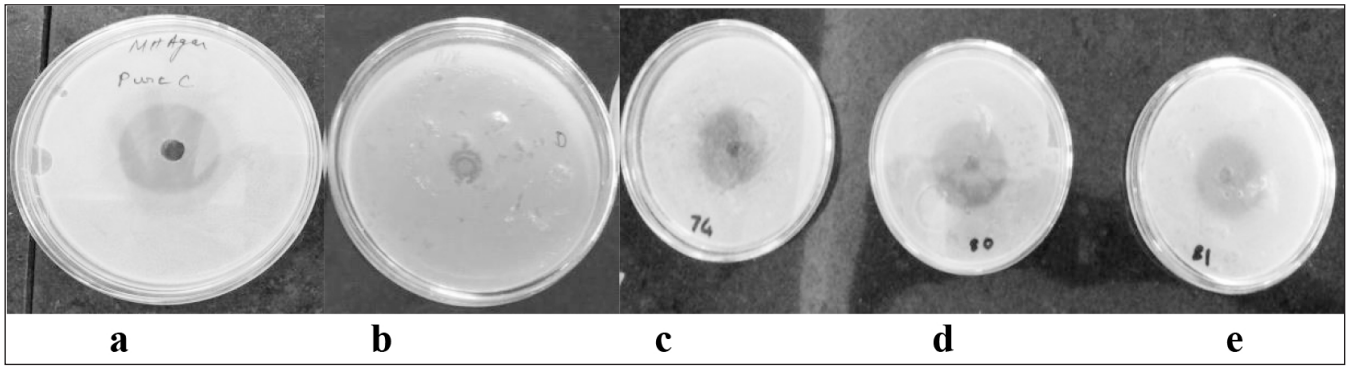


Fig. 7: Zone of inhibition a) ciprofloxacin b) dexamethasone c) CD 74 d) CD 80 and e) CD 81

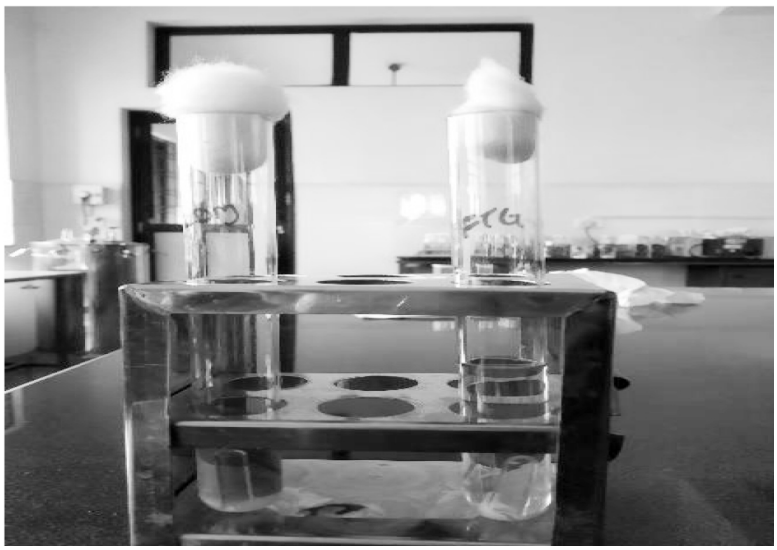


Fig. 8: a) Soyabean casein media and b) fluid thioglycolate media

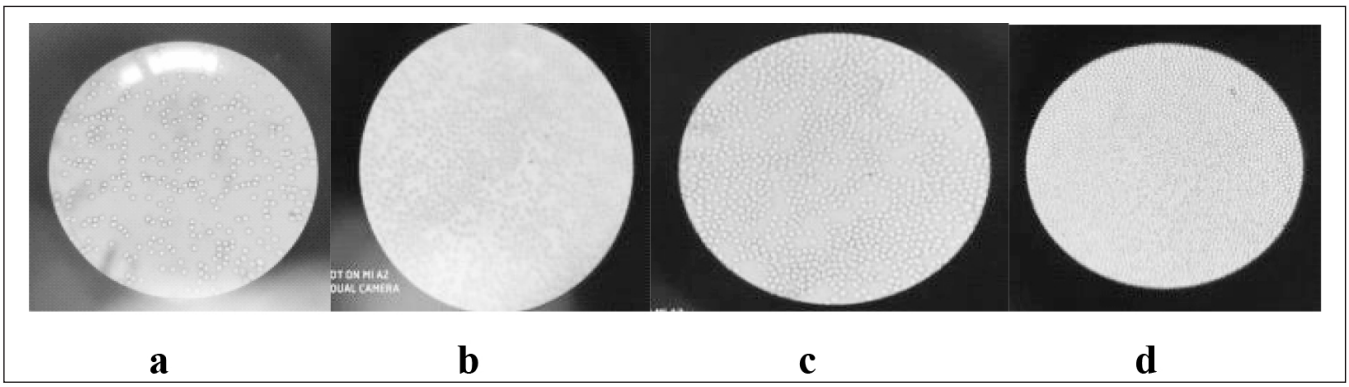


Fig. 9: a) Isotonic std b) isotonic film c) hypertonic std and d) hypotonic std

In vitro drug release study

Franz Diffusion Cell i.e the bi-chambered donor-receiver compartment model, was employed to determine the *in vitro* drug release. This cell was placed on the magnetic stirrer at ambient room temperature. Semi-permeable membrane (dialysis membrane 50, HiMedia) was used at the receptor site. Eye blinking movement was artificially designed by the magnetic stirrer and the rpm at which it swirled. 1 mL of the sample from the receptor compartment was withdrawn at periodic intervals and replaced by 1 mL phosphate buffer. Withdrawn samples were analysed and drug release was calculated[17].

Antimicrobial activity

The cup-plate technique with agar diffusion medium was used. The cup was bored at the centre of the plate. The developed film and standard solution of pure drug were taken separately into soybean casein digest medium earlier seeded with *Staphylococcus aureus* organism. The plates were incubated for a day at 37 ^{0}C after placing the film and standard solution in the plate. Zone of inhibition (ZOI) of the samples and standard were calculated and compared[18].

Sterility testing

Indian Pharmacopoeia 1996 procedure was employed, wherein fluid thioglycolate and soybean casein digest media were used. Experiment should be strictly performed under laminar air flow; the formulated films were cut into two equal halves and dropped in the two test tubes simultaneously. Incubation time was 7 days at 37 ^{0}C. The results were compared with positive and negative control samples[19].

Isotonicity evaluation

Tonicity of the film is a must as tissue damage can result if tonicity of the film is not maintained. Sodium chloride solutions of three different concentrations, namely, hypertonic (HT – 3 % w/V), hypotonic (HP - 0.2 % w/V) and isotonic (IS - 0.9 % w/V) concentrations, were prepared. Four clean slides were taken and labelled as HT, HP, IS and Test. A drop of blood with heparin (1 % w/V) was taken to prevent coagulation and further placed on all slides. Optimized film drop was placed on test slide and all four slides were covered with cover slip and checked under 45 x magnification microscope. Morphology of RBC's was studied[20, 21].

Antibacterial activity

Serial dilution method was employed to carry out the microbiological assay. *S. aureus* was the test organism employed. Two samples tested for minimum inhibitory concentration (MIC) were coded as A (film) and B (pure sample). The concentration of pure drug taken was 5 mg mL^{-1}. 51 µL of this drug solution contains 256 µg of the drug. Series of 14 test tubes was taken and numbered as 1 – 14. In 1st test tube, 2000 µL of broth was added and from 2nd test tube till 14th test tube, 1000 µL broth was added. 51 µL of broth from test tube 1st was withdrawn and discarded and replaced with drug solution. Concentration corresponds to 128 µg of drug. 1000 µL of the content from 1st test tube was transferred to 2nd and so on. This procedure was repeated till second last test tube corresponding to 128, 64, 32, 16, 8, 4, 2, 1, 0.5 and 0.25 µg mL^{-1}. The last test tube served as negative control. 10 µL of *S. aureus* broth was added in each tube except negative and kept for incubation at 37 ^{0}C for 24 h. Further MIC was calculated, and results are tabulated in Table VII[22].

Short term stability studies

Efficacy, safety and quality of the fabricated film was checked through stability testing. Optimized film was subjected to stability studies at room temperature (25 ^{0}C) for a period of 3 months. The samples were withdrawn at 30, 60 and 90 days' time period, and evaluated for parameters like surface pH, drug content and *in vitro* drug release[23].

RESULTS AND DISCUSSION

Results of preformulation studies performed on drug are represented in Table II.

IR Spectra

IR spectra of pure drugs and excipients were plotted and compared with standard samples. Drugs and excipients were found to be compatible with each other, as represented in Table III.

DSC

DSC was employed to understand thermal properties of dexamethasone and β – cyclodextrin. Due to glass transition, endothermic peaks were observed for dexamethasone at 267.2 ^{0}C, for β – cyclodextrins at 121.1 ^{0}C and for complex of dexamethasone with beta cyclodextrins at 105.7 ^{0}C, 291.5 ^{0}C, respectively, as shown in Figs. 1, 2, 3.

Determination of linearity and range

For ciprofloxacin and dexamethasone, linear calibration curve were obtained in the concentration

range of 3-15 µg mL^{-1} at λ_{max} 273.2 nm and 242 nm, respectively. It followed Beer-Lamberts law with regression coefficient (R^2) value of 0.999 for both ciprofloxacin and dexamethasone.

Preparation of beta cyclodextrin and dexamethasone complex

Dexamethasone and β-cyclodextrins in complex were prepared in six different molar ratios before incorporating in the ocusert development. The solubility profile of the drug was checked and the ratio 1:1.5 (drug: β- cyclodextrin) was finalized based on % cumulative drug release as shown in Fig. 4.

UV estimation method was employed to calculate the drug content. UV-simultaneous estimation method was also developed for the combined dosage form. Other parameters like surface pH, tensile strength and thickness were recorded of the formulated ocuserts as depicted in Table IV.

In vitro release study

It was performed using Franz diffusion cell and it was found that formulation CD 80 gave best results compared to the other two formulations. The values of cumulative release for ciprofloxacin and dexamethasone are shown in Table V and graphical representation in Figs. 5 and 6 respectively.

ANTIMICROBIAL ACTIVITY

Measurement of ZOI by cup plate method

Zones of inhibition (ZOI) of the formulated films were compared with that of pure drug against a positive and negative control. Readings of this study are tabulated and images on ZOI's are depicted in Table VI and Fig.7.

Sterility testing

When the formulation was incubated for the prescribed time and temperature, no turbidity was observed. This indicates that it passes the test for sterility, as shown in Fig. 8.

Isotonicity evaluation

Isotonicity test proved that the optimized film produces no change in the blood cells, neither bulging nor shrinking, as shown in Fig. 9. This proves that the formulated film is isotonic in nature.

Antibacterial activity

The MIC concentration was found to be 0.25 µg mL^{-1} for the film and 4 µg mL^{-1} for the pure drug, as shown in Table VII. Turbidity in the mentioned concentration indicates growth of organism.

Short term stability studies

Stability studies proved that the formulations CD 74, CD 80 and CD 81 showed no significant variations. Drug content varied slightly but were not significant, this variation in drug content can be attributed to moisture content. Thus, from the results we can interpret that films can be stored at room temperature for a short term period.

CONCLUSION

The formulated ocular films prove to be a novel drug delivery system with a promising approach in achieving greater drug absorption in comparison to the conventional ocular drops. CD 80 was the best amongst the three formulations in terms of drug content, *in vitro* drug release and anti-microbial activity. No interactions between drugs, excipients and β – cyclodextrin were observed in the optimized film when characterized with IR and DSC studies. Hence, combination of ciprofloxacin and dexamethasone as an ocular film could serve as a boost for the researchers, and as a boon to the patients in the future over the conventional ocular dosage forms.

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