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FORMULATION, DEVELOPMENT, AND EVALUATION OF OCUSERTS FOR QUINOLONE ANTIBIOTICS

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ABSTRACT

Ophthalmic films are the best sustained-release ocular drug delivery system. The present study targets developing such ocular films of antibacterial with corticosteroid and evaluating its potential. Conventional ocular dosage forms have low bioavailability with low therapeutic response. The solvent cast method employed in ophthalmic films has triggered the minds of researchers to design sustained drug delivery systems overcoming pre-corneal constraints. The compatibility of antibacterial with Loteprednol Etabonate added polymers, excipients were evaluated through preformulation studies. Different combinations of ocular inserts with antibacterial drug and corticosteroid, excipients were formulated and evaluated. Formula CLE 80 (Ciprofloxacin Hydrochloride with Loteprednol Etabonate) fulfilled the needs of all organoleptic parameters like appearance, texture, surface pH, drug content, and *in-vitro* studies. The results predicted CLE 80 ophthalmic film would be a promising controlled-release formulation for better patient compliance.

Keywords: Gemifloxacin Mesylate, Ciprofloxacin Hydrochloride, Moxifloxacin, Loteprednol Etabonate, Carbopol, Ocular Inserts, And Beta-Cyclodextrin Complex.

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INTRODUCTION

The human eye serves as a portal for delivering ocular therapeutic agents for the local effects. The formulators face a constant challenge to understand the anatomy, physiology, and biochemistry of the human eye for being impervious to foreign matter. Bioavailability is poor and so is the therapeutic response of eye drops as the drug gets eliminated resulting in poor patient compliance. Fabrication and technology of ophthalmic insert in the field of controlled and sustained ocular delivery systems are gaining popularity to overcome the barriers imposed by conventional ocular dosage forms. The present work aims at formulating ophthalmic film with a definite concentration of Gemifloxacin Mesylate, Ciprofloxacin Hydrochloride, and Moxifloxacin with Loteprednol Etabonate separately for the treatment of ocular conjunctivitis and further compare for the sustained release of the active. This was designed to increase the residence time of the drug and reduce the dosing frequency which was achieved by combining it with Carbopol 974, 980, 981, PEG 400, polyvinyl alcohol, and glycerine.[1, 2]

EXPERIMENTAL

Gemifloxacin Mesylate was gifted by Glenmark Pharmaceuticals, Solan, Himachal Pradesh, Ciprofloxacin hydrochloride, and Moxifloxacin was obtained from Indoco Remedies, Verna, Goa, and Loteprednol Etabonate was from Ajanta Pharma, Pvt, Ltd. Carbopol 974, 980 and 981 were gifted by Lubrizol Pvt, Ltd, Mumbai. PVA, PEG 400, and Beta cyclodextrins used were procured from Hi-Media. Analytical-grade chemicals were used for analytical purposes.

Preformulation Studies

Preformulation studies were performed on each pure drug procured and excipients used to formulate the ocular inserts concerning the description, solubility, and ultraviolet (UV) spectroscopic studies [3].

UV Spectroscopy Study

Determination of wavelength of Maximum Absorption

Pure Gemifloxacin Mesylate, Ciprofloxacin Hydrochloride, Moxifloxacin, and Loteprednol Etabonate were weighed separately and diluted in distilled water. All solutions were scanned in the wavelength region of 200 – 400 nm using a UV-visible spectrophotometer (UV- Shimadzu make).

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Determination of Linearity and Range

25mg of each pure drug were weighed separately and dissolved in a solvent. From the above stock, aliquots of working standard solution of Antibacterial and Loteprednol Etabonate were transferred to a series of 10 ml standard volumetric flask and diluted with Phosphate buffer pH 6.8 to get 3.0 µg /ml till 15µg /ml for antibacterial drugs and 2.5µg/ml till 20µg/ml concentration for Loteprednol Etabonate. Each solution was estimated in a UV- spectrophotometer at a wavelength specified for each pure drug. A graph of concentration against absorbance was plotted and the Beer's Lambert law was verified.[4] The absorbance measured is tabulated in Table-1 and 2 and the standard curve is shown in Fig.-1, 2, 3, and 4.

Table-1: Absorbance of Gemifloxacin Mesylate, Ciprofloxacin Hydrochloride, and Moxifloxacin

Concentration	Gemifloxacin Mesylate Absorbance (263.8nm)	Ciprofloxacin Hydrochloride Absorbance (273.20nm)	Moxifloxacin Absorbance (292.7nm)
0	0	0	0
3 µg/ml	0.343 ± 0.015	0.29 ± 0.015	0.3 ± 0.015
6 µg/ml	0.635 ± 0.02	0.54 ± 0.02	0.590 ± 0.02
9 µg/ml	0.980 ± 0.02	0.872 ± 0.02	0.92 ± 0.025
12 µg/ml	1.323 ± 0.01	1.169 ± 0.01	1.21 ± 0.023
15 µg/ml	1.633 ± 0.015	1.462 ± 0.015	1.47 ± 0.023

Table-2: Absorbance of Loteprednol Etabonate

Concentration	Loteprednol Etabonate Absorbance (245.8nm)
0	0
2.5 µg/ml	0.09 ± 0.015
5 µg/ml	0.171 ± 0.02
7.5 µg/ml	0.263 ± 0.015
10 µg/ml	0.345 ± 0.015
12.5 µg/ml	0.429 ± 0.02
15 µg/ml	0.53 ± 0.02
17.5 µg/ml	0.622 ± 0.01
20 µg/ml	0.688 ± 0.015

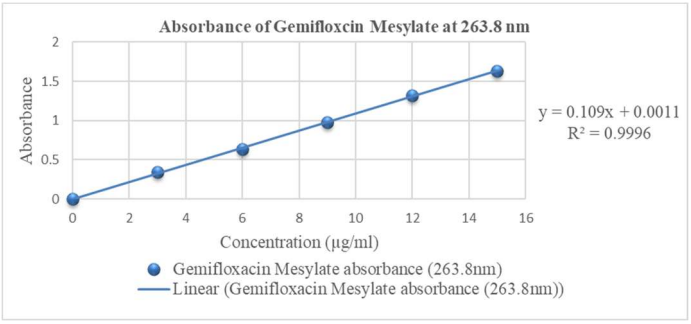

Fig.-1: Calibration Curve of Gemifloxacin Mesylate

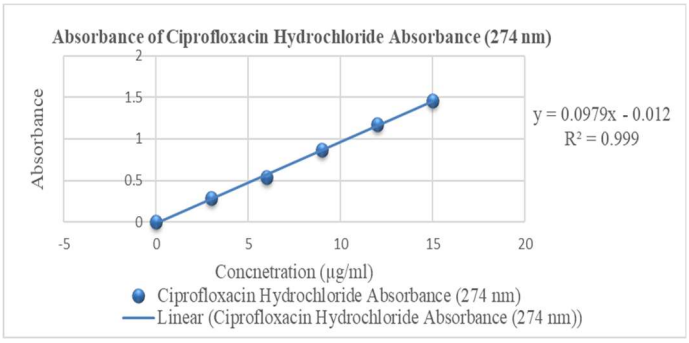

Fig.-2: Calibration Curve of Ciprofloxacin Hydrochloride

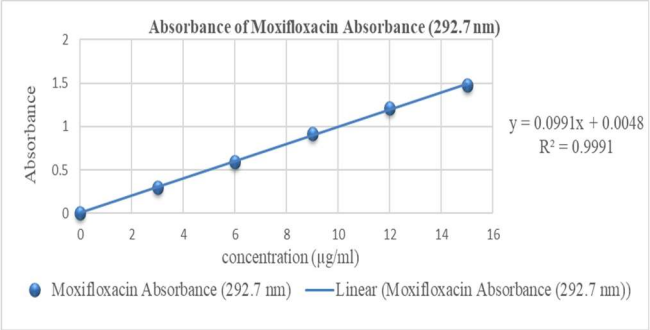

Fig.-3: Calibration Curve of Moxifloxacin

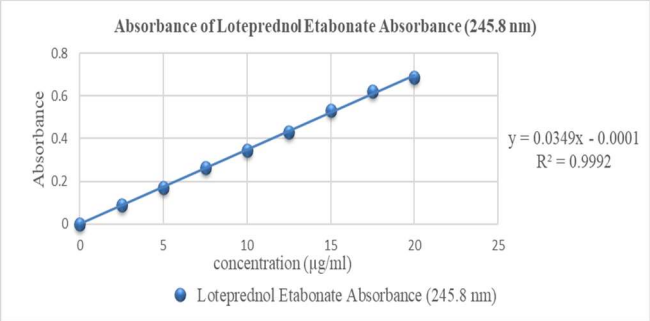

Fig.-4: Calibration Curve of Loteprednol Etabonate

DSC

The thermal property of the drug and excipients alone and in combination was studied using DSC (DSC-60 Shimadzu, TA-60 WS collection software).

IR

The FT-IR spectrum of the obtained pure drug sample was compared with the reference standard FT-IR spectrum of Gemifloxacin Mesylate, Ciprofloxacin Hydrochloride, Moxifloxacin, and Loteprednol Etabonate by potassium bromide method.

Preparation of Beta Cyclodextrin and Loteprednol Etabonate complex

A poorly water-soluble drug, Loteprednol Etabonate was complexed with β-Cyclodextrin, and six different molar ratios were prepared and evaluated based on % cumulative drug release.[5]

Preparation of Ocusert of Gemifloxacin Mesylate and Loteprednol Etabonate

Drug quantity was calculated as per the area of the petri dish taken (Table-3, 4, and 5). Proportion of 1: 9 (Carbopol: PVA) was soaked in 20ml of distilled water for 24 hours. PEG 400 and glycerin with the drug were incorporated in the above mixture and stirred for 6 hours on a magnetic stirrer. At the end of 6 hours, the mixture was poured into the mentioned Perti dishes and dried at 50^0C in a hot air oven for 4 hours. Films of dimensions 1cm^2 x 1cm^2 area were used for the evaluation purpose.[6]

Table-3: Composition of Ophthalmic Film (Gemifloxacin Mesylate with Loteprednol Etabonate)

Ingredients	Quantity		
	GLE74	GLE 80	GLE 81
Gemifloxacin Mesylate	19 mg equivalent to 0.3mg Gemifloxacin		
Loteprednol Etabonate: β CD	40 mg equivalent to 0.3mg Loteprednol Etabonate		
Carbopol 974	60mg	--	--
Carbopol 980	--	60mg	--
Carbopol 981	--	--	60mg
Poly Vinyl Alcohol	540mg	540mg	540mg
Poly Ethylene Glycol 400	0.5ml	0.5ml	0.5ml
Glycerine	25mg	25mg	25mg
Distilled Water	20ml	20ml	20ml

Table-4: Composition of Ophthalmic Film (Ciprofloxacin with Loteprednol Etabonate)

Ingredients	Quantity		
	CLE 74	CLE 80	CLE 81
Ciprofloxacin	11.5 mg equivalent to 0.18mg Ciprofloxacin		
Loteprednol Etabonate: β CD	40mg equivalent to 0.3mg Loteprednol Etabonate		
Carbopol 974	60mg	--	--
Carbopol 980	--	60mg	--
Carbopol 981	--	--	60mg
Poly Vinyl Alcohol	540mg	540mg	540mg
PEG 400	0.5ml	0.5ml	0.5ml
Glycerine	25mg	25mg	25mg
Distilled Water	20ml	20ml	20ml

Table-5: Composition of Ophthalmic Film (Moxifloxacin with Loteprednol Etabonate)

Ingredients	Quantity		
	MLE 74	MLE 80	MLE 81
Moxifloxacin	19 mg equivalent to 0.3mg Moxifloxacin		
Loteprednol Etabonate: β CD	40mg equivalent to 0.3mg Loteprednol Etabonate		
Carbopol 974	60mg	--	--
Carbopol 980	--	60mg	--
Carbopol 981	--	--	60mg
Poly Vinyl Alcohol	540mg	540mg	540mg
PEG 400	0.5ml	0.5ml	0.5ml
Glycerine	25mg	25mg	25mg
Distilled Water	20ml	20ml	20ml

Drug Content

Prepared films of dimension 1cm x 1 cm were dissolved in 10 ml phosphate buffer pH 6.8. 1ml from this stock was diluted to 10ml and analyzed using a UV-visible spectrophotometer at the absorbance values of 263.8 nm, 274 nm, 292.7 nm, and 245.8 nm respectively.[7]

***In-vitro* Drug Release Study**

Release kinetics were studied using Franz Diffusion Cell. Semi-permeable membrane (dialysis membrane 50, HIMEDIA) was used at the receptor site. 1 ml sample from the receptor compartment was withdrawn at periodic intervals and subsequently replaced with 1 ml Phosphate buffer. Withdrawn samples were analyzed and drug release was calculated using the UV simultaneous equation method.[8]

Antimicrobial Activity

Cup-plate technique with agar diffusion medium was employed to determine the zone of inhibitions. The cup was bored at the center of the plate and the developed films with drug combination and respective pure drug were taken separately into soyabean casein digest medium seeded with *Staph. Aureus* organism. Incubated for a day at 37 ^{0}C and compared with the standard.[9]

Antibacterial Activity

Serial dilution method was employed to carry out the microbiological assay. The test organism employed was *Staph. aureus.* Two samples for testing were coded as A (film) and B (pure sample) for minimum inhibitory concentration (MIC). The concentration of pure drug taken was 5mg/ml. 51µl of this drug solution contains 256µg of the drug. A series of 14 test tubes were taken numbered and kept for incubation at 37 ^{0}C for 24 hours. Further MIC was calculated and results were tabulated.[10]

RESULTS AND DISCUSSION

Results of preformulating studies performed on drug and excipients showed no incompatibilities between drug and excipient.

Drug Content

The drug content of the individual drugs in the ocular inserts was determined based on the UV-simultaneous estimation method developed and validated. Other evaluated parameters are recorded in Table-6, 7, and 8.

Table-6: Evaluated Parameters - Gemifloxacin Mesylate with Loteprednol Etabonate

Formulation code	Surface texture	Thickness (mm)*	Weight (mg)*	Tensile strength (g/cm^2)*	% Drug Content (±SD*)	
					Drug A	Drug B
GLE 74	Smooth	0.115 ± 0.03	188 ± 0.02	410 ± 0.08	96	93
GLE 80	Smooth	0.111 ± 0.01	184 ± 0.04	415 ± 0.03	70	93
GLE 81	Smooth	0.113 ± 0.02	186 ± 0.06	420 ± 0.05	90	80

Table-7: Evaluated Parameters – Ciprofloxacin Hydrochloride with Loteprednol Etabonate

Formulation code	Surface texture	Thickness (mm)*	Weight (mg)*	Tensile strength (g/cm^2)*	% Drug Content (±SD*)	
					Drug A	Drug B
CLE 74	Smooth	0.112 ± 0.04	198 ± 0.05	415 ± 0.05	70	83.33
CLE 80	Smooth	0.109 ± 0.02	185 ± 0.03	425 ± 0.08	73.33	77.78
CLE 81	Smooth	0.117 ± 0.01	192 ± 0.08	430 ± 0.03	76.66	66.67

*Data is expressed as Mean ± S.D. (n=3)

Table-8: Evaluated Parameters – Moxifloxacin with Loteprednol Etabonate

Formulation code	Surface texture	Thickness (mm)*	Weight (mg)*	Tensile strength (g/cm^2)*	% Drug Content (±SD*)	
					Drug A	Drug B
MLE 74	Smooth	0.117 ± 0.03	178 ± 0.02	400 ± 0.08	73.33	100
MLE 80	Smooth	0.113 ± 0.01	174 ± 0.04	403 ± 0.03	73.33	100
MLE 81	Smooth	0.115 ± 0.02	176 ± 0.06	402 ± 0.05	90.00	100

*Data is expressed as Mean ± S.D. (n=3)

In vitro Release Study

It was performed using Franz Diffusion Cell on the optimized films (GLE 81, CLE 80, and MLE 81) from each lot of antibacterial and Loteprednol batch. It was found that formulation CLE 80 gave the best results compared to the other two formulations. Tabulated in Table-9 and represented as Fig.-5 and 6

Table-9: Percentage Cumulative Drug Diffusion Profile

Time (hrs.)	GLE 81		CLE 80		MLE 81	
	263.8 nm	245.8 nm	274 nm	245.8 nm	292.7 nm	245.8 nm
01	14.39	8.62	14.16	8.88	12.29	17.22
02	14.86	29.72	29.02	18.21	19.47	30.70
03	18.47	32.42	36.24	18.87	24.64	41.54
04	48.38	85.32	49.56	19.27	31.79	54.94
05	63.09	89.86	56.47	28.79	39.22	66.95
06	74.37	98.82	64.22	40.23	44.98	76.21
07	100.70	100.47	67.75	46.88	51.60	89.65
08	--	--	73.84	51.22	65.55	91.43
09	--	--	74.14	52.57	80.02	95.39
10	--	--	85.64	70.50	99.19	95.42
11	--	--	93.40	78.71	--	--
12	--	--	103.66	101.74	--	--

Measurement of ZOI by Cup Plate Method

The zone of inhibitions of the optimized films was compared with that of the pure drug against a positive and negative control. CLE 80 film showed a maximum zone of inhibition of 4.1 cm.

Antibacterial Activity

The MIC concentration was found to be (0.5 µg/ml) for the Ciprofloxacin Loteprednol film and (4 µg/ml) for ciprofloxacin in comparison to other drugs and optimized films.

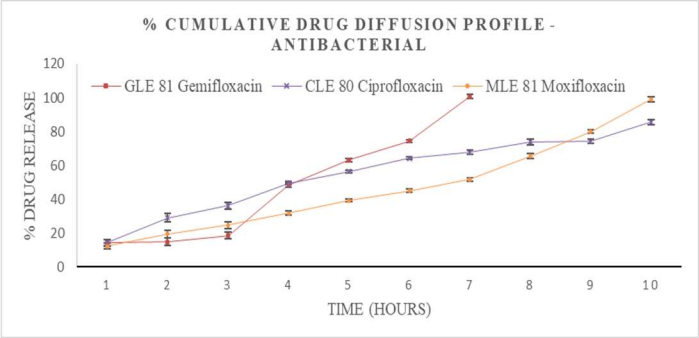

Fig.-5: Percentage Cumulative Release of (GLE 81), (CLE 80) and (MLE 81)

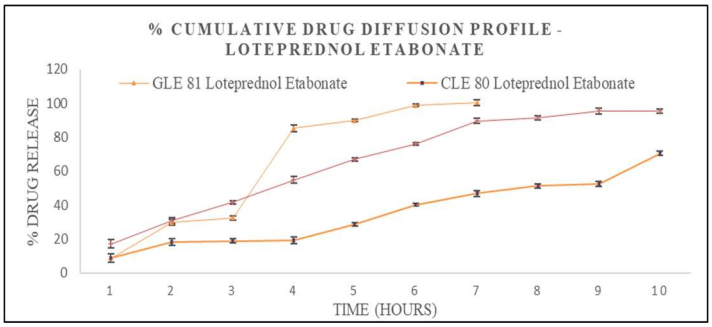

Fig.-6: Percentage Cumulative Release of Loteprednol Etabonate (GLE 81), (CLE 80) and (MLE 81)

CONCLUSION

The designed film CLE 80 (ciprofloxacin with Loteprednol Etabonate and Carbopol 980) proved to be the best amongst the three formulations Gemifloxacin with Loteprednol Etabonate and Carbopol 981 (GLE 81) and Moxifloxacin with Loteprednol Etabonate and Carbopol 981 (MLE 81), in terms of drug content, *in vitro* drug release and anti-microbial activity. Hence ocular film with Ciprofloxacin Hydrochloride and Loteprednol Etabonate serves as a boost for the researchers and a boon to the patients in the future over the conventional ocular dosage forms.

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTIONS

Swati Mayur Keny, corresponding author for this publication, is a faculty of PES's Rajaram and Tarabai Bandekar College of Pharmacy, Goa. All the above research work has been carried out on the same premises.

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[RJC- 8453//2023]