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SOLUBILITY ENHANCEMENT OF LOTEPREDNOL ETABONATE FOR DESIGNING OCULAR FILM WITH FLUOROQUINOLONE ANTIBACTERIAL

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ABSTRACT

Purpose: To formulate a novel drug delivery system for ophthalmic use for the treatment of bacterial conjunctivitis. To enhance solubility of poorly water-soluble drug loteprednol etabonate and formulate ocular inserts of fluoroquinolone moxifloxacin with loteprednol etabonate and evaluate its potential as a sustained ocular delivery system for the treatment of bacterial conjunctivitis.

Methods: Moxifloxacin and loteprednol etabonate compatibility individually and with the polymer and excipients were checked based on preformulation studies. Three different combinations of moxifloxacin and loteprednol etabonate incorporated with carbopol 974, 980, 981, PEG 400 and glycerine were formulated by solvent cast method and evaluated for clarity, smoothness, surface pH, drug content, and in-vitro drug release study.

Results: Clarity, smoothness, surface pH, drug content, and in-vitro drug release study were the various parameters evaluated on the formulated ocusert of moxifloxacin and loteprednol etabonate.

Conclusion: Formula MLE 81(Moxifloxacin with Loteprednol Etabonate and Carbopol 981) fulfilled the needs of all organoleptic parameters and also the invitro release study. Based on in vitro correlation stability

studies, it was concluded this ocular insert is a promising ocular drug delivery system for pharmaceutical researchers.

Keywords: Moxifloxacin, Loteprednol Etabonate, Carbopol 974, 980, 981, Ocular inserts and Beta-cyclodextrin complex

INTRODUCTION

Eye is a complex organ where administration of drug is a tedious job due to precorneal constrains. In the recent years, attention has been increased on two main objectives:

(A) To instil drug molecules for various ocular conditions and diseases that are poorly controlled.

(B) Improvising the existing ocular dosage forms and developing newer delivery systems in order to enhance the ocular bioavailability of existing molecules **[1, 2]**.

Ophthalmic device is used to instil ophthalmic preparations onto the external surface of the eye (topical), administered inside (intraocular) or adjacent (periocular) to the eye. In terms of sterility and osmotic pressure values, ophthalmic preparations are at par with the parental dosage forms. These also includes use of preservatives, tissue compatibility, pyrogen free intraocular dosage forms, particulate matter and suitable packaging **[3]**.

It has been studied that only a small fraction of the administered drug effectively 1% or even less of the instilled dose is ocularly absorbed as eye imposes various physiological and anatomical constraints. This factor pressurises the researchers and the clinician to develop a frequent dosing at an extremely high concentration which may results in several side effects of ophthalmic products. This above fact has triggered the researchers mind to explore and focus on specific areas in order to formulate newer ophthalmic dosage forms which will overcome the problems of conventional ocular therapy and improve the ocular bioavailability of the drug.

Conventional therapeutic dosage forms which are instilled in the ye includes solutions, suspensions and ointments. But with recent advancements in material science, the range of ophthalmic dosage forms has expanded significantly which includes gels, either preformed or spontaneous gels responsive to the ocular environment and ocular inserts, both forms reducing dosage frequency **[4, 5]**.

This present work aims at formulating ocusert with a definite concentration of moxifloxacin and beta-cyclodextrin complexed loteprednol etabonate for the treatment of ocular conjunctivitis and compared for the sustained

release of the active. The formulation was developed with the prime objective of increasing the residence time of the drug, reducing the dosing frequency by combining with Carbopol 974, 980, 981, PEG 400, Polyvinyl alcohol and glycerine **[6, 7]**.

MATERIALS AND METHODS

Materials:

Moxifloxacin was obtained as a gift sample from Indoco Remedies, Pvt Ltd, Verna, Goa and Loteprednol Etabonate from Ajanta Pharma Private Limited. Carbopol 974, 980 and 981 were gifted by Lubrizol Pvt, Ltd, Mumbai. PVA, PEG 400 and Beta cyclodextrins used were procured from Hi-Media Laboratories Pvt Ltd, Nashik. All chemicals used were of analytical grade.

Methods:

Preformulation Studies:

Preformuation studies like description, melting point, solubility, IR spectra's, UV spectroscopic studies and Differential Scanning Calorimetry (DSC) were performed on the procured drug samples and excipients to confirm its compatibilities **[8]**.

UV Spectroscopy Study:

Determination of wavelength of maximum absorption.

Pure moxifloxacin and loteprednol etabonate were weighed separately and diluted with methanol and subsequently with phosphate buffer. The prepared solutions were scanned in the wavelength region of 200 – 400 nm. Shimadzu make UV-visible spectrophotometer was employed for the procedure.

Determination of Linearity and Range:

Moxifloxacin and loteprednol etabonate 25 mg each were weighed separately and transferred in two different 25-ml volumetric flask. Methanol was used to solubilise the drug and was diluted up to mark to give a stock solution having strength of 1 mg/mL. It is further diluted to get a stock solution of strength 100 µg/mL.

Aliquots of 0.3 ml, 0.6 ml, 0.9 ml, 1.2 ml, 1.5 ml of working standard solution of individual drugs were transferred to a series of 10 ml standard volumetric flask and diluted with phosphate buffer pH 6.8 to get 3, 6, 9, 12 and 15 µg/mL of moxifloxacin and loteprednol etabonate respectively.

Wavelength selected were 292.7 nm and 248.5 nm for moxifloxacin and loteprednol etabonate respectively, against the blank solution prepared using methanol and phosphate buffer pH 6.8. The beer's lambert law was verified from the calibration curve by plotting the graph of concentration against absorbance **[9]**.

DSC:

The developed complex of beta-cyclodextrins and loteprednol etabonate and its thermal

property of alone and in combination were carried out using DSC (DSC-60 Shimadzu, TA-60 WS collection software). Endothermic and exothermic parameters of the drug and polymer were subsequently obtained **[10]**.

IR:

The FT-IR spectrum of the obtained sample was compared with the reference standard FT-IR spectrum of moxifloxacin and loteprednol etabonate by potassium bromide method.

Preparation of Ocusert:

Preparation of Beta cyclodextrin and Loteprednol Etabonate complex.

Loteprednol etabonate is a poorly-water soluble drug belonging to corticosteroid class. Solubility was thus enhanced by complexing it with β-cyclodextrin. Six different molar ratios were prepared and evaluated. The solubility profile of the drug was checked and the ratio to be used (Drug: β cyclodextrins) was finalized based on % cumulative drug release **[11]**.

Preparation of Ocusert of Fluoroquinolone Antibacterial with Loteprednol Etabonate.

Area of the chosen 9 cm petri dish was calculated and based on this area, drug to be incorporated was estimated. Carbopol to PVA (cold water dissolving) ratio of 1:9 was dissolved in 20ml of distilled water and kept undisturbed for 24 hours. After the completion of 24 hours the weighed amount of drug moxifloxacin and loteprednol etabonate complexed with beta-cyclodextrin, PEG 400 and Glycerine were subsequently incorporated in it with continuous stirring on magnetic stirrer for 6 hours at approximately 100 rpm. At the end of 6^{th} hour, the entire mixture was poured in the mentioned perti dishes and dried at 50^0 C for 4 hours. Thin films of 1cm x 1cm area were utilised for the various evaluation parameters **[12]**. The composition of the ocular film is tabulated in **Table 1**.

Table 1: Composition of Ocusert

Ingredients	Quantity		
	MLE 74	MLE 80	MLE 81
Moxifloxacin	19 mg equivalent to 0.3 mg Moxifloxacin		
Loteprednol Etabonate: βeta Cyclodextrin	40mg equivalent to 0.3mg Loteprednol Etabonate		
Carbopol 974	60 mg	--	--
Carbopol 980	--	60 mg	--
Carbopol 981	--	--	60 mg
Poly Vinyl Alcohol	540 mg	540 mg	540 mg
Poly Ethylene 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

Surface pH: Compatibility between the developed ocular insert with lachrymal fluid has to be established as to confirm non irritancy to eye. The prepared films were dissolved in 0.1 ml of double distilled water at room temperature. The surface pH was recorded by placing digital pH meter on the swollen films **[13-15]**.

Drug Content: Prepared film of the dimension 1cm x 1 cm film was cut and dissolved in 10 ml phosphate buffer pH 6.8. 1ml was further diluted to 10 ml and analysed using UV-visible spectrophotometer at the absorbance value of 292.7 nm and 248.5 nm respectively **[16-18]**.

In Vitro drug release study: Franz Diffusion Cell, bi-chambered donor-receiver compartment model was employed to determine the in vitro drug release of the developed ocular insert. Magnetic stirrer was used with ambient room temperature to study the release kinetics. Receptor compartment was created using semi-permeable membrane (dialysis membrane 50, HIMEDIA). Stirring rpm was minimum just closely relating to eye blinking movement. At periodic intervals 1ml sample was withdrawn and were simultaneously replaced by 1 ml Phosphate buffer. Withdrawn sample were analysed and drug release was calculated **[19, 20]**.

Antimicrobial activity: Agar diffusion medium using a cup-plate technique was employed wherein cup was bored at the centre of the plate. The developed film, standard solution of pure drug was taken separately into soyabean casein digest medium earlier seeded with Staphylococcus aureus organism. On placing the film and standard solution in the plate, they were incubated for a day at 37^0 C. Zone of inhibition were calculated and compared with the standard **[21]**.

Sterility Testing: Standard procedure of Indian Pharmacopoeia 1996 for sterility testing was employed, wherein fluid thioglycolate and soyabean casein digest media were used. Procedure was strictly followed under laminar air flow, wherein the formulated films were cut into two equal halves and dropped in the two test tubes simultaneously. It was further incubated at 37^0 C for seven days and checked for microbial growth. Positive and negative control samples were employed to compare the results **[22]**.

Isotonicity evaluation: Tissue damage possibility increase if the 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. 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, 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 45x magnification microscope. Morphology of RBC's was studied **[23]**.

Antibacterial Activity:

Serial Dilution method was employed for carrying out microbiological assay. Test organism employed was Staphylococcus aureus. Two samples tested for minimum inhibitory concentration (MIC) were coded as A (film) and B (pure sample). For MIC the compounds are tested against Staphylococcus aureus. The concentration of pure drug taken was 5 mg/ml. 51 µl of this drug solution contains 256 µg of the drug. Series of 14 test tubes were taken and numbered as 1 – 14. To 1st test tube 2000 µl of broth is added. And from 2nd test tube till 14th test tube 1000µl 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 is transferred to 2nd and so on. This procedure is repeated till second last test tube corresponding to 128 µg/mL, 64 µg/mL, 32 µg/mL, 16 µg/mL, 8 µg/mL, 4 µg/mL, 2 µg/mL, 1 µg/mL, 0.5 µg/mL and 0.25 µg/mL. The last test tube serves as negative control. 10 µl of Staphylococcus aureus broth is added in each tube except negative and kept for incubation at 37^0 C for 24 hours. Further MIC was calculated and results were tabulated **[24]**.

Short term Stability Studies: Stability testing was done to check the efficacy, safety and quality of the active used in the product. Optimized film was subjected to stability studies at room temperature 25^0 C for a period of three months. The samples were withdrawn at 30 days, 60 days and 90 days' time period and evaluated for parameters like surface pH, Drug content and in vitro drug release **[25, 26]**.

RESULTS AND DISCUSSION

Results of preformulation studies performed on drug and excipients are displayed in **Table 2**.

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 3**.

DSC was employed to understand thermal properties of Loteprednol Etabonate and Beta cyclodextrins. Due to glass transition endothermic peaks were observed for Loteprednol Etabonate 240.14 ^{0}C, Beta cyclodextrins at 121.1 ^{0}C and Complex of Loteprednol Etabonate with Beta

cyclodextrins at 108.83 ^{0}C, 232.40 ^{0}C respectively as depicted in **Figure 1-3**.

Linear calibration curve was obtained for Moxifloxacin and Loteprednol Etabonate in the concentration range of 3-15 µg/mL at λ_{max} 292.7 nm and 245.8 nm respectively. It followed Beer's Lamberts Law with regression coefficient (R^2) value of 0.999 for both Moxifloxacin and Loteprednol Etabonate.

UV-simultaneous estimation method was employed to determine the drug content from the combined dosage form. Other evaluated parameters of the prepared ocular films with respect to surface pH, Tensile strength, thickness was recorded as shown in **Table 4**.

In vitro Release Study was performed using Franz Diffusion Cell on all three films of Moxifloxacin Loteprednol Etabonate (MLE 74, MLE 80 and MLE 81) and it was observed that Formulation MLE 81 gave best results amongst the two optimised films. The values are depicted in **Table 5** and graphically represented as shown in **Figure 4, 5**.

Antimicrobial activity was demonstrated as zone of inhibitions of the formulated films and compared to that of pure drug against a positive and negative control. Results are displayed in **Table 6**. Zone of inhibitions of the ocular films are represented as **Figure 6**.

Sterility Testing was performed where the films were incubated for prescribed time and temperature, no turbidity was observed. This indicates it passes the test for sterility as shown in **Figure 7**.

Isotonicity test proved that the optimized film produces no change in the blood cells, neither bulging nor shrinking as shown in **Figure 8**. This proves the formulated film is isotonic in nature.

The Minimum Inhibitory Concentration was found to be 1µg/mL for the optimised film (MLE 81) and 2µg/mL for the pure drug as displayed in **Table 7**. Turbidity below the mentioned concentration indicates growth of organism.

Table 2: - Preformulation study on Drug and Excipients

Observed parameters	Moxifloxacin	Loteprednol Etabonate
Description	Moxifloxacin is pale yellow colour amorphous powder	It is white – off white amorphous powder
Melting Point	324.5 °C	221°C
Solubility	Freely soluble in water, DMSO, DMF, Methanol and Ethanol	0.0336mg/ml soluble in water, low solubility in DMSO, DMF, Ethanol and Methanol

Table 3: IR interpretation

Functional groups	Moxifloxacin (a)	Loteprednol etabonate (b)	A + B + Carbopol 974	A+ B+ Carbopol 980	A+ B+ Carbopol 981
O-H	3257.80	3419.79	3334.92	3421.72	3421.72
Aromatic C-H	3066.82	2941.44	2966.52	3066.82	3064.89
Aliphatic C-H	2953.02	2875.86	2931.80	2931.80	2927.94
C=O(Ketone)	1708.93	1714.72	1745.48	1745.48	1745.48
C - F	1454.33	1107.14	1107.14	1107.14	1107.14

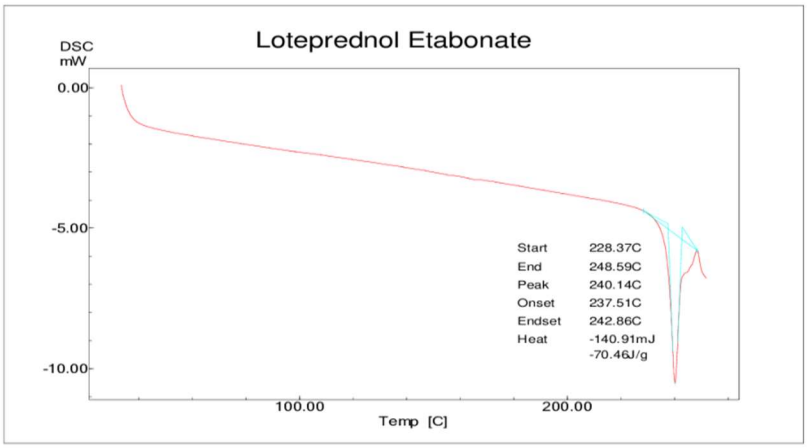

Figure 1: Thermal Analysis of Loteprednol Etabonate

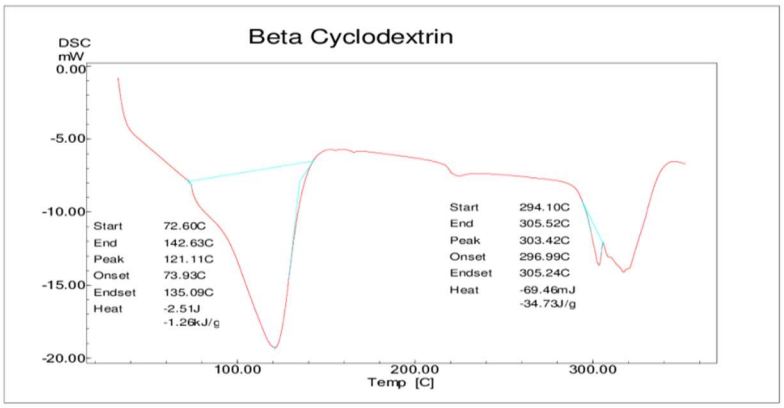

Figure 2: Thermal Analysis of Beta Cyclodextrin

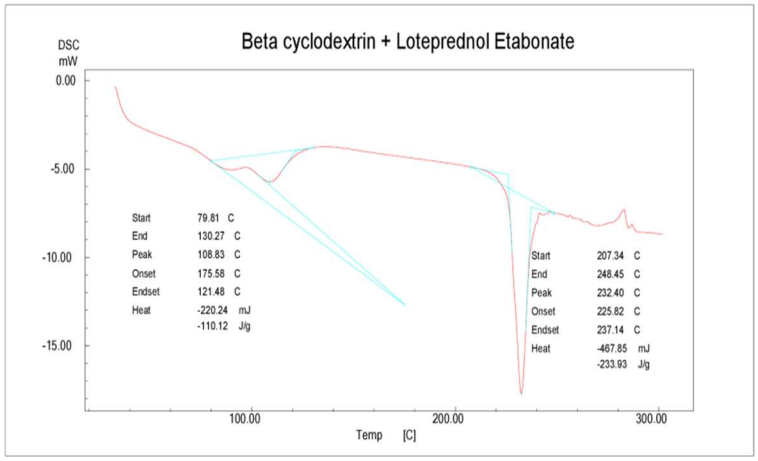

Figure 3: Thermal Analysis of Dexamethasone + Beta Cyclodextrin

Table 4: Evaluated parameters

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

Table 5: % Cumulative drug diffusion profile

% Cumulative drug diffusion profile						
Time (hours)	MLE74		MLE 80		MLE 81	
	294 nm	245.8 nm	294 nm	245.8 nm	294 nm	245.8 nm
01	16.33	7.07	17.84	13.65	12.29	17.22
02	23.28	10.6	21.96	26.08	19.47	30.70
03	27.63	19.31	28.91	39.70	24.64	41.54
04	33.89	24.13	39.08	50.45	31.79	54.94
05	42.37	37.67	54.96	53.10	39.22	66.95
06	49.57	46.51	65.88	60.85	44.98	76.21
07	56.69	57.32	74.57	69.74	51.60	89.65
08	65.55	70.11	85.52	77.67	65.55	91.43
09	74.14	88.616	96.92	83.81	80.02	95.39
10	97.49	98.92	100.34	94.68	99.19	95.42

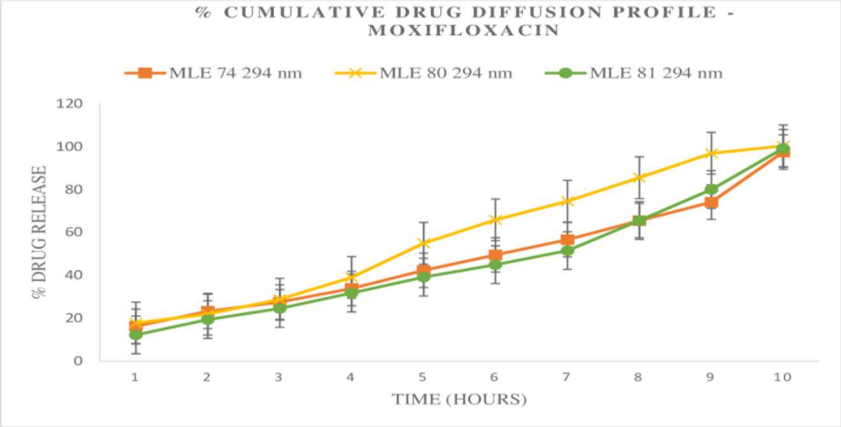

Figure 4: % Cumulative release of Moxifloxacin

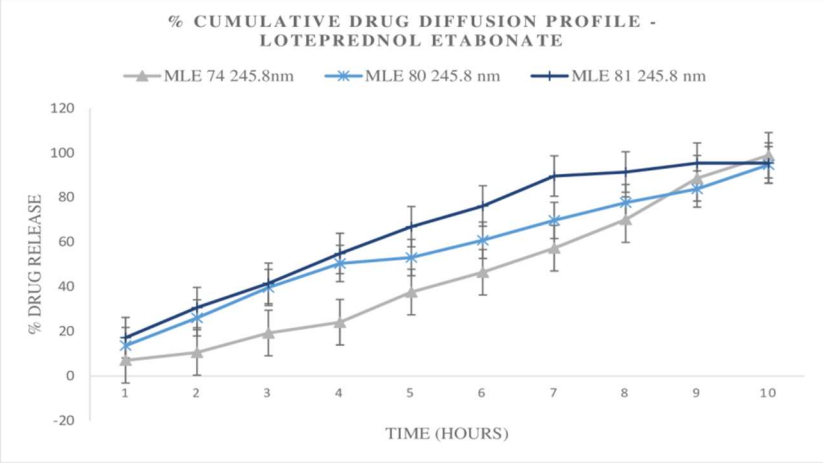

Figure 5: % Cumulative release of Loteprednol Etabonate

Table 6: Zone of Inhibition value

Formula	Zone of inhibition	
Negative Control	--	--
Positive Control	--	--
Moxifloxacin	Present	3.5 cm
Loteprednol Etabonate	Absent	0 cm
Moxifloxacin + Loteprednol Etabonate	Present	3.6 cm
MLE 74	Present	4.0 cm
MLE 80	Present	4.2 cm
MLE 81	Present	4.5 cm

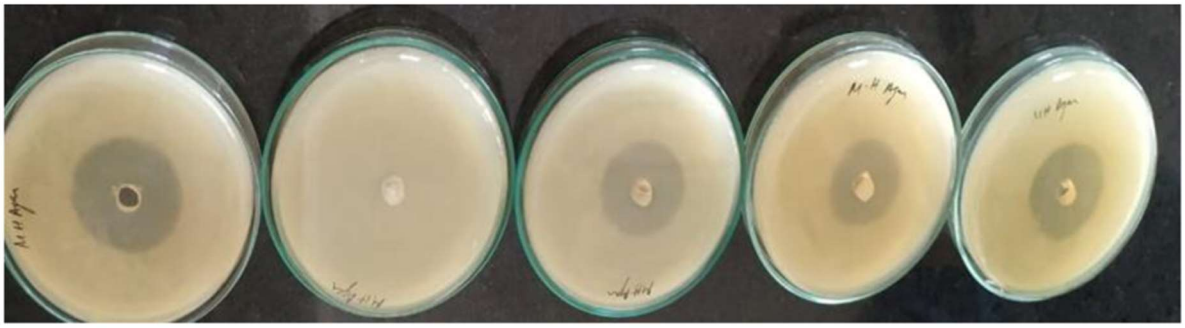

Figure 6: a) Moxifloxacin, b) Loteprednol Etabonate, c) MLE 74, d) MLE 80 and e) MLE 81

Figure 7 a) fluid thioglycolate media, b soyabean casein media

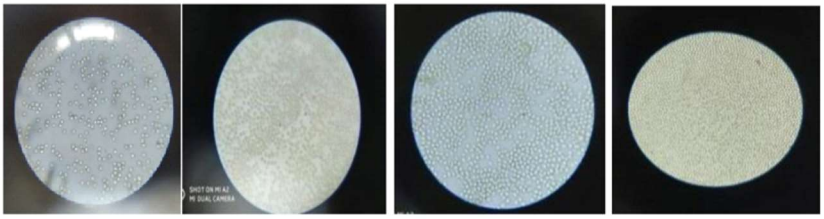

Figure 8: a) Isotonic standard, b) Isotonic film, c) Hypertonic standard and d) Hypotonic standard

Table 7: MIC determination of film MLE 81 and the pure drug Moxifloxacin

Concentration µg /mL	128	64	32	16	8	4	2	1	0.5	0.25	0.125	NC	MC	PC
Turbidity in MLE81	-	-	-	-	-	-	-	-	+	+	+	-	-	+
Turbidity in pure drug	-	-	-	-	-	-	-	+	+	+	+	-	-	+

NC- NEGATIVE CONTROL, MC – MEDIA CONTROL, PC – POSITIVE CONTROL

Short term stability studies proved that the formulation MLE 81 showed no significant variations. Minor changes were observed in terms of drug content but were not significant. The 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.

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.

The results concluded that film MLE 81 was the best amongst the three formulations in terms of drug content, *in vitro* drug release and anti-microbial activity. The optimized film showed no interactions between drugs, excipients and also beta cyclodextrin complex when characterized with IR and DSC studies. Hence combination of Moxifloxacin and Loteprednol Etabonate as an ocular film serves as a boost for the researchers and boon to the patients in the future over the conventional ocular dosage forms.

Ethical Issues

Not applicable.

Conflict of Interest

The authors report no conflicts of interest.

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