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THE PHARMA RESEARCH
An International Journal of Pharmacy Research

FORMULATION AND OPTIMIZATION OF OCULAR FILMS OF OFLOXACIN AND KETOROLAC TROMETHAMINE

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ARTICLE INFO

Published on: 15-03-2015
ISSN: 0975-8216

Keywords:
Ofloxacin; ketorolac tromethamine; ocular; films, carbopol, HPMC, PVPK30, ethyl cellulose

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ABSTRACT

Ocular films of ofloxacin and ketorolac tromethamine were prepared with objectives of reducing the frequency of administration, obtaining controlled release and greater therapeutic efficacy in the treatment of eye infections such as conjunctivitis, keratitis, corneal ulcers, etc. Films were prepared by solvent casting method. The ocular films were evaluated for drug-excipient interaction, physico-chemical characteristics and *in vitro* release studies by RP-HPLC method. There was no interaction between drug and excipients as revealed by IR spectra of the pure drug, medicated and placebo formulations.

All the formulations were subjected to evaluation of thickness, weight variation, folding endurance, drug content uniformity, in vitro release study, Surface pH, % Moisture absorption, Release Kinetics, and Ocular Irritation Studies. On the basis of *in vitro* drug release studies, the formulation PCP was found to be better than the other formulations and it was selected as an optimized formulation.

Introduction

Flouroquinolone is one of the promising group of antibiotics currently being used topically to treat conjunctivitis and corneal ulcers. Ofloxacin has proved to possess superior antibacterial activity *in vivo* and has better pharmacokinetic properties as compared with ciprofloxacin and norfloxacin.

Ofloxacin is a broad-spectrum antibacterial agent with activities against gram negative bacteria (*E. coli*, *Klebsiela pneumoniae*, *Serratia* species, *Proteus* species, *Pseudomonas aerogenosa* and *H. influenzae*) and gram-positive bacteria (*Staphylococcus* species, *Streptococcus enterococci*). It is used in the treatment of keratoconjunctivitis, blepharo-conjunctivitis, corneal ulcer, preoperative prophylaxis and other ocular infections. Topical application of ophthalmically active drugs is the most prescribed route of administration for treatment of various ocular disorders. It is generally agreed that the intraocular bioavailability of topically applied drugs is extremely poor. This is mainly due to drainage of the excess fluid by the nasolacrimal duct as well as dilution and elimination of the solution by tear turnover. Ocular bioavailability of

drugs is an important parameter influencing the efficacy of ophthalmic preparations.

Ketorolac tromethamine is a nonsteroidal anti-inflammatory drug, used to treat seasonal allergic conjunctivitis. At present, it is available in the form of eye drops, which need to be administered 1 or 2 drops every 15 to 30 min. initially in acute infection and 1 or 2 drops administered 4 times daily or more in severe conditions. To overcome these limitations associated with dosage regimen, an attempt has been made to formulate ocular films that may not only improve the efficiency of the therapy but also patient compliance. Several polymeric systems have been used to fabricate ocular films for better ocular bioavailability and retention to drug of which gelling systems have shown advantages of convenient administration and increased contact time.

Material and methods

Materials

Ketorolac tromethamine and ofloxacin was purchased from local vendor. HPMC K 100 M, Carbopol 934, Ethyl cellulose, PVP K 30, PEG 400, Nitroglycerin, dichloromethane, Ethanol all chemicals were of analytical grade.

Methods

The ketorolac tromethamine & ofloxacin containing ocular films were prepared based on HPMC K 100 M, Carbopol 934 & Ethyl cellulose by using solvent casting technique. Polymeric solutions were prepared by dissolving HPMC K 100 M, Carbopol 934 & Ethyl cellulose at distinct compositions along with ketorolac tromethamine, ofloxacin, PVPK30, PEG400 and nitroglycerin in dichloromethane and ethanol. Solvent was allowed to evaporate by placing the Petri dishes in oven (40 ± 2°C). Dried films were carefully removed from the Petri dish and then cut into oval shaped films with the help of a sharp edged die.

Evaluation Parameters

Prefomulation Studies:

Physical description:

Ofloxacin - a pale yellow crystalline powder.

Keterolac tromethamine - offwhite crystalline powder.

Melting point:

Melting point of the individual drugs were analysed using digital precision melting point apparatus.

HPLC analysis:

A solution of ofloxacin & ketorolac tromethamine was prepared in methanol and analysed over HPLC (Agilant) (Model 1120, compact) G4288A - gradient, low pressure binary gradient pump with UV detector. The Octadecyl Silane (ODS-3) Hypersil, (C 18 column size- 250 mm x 6 mm x 5 um) was used as the stationary phase with methanol and phosphate buffer pH 3.0 (55 : 45 v/v) as the mobile phase at a flow rate of 0.8 mL min^{-1}. Detection is performed at 270 nm.

Compatibility study by Infra-Red Spectroscopy

The spectrum was recorded with FTIR instrument (Perkin-Elmer RX-1) in the range of 400-4000 cm^{-1} using a resolution of 4 cm^{-1}, to evaluate the characteristic peaks of functional groups for both drugs separately. Individual drug samples were mixed with potassium bromide (KBr) and were pressed (hydraulic press) to obtained transparent/ semitransparent disks. All the combinations with drugs and ingredients were also analysed.

Formulation Evaluation

Physical appearance

The visual appearance of the film was conducted. The color of the film as well as the texture was observed. Drug distribution within the film was also visualized.

Thickness

The films were evaluated for the thickness of each film using a micrometer of sensitivity of 0.001 mm. The average of 10 readings was taken. The mean thickness of standard deviation was calculated.

Folding endurance

The folding endurance was expressed as the number of folds number of times the film was folded at the same place, either to break the specimen or to develop visible cracks. This test was important to check the ability of the sample to withstand folding. This also gives an indication of brittleness. The specimen was folded in the center, between the fingers and the thumb and then opened. This was termed as one folding. The process was repeated until the film showed breakage or cracks in the center of film. The total folding operations were named as folding endurance value.

Weight uniformity

The weight variation test was carried out by weighing three patches cut from different places of the same formulation and their individual weights were determine by using the digital balance. The mean value was calculated. The standard deviation of weight variation was compute from the mean value.

Surface pH determination

Films were left to swell for 5 h on an agar plate prepared by dissolving 2 % g/ml agar in warm simulated tear fluid, sodium chloride: 0.670 g, sodium bicarbonate: 0.200 g, calcium chloride 0.008 g, and purified water quantity sufficient. 100 g of pH 7.4 under stirring and then pouring the solution into Petri dish until gelling at room temperature. The surface pH was measured by means of a pH paper placed on the surface of swollen patch.

Swelling index

Swelling of the polymer depends on the concentration of the polymer, ionic strength and the presence of water. To determine the swelling index of prepared ocular films, initial weight of the film was taken, and then placed in freshly boiled and cooled artificial tear fluid pH 7.4 at 37°C. The film was removed from plate after every 1 h and surface water was removed with the help of filter paper, and film was reweighed. Swelling index was calculated.

Percentage moisture loss

The percentage moisture loss was carried out to check the integrity of the film at dry condition. The ocular films were preweighed accurately and kept in desiccators containing 100 ml of saturated solution of aluminum chloride. After 3 days, the films were taken out and weighed.

Percentage moisture absorption

The percentage moisture absorption was carried out to check the integrity of the film at dry condition. The ocular films were preweighed accurately and kept in desiccators containing 100 ml of saturated solution of aluminum chloride. After 3 days, the films were taken out and weighed.

In vitro drug release

In vitro drug release study was carried out by using biochemical donor-receptor compartment model. The commercial semi permeable egg membrane, presoaked overnight in the freshly prepared dissolution medium (STF pH 7.4) and was tied to one end of a cylinder (open at both the sides), which acted as donor compartment. The ocular film was placed inside the donor compartment in contact with the semi-permeable membrane. The donor compartment was attached to a stand and suspended in 25 ml of the dissolution medium maintained at 37 ± 1°C in the way that touches the receptor medium surface. The dissolution medium was stirred at a low speed using magnetic stirrer. The aliquots of 5 ml were withdrawn at regular intervals for 12 h. and replaced by an equal volume of dissolution medium every time. The samples were analyzed on UV spectrophotometer.

Result & Discussion

Prefomulation Studies

Physical description

Ofloxacin - a pale yellow crystalline powder.
Keterolac tromethamine - offwhite crystalline powder.

Melting point

Reported Melting point of Ofloxacin & Keterolac tromethamine standard drug is 270-275 ^{0}C & 162 ^{0}C and determined by capillary

method was 271- 272 ^{0}C & 161.5 ^{0}C, respectively.

HPLC analysis:

A solution of ofloxacin and ketorolac tromethamine was prepared in mobile phase and injected using 50µl injector loop. The retention time for the ofloxacin and ketorolac tromethamine was obtained at 4.49 min. and 5.61 min. The response obtained was a linear function of concentration over the range of 10-60 µgmL^{-1} for ofloxacin and 25-125 µgmL^{-1} for ketorolac tromethamine. (Fig. 1).

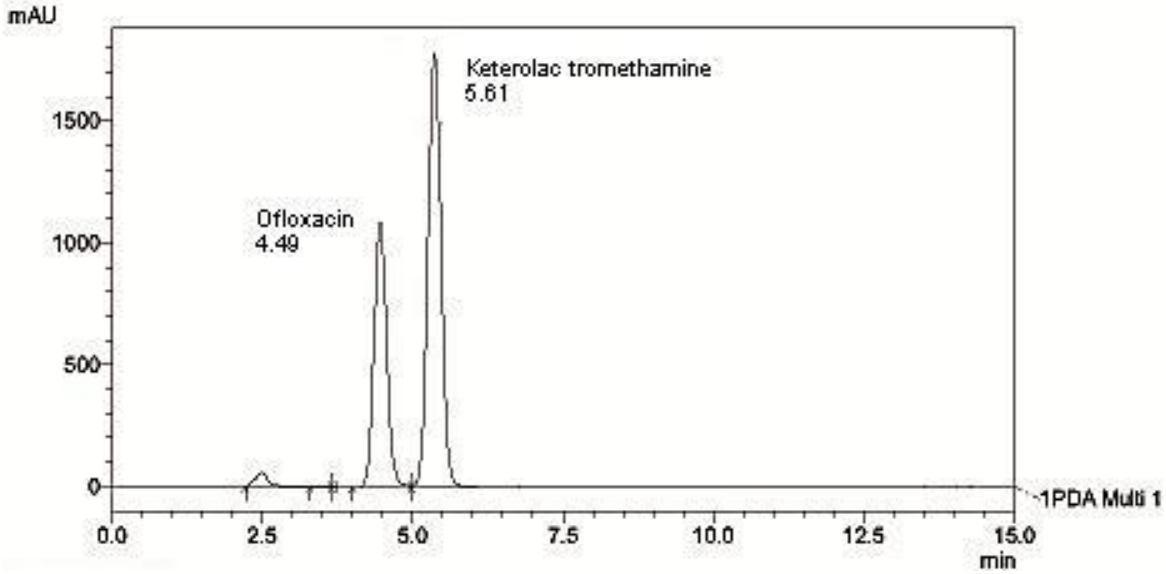

Fig 1: HPLC chromatogram of ofloxacin and keterolac tromethamine.

Compatibility studies of Ofloxacin and Keterolac tromethamine

The spectrum were recorded with FTIR instrument (Perkin-Elmer RX-1) in the range of 400-4000 cm^{-1} using a resolution of 4 cm^{-1}, to evaluate the characteristics of functional groups for both drugs separately. Individual drug samples were mixed with potassium bromide (KBr) and were pressed (hydrolic press) to obtained transparent/ semitransparent disks. All the combinations with drugs and ingredients inferred that the predicted formulation was compatible. The spectra of Ofloxacin and Keterolac tromethamine spectra is shown in fig 2 & 3.

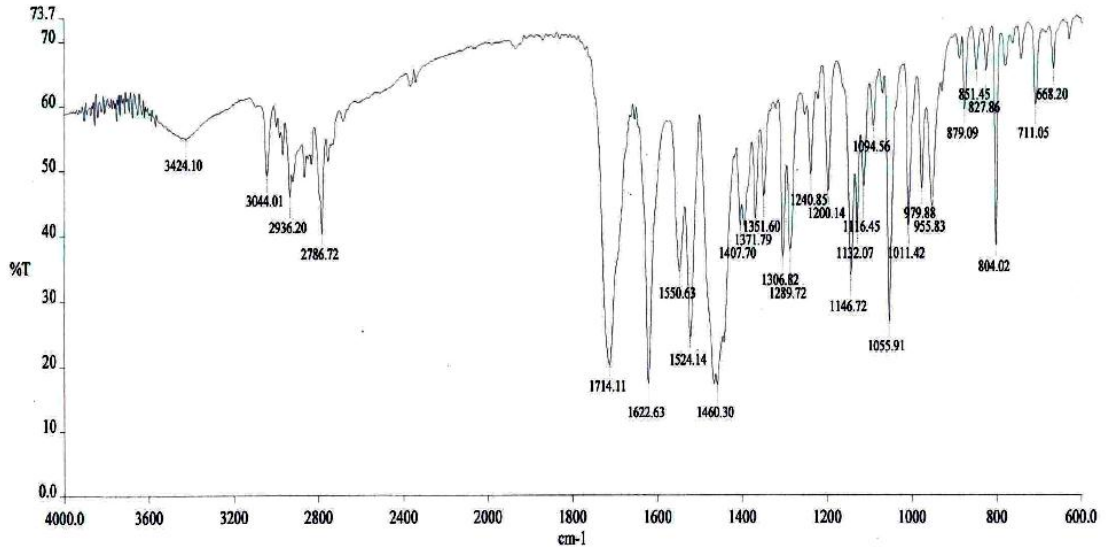

Fig. 2. FTIR spectra of Ofloxacin

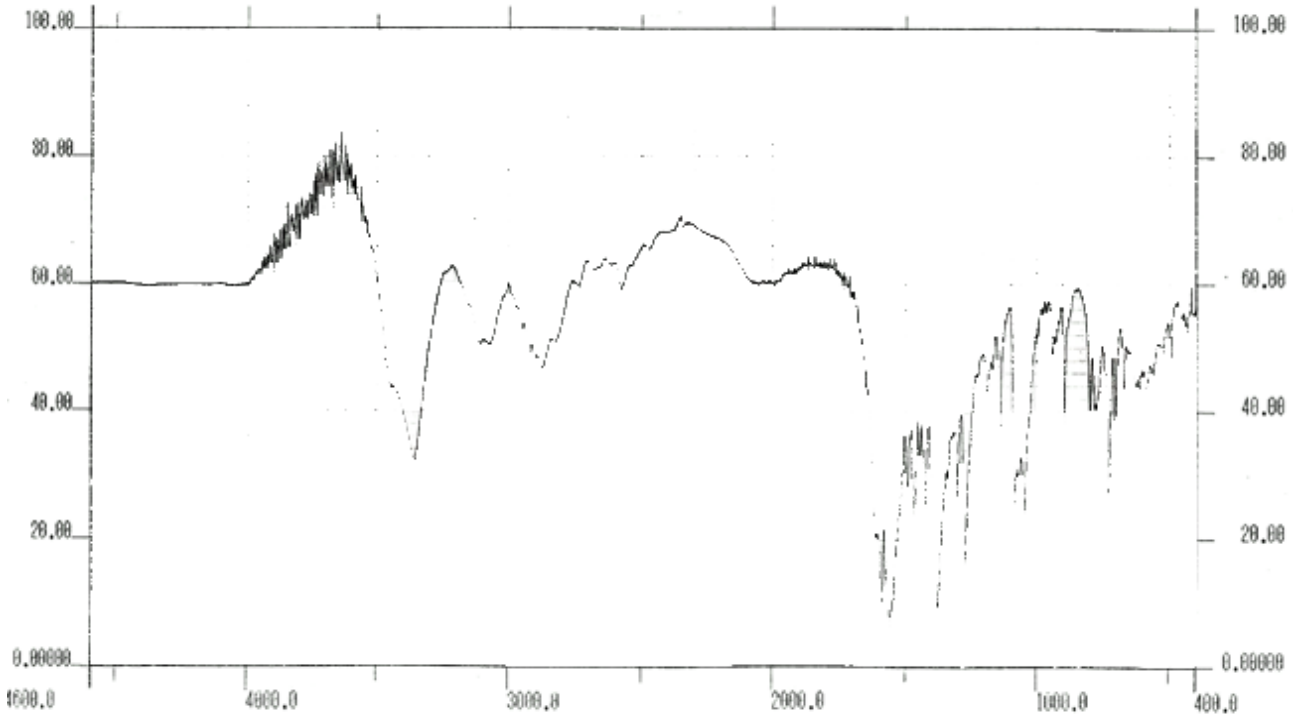

Fig. 3. FTIR spectra of Keterolac tromethamine

Evaluation Parameters

All the formulations with each polymers were evaluated for various parameters such as folding endurance, thickness, surface pH, weight variation, % moisture absorption, % moisture loss, drug release of Ofloxacin (OFL) and Ketorolac tromethamine (KT).

The results showed that the minimum folding endurance 37 with CP7 while maximum 98 with BE2, minimum thickness 3.0 with BE9 while maximum 7.2 with DC3, minimum surface pH 6.4 with BE6 while maximum 7.4 with CP3 & DC7, minimum weight variation 43.18 with CP2 while maximum 51.31 with AH9, minimum % moisture absorption 4.17 with BE1 while maximum 7.03 with AH8, minimum % moisture loss 0.92 with CP8 while maximum 1.97 with DC5, minimum drug release of Ofloxacin 80.3 with DC9 while maximum 98.9 with AH3 and minimum drug release of Keterolac tromethamine 80.2 with CP7 & DC7 while maximum 97.9 with CP2.

Table 1: Evaluation data of batches from AH1-AH9 containing HPMC K100M

Formulation Code	Folding endurance (no. of folds)	Thickness (µm)	Surface pH	Weight variation	% moisture Absorption (w/w)	% moisture loss (w/w)	% Drug Release (OFL)	% Drug Release (KT)
AH1	70	5.6	6.9	47.08	6.72	1.02	98.4	95.3
AH2	78	5.9	6.8	47.18	6.45	1.05	97.1	96.6
AH3	75	6.2	7.0	47.12	6.82	0.97	98.9	95.4
AH4	62	6.1	6.9	49.17	6.90	0.93	90.3	86.1
AH5	68	6.8	7.2	49.23	6.46	1.01	91.7	82.6
AH6	65	6.3	7.3	49.2	6.23	0.99	90.5	83.5
AH7	53	5.4	6.9	51.25	6.78	0.95	86.7	83.9
AH8	55	5.2	6.8	51.2	7.03	1.08	84.2	81.8
AH9	52	6.1	6.9	51.31	6.98	1.05	81.0	80.3

Table 2: Evaluation data of batches from BE1-BE9 containing Ethyl cellulose

Formulation Code	Folding endurance (no. of folds)	Thickness (µm)	Surface pH	Weight variation	% moisture Absorption (w/w)	% moisture loss (w/w)	% Drug Release (OFL)	% Drug Release (KT)
BE1	97	4.1	7.0	45.39	4.17	1.12	96.8	97.3
BE2	98	4.3	7.2	45.28	4.56	1.03	96.1	97.6
BE3	95	4.0	6.8	45.21	4.41	1.05	97.9	96.5
BE4	82	3.8	6.9	48.11	4.24	0.99	92.5	90.1
BE5	88	3.7	7.2	48.17	5.09	0.98	91.5	92.4
BE6	85	3.5	6.4	48.21	4.89	0.94	93.4	90.3
BE7	73	3.2	7.0	50.15	4.98	1.07	85.2	87.2
BE8	75	3.1	6.8	50.2	5.11	1.02	82.5	85.8
BE9	72	3.0	7.1	50.18	5.08	1.01	80.9	82.7

Table 3: Evaluation data of batches from CP1-CP9 containing PVP K30

Formulation Code	Folding endurance (no. of folds)	Thickness (µm)	Surface pH	Weight variation	% moisture Absorption (w/w)	% moisture loss (w/w)	% Drug Release (OFL)	% Drug Release (KT)
CP1	47	6.5	6.6	43.25	4.88	0.98	95.9	97.4
CP2	48	6.3	6.9	43.18	4.97	0.96	95.1	97.9
CP3	45	6.4	7.4	43.26	4.92	1.15	96.3	96.4
CP4	42	5.5	7.2	46.13	5.25	1.08	92.3	90.1
CP5	40	5.4	6.8	46.18	5.46	1.03	91.9	90.6
CP6	43	5.3	7.1	46.23	5.57	1.11	91.5	89.5
CP7	37	5.0	7.0	49.08	5.63	1.02	87.2	80.2
CP8	38	4.8	7.2	49.1	5.22	0.92	85.8	81.8
CP9	35	4.9	6.7	49.16	5.34	0.99	83.2	80.6

Table 4: Evaluation data of batches from DC1-DC9 containing Carbopol 934

Formulation Code	Folding endurance (no. of folds)	Thickness (µm)	Surface pH	Weight variation	% moisture Absorption (w/w)	% moisture loss (w/w)	% Drug Release (OFL)	% Drug Release (KT)
DC1	87	7.1	7.3	45.08	5.59	1.78	97.4	96.9
DC2	83	7.0	7.0	45.15	5.89	1.34	97.9	97.7
DC3	81	7.2	6.9	45.32	5.47	1.19	96.5	96.4
DC4	62	7.0	7.2	47.44	5.79	1.83	90.7	89.3
DC5	58	6.9	7.2	47.25	6.05	1.97	89.4	87.6
DC6	65	6.5	7.1	47.34	6.15	0.98	89.5	88.2
DC7	49	6.1	7.4	49.29	5.96	1.47	83.7	80.2
DC8	45	6.3	7.2	49.06	5.77	1.72	83.2	81.4
DC9	52	6.0	7.3	49.32	6.12	1.23	80.3	81.1

Factorial Design Study

All the formulations were further studied for factorial analysis.

The "Pred R-Squared" of 0.8946 was in reasonable agreement with the "Adj R-Squared" of 0.9674; i.e. the difference was less than 0.2.

"Adeq Precision" measured the signal to noise ratio. A ratio greater than 4 was desirable. Resulted ratio of 16.312 indicated an adequate signal.

Predicted Value

The predicted batches were further prepared and evaluated for comparative release studies.

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