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Ocular delivery systems of pefloxacin mesylate

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Ocular films of pefloxacin mesylate were prepared with the objectives of reducing the frequency of administration, to improve patient compliance, obtaining controlled release and greater therapeutic efficacy in the treatment of eye infections such as conjunctivitis, keratitis, kerato conjunctivitis, corneal ulcers etc. Polymers such as HPC, HPMC, PVP and PVA were used in different ratios to prepare the ocular films. They were evaluated for drug content which varied from 96–104%. Those which consisted of flexible and transparent films were subjected to *in vitro* release studies. The formulations which prolonged the release for eight hours were selected. The average weight and thickness of these were found to be 38.92–49.71 mg and 31.68–46.08 μm, respectively. The intactness of the formulations was confirmed by IR and TLC studies. *In vivo* studies carried out in the eyes of rabbits showed controlled release upto 8–9 h. There was a good correlation between the *in vitro* and *in vivo* data (r = 0.97–0.995). A minimum of 1 Mrad was found to be necessary for the sterilization of ocular films by gamma radiation. They were found to be stable at temperatures below 45 °C.

1. Introduction

Pefloxacin mesylate is a broad spectrum antibacterial agent useful in the treatment of eye infections [1] such as conjunctivitis, keratitis, kerato conjunctivitis, corneal ulcers etc. It is presently available as 0.3% eye drops. 1–2 drops every 15–30 min to 2–6 times daily have to be instilled for acute and moderately severe infections. Due to the increased frequency of administration, there is patient non-compliance and there is loss of drug from eye tear flow and nasal drainage in case of eye drops. Controlled release drug delivery systems such as ocular films of the drug were prepared with the objective of delivering the drug in a controlled manner for a specific period of time to obtain greater therapeutic efficacy by an increased contact time and to improve patient compliance by decreasing the frequency of administration.

Thus, in the present work, the ocular delivery systems of pefloxacin mesylate were prepared using HPC [2], HPMC [3], PVP [4], MC [5] and PVA [6] as the polymers and PEG-400, glycerine as plasticizers in different ratios and combinations. The formulations were evaluated for drug content, film characteristics, *in vitro* release, average weight and thickness, *in vivo* release characteristics and ocular irritancy. *In vitro/in vivo* correlation was investigated and sterility testing of the polymer films after gamma radiation sterilization was carried out followed by stability studies.

2. Investigations, results and discussion

The formula used for the preparations consisting of polymer and plasticizers and the formulation codes are shown in Table 1. Table 2 shows the formulations using mixture of polymers. Glycerine was used instead of PEG-400 in formulations F, G and H.

Among the 28 formulations prepared the following 18 were selected as they yielded good, transparent and flexible films. A-1 to A-4, B-1 to B-3, E-1 to E-5, F-1 to F-5 and H-3. The others yielded rigid and brittle films and hence were rejected. The drug content was determined

Table 1: Formulations using different polymers with different ratios

Drug	0.250 g	0.250 g	0.250 g	0.250 g	0.250 g
Polymer	0.250 g	0.500 g	0.750 g	1.0 g	1.250 g
Plasticizer (20% of Polymer weight)	0.05 ml	0.10 ml	0.15 ml	0.20 ml	0.25 ml
Distilled water q.s.	10 ml	10 ml	10 ml	10 ml	10 ml
Drug: Polymer ratio	1:1	1:2	1:3	1:4	1:5
Polymer	Formulation code				
HPC 25 cps	A-1	A-2	A-3	A-4	A-5
HPMC 15 cps	B-1	B-2	B-3	B-4	B-5
MC 10 cps	E-1	E-2	E-3	E-4	E-5
PVA 25 cps	F-1	F-2	F-3	F-4	F-5
PVP 30 cps	G-1	G-2	G.3	G-4	G-5

The plasticizer PEG-400 was used for the formulations containing HPC, HPMC and MC as the polymer and glycerine was used for the formulations containing PVA, PVP as the polymers

Table 2: Formulations using mixture of polymers

Codes →	H-1	H-2	H-3
Drug	0.250 g	0.250 g	0.250 g
HPC 25 cps	0.50 g	0.125 g	0.250 g
HPMC 15 cps	0.125 g	0.375 g	0.250 g
PVA 25 cps	0.125 g	0.250 g	0.250 g
Glycerine (30% of polymer weight)	0.15 ml	0.15 ml	0.15 ml
Distilled water q.s. to	10 ml	10 ml	10 ml
Drug: HPC:HPMC:PVA ratio	1:2:0.5:0.5	1:0.5:1.25:1	1:1:1:1

and it varied from 96% to 104%. The *in vitro* release studies were carried out in isotonic sodium phosphate buffer of pH 7.4 for a period of 8 h using a fabricated "through flow apparatus" [6]. The following observations were made: A-1 and A-3, the formulations of A with HPC as the polymer, retarded the release of the drug for 7 h whereas A-2 showed 99% release at the 6th hour. The release pattern from A-4 was much more prolonged and lasted for 8 h. The formulations of B with HPMC as the carrier showed complete release in 7–9 h, B-3 having a greater sustained release up to 9 h. The formulations with MC as the polymer showed complete release within 4–5 h. The formulation F with PVA as the polymer released the drug between 6 and 8 h. Among F-3, F-4 and F-5 which showed release up to 8 h, F-3 was chosen, as the drug to polymer ratio (1:3) is lesser compared to F-4 and F-5.

Table 3: *In vitro* t_{50}% release of pefloxacin mesylate from various ocular films

Polymer	Formulation code	t_{50}%* (min) ± S.D.
HPC	A-1	89 ± 1.160
	A-2	90 ± 1.246
	A-3	90 ± 0.983
	A-4	176 ± 1.431
HPMC	B-1	117 ± 0.053
	B-2	117 ± 0.332
	B-3	173 ± 0.982
PVA	F-1	118 ± 0.926
	F-2	152 ± 0.988
	F-3	224 ± 1.563
	F-4	156 ± 1.510
	F-5	90 ± 0.318
Combination of polymers	H-3	118 ± 0.074

No. of trials = 3
* The Average value of three readings is shown in the Table

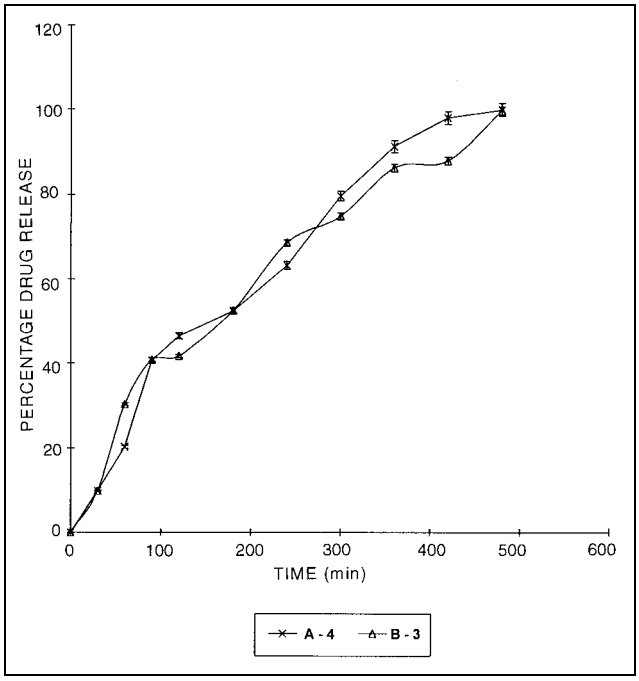

Fig. 1: *In vitro* release profile of pefloxacin mesylate from formulations A-4 and B-3

The formulation with combination of polymers, H-3, showed a slow release up to 8 h. Therefore by the t_{50}% values of the formulations as shown in Table 3 and also by the *in vitro* drug release profile (Figs. 1, 2), the best formulations A-4, B-3, F-3 and H-3 were selected for further studies.

The average weight and thickness of these best formulations were determined by randomly selecting ten samples from the batch. Weight and thickness ranged from 38.92–49.71 mg and 31.68–46.08 μm, respectively. IR and TLC studies were carried out. IR spectra of all the formulations showed the principal peaks of pefloxacin mesylate at the wave numbers 2950 cm^{-1}, 1720 cm^{-1}, 1630 cm^{-1},

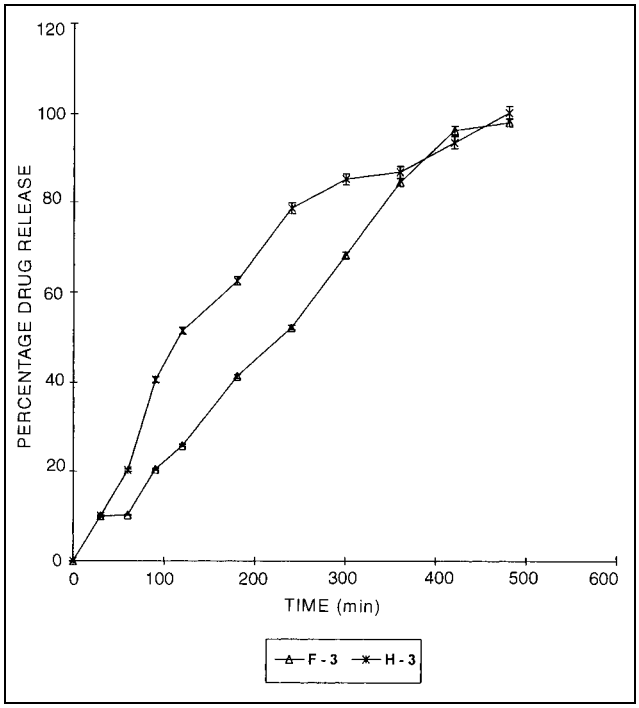

Fig. 2: *In vitro* release profile of pefloxacin mesylate from formulations F-3 and H-3

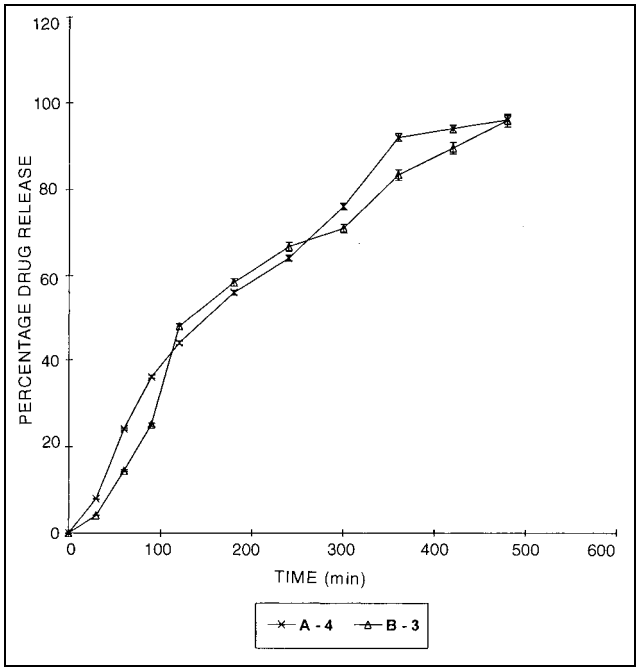

Fig. 3: *In vivo* release profile of pefloxacin mesylate from formulations A-4 and B-3

$1520\ cm^{-1}$, $1270\ cm^{-1}$ and there were no extra peaks. TLC studies indicated the same or comparable R_f values (0.74–0.76).

In vivo studies were carried out in albino rabbits. The comparative *in vivo* drug release profiles of different formulations are shown in Fig. 3, 4. It can be observed that the formulations showed a slow release up to 8–9 h. Also the polymer films did not show any ocular irritation throughout the period of study.

In vitro/In vivo correlation was determined by plotting the percent *in vitro* drug release against the percent *in vivo* drug release for the same period of time. The equations for best fit line and correlation coefficients are shown in Table 5. There was a good correlation between the in vitro and *in vivo* drug release.

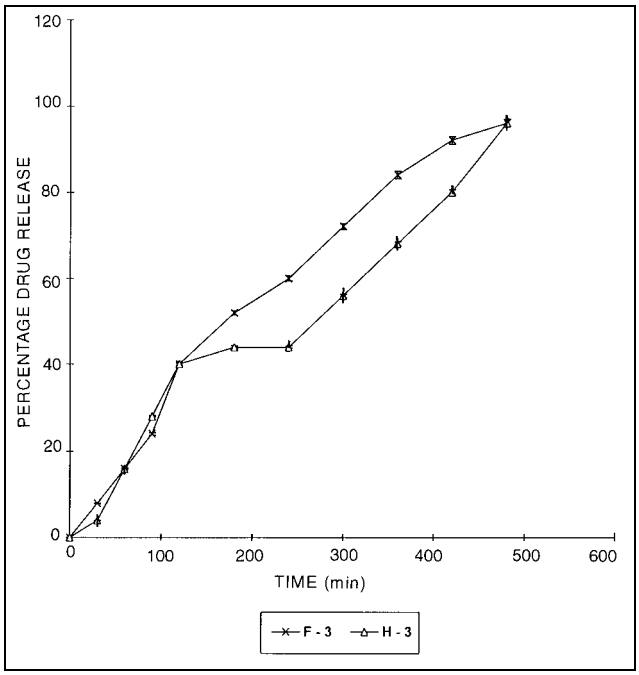

Fig. 4: *In vivo* release profile of pefloxacin mesylate from formulations F-3 and H-3

Table 4: *In vivo* $t_{50}\%$ release of pefloxacin mesylate from ocular films

Formulation code	$t_{50}\%$* (min) ± S.D.
A-4	146 ± 0.926
B-3	168 ± 1.449
F-3	177 ± 1.629
H-3	270 ± 0.641

No. of trials = 3
* Average value of three readings

Table 5: *In vitro/In vivo* correlation

Formulation code	Equation for best fit line	r
A-4	$y = 0.369 + 0.981x$	0.995
B-3	$y = -7.533 + 1.061x$	0.977
F-3	$y = 8.139 + 0.91x$	0.987
H-3	$y = -6.153 + 0.856x$	0.946

Sterilization and sterility testing were carried out and it was found that minimum of 1 Mrad was necessary for sterilization. IR and TLC studies carried out for formulations after sterilization indicated that they did not undergo any instability during sterilization.

Stability studies were carried out for a period of 2 months. The ocular films were stable both physically and chemically at the temperatures of 25 °C, 37 °C and 37 °C + 75% RH but showed slight physical changes and a loss of about 4% drug at temperatures of 45 °C and above. Thus, it is advisable to store them at temperatures below 45 °C.

From the results, the following conclusions can be drawn: The polymer films have distinct advantage over existing conventional dosage forms whose drug release is slow and uniform for a period of 8 h. This indicates that the frequency of administration can be decreased, thus achieving improved patient compliance and therapeutic efficacy.

The methodology used in the preparation is simple and reproducible. The polymers used are inexpensive and easily available. As the polymers used in the present work are water soluble, the films do not need to be removed after the time period as in the case of ocular films made of water insoluble polymers.

3. Experimental

3.1. Preparation of ocular films

Required quantity of the polymer was weighed and dissolved in distilled water stirring gently (slight warming was necessary to dissolve PVA). Required amount of the plasticizer was added followed by the drug and stirring was continued to form a homogeneous solution. Alumination foil cups (diameter 5 cm) were prepared by pressing the foil between the metal ring and the round wooden piece on a horizontal surface [7].

The prepared solution (2 ml) containing 50 mg of the drug was casted on the foil cup of 5 cm^2. The cup containing the solution was dried at 40 °C for 12 h and the films of 1 cm^2 area were cut containing 10 mg of the drug and taken for further studies.

3.2. Evaluation of ocular films

3.2.1. Estimation of the drug content

Each ocular film was taken in a 10 ml volumetric flask and dissolved completely in distilled water using cyclomixer. This solution 0.5 ml was pipetted into another 10 ml volumetric flask and estimated by a colorimetric method [8].

3.2.2. In-vitro release studies

An in-vitro "through flow apparatus" was fabricated for the *in vitro* drug release studies stimulating the eye.

An ocular film was placed in the "through flow cell" and 100 ml isotonic sodium phosphate buffer solution of pH 7.4 was taken in the conical flask. Necessary arrangements and connections were done in a way that the solution from the conical flask is continuously circulated to the "through

flow cell" and back to the conical flask with a flow rate of 11 drops/min at 37 ± 1 °C temperature. At specified intervals of time, 2 ml of the sample solution was withdrawn from the conical flask and replaced with fresh buffer solution. The samples were analyzed after necessary dilutions by colorimeter.

3.2.3. Determination of average weight and thickness

The average weight was determined by randomly selecting 10 samples from the batch. They were weighed individually and the value was reported. The thickness of the ocular films was determined by microscopy [9].

3.2.4. IR and TLC studies

IR spectra of the pure drug and formulations were determined using a Shimadzu Fourier Transform IR Spectrophotometer by the KBr disc method, TLC studies were carried out using chloroform/methanol/toluene/diethylamine/water (40:40:20:14:8) as the developing solvent and observing under UV radiation at 254 nm.

3.2.5. In vivo studies

Six albino rabbits of age 6–8 months weighing between 2.5–3.0 kg with normal diet were used for the study. The studies were set up using a single blind randomized cross-over design with a wash out period of 2 weeks. One film was placed below the upper eye lid of each eye of the rabbit [10]. At specific time intervals, the films were carefully removed and analyzed for the remaining drug content. The drug content obtained was subtracted from the initial drug content in the ocular film which gave the amount of drug released in the rabbit's eye at that particular time. The animals were also observed for any ocular irritation through out the period of study.

3.2.6. Sterilization and sterility testing

Sterilization was carried out by gamma radiation at doses of 0.5, 1.0, 1.5 and 2.0 Mrads and sterility testing was carried out according to the literature [11].

3.2.7. Stability studies

The formulations were stored at temperatures of 25 °C, 37 °C, 45 °C, 60 °C and 37 °C/75% RH for a period of 2 months [12]. The samples were assayed for drug content and observed for any physical changes in the film.

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Loteprednol etabonate, a new soft steroid is effective in a rabbit acute experimental model for arthritis

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Loteprednol etabonate, a new soft steroid designed for use as a local therapeutic, was compared to dexamethasone in rabbit experimental model for arthritis. Methods: Joint inflammation was induced by local injection of antigen into the patellofemoral articulation in sensitized rabbits. Co-administration of either dexamethasone or loteprednol etabonate directly into the joint effectively blocked the inflammatory response. Both the synovial fluid cellular content and synovium histology were examined. The steroid treatments prevented the adverse inflammatory effects of antigen action. These results, together with previous studies showing decreased systemic activity of the soft steroid, indicate that the loteprednol etabonate could provide a therapeutic advantage over currently used intra-articular steroids for alleviating rheumatoid arthritis.

1. Introduction

Arthritis has plagued mankind since the beginning of time and the pailful, debililtating condition can affect any joint. However, the associated stresses from our upright posture make the leg and back articulations particularly vulnerable to damage from inflammation. Unchecked, the condition can progress and result in partial or complete disability. Statistics gathered in the United States show that osteoarthritis followed cardiovascular disease as the leading cause of disability in individuals over 50 years of age [1]. Rheumatoid arthritis is an inflammation condition that often precedes the more severe osteoarthritis. The pathogenesis involves an immunological response with initial synovial membrane inflammation contributing to fibrinoid degeneration and white blood cell infiltration into the synovial fluid. Continued irritation can progress from slight morning stiffness to total joint failure from severe cartilage destruction [1, 2].

Current therapies for rheumatoid arthritis are designed to control inflammation. Nonsteroidal anti-inflammatory drugs are first choice therapeutic options to control the disease. More advanced cases can be treated with immune