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Research Article

IMPROVED BIOAVAILABILITY OF PEFLOXACIN USING CONTROLLED RELEASE OCULAR INSERTS

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ABSTRACT

Several polymeric systems have been used to fabricate ocular inserts for better ocular bioavailability and retention to drug, in which inserts have shown advantages to overcome the disadvantages of conventional dosage forms and increased contact time. Pefloxacin is a flouroquinolone antibacterial drug effective in the treatment of bacterial conjunctivitis. The objective of the present work was to develop ocular inserts of pefloxacin and evaluate their potential for controlled ocular delivery. Reservoir type ocular inserts containing different combination of HPMC 50 cps and HPMC 4 cps which was suitable sandwiched between rate controlling membranes made up of different ratio of Eudragit RS 100 and Eudragit RL100. The film were prepared by solvent casting technique using teflon coated Petri dishes and characterized thickness, hardness, weight variation, drug content, moisture loss, moisture absorption, ocular irritation study, *in vitro* and *in vivo* release studies. All the inserts were found to be stable, non irritant and had a thickness ranging from 0.285 mm - 0.328 mm. The drug content in the inserts was found to be varied between 92.55 % - 96.82 %. The formulation showed cumulative drug release for 48-50 hrs. The influence of rate controlling membranes of different concentrations in the release kinetics was studied. All the formulations followed a zero order release pattern. A high correlation coefficient (r = 0.9863) was existed between the *in vitro* and *in vivo* release. On the basis of *in vitro* drug release studies, the formulation with HPMC 50cps as reservoir along with Eudragit RS 100/Eudragit RL 100 (4:1) was found to be better than the other formulations and provided the desired drug release *in vitro* for 3 days and remained stable and intact at ambient conditions.

Keywords : Ocular inserts, Pefloxacin, Eudragit, HPMC.

INTRODUCTION

A polymer, natural or synthetic is a substance that is combined with a drug or other active agent to release drug in a predesigned manner[1,2]. The basic objective of controlled drug release is to achieve more effective therapies by eliminating the potential for both under and overdosing. Other advantages are the maintenance of drug concentration within a desired range, fewer administrations, optimal drug use and increased patient compliance[3]. Among anatomical locations, eye is one of the important sites that has been treated and studied for the optimum drug delivery by controlled drug delivery devices. Conventional dosage forms such as solutions, suspensions, gels and ointments are used but face incompetence due to rapid drug drainage from site of application and different physiological factors of the eye[4]. Polymeric inserts and discs have been developed to overcome such difficulties. Inserts allow for accurate dosing, reduced systemic absorption and better patient compliance resulting from reduced frequency of administration and lower incidence of systemic side effects. Moreover, inserts are least affected by nasolacrimal drainage and tear flow thus provide reliable drug release and longer residence in cul-de-sac[5]. Yasmin sultana et al., developed ocular inserts for controlled delivery of pefloxacin mesylate[6]. Sasaki and

co-researchers prepared a unique one-side-coated insert that releases drug from uncoated side only. Ocular application of the one-side-coated insert produced constant concentrations of tilisolol in the tear fluid over 180 min[7].

The aim of the present investigation was to prepare ocular inserts of pefloxacin and to demonstrate sustained antimicrobial action *in vitro* up to 3 days.

MATERIALS AND METHODS

Pefloxacin obtained from Stan Max pharmaceuticals, Hyderabad, HPMC 50CPS and HPMC 4CPS was purchased from CDH (P) Ltd. New Delhi. Eudragit RS100 and Eudragit RL 100 purchased from Degussa India Pvt. Ltd., Mumbai. All other chemicals used were of analytical grade.

Preparation of ocular inserts

Ocular inserts of pefloxacin was prepared by solvent casting technique by modified method reported in the literature[8]**.** A flat square shaped glass molds having surface area 25 cm^2 were fabricated for casting the patches. The formulation of insert involves three step viz.

(i) Preparation of drug reservoir (ii) Preparation of rate controlling membrane (iii) Sealing of drug reservoir.

For Preparation of drug reservoir 2 % w/v solution of HPMC 4K and HPMC 50 cps was prepared by heating approximately 1/3rd of the required volume of water to about 90 °C. To this HPMC powder was added with stirring until the particles are thoroughly wetted and evenly dispersed. The remainder of the water was then added to the above solution as cold water to lower the temperature of the dispersion. 50 mg of pefloxacin was added to the above solution and stirred well. The volume was made up to 10 ml with water. 10 % v/v propylene glycol was added as plasticizer. Then 4.5 ml of the above polymeric solution was transferred to sterilized glass molds of 5 × 5 cm for HPMC 4K and 7 ml was used in case of HPMC 50 cps. The films were dried in a hot air oven at 40° C and removed from the die and was stored in desiccator until further studies.

Preparation of rate controlling membranes was prepared using Eudragit RS100 and Eudragit RL100, concentration of which is shown in table 1. Required amount of Eudragit RS100 (ERS100) and Eudragit RL 100 (ERL100) was weighed and transferred to a beaker. To this ethanol: acetone solution (60: 40 % v/v) was added and stirred well. The volume was made up to 10 ml using the same solution. 20 % v/v propylene glycol was added as plasticizer. The prepared solutions were then transferred into sterilized glass molds and placed in hot air oven at 50 °C to evaporate the solvent. The composition of various formulations is given in table no 1.

Sealing of Drug reservoir films obtained were done by cutting in to suitable dimensions and then sandwiched between two rate controlling membrane and placed it over an aluminum foil and transferred to a beaker containing saturated with vapours of ethanol: acetone solution (60:40 % v/v) for 1-2 minutes for sealing to occur. The final ocular inserts consisted of three layers (mass: 8.332 mg to 6.75 mg (n = 6); thickness: 0.184 ± 0.0081 mm). Each ocular insert contained 0.82 mg of the drug. The ocular inserts were stored in an airtight container under ambient conditions.

Table 1: Composition of various formulations

Code	Drug reservoir (Plasticizer - 10 % V / V Propylene Glycol)		% of Drug	Rate controlling membrane (Plasticizer-20% v/v-PEG)	
				ERS 100	ERL 100
P1	HPMC 4K	2 %	0.5 %	4 %	1 %
P2	HPMC 4K	2 %	0.5 %	3 %	2 %
P3	HPMC 4K	2 %	0.5 %	2.5 %	2.5 %
P4	HPMC 4K	2 %	0.5 %	2 %	3 %
P5	HPMC 4K	2 %	0.5 %	1 %	4 %
P6	HPMC-50CPS	2 %	0.5 %	4 %	1 %
P7	HPMC-50CPS	2 %	0.5 %	3 %	2 %
P8	HPMC-50CPS	2 %	0.5 %	2.5%	2.5 %
P9	HPMC-50CPS	2 %	0.5 %	2 %	3 %
P10	HPMC-50CPS	2 %	0.5 %	1%	4 %

Drug-excipient interaction studies

In order to find out the possible interactions between pefloxacin and the polymer, differential scanning calorimetry (DSC) analysis was carried out on the pure substance, their physical mixtures, and the final films.

Evaluation parameters

Hardness

The apparatus designed in our laboratory to study the hardness of the insert (Figure1) consists of a wooden stand of 11 cm height and top area of 16×16 cm. A small pan was fixed horizontally on one end of the 2 mm thick iron rod whose other end is reduced to sharp point. A hole of 0.2 cm diameter was made at the centre of the top area of wooden stand for supporting the pan rod. An electric circuit was made through a 3 volt battery in such a way that the bulb lights up only when circuit is completed through the contact of the metal plate and the sharp end of the rod. The insert was placed between the metal plate and the sharp end of the rod. The weights were gradually added to the pan at an interval of 10 seconds and for the stabilization of force till the bulb was glown. The final weight was considered as the measure of hardness[9].

Fig. 1: Apparatus for Hardness Measurement

Estimation of percentage moisture absorbed

Ocular inserts were weighed and kept in a desiccator containing aluminum chloride and 79.5 % humidity was maintained. After three days inserts were taken out and reweighed. Percentage moisture absorbed was calculated using the equation[9].

$$\% \text{ Moisture Absorbed} = \frac{\text{Final wt} - \text{Initial wt}}{\text{Initial wt}} \times 100$$

***In Vitro* drug release studies**

The *in vitro* release studies were carried out using a fabricated flow through apparatus, simulating the conditions of ocular cavity. The ocular insert was placed between ring shaped plastic mesh. The arrangement of the mesh was clamped with two baby nipples. The arrangement was fixed to the stand. From one end of the nipple was connected to the reservoir containing phosphate buffer (pH 7.4). A small orifice is made on the other side of the nipple which acts as an outlet for collecting the sample. The arrangement is done in such a way that the dissolution medium will continuously flow on the ocular insert at a rate of 0.4 ml / minute. The temperature of the medium for the entire process was maintained at 37 ± 0.5° C. Samples were withdrawn at different time intervals and subjected to spectrophotometric analysis at 275 nm to find out the amount of drug released[10].

***In vivo* drug release studies**

In vivo studies were carried out using healthy albino rabbits with prior approval of institutional animal ethical committee. The inserts were placed into the conjunctival cul de sac of six healthy rabbits, at the same time the other eye was served as the control. At specific time intervals the inserts were carefully removed and analyzed for the residual drug content. Each insert was dissolved in 5 ml of phosphate buffer (pH 7.4), filtered the content and estimation of drug was done spectrophotometrically at 275 nm after suitable dilutions. The amount of drug remaining in the insert was subtracted from initial drug content of the insert to give the amount of drug released into the rabbit's eye**.** After a wash out period of one week the experiment was repeated for two times as before.

Ocular irritation study

Six albino rabbits were used in the study and were examined thoroughly for any preexisting ocular damage. The selected formulation was then placed in one eye of each animal by gently pulling the lower eyelid away from the eye ball (conjunctival cul-de-sac). The lids were then being gently held together for one second and the animal is released. The other eye, remaining untreated was served as the control. The eyes of each rabbits were examined 24, 48 and 72 hrs after treatment for irritation, inflammation etc by naked eye or by means of a pen torch. At the time of examination period each rabbit was scored for ocular reaction. The test may considered positive if three or more animal exhibit positive reactions at any observation period.

RESULTS AND DISCUSSION

The reservoir type of the ocular insert consisted of three layers of film, the inner reservoir film containing the drug and two-rate controlling films surrounding the reservoir. The ocular inserts are composed of a central reservoir of drug enclosed in specially designed semi permeable or micro porous membranes that allow the drug to diffuse from the reservoir at a precisely determined rate. For the preparation of the drug containing reservoir film, HPMC 4K and HPMC 50 CPS was chosen as the polymer. Propylene glycol used as plasticizer was found to be compatible with the drug and polymers used in the study. A mixture of ethanol: acetone (6: 4) was used for preparing rate controlling membranes of ERS 100 and ERL 100. Effect of polymer ratios on the drug release was determined by formulating ocular inserts using rate controlling polymers of different ratios. In all formulation 2 % w/v of HPMC and 50 mg of drug was maintained in matrix film. Best films were obtained

when the plasticizer concentration was 10 % v/v of the dry mass of the polymer. The content of the drug of 20 ocular inserts was estimated to be 0.84 ± 0.0012 mg.

The DSC thermograms of pefloxacin pure drug, physical mixture with HPMC and the formulation are represented in figure 2. The DSC thermogram of pefloxacin displayed the characteristic peak at 278^{o}C corresponding to its melting point. The drug peak appeared in the thermogram for all the drug-loaded films, confirming the chemical integrity of the drug. A slight shift in the pefloxacin peak in the thermograms of the drug-loaded films could be due to the presence of moisture in the film samples.

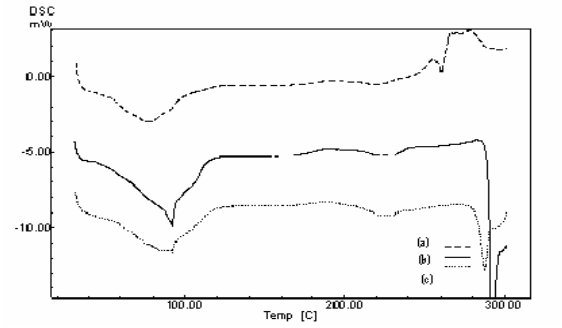


Fig. 2 : (a) Differential scanning calorimetry (DSC) spectra of pefloxacin (b) DSC spectra of physical mixture drug + pefloxacin (c) DSC spectra of formulation

The hardness of the formulations was in the range of 0.398 kg – 0.432 kg (Table 2) and found to be uniform. It was observed that the hardness of the inserts was slightly increased with increase in concentration of ERS 100. It was also observed that the hardness of the formulations containing HPMC 4K is higher than HPMC 50 CPS due to more viscosity.

Moisture absorption and moisture loss studies were conducted in all the formulations in triplicate and reported in Table 2. It was observed that as the concentration of ERL 100 increased the percentage of moisture absorption and loss was increased, which may be due to the hydrophilic nature and more permeability of ERL 100. The percentage moisture absorption and moisture loss was also influenced by polymer used in the matrix film. The moisture loss and gain was less in case of HPMC 4K formulations as compared to HPMC 50 CPS. The moisture loss or gain is contributed by viscosity of the polymer.

Table 2: Physicochemical properties of the formulations

Code	Weight* (mg)	Hardness* (Kg)	Thickness* (mm)	% moisture loss*	% moisture absorbed*
P1	8.332 ± 0.324	0.432 ± 0.145	0.328 ± 0.089	5.51 ± 0.864	4.68 ± 0.685
P2	7.952 ± 0.314	0.435 ± 0.334	0.318 ± 0.029	5.52 ± 0.201	4.86 ± 0.065
P3	7.856 ± 0.054	0.419 ± 0.154	0.315 ± 0.207	5.74 ± 0.142	4.92 ± 0.924
P4	8.235 ± 0.087	0.415 ± 0.614	0.314 ± 0.257	5.87 ± 0.987	5.01 ± 0.425
P5	7.625 ± 0.045	0.407 ± 0.084	0.308 ± 0.035	5.86 ± 0.547	5.32 ± 0.462
P6	6.842 ± 0.065	0.421 ± 0.324	0.312 ± 0.216	5.92 ± 0.395	5.23 ± 0.220
P7	7.290 ± 0.521	0.416 ± 0.064	0.310 ± 0.048	6.21± 0.914	5.35 ± 0.324
P8	7.11 ±0.065	0.412 ± 0.321	0.307 ± 0.088	6.28 ± 0.884	5.36 ± 0.213
P9	6.89 ± 0.215	0.403 ± 0.402	0.303 ± 0.125	6.34 ± 0.065	5.41 ± 0.447
P10	6.75 ± 0.026	0.398 ± 0.189	0.299 ± 0.067	6.47 ± 0.665	5.43 ± 0.336

*Average of three reading

The *in vitro* drug release studies of all the formulations containing HPMC 4K (P1-P5) showed prolonged release of drug than those prepared with HPMC 50 CPS. (P6-P10). The formulations P1 showed nearly 86.56 % of drug release at the end of 48 hours whereas for formulations P2, P3, P4 and P5 , it was found to be 90.40 %, 90.66 %, 90.79 %, and 91.29 % respectively. The formulations P6 to P10 showed 85 - 95 % drug release at the end of 12 hours. It was also observed that the rate of drug release was found to be increased with increase in the concentration of ERL 100. This may be due to the more permeable character of ERL 100 compared with ERS 100. The comparative plots of *in vitro* drug release of formulations P1 to P5 and P6 to P10 were plotted Figure 3 and Figure 4 respectively.

Fig. 3: Comparative *In vitro* drug release profile of formulations P1- P5

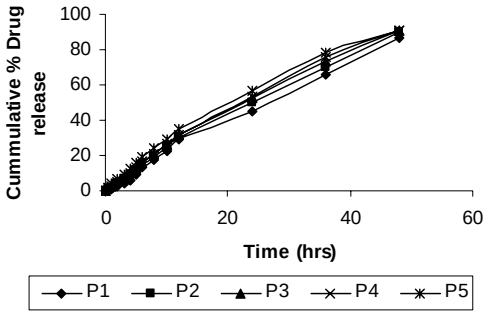
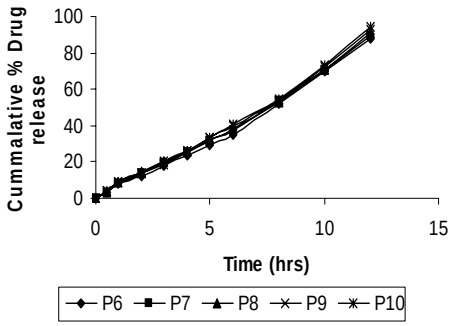


Fig. 4: Comparative *In vitro* drug release profile of formulations P6- P10



In vivo drug release studies were carried out on selected formulation (P2) to reduce the number of animals in the experiment. The studies were conducted in white albino rabbits in triplicate and amount of drug released was measurement by calculating the drug remaining (residual drug content) in the ocular inserts at periodic intervals. The drug release from the formulation after 48 hour was found to be 80.54 %. There was no drag out of the insert at the time of experiment which suggests that the dimensions of the insert were suitable for ocular use. Rabbits which are subjected for *in vivo* studies didn't show any irritation, inflammation and abnormal discharge which confirmed the safety of the polymers used in the formulation. A plot of *in vivo* drug release was plotted against *in vitro* drug release for the same formulation should a high Correlation coefficient (r) values (r = 0.9809).

To know the mechanism of drug release from these formulations, the data were treated according to first-order (log cumulative percentage of drug remaining vs time), Higuchi's (cumulative percentage of drug released vs square root of time), and Korsmeyer et al's (log cumulative percentage of drug released vs log time) equations along with zero order (cumulative percentage of drug released vs time) pattern.

The data from *in vitro* release studies of inserts did not fit first order kinetics (Table 3). When the data was plotted for First order equation, it showed non linearity indicating biphasic release pattern. Further to know the mechanism of release, we plotted Higuchi and Peppas plots, which indicated that diffusion was not dominating mechanism of drug release. The release of drug from the

inserts, when plotted against square root of time, did not show linearity, it indicates that the release pattern is not obeying Higuchi's kinetics. In our experiments, *in vitro* release profiles of all the formulations of patches truly fit zero-order behavior, and they could be best expressed by Peppas plots for the release of drug from a homogeneous-polymer matrix-type delivery system, which has been encapsulated by a polymer membrane.

The kinetic treatment reflected that release data of all the formulations showed R^2 value ranging from 0.8251- 0.9784 and 0.9783 - 0.9918 of and for first order and zero order equation respectively, indicating that release of drug follows zero order kinetic (reported in Table 3). Further Korsmeyer and Peppas equation resulted into the value of n, which is close to 1, indicating that the drug release was followed zero order kinetic. When n is approximate to 0.5, a Fickian / diffusion controlled release is implied, where $0.5 < n < 1.0$ non-Fickian transport and $n = 1$ for zero order (case II transport)[12].

Table 3: Drug release kinetics of formulations

Code	First order	Zero order	Higuichi	Koresmayer-Peppas	
	r	r	r	n	r
P1	0.9532	0.9907	0.9430	0.8260	0.9848
P2	0.9462	0.9918	0.9534	0.9799	0.9783
P3	0.9587	0.9898	0.9567	0.9528	0.9664
P4	0.9669	0.9876	0.9598	0.9470	0.9659
P5	0.9781	0.9783	0.9711	0.8809	0.9419
P6	0.9784	0.9870	0.8660	0.9692	0. 8362
P7	0.8552	0.9911	0.8817	0.9861	0.8362
P8	0.8326	0.9908	0.8788	0.9934	0.8390
P9	0.8223	0.9905	0.8799	0.9401	0.8127
P10	0.8251	0.9891	0.8697	0.9931	0.8103

CONCLUSION

Reservoir type ocular insert consisting of a HPMC reservoir with pefloxacin and rate-controlling membranes of Eudragit RS 100 and Eudragit RL 100 mixtures demonstrated sustained release of the drug in the eye for 2 days. The *in vivo* and *in vitro* results suggest that the lower hydrophilicity of rate-controlling membrane plays an important role in retarding the release of the drug from reservoir ocular inserts.

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