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Formulation and Evaluation of Dorzolamide hydrochloride Polymeric Film

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Abstract: The dorzolamide hydrochloride is a potential drug that can reduce intraocular pressure. The aim of the present study was to formulate and evaluate ophthalmic film of dorzolamide hydrochloride. The matrix drug delivery system of dorzolamide hydrochloride were prepared by evaporation (moulding) technique. Twelve formulations were developed, which differed in the combinations of polymers HPMC, Eudragit RS 100 and Eudragit RL 100 and at their different concentrations. PEG 400 was incorporated as a plasticizer. The prepared inserts were evaluated for various parameters like drug interaction studies, physicochemical characteristics, *in vitro* release studies, transcorneal permeation study by using excised goat cornea. There was no interaction between drug and polymer as revealed by UV absorption spectra and IR spectra of pure drug and drug and polymers mixture. The values of physicochemical evaluation were considered satisfactory and reveals good film properties. The surface pH of the prepared insert were between 6 to 7 which shows that the prepared insert would not alter the pH of tear fluid and does not have irritation potential as pH is within the accepted ocular range. The formulation DH 10 (1.25%w/v Eudragit RS 100, 2.50% w/v Eudragit RL 100, 40mg drug) sutained the drug release upto 24 hrs.
Keywords : Glaucoma, *in vitro* release, transcorneal release studies.

Introduction

The main goal of ocular drug delivery is controlled release of therapeutic concentrations to specific tissues. Topical ophthalmic drops are the most common method used to administer treatments for ocular disease. Various precorneal factors, such as drainage of the instilled solution, non-corneal absorption and induced lacrimation limit ocular absorption by shortening the cornea contact time of the applied drug [1]. These factors, and the corneal barrier itself, limit permeation of topically administered ophthalmic drugs. As a result, only a few percent of the applied drug dose is actually delivered into the intraocular tissues, and the major part (50–99%) is absorbed into the systemic circulation which can cause various side effects. Ophthalmic inserts offer several advantages like increased ocular residence, accurate dosing, possibility of releasing drugs at a slow, constant rate [2,3]. The aim of the present work was to formulate and evaluate polymeric ocular drug delivery system of dorzolamide to overcome the disadvantages associated with conventional ophthalmic dosage forms (eye drops and suspensions), to achieve long duration of action and to improve ocular bioavailability. Carbonic anhydrase inhibitors are one of promising group of drug currently being used to treat glaucoma [4,5]. Dorzolamide is a topical Carbonic anhydrase inhibitor currently being used to treat glaucoma. It is

relatively specific against carbonic anhydrase types II and IV [6]. Carbonic anhydrase types II and possibly, carbonic anhydrase types IV are key enzymes for the production of aqueous humor in the eye [7]. Dorzolamide hydrochloride is first U.S. FDA approved topical CAI [8]. As monotherapy, it reduces IOP by 18-26%. In glaucoma patients who were intolerant to systemic CAI, dorzolamide offers similar efficacy and better tolerability. Glaucoma is a group of eye diseases that damage the optic nerve. Damage usually occurs as a result of elevated pressure of the fluid (aqueous humor) in the eye [9,10]. Glaucoma remains a leading cause of blindness in adults over age 60, according to the National Eye Institute (NEI), a division of the National Institutes of Health [11].

Materials and Methods :

Materials

Dorzolamide hydrochloride was obtained from FDC, Waluj, Aurangabad. Hydroxypropyl methyl cellulose was procured from Sigma , Mumbai. Eudragit RS 100 and Eudragit RL 100 were obtained from Wackhardt, Aurangabad. All the other chemicals and solvents used were of analytical grade.

Polymeric film preparation

The polymeric films of dorzolamide hydrochloride were prepared by evaporation (moulding) technique. Required quantities of the polymers were weighed accurately and to this 10 ml of methanol (Table No.1). Add PEG-400 30% w/w followed by addition of drug(40 mg). The mixture was kept for stirring in a magnetic stirrer till the polymer dissolved. Then add polymeric drug solution into pre lubricated glass mould having a diameter 3.5 cm and keep it for 24 hrs at room temperature The films were then cut into 1.0 cm^2 size pieces having 2 mg drug . It was finally packed in self sealing poly glassine foils. These formulations were sterilized separately by exposing to UV radiation for 90 minutes in a cabinet under aseptic conditions and were finally packaged in pre-sterilized aluminum foil and stored in a desiccator for further studies.

Evaluation of prepared ophthalmic film

Drug-excipient interaction studies

Drug- excipient interaction studies were carried out by infrared spectroscopy and ultraviolet visible spectroscopy. The FT-IR spectrum of pure drug and Physical mixture of pure drug and polymers were analyzed to check the incompatibility between the pure drug and polymers using JASCO FTIR-4100 by potassium bromide method.

The sterilized formulations were dissolved in phosphate buffer pH 7.4 and the solution then scanned between 200 to 400 nm. UV scans of the placebo formulations also were run and were compared with those of medicated formulations. An accurate amount of dorzolamide was dissolved in phosphate buffer pH 7.4 and the absorbance of the resulting solutions were determined at 254 nm. Drug content were calculated to estimate the percentage recovery of loaded drug [12].

Table No. : 01, Formulation of ophthalmic film/insert

Formulation code	Drug (mg)	Polymers used(%w/v)			Plasticizer(%w/w) Polyethylene glycol 400
		HPMC	Eudragit RS100	Eudragit RL100	
DH 1	40	1.25	1.25	-	30
DH 2	40	1.25	2.50	-	30
DH 3	40	2.50	1.25	-	30
DH 4	40	2.50	2.50	-	30
DH 5	40	1.25	-	1.25	30
DH 6	40	1.25	-	2.50	30
DH 7	40	2.50	-	1.25	30
DH 8	40	2.50	-	2.50	30
DH 9	40	-	1.25	1.25	30
DH 10	40	-	1.25	2.50	30
DH 11	40	-	2.50	1.25	30
DH 12	40	-	2.50	2.50	30

Physicochemical evaluations

The ophthalmic film of dorzolamide were evaluated for physico-chemical characteristics such as thickness, weight variations, percentage moisture absorption, percentage moisture loss, folding endurance, surface pH and drug contents. The ophthalmic films were evaluated for film thickness by optical microscopy technique [13]. The ophthalmic films were kept vertically standing and thickness measured using the eye piece micrometer. The film thickness was measured at three different points along the film in triplicate and the mean thickness values were calculated. Determination of average weight and weight variation was carried out by individually weighing 10 films in an electronic balance [14]. For percentage moisture absorption test ophthalmic films were weighed and placed in a desiccator containing 100 ml of saturated solution of Sodium chloride. After three days the ophthalmic inserts were taken out and reweighed, the percentage moisture absorption was calculated [15]. For determination of percentage moisture loss ophthalmic films were weighed and kept in a desiccator containing anhydrous calcium chloride [15]. After three days the ophthalmic inserts were taken out and reweighed, the percentage moisture loss was calculated. The folding endurance is expressed as the number of folds (number of times the insert is folded at the same place, either to break the specimen or to develop a visible cracks. The specimen was folded at the center, between the fingers and the thumb and then opened. This was termed as one folding. This process was repeated till the insert show breakage or cracks in the center of the insert. The total operations were named as folding endurance value. The average of ten ophthalmic inserts was calculated and determined for folding endurance [16].The surface pH of ophthalmic film was determined by allowing them to swell in a closed petridish at room temperature for 30 minutes in 0.1 ml double distilled water. The swollen devices were removed and placed on pH paper to determine the surface pH. After 60 seconds the color developed was compared with the standard color scale [17]. For determination of drug content the ophthalmic film was transferred into a graduated glass stopper flask which contained 10 ml of phosphate buffer pH 7.4. It was closed and shaken vigorously. The solution was then filtered. 1ml of filtrate solution was taken and diluted to 5ml with phosphate buffer pH 7.4 and solution was analyzed by using UV spectrophotometer at 254 nm. The procedure was repeated for three times and average of three ophthalmic inserts was calculated[18].

***In vitro* release study using a fabricated dissolution cell**

In order to determine the drug release from the formulated ophthalmic inserts, a study was conducted using a fabricated dissolution cell. The cell consisted of a semi-circle reservoir, with 1 mm thickness and 25 mm internal diameter. The dissolution fluid was delivered from the left corner of the reservoir through a hypodermic needle, attached to a flow-regulator at 20 drops/ min flow rate. The outlet from the cell was collected through the groove made on the top right of the cell. A formulated film was placed on the concave surface and the phosphate buffer pH 7.4, maintained at 37°C was dropped at a flow rate of 20 drops/ min at the middle of the film. The amount of drug release from the film at different time intervals was determined by UV visible spectrophotometrically at 254nm. The in vitro release of the ophthalmic inserts was studied for each batch in triplicate[19].

Sterility testing

The sterility testing of formulated films were done according to I. P. Direct inoculation method as described in Indian Pharmacopoeia. Ideal batches of film were used for sterility testing. All the samples were inoculated separately in to ATGM and SBCD media and incubated at 35^0C and 20-25^0C, respectively for 7 days. Similarly unsterilized samples of films were also inoculated separately in to ATGM and SBCD media and incubated at 35^0C and 20-25^0C, respectively for 7 days. A control evaluation was also carried out [20].

Transcorneal permeation studies by using excised goat cornea

The in vitro transcorneal permeation study of the best formulation was carried out using bichambered donar receptor compartment model using excised goat cornea. The formulations selected for this study are DH2 (containing 1.25% of HPMC and 2.50% of Eudragit RS 100), DH6 (containing 1.25% of HPMC and 2.50% of Eudragit RL 100), DH11 (containing 2.50% of Eudragit RL 100and 1.25% of Eudragit RS 100) since they gave highest drug release. Whole eye ball of goat was transported from the local butcher shop to the laboratory in cold (40^oC) normal saline within 1 hour of slaughtering of the animal. The cornea was carefully excised along with 2-4 mm of surrounding scleral tissue and was washed with cold normal saline till the washing was free from proteins. Isolated cornea was mounted by sandwiching surrounding scleral tissue between clamped donor and receptor compartments in such a way that its epithelial surface faced the donor compartment. The donor compartment was filled with 0.7ml of pH 7.4 isotonic

phosphate buffer. The test formulation was placed on the cornea in the donor compartment and opening of the donor compartment was sealed with a glass cover slip. The surface of the cornea is in contact with receptor compartment, which contain 20ml of pH 7.4 isotonic phosphate buffer and stirred continuously using a magnetic stirrer. Samples were withdrawn from the receptor compartment at periodic intervals and replaced with equal volume of pH 7.4 isotonic phosphate buffer. The drug permeated was analyzed by UV- visible Spectrophotometer at 254 nm against reference standard using phosphate buffer pH 7.4 as blank [21].

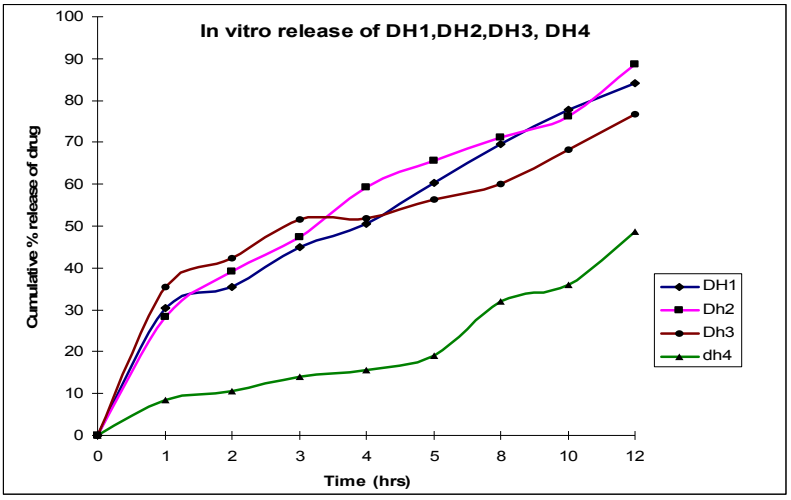
***In vitro* drug release pattern studies**

In order to study the, effect of different concentration and nature of polymers in mechanism of drug release, the obtained in vitro release data was fitted into the Higuchi [22] and Peppas [23] diffusion models to find the mechanism of release.

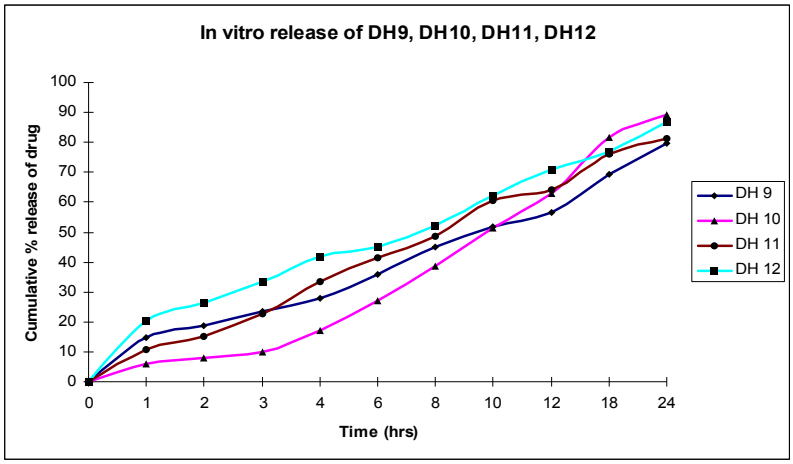
Table No. : 02, Evaluation of Dorzolamide HCl ophthalmic insert

Batch code	Evaluation Parameters					
	Thickness (µm)	Average weight and weight variation(mg/c^2)	Percentage Moisture absorption	Percentage Moisture loss	Folding endurance	Drug content (mg/cm^2)
DH 1	12.40 ± 0.57	13.37± 0.03	6.24± 0.11	18.18± 0.63	81± 3.51	1.885± 0.01
DH 2	14.79± 0.57	16.84± 0.21	6.53± 0.15	15.87± 2.73	87± 2.64	1.9766± 0.02
DH 3	14.79± 0.57	17.15± 0.06	6.15± 0.11	19.70± 1.77	76 ±3.00	1.7466± 0.03
DH 4	19.20± 1	23.45± 0.11	7.07± 0.66	13.69± 1.00	72± 3	1.652± 0.07
DH 5	14 ±0.57	14.10± 0.11	7.07± 0.03	22.82± 1.51	78 ±4.04	1.866± 0.02
DH 6	16.80± 1	17.26± 0.39	7.35± 0.13	18.89± 0.82	83± 1.52	1.994± 0.01
DH 7	16 ±1.52	18.82± 0.67	7.18± 0.04	23.33± 2	75± 2.51	1.9666± 0.02
DH 8	21.20 ±1.52	26.89± 0.28	7.73± 0.18	17.20± 1.58	71± 3.51	1.990± 0.01
DH 9	19.59± 1.52	18.49± 0.61	5.20± 0.14	16.44± 0.57	80 ±2.88	1.9255± 0.02
DH10	21.20 ±2	24.10± 0.11	4.33 ±0.11	15.45± 0.85	75± 4.5	1.95± 0.07
DH 11	24.39± 2.08	23.78± 0.29	3.06± 0.1	10.75± 1.52	78± 2.08	1.8466± 0.02
DH 12	32.40± 1	29.49± 0.59	3.62± 0.05	11.16± 0.16	73± 2	1.8266± 0.07

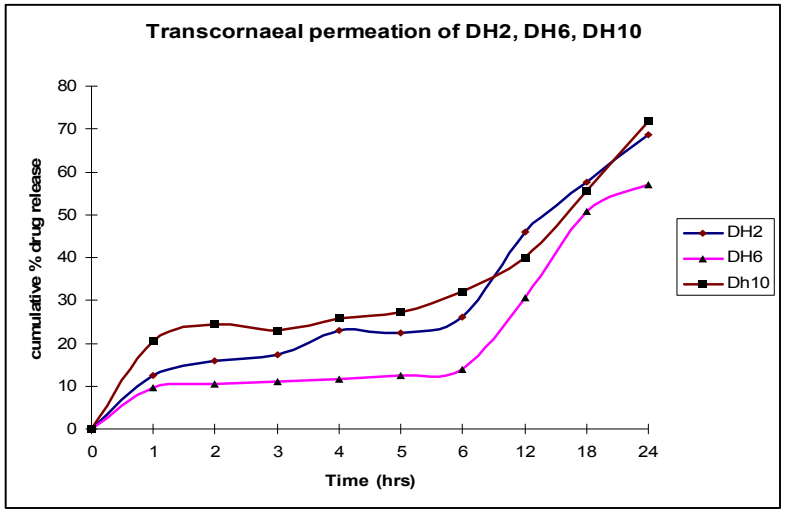
Graph No: - 01, *In vitro* drug release of DH1, DH2, DH3, DH4



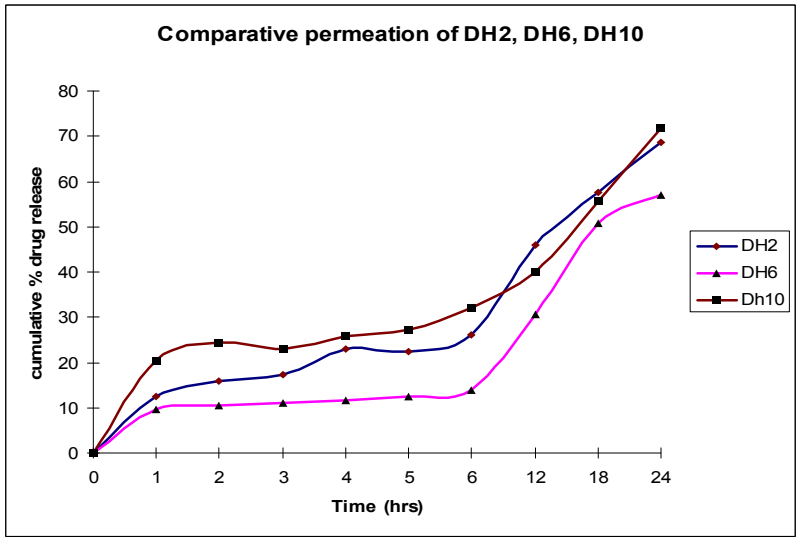
Graph No: - 02, *In vitro* drug release of DH5, DH6, DH7, DH8



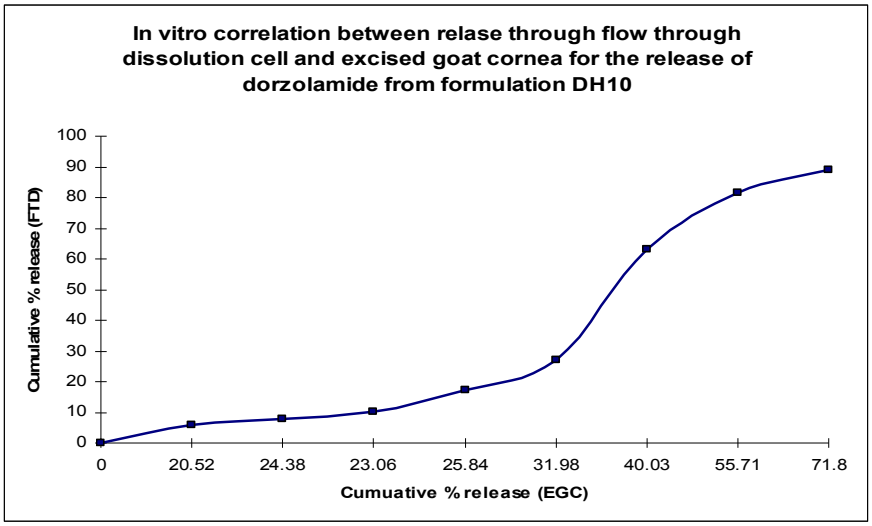
Graph No: - 03, *In vitro* drug release of DH9, DH10, DH11, DH12



Graph No.4 : Comparative permeation study of DH2, DH6, DH10 from excised goat cornea



Graph No: - 05. The *in vitro* correlation between drug release from flow through dissolution cell and drug release through excised goat cornea of formulation DH10



Result and Discussion

The ophthalmic film of dorzolamide were prepared by evaporation (moulding) technique and characterized on the basis of interaction studies, physico-chemical characteristics, sterility testing, *in vitro* release study, transcorneal permeation studies and in vitro drug release pattern studies.

In drug interaction studies, the spectra recorded were taken as qualitative in order to assess the changes in peak, pattern of peaks etc. No major differences were observed in IR spectra of pure drug and physical mixture of drug and polymers. The IR spectral analysis shows that all types of formulations have shown all peaks of dorzolamide hydrochloride indicating that there was no interaction between drug and polymer. By the IR studies it may be concluded that the drug found to be compatible with polymer used for the preparation of ophthalmic film. The UV spectra of the medicated formulations exhibited the absorption peak similar to those of pure drug sample. The λ_{max} of the pure drug and medicated formulations were found to be at at 254 nm. The UV spectra of the placebo formulations showed absorption profiles exclusive of those pure drug and medicated formulations. The result of the assay showed that 87.16 to 98.03 % of the loaded drugs from the different formulations were recovered unchanged.

The physicochemical characteristics of different formulations are shown in Table 2. The ophthalmic films of dorzolamide hydrochloride were found to be elastic and flexible. The results indicate that as the concentration of the polymers increased, there was increase in the thickness of the ophthalmic film. The films shows uniformity of weight and weight variation within the formulations. The minimum standard deviation values revealed that the process is reproducible in its capability of giving films of uniform magnitude.

The moisture absorption is more in films having the the high amount of hydrophilic polymer like HPMC while low in films having the high amount of hydrophobic polymers like ERS 100 and ERL 100.

The moisture loss was highest in films having the less concentration of hydrophobic polymer i.e. ERL 100 which offered minimum hindrance for the transfer of moisture while lowest in films having to high amount of hydrophobic polymers present i.e. ERS 100 and ERL 100.

The presence of plasticizers in the form of PEG 400 imparts flexibility to the polymers. PEG 400 forms hydrogen bond with polymers molecule thereby imparting flexibility to the film strips. The folding endurance test is important to check the ability of sample to withstand the folding. This also gives an indication of brittleness. The folding endurance values were found to be within the range of 71± 3.51 to 87± 2.64 which is considered satisfactory and reveals good film properties. The surface pH of the prepared films were found between 6 to 7. This shows that the prepared insert would not alter the pH of tear fluid and does not have irritation potential as pH is within the accepted ocular range. Reliability of the process in the purview of getting uniform drug loading was confirmed by drug content analysis data. The mean drug content was found to be in the range of 1.652 ± 0.02 to 1.994 ± 0.01 and independent of solid content. No significant difference in drug content was noted

when increase in polymer concentrations. The drug content uniformity values owed the fact that the process used in the study is capable of giving films with uniform drug content, with unsubstantial differences in targeted drug loading.

The *in vitro* release of dorzolamide hydrochloride ophthalmic film was found to be in the range of 48.56 % to 89.96%. The formulation DH4 shows lowest release of drug which may be due to the high concentration of polymer present (Fig.1). The formulation DH6 shows high drug release which may be due to the high permeability of ERL 100 as compared to ERS 100 (Fig. No.2). The in vitro drug release of formulations having the combination of ERS 100 and ERL 100 polymers i.e. DH9, DH10, DH11, DH12 shows the sustained drug release for 24 hrs (Fig.3). The formulations which gave good result with highest drug release i.e. DH2, DH6 and DH10 were selected for further studies like sterility testing, transcorneal permeation study through excised goat eye and kinetic release pattern studies.

Ultra violet radiation was used to sterilize the ophthalmic film and sterility testing was carried out under aseptic conditions. It was found visually that the Alternate thioglycolate, Soyabean casein digest media, Fluid thioglygolate media containing sterilized ocular inserts were free from turbidity. This confirmed the absence of aerobic organism, anaerobic organism and fungi. From this it confirms the sterility of ophthalmic inserts.

In transcorneal permeation studies, the cumulative percent release found to be in the range of 68.74 to 71.80 % after 24 hrs (Fig.4). The formulation DH10 (containing 2.50% of Eudragit RL 100and 1.25% of Eudragit RS 100) shows highest drug release i.e. 71.80 afetr 24 hrs . Probably the reason for less release rate in case of Excised Goat Cornea in comparision to drug release from flow through dissolution cell is that the cornea carries charged groups and therby becomes less permeable to charged species or ions. Hence there is decrease in the percentage of drug permeation. *In vitro* release study of the formulations through excised goat cornea has confirmed the fact that the formulation is capable of releasing the drug for the extended period of 24 hrs. The in vitro correlation between drug release from flow through dissolution cell and drug release through excised goat cornea of formulation DH10 was good (Fig.5). This was confirmed by the regression analysis of data. The correlation value was found to be 0.8693.

Conclusion

In the Present study, efforts have been made to prepare ophthalmic films of dorzolamide hydrochloride using different polymers such as HPMC, ERS100 and ERL 100. The *in vitro* release study shows that the formulation DH 10 containing 1.25 %w/v of ERS 100 and 2.50 % w/v of ERL 100 shows highest drug release after 24 hrs and hence capable of sustaining the drug release for 24 hrs. The matrix drug delivery system of dorzolamide hydrochloride may be effective drug delivery with increased corneal residence time for the treatment of glaucoma.

References

1. Chien YW. Ocular drug delivery and delivery systems. In: Novel drug delivery systems. 2nd edition. New York: Marcel Dekker; 1992: 269-70.
2. Karthikeyan D., Bhowmick M., Pandey V.P., Nandhakumar J., Sengottuve S., Sonkar, S., Sivakumar T. The concept of ocular inserts as drug delivery systems: An overview. Asian Journal of Pharmaceutics 2008: 460-468.
3. Saettone M.F., Salminen L. Ocular inserts for topical delivery. Advanced Drug Delivery Reviews ., 1995, 16: 95-106.
4. Saxena R., Prakash J.,Mathur P.,Gupta S.K. Pharmacotherapy of Glaucoma. Indian Journal of Pharmacology ., 2002, 34 : 71-85.
5. Resch H., Garhofer G. Topical drug therapy for glaucoma. Wien Med Wochenschr, 2006, 156/17-18 : 501-507.
6. Colin Dollery, Therapeutic Drugs, 2nd edition, Churchill Livingstone: D211-213.
7. Mortimer M.C., Formation of the Aqueous Humor :Transport Components and Their Integration. Current Topics in Membranes 62: 1-45.
8. Michael F.S. Pharmacological and ocular hypotensive properties of topical carbonic anhydrase inhibitors. Progress in retinal and eye research 2000, 19(1) : 87-112.
9. Gary H., Marilyn H., Glaucoma. Googlesearch.Avialable from http://www. allaboutvision.com. Accessed on 08/03/2009.
10. Lucy Titcomb. An update of Glaucoma. The Pharmaceutical journal 2008 : 219-222 .

11. Titcomb L. An update of Glaucoma. The Pharmaceutical journal. 2008, 280:219-222 .
12. Tanwar Y.S., Patel D., Sisodia S.S. In vitro and in vivo evaluation of ocular inserts of ofloxacin. DARU. 2007, 15(3) : 139-145.
13. Yung C. L., Jeffrey W. M., Gerald J. N., Salim I. B., Samuel H. Y. Formulation and in vivo evaluation of ocular insert containing phenylephrine and tropicamide. International Journal of Pharmaceutics 1999, 182: 121–126.
14. Yasmin S., Mohammad A., Asgar A. Ocular inserts for controlled delivery of pefloxacin mesylate: Preparation and evaluation. Acta Pharm., 2005 : 305–314.
15. Sadashivaiah R., Dinesh B.M., Patil U.A., Desai B.G., Raghu K.S. Design and in vitro evaluation of haloperidol lactate transdermal patches containing ethyl cellulose-povidone as film formers. Asian journal of Pharmaceutics 2008, 2(1) : 43-49.
16. Mishra D.N., Gilhotra R.M. Design and characterization of bioadhesive in-situ gelling ocular inserts of gatifloxacin sesquihydrate. DARU, 2008, 16(1): 1-8.
17. Balasubramaniam J., Srinatha A., Pandit J.K., Nath G. In vitro microbiological evaluation of polyvinyl alcohol-based ocular inserts of Ciprofloxacin hydrochloride. Indian journal of pharmaceutical sciences 2006, 68(5) : 626-630.
18. Sofia P., Dimitrios B., Konstantinos A., Evangelos K., Manolis G. Chitosan nanoparticles loaded with dorzolamide and pramipexole. Carbohydrate Polymers 2008, 73: 44–54.
19. Rao V., Shyale S. Preparation and Evaluation of Ocular Inserts Containing Norfloxacin. Turk J Med Sci. 2004, 34 : 239-246.
20. Indian Pharmacopoeia, Ministry of Health and Family Welfare, Govt. of India, New Delhi. 3rd edition, 1996 : A- 117 - A- 124.
21. Karthikeyan D.,Bhowmick M., Pandey V.P., Sengottuvelu S., Sonkar S., Gupta N., Mohod V., Shivakumar T. Design and Evaluation of Ofloxacin Extended Release Ocular Inserts For Once a Day Therapy. Research J. Pharm. and Tech. 2008, 1(4) : 460-468.
22. Higuchi, T. Mechanism of sustained-action medication theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J. Pharm. Sci. 1963, 52 :1145-1149.
23. Peppas N.A. Analysis of Fickian and non-Fickian drug release from polymers.Pharm. Acta Helv. 1985, 60 : 110-111.

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