ISSN 0975-6299

Vol 2/Issue 2/Apr-Jun 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

PHARMACEUTICS

IN SITU GELLING OPHTHALMIC DRUG DELIVERY SYSTEM FOR GLAUCOMA

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ABSTRACT

Elevated intraocular pressure (IOP) is accepted as the single most important risk factor for glaucoma. The present work describes the formulation and evaluation of an ophthalmic delivery system of an antiglaucoma agent, dorzolamide hydrochloride, based on the concept of ion-activated *in situ* gelation. Sodium alginate was used as the gelling agent in combination with HPC (Hydroxy Propyl Cellulose) that acted as a viscosity-enhancing agent. In vitro release studies indicated that the alginate/HPC solution retained the drug better than the alginate or HPC solutions alone. The formulations were therapeutically efficacious, stable and provided sustained release of the drug over a period 10 hrs. These results demonstrate that the developed system is an alternative to conventional ophthalmic drops in view of patient compliance, industrially oriented and economical.

KEYWORDS

Intra ocular pressure, Dorzolamide hydrochloride, *in situ* gelation, Sodium alginate

INTRODUCTION

Ophthalmic drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. The anatomy, physiology and biochemistry of the eye render this organ exquisitely impervious to foreign substances. The challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage. The primitive ophthalmic solutions, suspensions and ointment dosage forms are clearly no longer sufficient to combat some present virulent diseases. Due to tear drainage, most of the administered dose passes via the naso-lacrimal duct into the GI tract, leading to side effects. Rapid elimination of the eye drops administered often results in a short duration of the therapeutic effect making a frequent dosing regimen necessary. Ocular therapy would be significantly improved if the precorneal residence time of drugs could be increased. Several new preparations have been developed for ophthalmic use, not only to prolong the contact time of the vehicle on the ocular surface, but also to slow down drug elimination. However, these preparations have some disadvantages such as poor compliance, especially by elderly people and many patients sometimes lose the device without noticing it. From the point of view of patient acceptability, a liquid dosage form is preferable. This problem can be overcome by the use of polymeric solutions, which change to a gel as a result of exposure to the physiological temperature, pH or ionic composition of the lacrimal fluid. Such a system can be formulated as a liquid dosage form suitable to be administered by instillation into the eye, which upon exposure to physiological conditions, changes to the gel phase thus increasing the pre-corneal residence time of the delivery system and enhancing ocular bioavailability.[1 2 3]

Sodium alginate, the sodium salt of alginic acid, is a natural hydrophilic polysaccharide containing two types of monomers, β-D-mannuronic acid (M) and α-L- guluronic acid (G). The polymer forms three-dimensional hydrogel matrices and the high G content alginate forms a low viscosity, free-flowing liquid at concentrations suitable for gel formation in the lacrimal fluid. Sodium Alginate was chosen as a vehicle for ophthalmic formulations since it exhibits several favorable biological properties such as biodegradability and non-toxicity. A prolonged precorneal residence of formulations containing alginic acid was looked for, not only based on its ability to gel in the eye but also because of its mucoadhesive properties.[4]

The objective of the present study was to develop an ion activated *in situ* gelling for dorzolamide hydrochloride, which is a non-bacteriostatic sulfonamide derivative devoid of systemic side effects as seen after oral administration of carbonic anhydrase inhibitors. It penetrates cornea, inhibits carbonic anhydrase-II in the ciliary body, slows the production of local bicarbonates and thus decreases sodium and fluid transport which in turn reduces the secretion of aqueous humor.[5] Sodium alginate was used as the gelling agent in combination with hydroxypropylcellulose (HPC) as the viscosity enhancer for the formulation of dorzolamide hydrochloride eye drops (2 % w/v), which undergo gelation when instilled into the cul-de-sac of the eye and provide sustained release of the drug.

MATERIALS AND METHODS

Materials

Dorzolamide hydrochloride was obtained as a gift sample from Precise Chemipharma Pvt. Ltd., Mumbai, India. Sodium alginate and HPC were obtained from Snap alginate, Tamil Nadu, India and H.D Fine Chemicals, Mumbai, India respectively. All other reagents

were of analytical grade. Rabbits (New-Zealand white albino, 10-12 months age, 2-2.5 kg body weight) used for the Intra ocular pressure study and Draize eye irritancy studies.

Preparation of formulations

The Table 1 shows the composition of all the formulations. The alginate and alginate/HPC solutions were prepared by dispersing the required amount in distilled water with continuous stirring. Dorzolamide hydrochloride (2% w/v) was dissolved in distilled water. Benzalkonium chloride (0.01% v/v) solution used as antimicrobial agent was then added to the above solution. The drug solution was then added to the alginate or alginate/HPC solution under constant stirring to obtain a uniform solution. Distilled water was then added to make the volume up to 30ml.[6]

Table 1

In situ gelling ophthalmic formulations of dorzolamide hydrochloride using sodium alginate and various viscosity modifiers

Code	Drug (%w/v)	Sodium alginate (%w/v)	HPC (%w/v)	Mannitol (%w/v)	BKC (%w/v)	Distilled water
SP1	2	1	0.1	5	0.01	q.s.to 30ml
SP2	2	1	0.2	5	0.01	
SP3	2	1	0.3	5	0.01	
SP4	2	2	0.1	5	0.01	
SP5	2	2	0.2	5	0.01	
SP6	2	2	0.3	5	0.01	

Evaluation studies

a. Clarity

The clarity of the formulations before and after gelling was determined by visual examination of the formulations under light alternatively against white and black backgrounds.

b. Differential Scanning Calorimetry (DSC) study of physical mixture of drug and polymer

The differential calorimetric scanning of physical mixture of drug and individual polymer were carried out using Differential Scanning Calorimeter (DSC 60 Shimadzu, Japan). Samples were placed in aluminum crucibles and DSC thermograms were recorded at the heating rate of 10^0C/min in the range of 0^0C to 300^0C. Air was purged at the rate of 10 mL/min.

c. pH

The pH of each of prepared ophthalmic formulations was determined by using pH meter (Toshniwal, Ajmer) and electrode (Sentek Ltd.). The pH meter was calibrated before each use with standard pH 4, 7 and 9.2 buffer solutions. The formulation temperature was maintained at 25^{o}C.[7]

d. Assay

The specified volume (1ml) of each of the ophthalmic formulations was taken and diluted with distilled water to make concentration 20µg/ml. The samples were analyzed spectrophotometrically at λmax of 253 nm. The concentration of dorzolamide hydrochloride in samples was determined from a previously prepared calibration curve. The study was done in triplicate.

e. Test for gelling ability

The individual ophthalmic formulations (100µl) were added into 2 ml of Simulated tear fluid (37°C ± 1°C) contained in glass vials. The transition of solution to viscous gel was observed visually.[6]

f. In vitro Diffusion studies

The Keshery-Chein glass diffusion cell was used for *in-vitro* study. The recipient compartment of diffusion cell was filled

with STF (20ml) which was stirred continuously using magnetic stirrer. The pretreated dialysis membrane (Hi-media, Mol. wt- cut off 10,000) of appropriate diameter (1.75 cm) was mounted carefully on the rim of the recipient compartment. Approximately 1 ml of ophthalmic solution (marketed preparation) and the semisolid gels formed with the same volume of each formulation were tested separately. The whole diffusion assembly was then placed on the thermostatically controlled hot plate so as to maintain the temperature of the STF at 37 ± 2°C. About 1 ml quantities of aliquots were withdrawn carefully from the side arm of the diffusion cell and were replaced immediately with the same volume of fresh STF (maintained at 37 ± 2 °C). The samples were diluted suitably and the content of dorzolamide hydrochloride in each sample was estimated from the absorbance using the previously prepared calibration curve of dorzolamide hydrochloride in STF. Placebo gel diffusion solution was used as blank. The study was done in triplicate.

g. Determination of viscosity of ophthalmic formulations

The viscosity values were estimated for both the preparations i.e. ophthalmic solutions of dorzolamide hydrochloride as well as the preformed gels.[8]

i. Determination of viscosity of ophthalmic solutions

The specified volume of prepared ophthalmic solution was transferred in sample cell which was placed carefully within the adaptor (Brookfield RVDV-II + PRO viscometer, Adapter spindle No-21). The water of 25^{o}C was circulated through jacket of the adaptor. The viscosity values were recorded.

ii. Determination of viscosity of preformed ophthalmic gels

The sodium alginate gel formulations were prepared by adding $CaCl_2$ solutions into the sodium alginate solution. The gels were formed due to the interaction between alginate ions and Ca^{2+} ions. The viscosity values were recorded using Brookfield RVDV-II + PRO (T- bar spindle No. 93).

h. Intra Ocular Pressure study and irritancy study

The optimized formulation was terminally sterilized and evaluated for in vivo performance in animal model (Albino Rabbits). The protocol was approved by college ethical committee (Ethical committee Registration number is DYPIPSR/IAEC/Reg. No.18/2009). Healthy New-Zealand white albino rabbits were selected for the test. Animals were randomly placed in cages upon receipt and then were randomized according to the body weights. The selected animals were administered the respective volume of ophthalmic formulations and intra ocular pressure were measured at 0hr, 1hr, 6hrs, 12hrs and after 24hrs. Rabbits were housed separately for 24 hrs prior to the experimentation. On the day of experiment all the animals were anaesthezised by instilling tetracaine (local anaesthetic 1% w/v). After anaesthetizing, the test solutions were instilled in to the *cul de sac* of both eyes of the rabbits and intra ocular pressure were measured by using Schiötz tonometer. Animals were anaesthetized each time prior to IOP measurement.[9]

For the irritancy study, about one drop of the test solution was instilled into lower *cul-de-sac* of one ofthe eyes while other eye of the same animal served as the control.Toxicity symptoms if any were observed in the treated eye at preselected intervals of 1, 4, 24, 48, and 72 hrs and suitable scores were assigned depending onthe severity of the symptoms for each animal.[10]

RESULT AND DISCUSSION

The appearances of formulations were found to be clear. The formulation showed good gelling ability and had phase transition within 60 seconds. pH of all the formulations were in the range of 6.7-7.2. Due to addition of Ca^{++} ions, solution form changes to gel form and there was drastic increase in viscosity. All the solution and gel formulations showed shear

thinning behavior which is required to remove solution from the bottle and spread due to eye blinking. In contact with simulated tear fluid at 37°C the solutions instantly transformed to gel form.

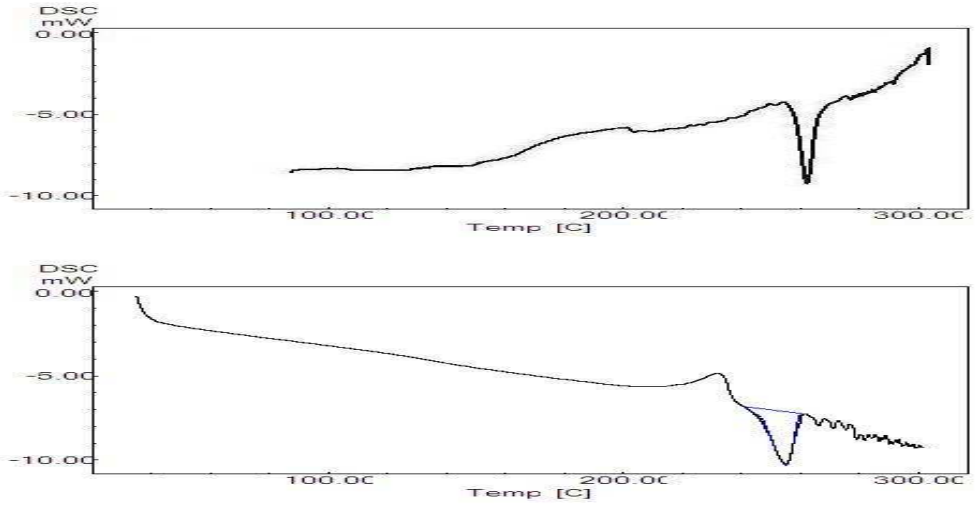
Table 2
Physical characteristics of ophthalmic formulations prepared with sodium alginate and Hydroxypropyl cellulose

Code	Dorzolamide hydrochloride (%w/v)	Sodium alginate (%w/v)	Hydroxypropyl cellulose (%w/v)	Appearance and Clarity	pH	Gelling ability
SP1	2	1	0.1	oo	6.7	++
SP2	2	1	0.2	oo	6.9	++
SP3	2	1	0.3	oo	7.0	++
SP4	2	2	0.1	oo	6.9	++
SP5	2	2	0.2	oo	6.9	++
SP6	2	2	0.3	oo	7.2	**+++**

Note: (-) No phase transition, (+) Phase transition within 60 sec, collapse of gel structure within 1-2 hrs, (++) Phase transition within 60 sec, collapse of gel structure within 3-4 hrs, (+++) Phase transition within 60 sec and gel structure stable for more than 6 hrs
Note: (*) Turbid, (o) slightly turbid, (oo) Clear solution, (ooo) Clear and transparent

Differential Scanning Calorimetry (DSC) study of physical mixture of drug and polymer

Figure 1
DSC thermogram of (A) drug and (B) drug and sodium alginate



The DSC thermograms of physical mixture showed characteristic endothermic peak corresponding to dorzolamide hydrochloride around 263°C. Therefore, there was no interaction between the drug and polymer (Figure 1).

Rheological Evaluation:
It was observed that there was corresponding increase in viscosity at solution phase of each formulationwith increasing concentration of bioadhesive polymer i.e. HPC from 0.1% w/v to 0.3% w/v.

The administration of ophthalmic preparation should influence as little as possible the pseudoplastic character of the precorneal tear film. Since the ocular shear rate is very large ranging from 0.03s^{-1} during interblinking period to 40000s^{-1} during blinking, viscoelastic fluids with viscosity that is high under conditions of low shear rate and low under conditions of high shear rate are preferred. These solutions are well tolerated at any viscosity because of blinking adaptation.

Table 3
Viscosity of ophthalmic formulations (both solutions and gels) of sodium alginate with HPC at 10, 50 and 100 rpm

Code	Viscosity of formulations at 10, 50 and 100 rpm both at 25 °C and 37 °C (after addition of Ca ++)					
	25 °C	37 °C	25 °C	37 °C	25 °C	37 °C
	10	10	50	50	100	100
SP1	100	20400	60	5600	45	2800
SP2	115	25780	95	6500	85	3400
SP3	160	31710	110	7840	90	3900
SP4	180	34900	140	9600	115	5800
SP5	200	38900	150	10250	120	6300
SP6	240	46000	180	12200	140	6900

For all the formulations studied, the viscosity at 37°C (after addition of Ca^{2+}) was much higher than those at 25°C suggesting the occurrence of phase transition between these two conditions.

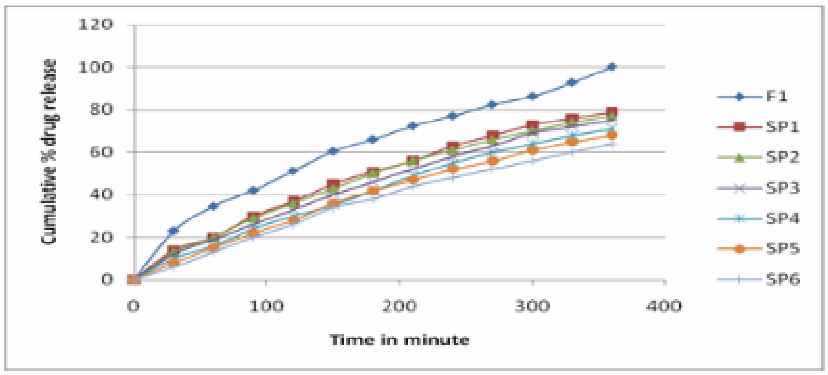
Sodium alginate contains two types of monomers β-D- mannuronic acid(M) and α-L-guluronic acid (G) when comes in contact with the calcium ions in tear fluid, G moieties interact with the calcium ions to form three dimensional ionotropic hydrogel matrices.[9]

Estimation of dorzolamide hydrochloride

Estimation of drug contents in ophthalmic solutions was carried out by spectrophotmetrically using UV spectrophotometer (UV 1700, Shimazdu). The contents of dorzolamide hydrochloride in the selected *in situ* gelling solution systems were found to be 97.2-100.6%w/v.

In vitro diffusion study

Figure 2
Diffusion of dorzolamide hydrochloride ophthalmic formulations compared with marketed formulation



**F1=marketed formulation(Trupost)*

Formulation SP1 to SP6 showed retardation of dorzolamide hydrochloride release as concentration of HPC increased from 0.1% to 0.3% and concentration of sodium alginate

increased from 1% to 2%. Total release in 6 hrs was decreased from 79.14 % to 64.78 %. Hydroxypropylcellulose is hydrophilic and swellable polymer, hence it caused increase in viscosity & decrease in diffusion of the drug over the period of more than 6 hrs. As the concentration of sodium alginate increases from 1% to 2%, more G molecules are available for interaction resulting in compact network which retard the drug release.

Test for efficacy of in situ gelling ophthalmic formulation of dorzolamide hydrochloride for reducing elevated IOP in rabbits

For the 1 hr of treatment, the reduction in IOP is evident for all the groups treated with marketed preparation F_1 (Standard) and the test solution [t_1] (SP5) when compared with animals belonging to the no treatment control. Subsequently the marketed preparation indicated gradual decrease in IOP upto 6 hrs of treatment and thereafter no effect on IOP was observed. But the test formulation was effective in reducing the IOP upto the 6 hrs of treatment like the marketed preparation. Moreover, this reduction in IOP was prolonged upto 12 to 16 hrs. (Table 5)

Table 5
Efficacy studies of in situ gelling ophthalmic formulation (SP5) of dorzolamide hydrochloride

Time	0 hr		1 hr		2 hr		6 hr		12 hr		24 hr	
Eye	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**
C	19.2	18.8	19.0	18.5	18.8	18.5	19.0	18.8	19.2	18.9	20.1	19.3
SP5	19.2	18.9	17.8	17.2	16.3	16.7	15.1	15.0	14.7	14.4	18.6	18.4

(Intraocular pressure measured in mmHg)

Acute Toxicity Study

The results of the ocular irritation studies (Table 6) shown that the optimized formulations were non-irritant. Hence, it was safe for administration. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctiva were visible.

Table 6
Ocular irritation scores of ophthalmic formulation of dorzolamide hydrochloride

Formulation code	SP5			
	1hr	24 hrs	48 hrs	72 hrs
Redness	0	0	0	0
Excessive Tearing	0	0	0	0
Inflammation	0	0	0	0

(0 - No redness, no inflammation or excessive tearing, 1 - Mild redness with inflammation & slight tearing, 2 - Moderate redness with moderate inflammation and excessive tearing, 3 - Severe redness with severe inflammation and excessive tearing)

CONCLUSION

Rationale of the present study was to improve the precorneal residence time, and sustain the drug release by utilizing the approach of in situ gelling systems using Sodium alginate as in situ gelling polymer. It was envisaged that this techniques would prove successful in case of formulations prepared with the drug (dorzolamide hydrochloride). Formulation of dorzolamide hydrochloride developed using ion sensitive

polymer - Sodium alginate and viscosity modifying agent – Hydroxypropyl cellulose is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer precorneal residence time and ability to sustain drug release. The polymers used are inexpensive and easily available. The formulation also promises to reduce the frequency of drug administration, thus improving patient compliance. As the concept involved is novel and the methodology used for the preparation is simple as that of conventional ophthalmic liquid dosage form, it is industrially oriented and economical.

ACKNOWLEDGEMENT

Authors are thankful to Dr. Sunil Sukhtankar of Pricise Chemipharma Pvt. Ltd., Mumbai, India for providing gift sample of drug and Dr. A. D. Deshpande for proving the facilities for the present work.

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