



ENHANCEMENT OF ANTI-GLAUCOMA POTENTIAL BY NOVEL OCULAR DRUG DELIVERY SYSTEM

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ABSTRACT

Brimonidine is an anti glaucoma agent useful in treatment of IOP. An attempt has been made to formulate ophthalmic insert of brimonidine tartrate using HPMC, PVA, Chitosan and Sodium alginate by solvent casting method. The prepared ophthalmic insert were then evaluated for uniformity of thickness, weight, drug content, % moisture absorption, % moisture loss, folding endurance and surface pH. *In vitro* drug release of formulated batches of ophthalmic insert was performed by studying the diffusion through artificial cellophane membrane. On the basis of all physicochemical parameters and *in vitro* drug release studies, the formulation (F-1) with Chitosan (3%) was found to be promising and it was selected as an optimized formulation. The result of *in vitro* diffusion study of F-1 exhibited zero order kinetic with Higuchi diffusion mechanism drug release. The pharmacodynamics evaluation of F-1 in rabbit eye model indicated higher and longer IOP lowering potential of ocular insert as compared to eye drops. Chitosan based brimonidine ocular insert could be a potential vehicle to enhance ocular bioavailability and patient compliance.

Keywords: Ocular Inserts, Glaucoma, Brimonidine, IOP lowering etc.

INTRODUCTION

The eye as a portal for drug delivery is generally used for local therapy against systemic therapy in order to avoid the risk of eye damage from high blood concentration of drug, which is not intended¹. Most ocular treatments like eye drops and suspensions call for the topical administration of ophthalmically active drugs to the tissues around the ocular cavity. These dosage forms are easy to instill but suffer from the inherent drawback that the majority of the medication they contain is immediately diluted in the tear film as soon as the eye drop solution is instilled into the cul-de-sac and is rapidly drained away from the pre-corneal cavity by constant tear flow and lacrimo-nasal drainage. For this reason, concentrated solutions and frequent dosing are required for the instillation to achieve an adequate level of therapeutic effect. One of the new classes of drug delivery systems, ophthalmic inserts, which offer many advantages over conventional dosage forms, like increased ocular residence, possibility of releasing drugs at a slow and constant rate, accurate dosing, exclusion of preservatives and increased shelf life^{1,2}.

Ophthalmic inserts are defined as sterile preparations, with a thin, multilayered, drug-impregnated, solid or semisolid consistency devices placed into cul-de-sac or conjunctival sac and whose size and shape are especially designed for ophthalmic application. They are composed of a polymeric support containing or not drug, the latter

being incorporated as dispersion or a solution in the polymeric support. The inserts can be used for topical or therapy³.

Glaucoma is an optic neuropathy characterized by acquired loss of retinal ganglion cells (RGCs) and atrophy of the optic nerve leading to vision loss. Elevated intra ocular pressure (IOP) is a primary risk factor both for the development of glaucoma and for progression of optic nerve changes and visual field loss in the disease⁴. Brimonidine tartrate is a highly selective α_2 -adrenoceptor agonist which reduces intra-ocular pressure (IOP) by reducing aqueous humour production and increasing aqueous humour outflow via the uveoscleral pathway⁴. In the present study brimonidine ocular inserts were prepared and evaluated to sustain drug release and enhance ocular bioavailability.

MATERIALS AND METHOD

Material

Brimonidine tartrate was obtained as gift sample from Centaur Pharmaceutical Ltd. Sodium alginate was obtained as gift sample from Snap natural and alginate products Ltd, Ranipet. Chitosan obtained as gift sample from Indian sea food, Cochin. HPMC and Polyvinyl alcohol were from CDH, Mumbai. Brimonidine eye drops 0.2% were purchased from local drug store. Tonometry was done by Brio Schiotz Tonometer.

Table 1: Composition of various brimonidine ocular insert in different formulation

Formulation code	Chitosan (%w/v)	PVA (%w/v)	HPMC (%w/v)	Sodium Alginate (%w/v)
F-1	3%	-	-	-
F-2	-	3%	-	-
F-3	-	-	3%	-
F-4	-	-	-	3%

*0.2% brimonidine was added in all the formulations

Preparation Of Ocular inserts

Ophthalmic inserts of brimonidine were prepared by solvent casting technique. 0.2% Brimonidine was dissolved in individual polymeric dispersion 3% concentration of HPMC, PVA, Chitosan, and Sod. Alginate dispersion (Table 1). This mixture was kept for stirring in a magnetic stirrer for 2 hrs. This was allowed to stand for 12 hours. The polymeric drug solution was then poured into pre lubricated glass moulds and kept for drying in a hot air oven at $40 \pm 2^\circ\text{C}$ till complete drying. Dried films were carefully removed from the Petri

dish and then cut into oval shaped inserts with the help of a sharp edged die (13.2mm in length and 5.4 mm in width). Each ocular insert contained 0.4mg of the drug⁵.

Thickness of Ocular inserts

The prepared films were evaluated for the thickness of each film using digital vernier caliper. The mean thickness and standard deviation were calculated. Weight variation test was done by weighing twenty inserts individually using a digital balance⁶.

Drug content measurement

The uniformity of drug content of the ophthalmic insert was determined. Formulations were dissolved separately in 5 ml STF pH 7.4 and the resulting solutions were filtered and analyzed at 270 nm⁷.

Surface pH determination

Surface pH Determination and standard deviation were calculated, inserts were allowed to swell for 5 hours on agar plate prepared by dissolving 2% (m/v) agar in warm simulated tear fluid pH 7.4. The

surface pH was measured by means of a pH paper placed on the surface of swollen patch⁷.

Folding endurance of inserts

Folding endurance 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 visible cracks. The process was repeated till the insert showed breakage or cracks in center of insert. The total folding operations were named as folding endurance value⁶.

Table 2: Physical characterization of ocular films: thickness, weight, surface pH, and drug content

FormulationCode	Thickness*(mm)	Weight# (mg)	SurfacepH	% Drug content	Folding endurance
F-1	0.158 ± 0.007	4.52 ± 0.20	6	99.02 ± 1.34	384
F-2	0.098 ± 0.012	5.04 ± 0.21	7	97.33 ± 1.11	367
F-3	0.120 ± 0.008	4.21 ± 0.45	5	98.41 ± 2.20	375
F-4	0.169 ± 0.014	4.81 ± 0.20	6	99.33 ± 1.27	326

Table 3: % Elongation, tensile strength and % equilibrium swelling of ocular films

Formulation code	(%) Elongation*	Tensile strength* in g/mm ²	(%) Equilibrium swelling*
F-1	16.62 ± 0.04	25.40 ± 0.07	38.0 ± 0.8
F-2	18.80 ± 0.01	22.16 ± 0.06	43.5 ± 0.8
F-3	09.30 ± 0.07	15.42 ± 0.04	48.0 ± 0.7
F-4	13.63 ± 0.01	14.51 ± 0.06	50.0 ± 0.4

Swelling behavior of prepared ocular inserts

To determine the swelling index of prepared ocular inserts (n=3), initial weight of insert was taken, and then placed in agar gel plate for brimonidine inserts, and incubated at 37°C ±1 for five hours, insert was removed from plate after every one hour, surface water was removed with help of filter paper, and insert was reweighed.

Tensile strength was studied for ocular insert, cut into strips (50 x 10mm). Tensile strength and elongation at break was determined by modified method⁷.

Determination of interaction studies

Interaction studies were done to confirm the compatibility of drug and polymer. IR studies were taken using the KBr pellet method. An IR spectrum of ocular inserts was compared with IR of pure drug¹.

UV spectra of pure drug, ocular inserts were done qualitatively in order to access the pattern of peaks and for comparison purpose. The prepared formulations were diluted by taking 2 ml sample and diluted up to 100ml, then taking 5ml of diluted sample and further dilute up to 50ml. Then solutions were scanned for absorption at 270 nm.

Chromatographic purity of pure drug and prepared inserts has been done by assayed the TLC using silica gel as the coating substance (stationary phase) and a mixture of acetonitrile and buffer solution as the mobile phase. R_f value of pure drug and formulations were compared for the interaction in between polymers and drug.

In-Vitro drug release study

The *in-vitro* drug release studied by using bi-chambered donor receiver compartment model¹ (Figure-1), designed using commercial semi-permeable membrane of transparent and regenerated cellulose type (Sigma dialysis membrane) was used. The insert was placed in the donor compartment. The entire surface of the membrane was in contact with the reservoir compartment that contained 25 ml of STF for Brimonidine inserts, which was stirred continuously using a magnetic stirrer at 20 rpm to simulate blinking action. Drug release was determined by withdrawing a defined quantity of sample 5ml from the sampling port at periodic intervals, which was replaced with equal volume of dissolution medium. Drug content was analyzed at 270 nm using respective dissolution mediums as blank on UV- Spectrophotometer. The release data were kinetically analyzed using different kinetic models (zero-order, first-order and Higuchi diffusion model) to determine the mechanism of drug release from the prepared ocular Inserts⁷.

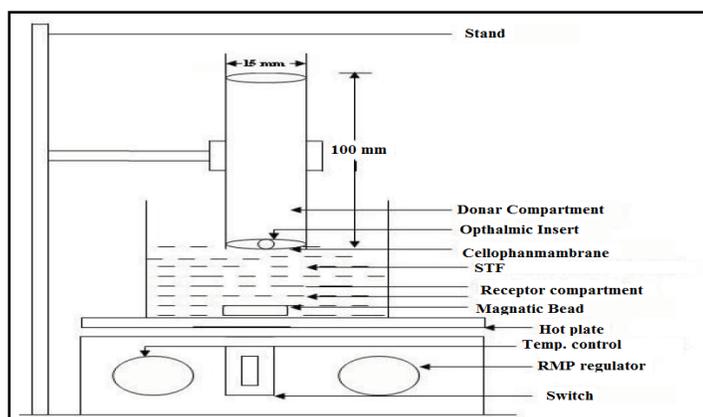


Fig. 1: Diagrammatic model of in-vitro drug release bi-chambered donor receiver compartment model

In-vivo study drug release study

The *in-vivo* studies are performed in albino rabbit, because the rabbit's eye stimulates an adult human eye with respect to size, shape, physiology, and composition of tears. All rabbits used in these experiments were normotensive and were housed under proper conditions. The animals were divided into 2 groups each comprising of 5 rabbits, one test group administered the marketed brimonidine eye drop and second group was administered ophthalmic inserts of brimonidine. Both eyes are used for the study one eye as an experimental and one eye as control. Intra Ocular Pressure (IOP) was measured with Schioetz Tonometer⁸.

IOP measurements were done at 0, 0.5, 1, 3, 7, 9, and 12 hours post administration. For measuring the IOP, rabbits were placed in restraining boxes and eyelids were retracted gently with one hand, without exerting pressure on the eye ball and the tonometer was placed in the horizontal position on the center of the cornea. The handle was in the midway between the top and foot plate of the cylinder, thereby the instrument might cut the independently with its own weight. The position of the pointer was noted and the tension in mm Hg was determined from the calibration scale. The observations of pattern of IOP and the changes in IOP were calculated⁹.

The IOP was measured immediately before giving the formulation and at a suitable time interval following the treatment. The formulation was tested on a group of at least five healthy male rabbits. Similar protocol was repeated for the marketed eye drops⁹.

Change in IOP for each eye is expressed as follows:

$$IOP = IOP_{\text{zero time}} - IOP_{\text{time}}$$

IOP is reported as the mean (\pm S.E.M.) for $n = 5$.

The result of *in vivo* study was presented as mean \pm SD. The results obtained from *in vivo* study were subjected to statistical analysis, using one-way analysis of variance (ANOVA) for which $P < 0.05$ ¹⁰.

RESULTS AND DISCUSSION

The results obtained from all over the studies were as following; the prepared inserts were translucent, colorless and smooth in texture, uniform in appearance and show no visible crack or imperfection. The inserts had a thickness varying from 0.098 ± 0.012 to 0.169 ± 0.014 mm and weight varying from 4.81 ± 0.20 to 5.04 ± 0.21 mg. The drug content was consistent in all batches and varied from 97.33 ± 1.11 to $99.33 \pm 1.27\%$. The surface pH of the ocular films was within the range of 5 to 7. The recorded folding endurance for all the films was from 326 to 384 (Table-2). Percent equilibrium swelling of all the formulations is shown in the table 1 which varied from 38.0 ± 0.8 to $50.0 \pm 0.4\%$, sodium alginate showed maximum degree of swelling i.e. $50.0 \pm 0.4 \%$ as compared to any other formulation. The tensile strength of the inserts ranged between 14.51 ± 0.06 to 25.40 ± 0.07 g/ mm² and elongations at break was between 9.30 ± 0.07 to $18.8 \pm 0.01\%$ (Table-3).

The interaction of Brimonidine Tartrate with polymer was studied using FTIR spectroscopy. It was found that drug had no interaction with polymer. The R_f value of TLC was found that drug had no interaction with the polymers. In order to understand the drug release mechanism, the release data was tested assuming common kinetic model (Table-4). It indicates that the release of drug from the patches might have followed Higuchi model kinetics. Drug release pattern from inserts is shown in figure-2. The formulation F-1 showed the potential of sustaining the drug release for 12 hr and hence formulation F-1 was selected as optimized formulation. The order of sustaining potential of drug release was found in following order **F-1<F-2<F4<F-3** (Figure 2).

It indicates that chitosan displayed maximum potential of prolonging drug release compared to other polymer at same concentration. This could be attributed to the comparatively poor solubility of chitosan as compared to other polymer which all hydrophilic/ water soluble in nature.

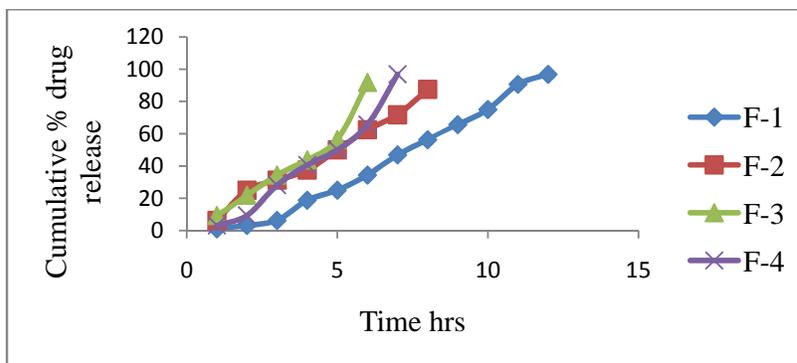


Fig. 2: Plot of cumulative % drug release vs. time hrs

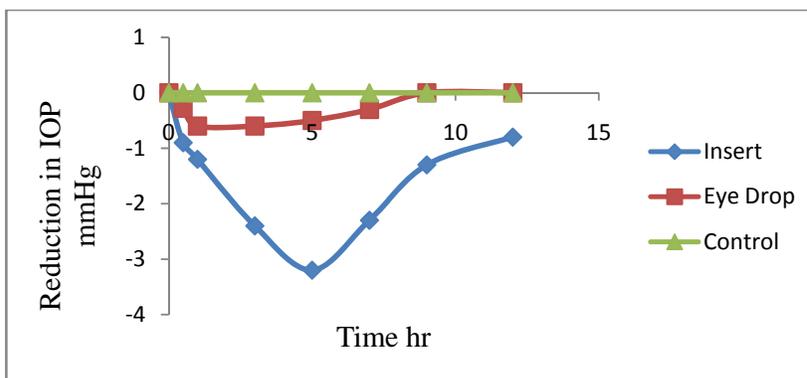


Fig. 3: Reduction in IOP after topical administration of brimonidine ocular insert compared to brimonidine marketed preparation

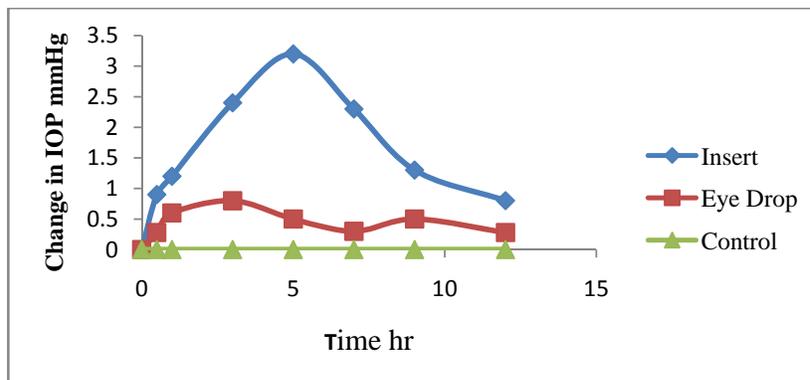


Fig. 4: Change in IOP after topical administration of brimonidine ocular insert compared to brimonidine marketed preparation

Pharmacodynamic evaluation was performed to interpret the efficacy of the developed formulation and the drug. After the application of ophthalmic inserts, there was a reduction in IOP. The insert showed a reduction of 2.4 mm Hg in IOP in 3 hours time period and the eye drop showed a reduction of 0.8 mm Hg IOP in 3 hours⁸. The activity was noticed over a period of 12 hour post administration for insert. IOP measurements with a marketed formulation of brimonidine eye drop, showed a peak effect at 3 h (compared to 5 h with insert). On the other hand chitosan based ocular insert showed an effect which was sustained for up to 12 hr (IOP ranging from 0.9 mmHg to 3.2 mmHg from 0.5 to 5h, while the eye drop was 0.3 at 0.8 mmHg h and reduced to 0.5 to 3h). Peak effect obtained with insert was 3.2 mmHg at 5 h. Similarly, a statistically significant lowering ($P < 0.0072$) in IOP reduction was noted with respect to the eye drops indicating a better potential of ocular inserts over the eye drops¹⁰.

CONCLUSION

The formulation of ophthalmic insert containing brimonidine tartrate (0.2% w/v) and chitosan (3% w/v) seems to be promising and further *in vivo* study must be carried out to check the efficacy of preparations. Developed insert achieved the targets of present study, such as increase residence time, prolonged zero order release, reduction in frequency of administration, and thus improve patient compliance.

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