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Full Length Research Paper

Formulation development, optimization and evaluation of once a day occuserts of brimonidine tartrate

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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 pathway. The objective of the present work was to develop ocular inserts of Brimonidine Tartrate and evaluate their potential for sustained ocular delivery. Matrix-type ocular inserts were prepared by solvent casting technique-employing mercury as substrate and characterized in vitro by drug release studies using a flow-through apparatus that simulated the eye conditions. Nine formulations were developed, which differed in the ratio and weight of polymers carbopol 934P and HPMC-K15M. All formulations carried PEG-400 (30 % w/w) plasticizers. The optimized formulation was subjected to interaction studies, all physico-chemical study, sterility test, in vivo studies, and stability studies to assess the effectiveness of the formulation. Cumulative drug released from the formulation ranged from 90-98% within 24 hours. On the basis of in vitro drug release studies, the formulation with Carbopol-934: HPMC K15M (80:20) was found to be better than the other formulations and it was selected as an optimized formulation. On the basis of interaction studies, all physico-chemical study, sterility test, in vivo studies, and stability studies, it can be concluded that this ocular insert formulation provided the desired drug release in vitro for one day and remained stable and intact at ambient conditions.

Keywords: Brimonidine Tartrate; ophthalmic inserts; *in vitro* release studies; *in vivo* studies.

INTRODUCTION

Continuous delivery of drugs to the eye offers major advantages over conventional therapies that involve administration of drug solutions or suspensions as eye drops. Eye drop administration often results in poor bioavailability and therapeutic response due to rapid precorneal elimination of the drug and is also associated with patient compliance problems (Schoenwald, 1990, Hume et al., 1994). For this reason, several approaches have been reported and various ophthalmic vehicles, such as suspensions, ointments, inserts and aqueous gels, have been investigated to extend the ocular residence time of topically applied medications (Dicolo et al., 2001). Ophthalmic inserts 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 (Kawakami et al., 2001; Sasaki et al., 1993; Baleens et al., 1998).

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 pathway (Julie and Julia, 1998). The aim of the present investigation is to develop and evaluate once a day ophthalmic insert of Brimonidine Tartrate as monolithic matrices using polymers in different combinations by solvent casting technique to achieve a controlled release of Brimonidine Tartrate.

MATERIALS AND METHODS

Materials

Brimonidine Tartrate was obtained as gift sample from.

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Table No. 1: Composition of formulations

Batch code	Carbopol-934: HPMC K15M	Total Polymer Weight (mg)
F1	20:80	250
F2	20:80	300
F3	20:80	350
F4	50:50	250
F5	50:50	300
F6	50:50	350
F7	80:20	250
F8	80:20	300
F9	80:20	350
All batches contain 90mg Brimonidine Tartrate and PEG-400 (30% w/w)* and 10 ml water used as a solvent		

* Based on Polymer weight

Sun Pharmaceutical Industries Ltd., Silvassa, Gujarat HPMC-K15M was obtained as gift sample from Colorcon Asia Pvt. Ltd., Goa and Carbopol 934P from Intas pharmaceuticals Ltd.

Preparation of Ophthalmic inserts

Ophthalmic inserts containing Brimonidine Tartrate (7.17 mg/cm^2) were prepared by solvent casting technique-employing mercury as substrate (Scirra and Gidwani, 1972). In the present study, total of nine formulations were formulated using Carbopol-934P and HPMC-K15M in different concentration. PEG-400 was incorporated at a concentration of 30 % w/w of polymer as plasticizers.

They are designated as F_1, F_2, F_3, F_4, F_5, F_6, F_7, F_8 and F_9 respectively. The detailed compositions of the inserts are given in Table No.1 above.

The polymer(s) were dissolved in distilled water. The polymer(s) solution was mixed thoroughly with the help of magnetic stirrer for 60 minutes to get a clear solution. PEG-400 was added as plasticizer to viscous polymer solution, and stirred for further 30 minutes. Weighed amount of Brimonidine Tartrate was added and stired for 2 hour to get uniform dispersion. 10 ml dispersion was poured in a glass bangle (4 cm diameter) placed on a mercury surface and dried at 40°C for 24 hours the dried films were carefully removed and cut into 0.5024 cm^2, wrapped individually in aluminum foil and stored in desiccator until further use.

Drug Excipients interaction studies

The drug-excipients studies were confirmed by infrared spectrophotometer using KBr disc method. The IR spectra obtained was elucidated for important groups. The identification peaks were found i.e. -NH streching at 3473.91 with a shoulder at 3437.26, -CN streching at 1300.07, 2362.88 and 2341.66 two band for the carboxlate ion,1718.63 is the peak for -C=O streching. The IR spectrum is depicted in figure 1 below.

Physicochemical Evaluation

Uniformity of thickness

Transverse sections of the insert at 5 different points were taken and the thickness was determined using optical microscopic technique.

Weight variation

Weight variation test was done by weighing five inserts individually using a digital balance. The average weight of the insert was taken as the original weight.

Surface pH[9]

Surface pH of the insert was determined by allowing them to swell in closed petridish at room temperature for 30 minutes in 0.1 ml of warm simulated artificial tear fluid (ATF; sodium chloride: 0.670 g, sodium bicarbonate: 0.200 g, calcium chloride 2H2O: 0.008 g, and purified water q.s. 100 g). The swollen devices were removed and placed under digital pH meter to determine the surface pH.

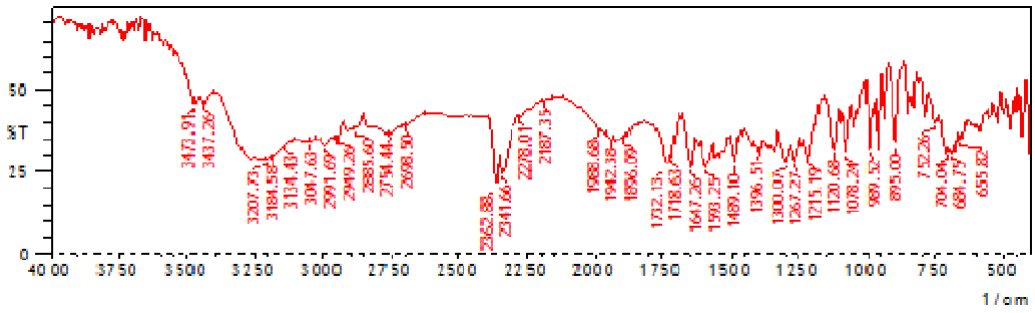

Figure 1. (a) In vitro release of Brimonidine Tartrate from F1, F4 and F7 formulations.

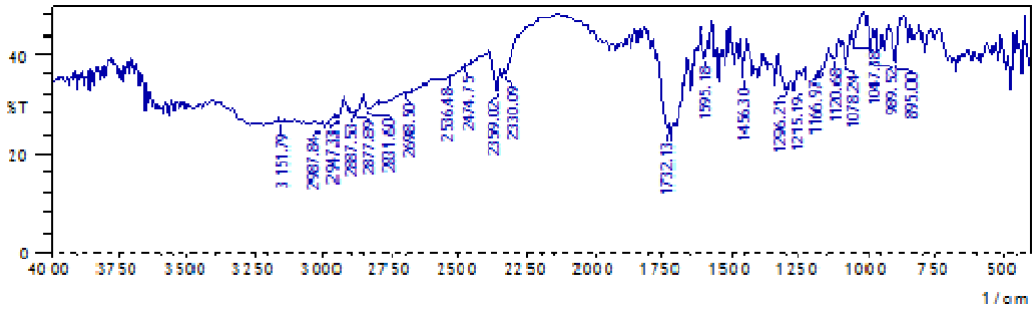

Figure 1. (b) In vitro release of Brimonidine Tartrate from F1, F4 and F7 formulations.

Folding Endurance[10]

The folding endurance was reassured manually for the prepared inserts. It is expressed as number of time the inserts are folded at the same place either to break the insert or to develop visible cracks. This is important to check the ability of sample to withstand folding. This also gives an indication of brittleness. The number of times the films could be folded at the same place without breaking gives the value of folding endurance.

Drug content uniformity

The uniformity of drug content of the ophthalmic insert was determined, based on the dry weight of drugs and polymers used by means of a UV spectrophotometric method Formulation were dissolved separately in 10 ml ATF pH 7.4 and stirred for 30 minutes on a magnetic stirrer at 100 rpm. The resulting solutions were quantitatively transferred to volumetric flasks, and diluted suitably with pH 7.4 ATF. The resulting solutions were filtered and analyzed for Brimonidine tartrate content at 270 nm.

Tensile Strength[11]

The tensile strength of the ophthalmic insert was measured using tensile strength instrument (locally fabricated instrument). Average reading of three inserts from each batch was taken as the tensile strength.

Tensile strength (g/mm^2) = break force (g)/cross-sectional area of the sample (mm^2)

Elongation at break (%) = increase in length at break point (mm) / Original length (mm) ×100

Hardness[11]

The hardness of the ophthalmic inserts was measured using fabricated hardness apparatus. Average reading of three inserts was taken as hardness.

Swelling index[12]

Swelling index was determined by immersing the insert in a preweighed stainless steel basket in 20 ml of freshly boiled and cooled artificial tear fluid pH 7.4 at 37^0 C. The designed for in vitro determination of drugs in ophthalmic

Table No. 2: Physico-chemical evaluation data of Ophthalmic Inserts

	Weight variation# (mg)	Thickness* (mm)	Folding Endurance*	Surface pH*	Tensile Strength* (gm/mm^2)	Elongation at Break (%)	Hardness (gm)	Swelling Index*	Drug Content
F1	13.12 ± 0.38	0.214 ± 0.0540	322±7	7.4 ± 05	0.234 ± 0.0067	29.2	253 ± 2.15	13.5 ± 0.220	97.5 ± 0.15
F2	15.12 ± 0.62	0.331 ± 0.0034	347±8	6.7 ± 00	0.245 ± 0.0020	31.4	234 ± 3.54	11.1 ± 0.615	98.5 ± 0.30
F3	17.29 ± 0.56	0.443 ± 0.0467	380±7	6.9 ± 00	0.224 ± 0.0031	28.9	227 ± 5.28	10.9 ± 0.145	99.3 ± 0.10
F4	13.35 ± 0.42	0.208 ± 0.0033	336±5	6.5 ± 00	0.239 ± 0.0045	34.5	267 ± 2.74	07.8 ± 0.325	97.8 ± 0.36
F5	15.42 ± 0.03	0.327 ± 0.0031	343±7	7.2 ± 05	0.263 ± 0.0054	29.8	233 ± 3.28	07.1 ± 0.210	98.2 ± 0.15
F6	17.54 ± 0.23	0.458 ± 0.0424	384±8	7.2 ± 05	0.241 ± 0.0046	28.9	242 ± 3.56	04.5 ± 0.430	99.3 ± 0.22
F7	13.22 ± 0.04	0.198 ± 0.0044	370±8	5.9 ± 00	0.234 ± 0.0022	33.2	253 ± 7.21	03.8 ± 0.235	97.7 ± 0.30
F8	15.12 ± 0.12	0.338 ± 0.0370	321±9	6.3 ± 00	0.226 ± 0.0056	29.6	225 ± 5.84	03.2 ± 0.154	98.5 ± 0.15
F9	17.35 ± 0.42	0.449 ± 0.0023	337±9	6.0 ± 05	0.258 ± 0.0020	35.3	261 ± 4.27	02.8 ± 0.265	99.1 ± 0.27

*Each reading is an average of three determinations

weight of the swelled insert was determined at specified time intervals (every 5 minutes). The procedure was continued till there was no increase in the weight. And the relative weight gain (water uptake) was calculated using the following relationship.

Swelling Index = $(W_t - W_o / W_o) \times 100$

Where W_t = weight of patch at time t

W_o = weight of patch at time zero.

IN VITRO Release studies[13]

To simulate the actual physiological conditions prevailing in eye, an in vitro open flow through assembly was inserts and was used in the present work.

Description of open flow through assembly

A 2 ml glass tube open at both ends was used as an in vitro diffusion cell. Two fluted glass adapters were fused at both open ends so that one formed the other fluted end was used to withdraw samples. The inlet of this tube was connected to a reservoir containing artificial tear fluid pH 7.4. The head of the reservoir was kept constant. Flexible PVC tubing was connected from this reservoir to the cell, in which 2 ml of ATF was maintained constant. The rate of flow of ATF was controlled with a valve.

Procedure

ATF pH 7.4 was put into the reservoir. A small volume of fluid was allowed to drain away, so as to remove any entrapped air bubbles in the cell. An ophthalmic insert was stuck onto a thin small, circular Teflon disc, so that only one surface was exposed to the diffusion fluid. This disc was steadily inserted into the cell containing 2 ml of fluid. The temperature of the fluid was kept at 35 ± 1ºC constantly. At regular intervals the diffusion fluid was taken to analyze for drug content using a UV spectrophotometer at 270 nm.

Sterilization and Test for Sterility[14]

In the present study, the optimized formulation was sterilized separately in their final packaged container by exposing them to UV radiations for 90 minutes. The irradiated ophthalmic insert was tested for their sterility as per the pharmacopoeial procedure, which is intended for detecting the presence of viable forms of Bacillus subtilis in or on sterilized preparations. The tests were carried out under aseptic conditions to avoid accidental contamination of the product during the test.

IN VIVO release study[15]

For the purpose, six male albino rabbits each weighing 2-2.5 kg were selected. They were fed on standard diet. In which one serves as a control by placing blank insert in cul-de-sac of both eyes. The ophthalmic inserts were placed in the cul-de-sac of both eyes of five rabbits. At regular time intervals, the remaining ocular inserts were removed carefully and analyzed for the drug content using an UV spectrophotometric method at 270 nm. The drug content obtained was subtracted from the initial drug content in the ophthalmic insert which gave the amount of drug released in rabbit's eye.

Table No. 3: Kinetic model for the formulations F1, F2, F3, F4, F5, F6, F7, F8 and F9.

Formulation										
		F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Higuchi	**R**	0.9874	0.9884	0.9883	0.9819	0.9827	0.3949	0.9801	0.9732	0.9672
	K	30.95	31.22	31.71	32.72	32.45314	77.38	28.83	28.92	28.97
Zero Order	**R**	0.9959	0.9979	0.9976	0.9991	0.9982	0.4125	0.9893	0.9894	0.9878
	K	06.32	06.19	06.11	06.35	06.12	14.65	4.92	4.97	5.01
Korsemeyer	**R**	0.9955	0.9977	0.9973	0.9979	0.9962	0.8906	0.9974	0.9958	0.9920
	K	0.12	0.11	0.09	0.07	0.06	0.03	0.02	0.02	0.01
	n	1.5436	1.6234	1.7569	1.9454	2.0118	2.6107	2.5000	2.7332	3.0669
Hixon Crowell	**R**	-0.9959	-0.9979	-0.9976	-0.9991	-0.9983	-0.4125	-0.9893	-0.9894	-0.9878
	K	-2.10	-2.06	-2.03	-2.11	-2.04	-4.88	-1.64	-1.65	-1.66
First Order	**R**	0.9514	0.9478	0.9344	0.9527	0.9504	0.8652	0.8985	0.9072	0.9118
	K	0.06	0.06	0.06	0.07	0.07	0.08	0.07	0.07	0.08
Peppas	**R**	0.9959	0.9979	0.9976	0.9991	0.9982	0.4125	0.9893	0.9894	0.9878
	K	0.06	0.06	0.06	0.06	0.06	0.15	0.05	0.05	0.05

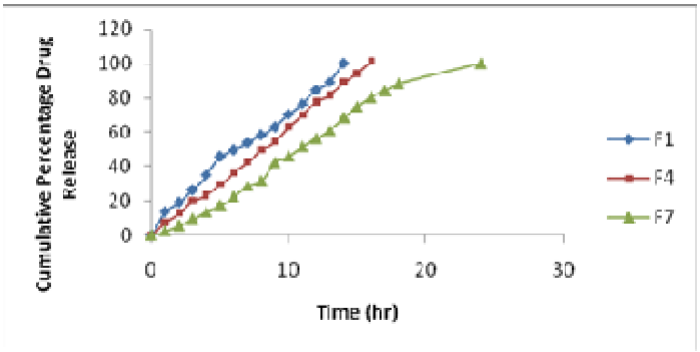

Figure 2. In vitro release of Brimonidine Tartrate from F2, F5 and F8 formulations.

Irritation Study

During *in vivo* study the rabbit eye was observed for sign of any irritation, redness, swelling or haziness.

Stability Studies

The optimized formulation was packed in aluminum foil. It was then stored at 40°C / 75 % RH according to ICH[16]. Samples were withdrawn after three month and evaluated for change in drug release pattern.

RESULTS AND DISCUSSION

Physicochemical data presented in table 2 above show weight variation, thickness, folding endurance, surface pH, tensile strength, elongation at break, hardness, swelling index and drug content of the prepared inserts. 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 0.198 ± 0.0044 to 0.449 ± 0.0023 mm and weight varying from 13.12 ± 0.38 to 17.54 ± 0.23 mg. It was found that the thickness and weight of the inserts

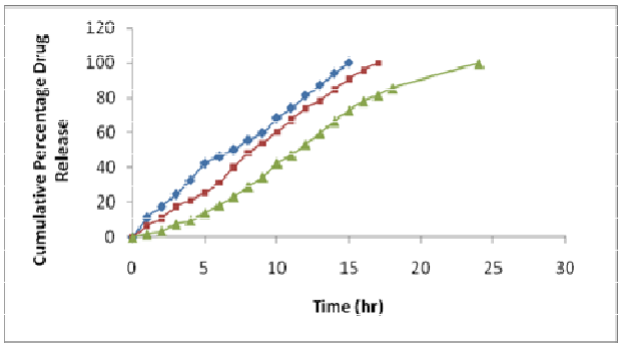

Figure 3. In vitro release of Brimonidine Tartrate from F3, F6 and F9 formulations.

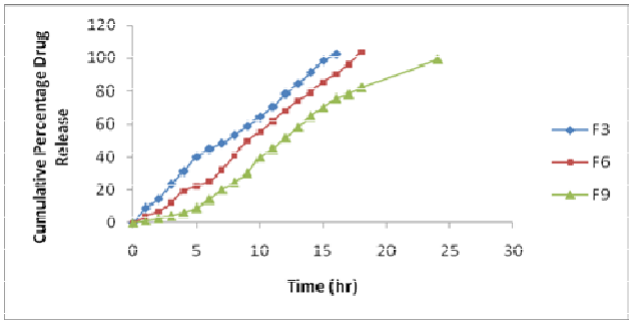

Figure 4. in vitro- in vivo correlation of F9.

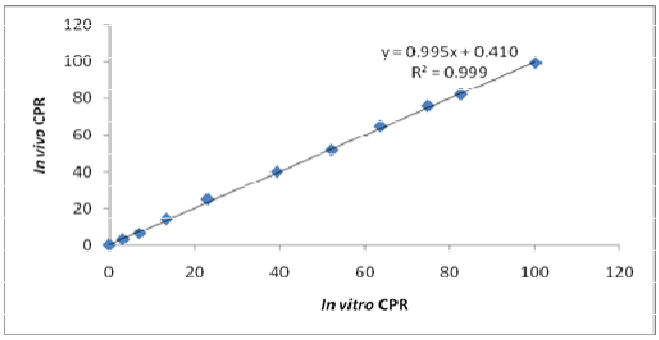

Figure 5. In vitro release of Brimonidine Tartrate from F9 evaluated for stability study.

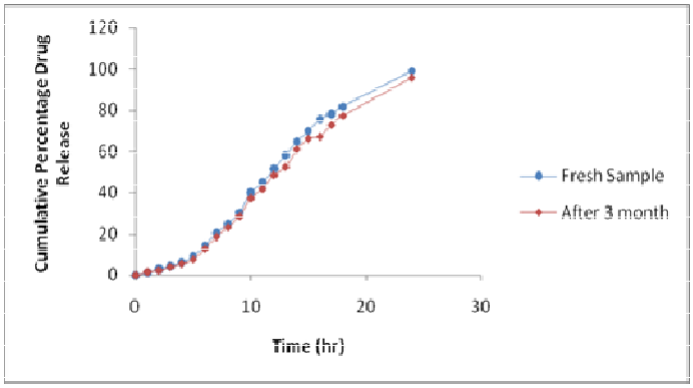

Figure 6: IR Spectra of (a) Brimonidine Tartrate, and (b) HPMC-K15M + Carbopol-934P + Brimonidine Tartrate.

were increased by increase in the total polymer concentration. The inserts were found to possess uniform weight and thickness within the batch. The recorded folding endurance for all batches was greater than 300, which is considered satisfactory and reveals good film properties. Surface pH was within range of 5.9 ± 00 to 7.4 ± 05. Tensile strength and elongation at break varying from 0.224 ± 0.0031 to 0.263 ± 0.0054 and 29.2 to 35.3 respectively. The hardness of the inserts varies between 227 ± 5.28 to 267 ± 2.74. The drug content was consistent in all batches and varied from 97.5 ± 0.15 % to 99.3 ± 0.22 %.The equilibrium swelling % varied from 02.8 ± 0.265 % to 13.5 ± 0.220 %. Increase in amount of carbopol-934P in formulation decreased swelling. In order to understand the drug release mechanism, the release data was tested assuming common kinetic model((((16)))) (Table 3 above). It indicates that the release of drug from the patches might have followed zero order kinetics. Influence of change in ratio on drug release in insert containing total polymer weight 250 mg, 300 mg and 350 mg as a function of time is shown in figure 2 above, figure 3 below and figure 4 below. The formulation F9 showed the potential of sustaining the drug release for 24 hrs and hence formulation F9 was selected as optimized formulation. *In vivo* release studies have shown that the formulation F9 is capable of replacing the drug for 24 hrs almost in same pattern, which was found in *in vitro* studies. It was found to release 100.07 % of loaded drug at 24 hrs. To establish the correlation between in vitro in vivo release data, regression analysis was carried out. The correlation value of 0.999 indicated correctness of the in vitro method followed and adaptability of the delivery system to the biological system where it can release the drug in concentration independent manner figure 5 above. Formulation F9 passed the test for sterility. There was no sign of any irritation, redness, swelling or haziness in the rabbit's eyes used for the study indicating that insert is free from ocular toxicity and safe for ocular use. Stability study performed show no significant changes (figure 6 above) in the film which suggest that the film was stable.

CONCLUSION

Formulation F9 has achieved the targets of present study such as prolonged zero order release, reduction in frequency of administration and thus may improve patient compliance.

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