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Research Article

PREPARATION AND EVALUATION OF OPHTHALMIC INSERTS OF BRIMONIDINE TARTRATE

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ABSTRACT

The purpose of this work was to develop an ocular insert for the treatment of glaucoma with α-agonist Brimonidine tartrate. Nine such ocular inserts were prepared by solvent casting method using polymers Hydroxyethyl methacrylate (HEMA), EUDRAGITRL-100,EUDRAGIT RS-100, plasticizers, Dibutyl phthalate(DBP), PEG 400 and Propylene glycol and water as solvent. These formulations were evaluated for mechanical properties like tensile strength, % elongation at break, strain, folding endurance, physicochemical properties like thickness, weight variation, surface pH, %moisture absorption, compatibility, and drug content. *In vitro* drug release was carried out and the release kinetics was studied which revealed case II transport. All the formulations were taken for sterilization and subjected to eye irritancy test on Rabbits. Inserts containing HEMA plasticized with DBP showed better shape retaining properties and more controlled release of drug. On the basis of evaluation it was found that tensile strength and folding endurance of the inserts prepared with plasticizer DBP was high compared to inserts prepared with other plasticizer and inserts containing HEMA showed good shape retaining properties than other polymers used in the study.

Keywords: Brimonidine tartrate, HEMA, EUDRAGIT RL-100,EUDRAGIT RS-100.

INTRODUCTION

Ophthalmic inserts are defined as sterile preparations, with a solid or a semi solid consistency, are essentially composed of a polymeric support containing drugs, the latter being incorporated as dispersion or a solution in the polymeric support. It also offers accuratedosing to overcome the side effects of pulsed dosing by conventional systems, increases the ocular bio availability of drugs byprolonging the corneal contact time, to circumvent the protective barriers like drainage, lacrimation and conjuctival absorption[1]. Therefore, the possibility of incorporating various novel chemicals, technological approaches and exclusion of preservatives, are reducing the risk of sensitivity reactions[2].The basic objective of ocular controlled drug release is to achieve more effective therapies by eliminating the potential for both under and overdosing, maintenance of drug concentration within a desired range, fewer administrations, optimal drug use and increased patient compliance[3].

Brimonidine tartrate is a highly selective a2-adrenoceptor agonist which reduces intra-ocular pressure (IOP) by reducing aqueous humour productionand thereby increasing aqueous humour outflow via the uveoscleral pathway.

Its selectivity towards alpha-2 adrenergic receptors and its neuroprotective activity on retinal ganglionic cells makes it as an important therapeutic agent for the treatment of open angle glaucoma[4]. The recommended dosage of Brimonidine tartrate for the treatment of glaucoma is 1 drop of 0.2% solution in the affected eyes three times a day.

The present formulation is intended to provide a sustained release of film over a period of 8 hrs to increase patients' compliance by incorporating novel chemicals without preservative.

MATERIALS AND METHODS

MATERIAL

Brimonidine tartrate was agift sample from Cipla Pvt.Ltd, Mumbai.Eudragit RL 100Eudragit RS andHydroxyethyl methacrylate (HEMA)were obtained from S.D. Fine Chemicals Mumbai. All other reagents and solvent used were of analytical grade.

METHOD

The inserts were prepared by solvent casting method[5]. Weighed quantity of polymer was dissolved in 10ml distilled water under continuous stirring as per the quantity mentioned in Table1. 150mg Brimonidine tartrate per ocular insert was added to the polymeric solution. The medicated polymer solution was sonicated for fifteen minutes to remove air bubbles. Then plasticizer was added under continuous stirring. Then resultant solution was stirred. After proper dispersion the casting solution was poured on glycerin coated Petri plate (Diameter 5 cm and area 19.625 cm^2) and covered with inverted funnel to allow slow and uniform evaporation of solvent at 40^0C for 24 h. The dried film thus obtained was punchedwith sharp edged dieinto ten pieces. Inserts thus prepared were packed in sterilized aluminum foil which was further stored in desiccators at room temperature[6].

EVALUATION OF OCULAR INSERT

Mechanical properties

Tensile strength

The tensile strength was measured using a tensile strength instrument.One end of the film was attached with an adhesive tape and the other end of the insert was fixed by adhesive tapes with a small pin placed in between the base plate.This fixing assisted in keeping the insert straight while stretching. In the adhesive tape a small hole was made near the pin in which a hook was inserted. The hook was attached with a thread, passed over the pulley where a small pan was attached to hold the weights. A small pointer was attached to the thread, which travelled over the graph paper affixed on the base plate. To determine the tensile strength, weights were gradually added to the pan to increase the pulling force until the patch broke. The distance travelled by the pointer on the graph paper before the breaking of the patch determined the elongation. The weight required to break the patch was noted as break Load[7].

Tensile strength was calculated using the following formula:

Tensile strength= Breaking load / Cross Sectional Area of the sample (1)

Percentage Elongation at break

Percentage elongation at break was calculated according to the following formula.

% Elongation at break= {Change in length (mm) / original length (mm)} X 100 (2)

Strain

Strain was calculated according to the following formula.

Strain= Change in length (mm) / Original length (mm) (3)

Folding endurance

Folding endurance for ocular inserts was calculated by folding the inserts repeatedly in the same position till a crack appeared. Number of folds required to produce the crack were counted. Folding endurance test was repeated using more sets of ocular inserts[8].

Weight of ocular insert

The ocular insert was taken out and weighed using digital balance and the average weight of each insert was determined[9].

Uniformity of thickness

The thickness of the insert was determined using Micrometer gauze (Mitotoyo, Japan) at five random points of each insert. The mean value was calculated[10].

% Moisture absorption

The percentage moisture absorption test was carried out to check physical stability or integrity of the film at humid condition. The inserts were weighed and placed in desiccators containing saturated solution of sodium chloride and 75±5% RH was maintained. After three days, the inserts were taken out and reweighed. The % moisture absorption was calculated using the following formula[11].

% Moisture absorption =
[(Final weight – Initial weight) / Initial weight] X 100 (4)

Surface pH

Surface pH test was carried out to investigate any possible eye irritation. The inserts were allowed to swell in a closed Petri plate at room temperature for 30 minutes in 0.1 ml of double distilled water. The swollen insert was removed and placed under digital pH meter to determine the surface pH[9].

Drug content

Drug content to check the uniformity of the drug in the circular inserts, five inserts were taken out from each film. Each insert was placed in a glass vial containing 5 ml of Phosphate buffer pH 7.4. The inserts were dissolved by the aid of a magnetic stirrer, the solution was then filtered through filter membrane of 0.45µm. 1 ml from the filtrate was withdrawn and assayed spectrophotometrically after suitable dilution at 248nm against a blank solution which was prepared by using a placebo film in the same solvent to prevent the interference of polymer and plasticizes[12].

In-vitro drug release studies

The in-vitro drug release studies were carried out using diffusion cell. The insert was placed on the cellophane membrane and isotonic Phosphate buffer of pH 7.4 was added to it. The entire surface of the membrane was in contact with the receptor compartment containing isotonic Phosphate buffer of pH 7.4. The content of the receptor compartment was stirred continuously using magnetic stirrer at 50 rpm and its temperature was maintained at 37°C ± 0.5°C. At certain time intervals, 1 ml of the solution in the receptor compartment was withdrawn and replaced with 1 ml of fresh Phosphate buffer. The withdrawn sample was and it was analyzed using UV spectrophotometer at 248nm[12].

Statistical analysis

The difference in the release data for the different formulation was done by one way analysis of variance of means (ANOVA) at 5 % significance level using Microsoft 2007 excel package. Invitro dissolution data i.e. drug release at 8 hrs was taken as the parameter for ANOVA analysis.

Sterility test

Sterility testing is intended for detecting the presence of viable form of microorganisms and was performed by using fluid Thioglycolate medium and Soybean casein digest medium, as per the Indian pharmacopoeia.

The formulations were sterilized separately under UV radiation for 30mins.The irradiated formulations were tested in aseptic conditions for viable forms of bacteria, fungi, Yeast in both the media prescribed by Indian Pharmacopoeia for 7 days[13].

Eye irritancy test

The sterilized ocular insertswere used for eye irritancy test. The Draize technique designed for testing ocular irritation of the ophthalmic product was used[14]. Clearance for the handling of experimental animals was obtained from the Institutional Animal Ethical Committee (IAEC) constituted for the purpose. According to the Draize test, the ocular insert was placed into the lower cul-de-sac with observation of the various criteria made at a designed time interval of 1hr, 24hrs, 48hrs, 72hrs and 1week after administration. Three male rabbits weighing 1.5 to 2kg were used for the present study. The sterile formulation was instilled twice a day for a period of 7. Rabbits were observed periodically for redness, swelling, watering of the eye[15].

RESULTS AND DISCUSSION

Mechanical properties

Mechanical properties like Tensile strength, % elongation at break, strain were investigated to determine the suitability and acceptability of the ocular inserts. The nature of the plasticizer and the polymers in combination affected the mechanical properties.Inserts made with plasticizer DBP showed higher tensile strength compared to Propylene glycol and PEG 400. Among the plasticizers the tensile strength of the inserts decreased in the following order DBP>PG>PEG400 (Table 2). On the other hand as thecross linking of the polymer increased the tensile strength also increased. Therefore inserts prepared with HEMA plasticized with DBP possessed high tensile strength. The tensile strength and strain were found to be greater in film contacting HEMA, than Eudragit RL100 or Eudragit RS 100.

Folding endurance

The folding endurance determined the ability of the film to rupture. It was found that the folding endurance was least for the formulation F3 (22.33±2.51) and found to be highest for the formulation F9 (37±2.64). It reveals that the folding endurance increases exponentially with DBP as plasticizer with any of the polymer than with PEG 400 and propylene glycol.

Weight of ocular insert

The weight of the ocular insert was determined using digital balance. The weight of ocular insert was found to be in between 0.95±0.07 and 1.03±.0.02 (Table 3).

Uniformity of thickness

The mean thickness of the insert was determined using Micrometer gauze. For all formulations the thickness was found to be between 0.14±0.05 and 0.15±0.01 (Table 3). The

low standard deviations indicate the uniformity of the thickness.

% Moisture absorption

The % moisture absorption was found to be between 1.02±0.75and1.54±0.42 (Table 3).F2 showed lowest moisture uptake and F3 showed highest moisture uptake. This was attributed to the type of plasticizer. Inserts prepared with PEG 400 showed more moisture uptake than Propylene Glycol and Dibutyl Phthalate.

Drug content

The drug content for all nine formulations was in the range of 95.53±0.41and 98±0.6 (Table 2). So it qualified the IP specifications for assay of drug content which should not be less than 90% and should not be more than 110%(Table 3).

Surface pH

The surface pH for all the nine formulations was within the range of 7.2 to 7.4. Generally the pH of ophthalmic formulations should be within 4.5 to 11.5. As the drug of choice wasbasic,the pH of all the formulation showed in the range of 7.2 to 7.4 (Table 3).

***In-vitro* drug release studies**

All the formulations have been able to release the drug above 80% in 8 hrs time. Formulations prepared with Eudragit RL 100 and RS 100 showed comparatively better releaseas in Fig.1. Formulations made with PEG 400with any polymer showed higher release compare to other plasticizers used in the study. This is attributed to the higher moisture uptake by PEG 400 inserts and thereby more drug releasesas both these are similar processes dependent on the film matrix hydrophilicity and diffusivity of water in the inserts. But F7, F8 and F9 which contain HEMA showed comparatively lesser release than the other formulationsas shown in Fig.2. This is due to the nature of the polymer which when subjected to water, swelled due to the molecule's hydrophilic pendant group. It formed a matrix from where drug diffused slowly without changing its shape[16].

Release kinetics of ocular inserts

The release data were subjected to kinetic analysis to show the mechanism of drug release from the ocular inserts. The KorsemeyerPeppas model helped to determine the pattern of drug release from the polymer matrix. As per this model n -diffussional exponent of drug release has a value 0.5< n >1. n=0.5 indicates Fickian diffusion. 0.5< n >1 indicates nonFickian diffusion and Value of n>1 indicates super case II transport.

Results of kinetics indicated all values are greater than 0.5 which indicates super case II transport, i.e. involvement of diffusion mediated and polymer chain relaxation mediated drug release. The data indicates zero order from kinetic analysis as the R^2 values are highest for the zero order (Table 4).

Statistical analysis

The differences in the *In-vitro* dissolution release of the formulations were done by one way analysis of variance of means (ANOVA) at 5% significance level using Microsoft 2007 excel package. % Drug release at 8 hrs was taken as the parameter for ANOVA analysis. The P-value was determined and the result is shown in the Table 5.

Sterility test

All the sterile inserts complied with the test for sterility with a positive control and a negative control test as per the Pharmacopoeial procedure. The formulations also did not show any growth of microorganisms, which suggest that the inserts were sterile.

Eye irritancy test

The sterilized formulations were placed into the lower cul-de-sac with observation of the various criteria made at a designed time interval of 1hr, 24hrs, 48hrs, and 72 hrs and 1week after administration. No redness, swelling, watering of the rabbit eye was observed. It indicates that the inserts were free form ocular irritancy and toxicity.

CONCLUSION

The inserts of Brimonidine Tartrate were successfully formulated with different polymers and plasticizers. All the inserts showed reasonably good physical, mechanical properties and drug release suitable for an ophthalmic inserts. In the experiment the different polymers and plasticizers combination showed varied mechanical properties and release of drug which was further proved by one way ANOVA at 5% significance level and % drug release at 8hrs as parameter yielded a P-value 1.37E-12, so it can be concluded all the formulations were found to be different (P-value < 0.001). Plasticizer DBP when combined with all the polymers used in the study showed good mechanical properties and folding endurance than Propylene glycol and PEG 400. Inserts prepared with plasticizer PEG 400 showed more water intake and thereby more release of drug. Inserts prepared with polymer HEMA showed good retention of shape of the insertand lesser release. This is attributed to the chemical nature

of the polymer HEMA and its water absorption property.The kinetic study revealed all the formulation followed zero order kinetics and Case II transport. The inserts showed no ocular toxicity and irritancy to rabbit eye. Considering all the Physical studies, it can be concluded thatan ophthalmic insert of Brimonidine tartrate can be prepared successfully.

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Table1: Composition of Ocular inserts of Brimonidine Tartrate

Formula	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug (mg)	150	150	150	150	150	150	150	150	150
Eudragit RL-100(mg)	200	-	200	-	200	-	-	-	-
Eudragit RS 100 (mg)	-	200	-	200	-	200	-	-	-
HEMA (mg)							200	200	200
Propylene Glycol (ml)	3	3	-	-	-	-	-	-	3
PEG 400 (ml)-	-	-	3	3	-	-	-	3	-
DBP (ml)	-	-	-	-	3	3	3	-	-
Water q.s 10 gm	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Table 2: Mechanical properties of the ocular inserts

Formulations	Tensile Strength (kg/mm²)	% Elongation at break (mm %)	Strain
F1	1.98±0.05	25±0.01	0.28±0.09
F2	1.94±0.02	23±0.03	0.27±0.02
F3	1.86±0.2	22±0.04	0.45±0.06
F4	1.64±0.23	21±0.28	0.24±0.02
F5	1.97±0.01	28±0.45	0.36±0.01
F6	2.02±0.01	30±0.25	0.26±0.01
F7	3.64±0.34	48±0.04	0.55±0.21
F8	2.96±0.54	41±0.01	0.64±0.03
F9	3.25±0.01	45±0.09	0.52±0.02

SD=Standard deviation and no of replicates (n) =3.

Table 3: Physicochemical properties of the ocular inserts

Formulations	Weight variation± S.D	Thickness (mm)±S.D	Folding endurance± S.D	Surface PH	% Moisture absorption± S.D	Drug content± S.D
F1	0.99±0.02	0.15±0.01	28.66±2.08	7.3	1.49±0.07	96.6±0.87
F2	0.95±0.07	0.14±0.05	29.67±4.35	7.4	1.02±0.10	97.63±0.66
F3	1.01±0.02	0.15±0.01	22.33±2.51	7.4	1.54±0.42	98±0.6
F4	1.00±0.02	0.15±0.01	25±6.65	7.2	1.5±0.45	99.3±0.1
F5	0.97±0.04	0.14±0.01	32±1.01	7.2	1.47±0.75	96.6±0.2
F6	1.03±0.02	0.15±0.05	30.33±3.21	7.2	1.14±0.17	96.66±1.18
F7	0.99±0.03	0.14±0.05	33.3±3.78	7.3	1.21±0.17	95.53±0.41
F8	0.99±0.05	0.15±0.01	32.3±0.57	7.3	1.43±0.18	96.8±0.44
F9	0.99±0.06	0.15±0.05	37±2.64	7.2	1.35±0.18	97.63±1.3

SD=Standard deviation and no of replicates (n) =3.

Table 4: *In-vitro* release constant and regression values for different model

Formulations	K_0	R^2_0	K_1	R^2_1	K_{HG}	R^2_{HG}	K_{KP}	R^2_{KP}	n
F1	19.54	0.97	0.26	0.85	72.82	0.92	2.04	0.99	1.08
F2	18.74	0.98	0.27	0.86	68.83	0.97	2.22	0.99	1.17
F3	19.2	0.988	0.31	0.88	77.58	0.97	2.52	0.99	1.36
F4	19.46	0.97	0.29	0.86	77.62	0.97	2.41	0.99	1.32
F5	18.44	0.98	0.34	0.89	78.55	0.96	2.65	0.99	1.35
F6	18.47	0.98	0.32	0.86	75.17	0.98	2.61	0.99	1.41
F7	16.03	0.96	0.33	0.89	66.63	0.94	3.01	0.98	1.56
F8	16.62	0.97	0.32	0.89	68.4	0.94	2.86	0.98	1.48
F9	15.9	0.96	0.34	0.91	67.24	0.93	3.03	0.97	1.53

K_0=Zero order rate constant, R^2_0= Zero order Regression constant
K_1= First order rate constant, R^2_1= First order Regression constant
K_{HG}= Higuchi rate constant, R^2_{HG}=Higuchi Regression constant
K_{KP}= KorsemeyerPeppas rate constant, R^2_{KP}=KorsemeyerPeppas Regression constant,
n= diffusion exponent of drug release.

Table 5: One way ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	42006.4	2	21003.2	88.62254	1.37E-12	3.354131
Within Groups	6398.896	27	236.9961			
Total	48405.3	29				

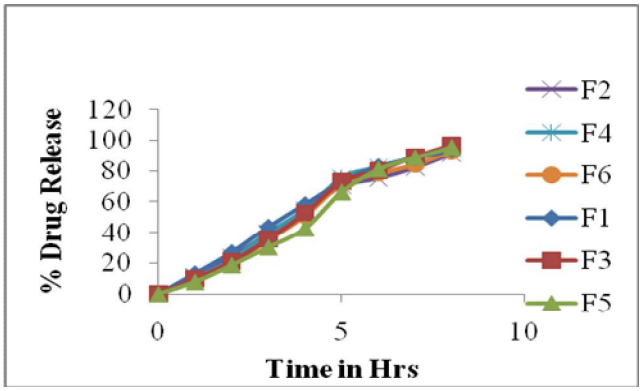

Fig. 1: Release of brimonidine tartrate from Eudragit RL-100 and RS-100 inserts

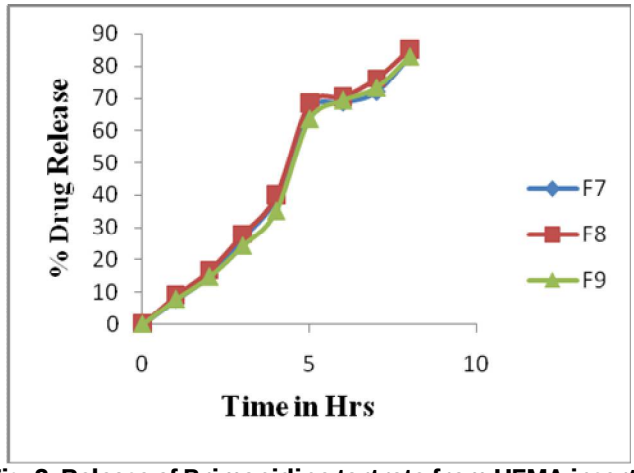

Fig. 2: Release of Brimonidine tartrate from HEMA inserts

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