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RESEARCH ARTICLE

Sustained release ocular inserts of brimonidine tartrate for better treatment in open-angle glaucoma

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Abstract Pathology of eye, especially in the case of glaucoma, requires optimal therapeutically effective concentration of the drug in the ocular tissues for prolonged period of time with decreased dosing frequency and improved patient compliance. In the present study, brimonidine tartrate (BRT) ocular inserts were designed based on hydrophilic and/or inert/zwitterionic polymer matrix to design mucoadhesive and extended release ocular inserts. Designed inserts were evaluated for their physicochemical properties such as crushing strength/hardness, friability, drug content and mucoadhesion, and erosion and in vitro drug release characteristics. The selected optimised formulations were compared with marketed preparation for in vivo ocular irritation in healthy rabbits and for in vivo pharmacodynamic efficacy on alpha-chymotrypsin-induced glaucomatous rabbits. The developed formulations showed good physicochemical properties and mucoadhesive strength, and a good correlation was seen between rate of erosion or swelling with drug release rate in case of formulations with higher proportion of polyethylene oxide (PEO). Modulation of drug release was achieved by incorporating Eudragit in PEO matrix. Addition of Eudragit resulted in shifting of drug release mechanism from erosion-controlled to diffusion-controlled mechanism. In vivo ocular irritation studies confirmed the absence of any irritation upon administration in rabbits. Intraocular pressure (IOP) measurement studies showed an improved IOP-lowering ability of ocular insert of BRT in comparison to eye drops.

Keywords Brimonidine tartrate · Glaucoma · Ocular inserts · Intraocular pressure · Polyethylene oxide · Eudragit

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Introduction

Glaucoma, a disease characterised by a rise in intraocular pressure (IOP), is the second largest cause of blindness worldwide and has now become not only a disease of elevated IOP but also as an optical neuropathy. It is a condition of elevated IOP associated with progressive death of retinal ganglionic cells (RGC) and subsequently, if untreated, results in progressive retinal damage, visual field loss and blindness [1]. Filtration surgery remains the choice of treatment in severe and drug-nonresponsive cases. However, topical drug treatment to decrease IOP is still preferred in mild to moderate cases. The current drug therapy of glaucoma includes topical administration of drugs such as beta blockers (timolol maleate, betaxolol), carbonic anhydrase inhibitors (acetazolamide, dorzolamide, brinzolamide), prostaglandins (latanoprost, brimoprost, unoprostone) or alpha-agonist (brimonidine tartrate) [2–4].

Brimonidine tartrate (BRT), a selective alpha-2 agonist, has been approved for the treatment of open-angle glaucoma, as monotherapy or in combination with timolol. It acts by reducing the IOP by decreasing the production of aqueous humour and increasing its outflow by uveoscleral pathway [3, 4]. It has also been shown to have neuroprotective activity on RGC cells located near the inner

layers of the cornea. It has been shown to increase the survival of RGC cells in post-glaucoma therapy by its neuroprotective mechanisms [5–7]. The IOP-reducing action of brimonidine tartrate together with its neuroprotective properties makes this an important drug in the class of antiglaucoma agents.

Currently, BRT is available as eye drop formulations, which has to be instilled multiple times a day (3–4 times a day) to elicit and maintain its antiglaucoma effect but results in patient incompliance along with inconsistent drug levels in the ocular tissues [2]. Eye drop preparations though widely used suffer from the drawback of rapid drainage of drug out of the eye or into nasolacrimal pathway due to rapid tear turnover resulting in loss or systemic absorption of the drug. While loss of drug results in compromised therapeutic efficacy, systemic absorption results in undesired systemic side effects.

Non-implantable ocular inserts are solid ocular mini discs with mucoadhesive property that are designed to be placed topically in the lower cul-de-sac. These inserts could help to overcome the ocular drug delivery limitations and enhance the ocular bioavailability of drugs by improving the precorneal residence time of drugs and also decrease the non-productive drug loss-related systemic toxicities [8, 9]. Dosing of drug using the ocular inserts is more accurate with a low risk of systemic adverse effects as the drug does not drain into the nasolacrimal passage. And their solid state offers enhanced shelf life, and presence of formulation additives such as preservative is not required. Also, once daily or weekly administration is possible which can be extended to once a month or beyond in the case of implantable ocular inserts formulated using biodegradable/bioerodible polymers.

Polyethylene oxide (PEO) is group of hydrophilic gel-forming polymers with molecular weight-dependent physicochemical properties such as rate of swelling and erosion. The polymer is shown to possess tendency of hydrating upon contact with water forming a superficial gel which eventually erodes slowly, releasing the incorporated drug at a sustained rate [10]. The drug release from PEO matrices is governed principally by polymer swelling and erosion and/or drug diffusion through the hydrated gel layer or all mechanisms together [11]. Due to its good mucoadhesion properties and excellent compressibility, it forms a choice of polymers in drug delivery including that of ocular applications. But due to rapid hydration, swelling and subsequent erosion property, designing a prolonged release formulation of drugs using PEO alone is challenging as the rate of release increases with time due to the rapid erosion of PEO matrices [12]. Addition or combination of polymers with different but complimentary properties could be a better and alternative approach to improve the ocular drug delivery applications of PEO. Eudragits (Eudragit RL 100 and Eudragit RS 100), derivatives of acrylic acid and methyl methacrylates, improve the duration of drug release while retaining the mucoadhesive properties in an optimal proportions levels. Eudragits are chemically inert/zwitterionic polymers and are non-hydrating and non-swellable upon contact with water with lesser erodibility. The bulk of drug release from Eudragit-based matrix occurs predominately by diffusion. Since they are poorly mucoadhesive, a prolonged residence of the ocular inserts based on Eudragit polymer is an issue. Ocular inserts with insufficient levels of mucoadhesive strength can slide on the ocular surfaces and can cause eye irritation and discomfort to the patient.

An appropriate grade of PEO with Eudragit (RL 100/RS 100) in optimum proportion can result in desired prolonged drug release profile while retaining optimum mucoadhesive strength throughout the duration of drug release such that an effective BRT delivery to the eye can be achieved.

The primary objective of the present study was to design and evaluate solid ocular inserts of BRT for once daily topical application using PEO 100 or 400 kDa either alone or in combination with Eudragit RS 100 (ERS 100) and Eudragit RL 100 (ERL 100). The designed ocular inserts would avoid multiple peak-trough drug profile in ocular tissue/fluid on a daily basis, thereby resulting in better therapeutic outcome in controlling intraocular pressure and better patient compliance. As the drug from the ocular insert will not drain off into the nasolacrimal passage, the systemic absorption-related side/toxic effect of the drug can be avoided. This is particularly useful while controlling intraocular pressure in patients with cardiovascular complications using adrenergic agents.

Experimental

Materials and equipments

BRT was obtained as a gift sample from FDC Ltd, Mumbai, India. Poly(ethylene oxide) of molecular weights 100 and 400 kDa were purchased from Sigma Aldrich, Bangalore, India. Eudragit RS 100 and Eudragit RL 100 were obtained as gift sample from Evonik Deggusa, Mumbai, India. Alpha-chymotrypsin (Type II, lyophilized powder, ≥40 units/mg protein) was purchased from Sigma Aldrich, Bangalore, India. All other chemicals and reagents used were of analytical or pharmaceutical grade.

A five-digit analytical balance (Mettler Toledo AG135, Mettler, GMBH, Greifensee, Switzerland) was used for all weighing purposes. Tablet compression machine (Rimek, Mohali, India) was used in the compression of ocular inserts. Texture analyser (TA-XT2, Stable Microsystems, UK) was used for determining crushing strength. Friability was determined in a Campbell Electronic Friabilator

(Campbell Electronics, Mumbai, India). Humidity chambers (Newtronics, India) were used to maintain ambient (25°C± 2°C/60±5% RH) and ATC (40°C±2°C/75±5% RH) conditions. High quality pure water was prepared using Millipore purification system (Model Elix SA 67120, Molsheim, France). In vitro release studies were carried out using USP Type I dissolution apparatus (basket type, Electrolab TDT-08L, Mumbai, India).

Methods

Preparation of ocular inserts

Weighed amounts of drug and polymers were passed through sieve No. 100 and dried in vacuum. The dried drug and polymer were blended together and granulated using isopropyl alcohol as granulating fluid. The resulting granules were dried, passed through sieve No. 60 and lubricated with 0.5% *w*/*w* magnesium stearate. The lubricated blend was compressed into ocular inserts using 4-mm die punches on tablet compression machine (Rimek, Mohali, India). The ocular insert-based formulations were designed to study the following: (a) effect of proportion and molecular weight of hydrophilic polymer (PEO 100 kDa and PEO 400 kDa), (b) effect of proportion and type of inert/zwitterionic polymers (Eudragits RL 100 or Eudragit RS 100), and (c) effect of combination of hydrophilic (PEO 100 kDa or PEO 400 kDa) with inert/zwitterionic polymers (Eudragit RL 100 and Eudragit RS 100) on the physicochemical properties, mucoadhesive strength erosion pattern and in vitro drug release profiles. The components of designed ocular inserts are enlisted in Tables 1 (single polymer-based ocular inserts) and 2 (polymer combination-based ocular inserts).

Evaluation of ocular inserts

Drug content estimation

For drug content estimation, 20 ocular inserts from three batches were accurately weighed and pulverised in mortar and pestle. An aliquot amount of triturate equivalent to 1 mg of BRT was taken, and drug was extracted using phosphate buffer (pH 7.4). It was then sonicated, filtered

Table 1 Formulation composition and physicochemical properties of designed ocular inserts based on single polymer

Formulation code	Ingredients (% *w*/*w*)				Physicochemical parameters				
	PEO		Eudragits		BRT content (mg)	Weight[a] (mg)	Assay[b] (%)	Crushing strength/ hardness[c] (N)	Friability[d] (%)
	100 kDa	400 kDa	ERL 100	ERS 100					
(a) Hydrophilic polymer-based ocular inserts									
BP1–20	20	–	–	–	1.0	3.20±0.14	99.4±2.1	25.2±1.1	0.4
BP1–60	60	–	–	–	1.0	7.60±0.12	100.1±1.0	26.6±2.8	0.3
BP1–100	100	–	–	–	1.0	12.09±0.11	98.0±2.1	28.2±2.2	0.2
BP4–20	–	20	–	–	1.0	3.14±0.15	100.2±1.2	26.2±2.1	0.5
BP4–60	–	60	–	–	1.0	7.52±0.13	101.2±1.3	26.2±1.8	0.4
BP4–100	–	100	–	–	1.0	12.76±0.30	98.4±2.0	28.0±2.1	0.2
(b) Inert/zwitterionic polymer-based ocular inserts									
BERL-20	–	–	20	–	1.0	3.36±0.19	99.1±1.1	18.2±2.2	0.3
BERL-60	–	–	60	–	1.0	7.89±0.20	100.1±1.0	24.5±2.8	0.7
BERL-100	–	–	100	–	1.0	12.11±0.11	98.0±2.1	25.2±2.2	0.5
BERS-20	–	–	–	20	1.0	3.44±0.13	101.2±1.2	23.3±2.1	0.4
BERS-60	–	–	–	60	1.0	7.59±0.23	100.7±2.3	23.9±1.8	0.7
BERS-100	–	–	–	100	1.0	12.36±0.40	98.1±2.0	26.0±2.1	0.3

PEO polyethylene oxide (molecular weight, 100 and 400 kDa)

Percentage of polymer refers to the % *w*/*w* calculated based on the polymer content in the ocular inserts out of total weight of ocular inserts

[a] Mean of 20 ocular inserts from three batches

[b] Mean of 10 ocular inserts from three batches

[c] Mean of three ocular inserts from three batches (expressed in Newtons)

[d] Based on 20 ocular inserts

Table 2 Formulation composition and physicochemical properties of designed ocular inserts with combination PEO and Eudragits

Formulation code	Ingredients (% *w/w*)				Physicochemical parameters				
	PEO		Eudragit		BRT content (mg)	Weight[a]	Assay (%)[b]	Crushing strength/ hardness (N)[c]	Friability (%)[d]
	100 kDa	400 kDa	ERS 100	ERL 100					
(a) Combination of PEO 100 kDa and Eudragit RL 100									
BP1–100	100	–		0	1.0	12.44±0.23	102.2±1.2	29.2±2.1	0.4
BP180ERL20	80	–	–	20	1.0	11.90±0.33	98.4±3.4	28.2±2.1	0.7
BP160ERL40	60	–	–	40	1.0	12.53±0.43	99.5±3.3	29.9±1.2	0.6
BP140ERL60	40	–	–	60	1.0	12.22±0.44	101.2±3.5	28.3±0.2	0.5
BP120ERL80	20	–	–	80	1.0	12.44±0.29	99.4±2.2	29.2±1.0	0.3
BERL-100	0	–	–	100	1.0	12.44±0.23	102.2±1.2	29.2±2.1	0.4
(b) Combination of PEO 100 kDa and Eudragit RS 100									
BP180ERS20	80	–	20	–	1.0	11.99±0.22	101.1±1.0	28.6±2.8	0.6
BP140ERS60	40	–	60	–	1.0	12.36±0.40	99.2±2.0	28.0±2.1	0.3
BP120ERS80	20	–	80	–	1.0	12.22±0.23	100.2±2.3	29.2±1.8	0.7
BERS-100	0	–	100	–	1.0	12.44±0.23	102.2±1.2	29.2±2.1	0.4
(c) Combination of PEO 400 kDa and Eudragit RL 100									
BP4–100	–	100		0	1.0	12.45±0.24	99.9±2.3	29.3±2.0	0.6
BP480ERL20	–	80	–	20	1.0	11.89±0.44	101.2±3.2	29.8±2.1	0.5
BP460ERL40	–	60	–	40	1.0	12.54±0.29	100.2±2.6	30.2±1.2	0.3
BP440ERL60	–	40	–	60	1.0	12.44±0.44	99.2±1.2	28.9±1.0	0.6
BP420ERL80	–	20	–	80	1.0	12.48±0.48	99.0±1.0	28.0±1.0	0.2
(d) Combination of PEO 400 kDa and Eudragit RL 100									
BP480ERS20	–	80	20	–	1.0	12.22±0.41	100.7±2.3	30.27±1.6	0.3
BP460ERS40	–	60	40	–	1.0	12.21±0.23	99.3±0.6	28.32±1.8	0.2
BP440ERS60	–	40	60	–	1.0	12.11±0.51	99.0±3.2	29.22±2.1	0.3
BP420ERS80	–	20	80	–	1.0	11.94±0.53	102.2±1.2	27.22±0.6	0.4

PEO polyethylene oxide (molecular weight, 100 and 400 kDa)

Percentage of polymer refers to the % *w/w* calculated based on the polymer content in the ocular inserts out of total weight of ocular inserts

[a] Mean of 20 ocular inserts from three batches

[b] Mean of 10 ocular inserts from three batches

[c] Mean of three ocular inserts from three batches (in Newtons)

[d] Based on 20 ocular inserts

and suitably diluted, and analysed spectrophotometrically at 248 nm [13].

Crushing strength/hardness

Crushing strength/hardness of the prepared ocular inserts was determined on three ocular inserts of each batch by texture profile analysis method using a Texture analyser (TA-XT2, Stable Microsystems, UK) which was connected with a 30-kg weight load cell, using a 4-mm diameter analytical probe. The instrument had a force of resolution of 0.1 g, measurement accuracy of 0.001% and distance resolution of 0.001 mm. The probe was programmed to penetrate the formulation at a speed of 0.1 mm/s and withdrawn at a speed of and distance of 1 mm. Crushing strength was calculated from the maximum force from the force time curve.

Friability studies

Friability of the formulated ocular inserts was determined for 20 ocular inserts using Roche's friabilator at falling shocks at 25 rpm, operated for 4 min. The weights were noted down before and after the experiment, and the percent friability was calculated from the weights before and after the study. The results of weight variation, drug content estimation, crushing strength/hardness, and friability are presented in Tables 1 and 2 for single polymer-based

ocular inserts and combination polymer-based ocular insert systems, respectively.

Mucoadhesive strength determination

Mucoadhesive strength of designed ocular inserts was determined by in house modification of reported methods [14, 15] using an accurate analytical balance. The left pan of the analytical balance was replaced with Teflon block (6 cm×6.2 mm) with a vertically down perpendicular extension of 2 cm×1.5 cm. Goat mucosal tissue was obtained from a local slaughter house at Pilani, India. The lower block was tied with a mucosal membrane and was maintained in STF (pH 7.4) at 37°C±0.5°C. The ocular inserts for mucoadhesive strength measurements were attached to the lower surface of the upper block using glue. The ocular inserts were kept in contact with the mucosal membrane with some weight (40 g) on for about 15 min. After 15 min, weights were removed, and the experiment was initiated. The water was added dropwise using a micropipette to the other side of the pan slowly until the ocular insert gets detached from the membrane. The rate of addition of water was kept constant for all the mucoadhesive strength determination study (about 3 min). The preliminary studies were performed to optimise the rate of addition of water, contact time of ocular inserts with the membrane before adding weights.

The mucoadhesion was calculated as the force in terms of weight required for the detachment, calculated as force per unit contact surface area of the ocular inserts, expressed in Newtons/cm^2.

In vitro release studies

In vitro drug release studies were performed using modified USP type I (basket type) apparatus. Small glass cylinders of 50 ml capacity were fitted in place of dissolution media vessel. Weighed ocular inserts were placed in the containers, while maintaining the cylinder in dissolution apparatus containing 25 ml of STF (pH 7.4) at 37±0.5°C while the speed was maintained at 50 rpm. Samples were withdrawn at predetermined time intervals, diluted suitably and analysed spectrophotometrically at 248 nm [13]. The percentage of drug released at each time interval was calculated as cumulative percent drug release.

The in vitro drug release data were analysed using Microsoft Excel 2003. In the case of polymer matrices that undergo swelling and subsequent erosion, Korsmeyer–Peppas (KP) model was considered to be suitable, as there might be several processes like polymer chain relaxation, swelling and hence change in matrix geometry and subsequent erosion. All the above processes might ultimately result in altered matrix geometry. Also, the KP model was applied to the release data up to 60% of the drug release.

The KP model is given by

$$M_t/M_\infty = Kt^n \quad (1)$$

where K is kinetic constant incorporating structural and geometric characteristics of the matrix, M_t is the amount of drug released at time t, M_∞ is the amount of drug released at infinite time, and n is the release exponent, indicative of release mechanism. If n=0.45, it indicates a Fickian diffusion-controlled release process. If n=0.45 to 0.89, it indicates non-Fickian anomalous, considered as combination of drug diffusion in the hydrated matrix and polymer relaxation and erosion. If n=1.0, it indicates zero-order release, and if n is more than 1.0, it indicates super-Case II release. The values of n, K and R^2 were used to determine the release rate mechanism and a best fit model. Based on the regression analysis of log % CDR vs. log time, data using Eq. 1, the value of n, K and R^2 were determined and are presented in Tables 3 and 4. Using n and K values, $t_{10\%}$, $t_{50\%}$ and $t_{90\%}$ (time for 10% *w/w*, 50% *w/w* and 90% *w/w* drug release, respectively) were calculated.

Erosion pattern studies

Measurement of erosion of ocular inserts was carried out after immersion in the test medium, in order to correlate the observed mechanisms of drug release with the rate of polymer hydration and swelling and subsequent erosion. Weighed ocular inserts (W_0) were placed in a closed plastic container with a mesh underneath the ocular inserts in a medium, STF freshly prepared and equilibrated at 37± 0.5°C and pH 7.4 on USP type I (basket type) apparatus at 100 rpm. At different time intervals, each container was taken out from the mesh. The wet ocular inserts were then dried in an oven at 55°C till a constant weight is obtained (W_1). The experiment was performed in triplicates for each time point, and fresh samples were used at each individual time points.

The percentage remaining of ocular inserts after erosion (ES) was calculated using the following equation:

$$\%\ \text{Remaining} = 100 - \text{ES} \quad (2)$$

$$\%\ \text{ES} = W_0 - W_1/W_0{*}100 \quad (3)$$

Batch reproducibility and stability studies

Two batches of each series of each of the formulations were prepared again separately and were evaluated as per the procedure mentioned above, and the results were compared

Table 3 Results of drug release kinetics studies for polymer combination (hydrophilic polymers (PEO 400 kDa and PEO 400 kDa) with inert/zwitterionic polymers (ERL 100 and ERS 100))-based brimonidine tartrate ocular inserts fitted into Korsmeyer–Peppas model

Batch code	Peppas model			$t_{10\%}$ (h)	$t_{50\%}$ (h)	$t_{90\%}$ (h)
	K	R^2	*n*			
BP1–100	0.32	0.9725	0.71	0.30	2.9	6.6
BP180ERL20	0.29	0.9908	0.67	0.31	3.4	8.4
BP160ERL40	0.26	0.9892	0.64	0.34	4.3	10.5
BP140ERL60	0.17	0.9852	0.61	0.51	7.1	13.5
BP120ERL80	0.16	0.9932	0.61	0.52	7.3	19.4
BERL-100	0.14	0.9825	0.58	0.53	9.0	25.6
BP180ERS20	0.28	0.9918	0.67	0.32	3.5	8.4
BP160ERS40	0.26	0.9892	0.64	0.35	4.3	10.7
BP140ERS60	0.22	0.9768	0.63	0.41	5.4	13.7
BP120ERS80	0.16	0.9938	0.61	0.52	7.3	19.2
BERS-100	0.17	0.9768	0.52	0.41	8.89	27.1
BP4–100	0.36	0.9725	0.61	0.19	2.7	7.1
BP480ERL20	0.32	0.9908	0.62	0.24	3.3	8.4
BP460ERL40	0.28	0.9949	0.55	0.25	4.5	12.9
BP440ERL60	0.20	0.9852	0.53	0.37	7.5	18.4
BP420ERL80	0.19	0.9938	0.51	0.39	8.7	26.4
BP480ERS20	0.32	0.9908	0.60	0.23	3.4	8.9
BP460ERS40	0.29	0.9892	0.56	0.26	4.6	13.3
BP440ERS60	0.20	0.9852	0.53	0.38	7.7	17.5
BP420ERS80	0.20	0.9938	0.52	0.38	8.4	26.0

K release rate constant (h^{-n}), R^2 regression coefficient, *n* release exponent indicates the mechanism of drug release, $t_{10\%}$, $t_{50\%}$ and $t_{90\%}$ time taken (in hours) for 10%, 50% and 90% drug release, respectively

with all the three batches prepared. Stability studies were carried out on ocular inserts formulation according to the International Conference on Harmonization guidelines [16]. A required quantity of ocular inserts was packed into small cellophane packets and were stored in a stability chambers (Thermo labs, Mumbai, India) maintained at 25±2°C/60±5% RH, and 40±2°C/75±5% RH. Samples were withdrawn at 0-, 1-, 2-, 3- and 6-months interval, and the physical parameters, drug content and in vitro release profiles were evaluated.

In vivo studies

Animals

New Zealand white rabbits, weighing 2.5–3.5 kg, were provided by the Central Animal House Facility of BITS Pilani and were housed under controlled and standardised conditions. They were fed a normal pellet diet, and water was given ad libitum. The animals were acclimatised to light and dark cycles for 12 h. All the animals met the

Table 4 Results of in vivo pharmacodynamic efficacy studies of brimonidine tartrate ocular insert formulations (PEO 100 kDa or PEO 400 kDa with ERS 100 or ERL 100) in comparison to commercial BRT eye drops (Iobrim® E/D) in glaucomatous rabbits

Formulation	I_{max} (mmHg)	t_{max} (h)	$AUC_{(\Delta IOP\ vs.\ t)}$ (h-mmHg)	Slope	Duration (h)	AUC_{Rel}
Eye drops (Iobrim® E/D)	8.55±0.21	1	38.40±4.22	0.4763	6	–
BP140ERS60	7.80±0.30	3	93.14±5.54*	0.1674*	24**	2.4
BP140ERL60	7.94±0.30	3	90.23±3.54*	0.1667*	24**	2.3
BP440ERS60	7.97±0.32	3	92.35±6.21*	0.1756*	24**	2.4
BP440ERL60	7.97±0.22	3	89.42±4.21*	0.1674*	24**	2.3

I_{max} maximum reduction in IOP (mmHg), t_{max} time taken for maximum reduction in IOP (h), $AUC_{(\Delta IOP\ vs.\ t)}$ area under the ΔIOP vs. time curve, *Slope* slope of terminal linear portion of ΔIOP vs. time curve, AUC_{Rel} ratio of AUC $_{(\Delta IOP\ vs.\ t)}$ test (designed formulations) to AUC $_{(\Delta IOP\ vs.\ t)}$ reference (marketed eye drops)

Each data point represents the average of three measurements per animal (number of animals=3) with standard deviation

*$p<0.01$, statistically significant difference from eye drops

**$p<0.001$, statistically significant difference from eye drops

following criteria: (a) both the eyes were completely healthy with no injury or history of injury, (b) the basal IOP was in the range of 22±3 mmHg, and (c) the IOP difference between contralateral eyes were not exceeding 2 mmHg. The animal handling and studies were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication No. 92–93, revised in 1985) and in conformation to Association for Research in Vision and Ophthalmology (ARVO) and was approved by the Institutional Animal Ethics Committee of BITS, Pilani (protocol No. IAEC/RES/12-04).

Ocular irritation studies

The objective of the ocular irritation studies was to assess qualitatively as well as quantitatively the ocular tolerance and irritability/toxicity of selected formulation upon administration to eye. Ocular irritation studies were performed on selected formulations showing promising in vitro results, according to Draize technique [17] on healthy New Zealand white rabbits each weighing 2.5 to 3.5 kg, divided into following three groups. The solutions (saline, marketed eye drop) and developed formulations (selected in situ gels, ocular inserts and nanoparticles) were administered once a day for a period of 7 days. At the time of formulation instillation, the animals were maintained in restrainer boxes, but allowed to move their heads freely. The evaluation was performed according to the Draize technique [17] by periodically observing for ocular redness, swelling, and watering conjunctival chemosis, discharge, and corneal lesions. The standard scoring system was followed up to ascertain the outcome of the experiment.

In vivo pharmacodynamic studies

Induction of glaucoma

Rabbits were anaesthetised by intramuscular injection of 4 mg/kg of xylazine and 35 mg/kg of ketamine. Chronic ocular glaucoma was induced by a single posterior injection of alpha-chymotrypsin (10 mg/ml, 0.1 ml) into posterior segment of eye in rabbits [18]. Care was taken to avoid the contact of alpha-chymotrypsin to the surface of the eye. A daily ocular examination was followed up for a few days. After 2–3 days of injection, one drop of ciprofloxacin eye drop (Ciplox® Cipla, India), dexamethasone eye drop (Dexacip®, Cipla India) and a drop of diclofenac sodium eye drop (Voltaren®, Novartis, India) were instilled to prevent topical inflammation. Animals that showed cases of severe inflammation and erratic or inconsistent IOP increase were excluded from the study. When the IOP was stabilised to 39±3 mmHg, for three successive days, the pharmacodynamic response studies were initiated.

Pharmacodynamic efficacy measurement studies

For IOP-lowering studies, the selected ocular formulations and conventional ophthalmic drops (2–3 drops; Iobrim® E/D (containing 2 mg/ml drug), FDC Ltd, Mumbai, India) were instilled carefully into the lower cul-de-sac of the left eye of the rabbits (n=3), while to the right eye, 2–3 drops of normal saline were administered. The saline-treated eye acted as control in the experiments. Immediately after instillation, eye lid was closed for 10 s in order to avoid spillage or movement of the preparation. IOP was measured by using calibrated Schiotz tonometer (Scope medical, Mohali, India) at different time intervals. The change in IOP (ΔIOP) at each time point from the stabilised IOP (zero time) was determined by

$$\Delta\mathrm{IOP} = \mathrm{IOP}_{\text{zero time}} - \mathrm{IOP}_{\text{time t}} \tag{4}$$

ΔIOP is reported as mean (±SEM) of three animals (n=3) for each treatment at each time point. The ΔIOP vs. time curve was plotted to compare the efficacy of prepared formulations with the conventional ophthalmic drops, and the comparison was done in terms of: (a) I_{max}: peak decrease in IOP, (b) t_{max}: time to reach peak IOP decrease, (c) $AUC_{(\Delta IOP\ vs.\ t)}$: area under the ΔIOP vs. time curve, (d) duration of IOP decrease, and (e) slope of terminal linear portion of the decrease in IOP vs. time curve [19]. The $AUC_{(\Delta IOP\ vs.\ t)}$ of ΔIOP vs. time curve was calculated using trapezoid rule (also calculated using Graph Pad Prism 4 software). The AUC_{Rel} was calculated using the following equation:

$$\mathrm{AUC_{Rel}} = \frac{\mathrm{AUC}_{(\Delta\mathrm{IOP\ vs.\ t})}\ \text{Test (designed formulations)}}{\mathrm{AUC}_{(\Delta\mathrm{IOP\ vs.\ t})}\ \text{Reference (marketed eye drops)}} \tag{5}$$

Results and discussion

Physicochemical characteristics

The prepared ocular inserts were slightly yellowish in colour, flat surfaced, and circular with 4 mm in diameter and thickness ranging from 0.3 to 0.6 mm. The thickness of ocular inserts prepared from single polymer system at 20% *w*/*w* polymer proportion was 0.3 mm while that with 100% *w*/*w* polymer proportion was 0.6 mm. The weight varied with the amount of polymer in the system, from 3.2±0.2 to 12±0.5 mg. Drug content was found to be 1.0±0.05 mg. The friability of designed formulations was within acceptable limits of NMT 1% (Tables 1 and 2). The components and physicochemical properties of designed ocular inserts are enlisted in Tables 1 and 2.

Effect of hydrophilic polymer proportion (single polymer ocular inserts)

The drug release from PEO matrices is elicited by instant water absorption into the matrix resulting in the formation of gel layer on the crystalline polymer. The water-soluble drugs incorporated in the matrices, released primarily by diffusion after the drug dissolves in the hydrated polymer and diffuses out of the swollen matrix. Meanwhile, with time, erosion supersedes diffusion, and gelled layer starts eroding. The polymer erosion is expected to play a major role in the drug release from the PEO matrices. The drug release was found to be extended as the proportion of PEO 100 kDa was increased in the ocular inserts matrix. The release rate, calculated using KP model, were found to be 0.48 $h^{-0.94}$, 0.45 $h^{-0.71}$ and 0.32 $h^{-0.71}$ for the formulations with 20% *w/w*, 60% *w/w* and 100% *w/w* of PEO 100 kDa. All the three formulations showed acceptable initial burst release with $t_{10\%}$ values of 0.2 to 0.3 h with the duration of the drug release prolonged with the increase in the percentage of PEO in the ocular inserts (Fig. 1a). The corresponding $t_{90\%}$ values were obtained as 2.6, 3.4 and 6.6 h, respectively, for 20%, 60% and 100% *w/w* PEO 100 kDa containing ocular inserts.

The rate of gel formation and surface erosion from the PEO matrix mainly depends on the molecular weight and hydrodynamics of the dissolution medium. In case of PEO 400 kDa, the swelling and subsequent stronger gel formation together with slow erosion of the polymer contribute to more extended duration of release of the drug in comparison to that of PEO 100 kDa (Fig. 1b).

The release rate for the formulations prepared with PEO 400 kDa alone was found to be 0.47 $h^{-0.81}$, 0.43 $h^{-0.70}$ and 0.29 $h^{-0.70}$ for 20% *w/w*, 60% *w/w* and 100% *w/w* of PEO 400 kDa, respectively (Table 3). Acceptable initial release was observed in the case of ocular inserts prepared using PEO 400 kDa alone, with $t_{10\%}$ values of 0.13, 0.17 and 0.33 h, while $t_{90\%}$ was found to be 3.1, 4.0 and 7.5 h, respectively, for 20% *w/w*, 60% *w/w* and 100% *w/w* PEO 400 kDa ocular inserts. The value of release exponent for both PEO 100 kDa and PEO 400 kDa indicated non-Fickian anomalous drug transport [10–12]. The drug release was expected to be governed by a combination of diffusion and polymer erosion.

To further establish the mechanism of drug release, the percent drug released was plotted against percent erosion of matrix for formulations prepared using 100% *w/w* of PEO 100 kDa and PEO 400 kDa alone. The results are depicted in Fig. 2a and b for BP1-100 (100% *w/w* PEO 100 kDa) and BP4-100 (100% *w/w* of PEO 400 kDa), respectively. Swelling studies were also conducted along with erosion studies, but the results were inconclusive. The ocular inserts prepared with PEO showed initial swelling followed by erosion of the matrix. The degree of swelling measured was inconclusive as both swelling and erosion occurred simultaneously.

In both cases, the percent drug release was found to correlate with very high goodness of fit (slope value approaching unity) with percent erosion of matrix indicating that the drug release is predominately governed by erosion process (Fig. 2) [20].

Effect of inert/zwitterionic polymer proportion

Eudragits are methacrylic and methyl methacrylate copolymers which are known to form a hard, compact and non-erodible matrix. Since ERL 100 and ERS 100 exist in salt form (with low content of quaternary ammonium groups), they exhibit a pH-independent permeability and release of incorporated drugs. In the case of BRT ocular inserts formulated using ERS 100 and ERL 100 as the release retardant matrix base, the drug release was extended beyond 24 h. However, the formulations prepared with ERL 100 showed comparatively faster rate of release as they are more permeable to water than ERS 100, thereby facilitating better penetration of dissolution media.

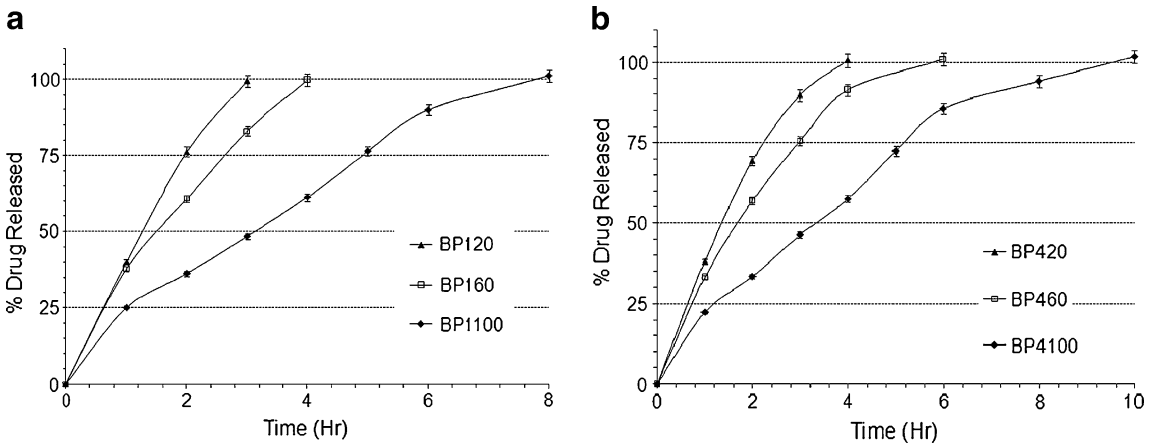

Fig. 1 In vitro drug release profile of PEO-based brimonidine tartrate ocular insert formulations prepared with different proportion of **a** PEO 100 kDa and **b** PEO 400 kDa. Each data point represents the average of two batches in triplicate with standard deviation

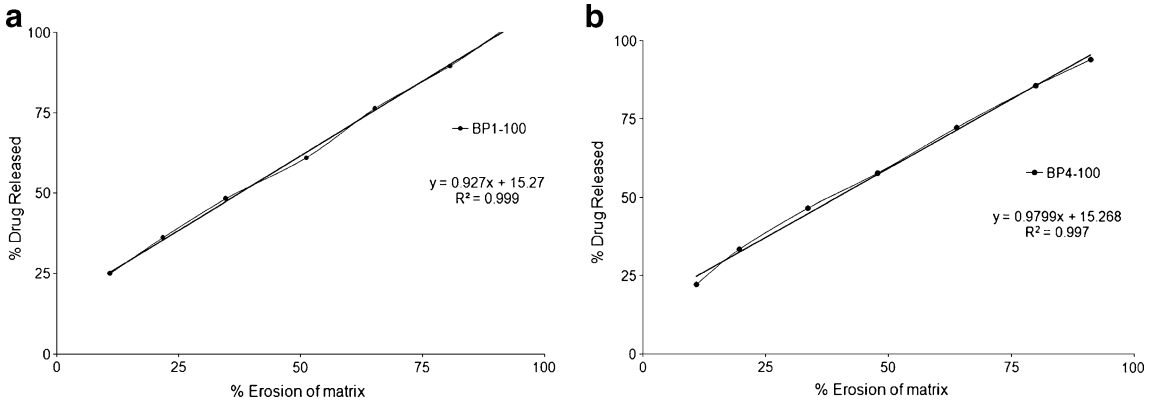

Fig. 2 Relationship between percent matrix erosion with percent drug released for PEO-based brimonidine tartrate ocular insert formulations prepared with **a** PEO 100 kDa and **b** PEO 400 kDa at 100% *w/w* proportion. Each data point represents the average of two batches in triplicate

The release rate was found to be 0.34 $h^{-0.54}$, 0.27 $h^{-0.58}$ and 0.14 $h^{-0.57}$ for the formulations with 20%, 60% and 100% *w/w* of ERL 100 proportion (Fig. 3) and 0.33 $h^{-0.57}$, $0.29^{-0.52}$ and 0.17 $h^{-0.52}$ for the formulations with 20%, 60% and 100% *w/w* of ERL 100, respectively. The initial release as indicated by $t_{10\%}$ value varied from 0.2 to 0.5 h (in the case of ERL 100) and from 0.2 to 0.4 h (in the case of ERS 100). The duration of release ($t_{90\%}$) value varied from 9.9 h (20% *w/w*) to 25.6 h (100% *w/w*) for ERL 100 ocular inserts and from 10.3 h (20% *w/w*) to 27.1 h (100% *w/w*) for ERS 100-based ocular inserts. The release exponent (*n*) was found to indicate non-Fickian anomalous drug transport.

The relationship between drug release vs. erosion showed that in the case of 100% *w/w* of polymer for both ERL 100 (Fig. 4a) and ERS 100 (Fig. 4b), showed considerably high percentage drug release in comparison to percentage erosion of matrix. The slope value of the best fit curve was found to be more than 2.0 in both the cases, suggesting that the drug release occurs predominately by diffusion. Eudragit-based matrices also showed initial swelling followed by erosion of the matrix. The degree of swelling measured was inconclusive as both swelling and erosion occurred simultaneously.

Effect of combination of hydrophilic and inert/zwitterionic polymers

PEO 100 kDa with Eudragits

The drug release from BRT ocular inserts designed using combination of PEO 100 kDa and ERL 100 or ERS 100 was found to vary depending upon the relative proportion of PEO and Eudragit in the matrix. The results of in vitro release studies performed on the formulations with varying proportions of PEO 100 kDa and ERL 100 are shown in Fig. 5. As the relative proportion of ERL 100 was decreased in the matrix from 100% *w/w* to 0 with the corresponding increase in PEO 100 kDa proportion, the rate of release was found to increase with lesser over all extension of duration of release.

In the case of formulations containing 100% *w/w* of PEO 100 kDa and no ERL 100, the BRT release was found to be extended for 6 to 7 h ($t_{10\%}$ of 0.3 h and $t_{90\%}$ of 6.6 h), and the release rate was found to be 0.32 $h^{-0.71}$ with *n* value of 0.71. Various combinations of PEO 100 kDa and ERL 100 were investigated in order to optimise formulations, such that the drug release was prolonged up to 24 h, and at the

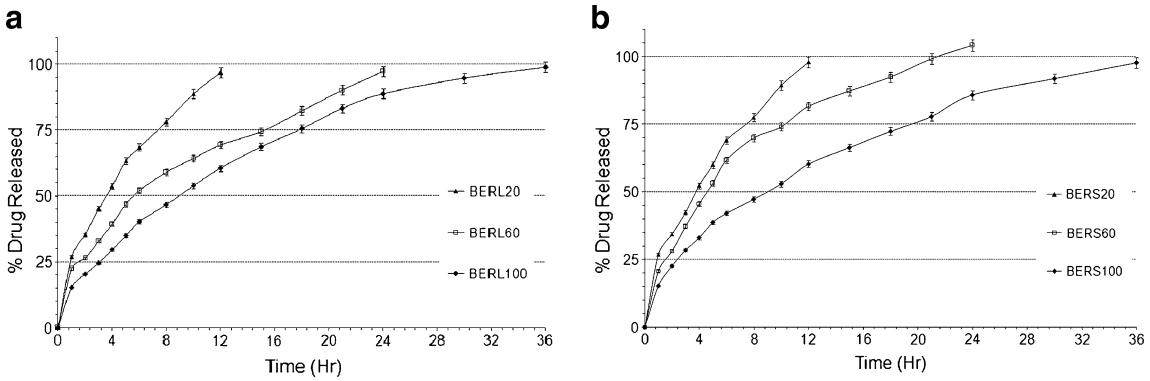

Fig. 3 In vitro drug release profiles of Eudragit-based brimonidine tartrate ocular insert formulations prepared with different proportions of **a** ERL 100 and **b** ERS 100. Each data point represents the average of two batches in triplicate with standard deviation

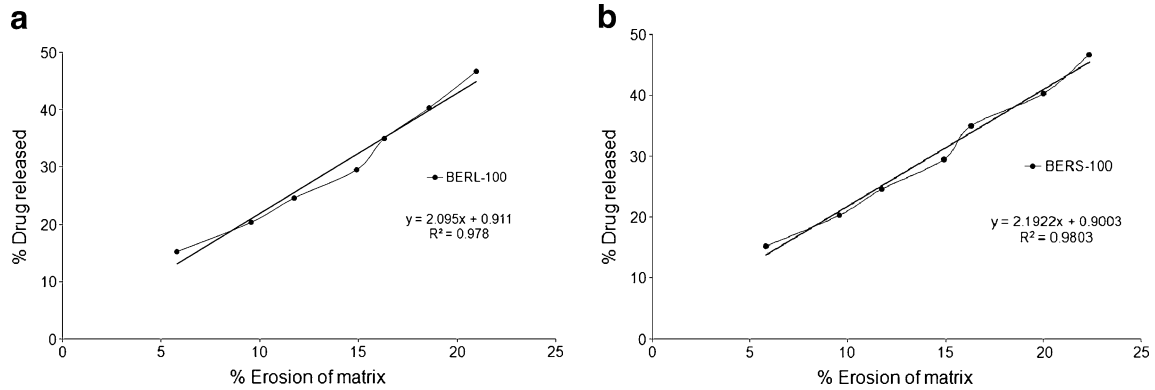

Fig. 4 Relationship between percent matrix erosion with percent drug released for Eudragit-based brimonidine tartrate ocular inert formulations prepared with **a** ERL 100 and **b** ERS 100 at 100 *w/w* proportion. Each data point represents the average of two batches in triplicate with standard deviation

same time, sufficient mucoadhesive strength was retained. It is expected that the relative proportion of PEO in the ocular insert matrix will have a major contribution to the mucoadhesive strength of the ocular inserts.

When the PEO 100 kDa proportion in the matrix was decreased with corresponding increase in ERL 100 proportion, $t_{90\%}$ value was found to increase (Table 3). A drastic increase in $t_{90\%}$ (13.5 h) and a substantial decrease in release constant (0.17) were observed with duration of drug release extended up to 24 h in the case of formulations prepared using PEO 100 kDa of 40% *w/w* and ERL 100 of 60% *w/w*. On further increasing the relative proportion of ERL 100 up to 100% *w/w*, the rate of release was found to decrease further with duration of release extending beyond 36 h.

Similar observation was seen in the case of formulations with combination of PEO 100 kDa and ERS 100. The results are shown in Fig. 6.

In both ERL 100 and ERS 100, because they exist as salts, the drug release from their matrices is pH-independent and very slow. In the case of ERL 100, the poly(ethyl acrylate, methyl methacrylate, trimethylammonioethyl methacrylate chloride) ratio is 1:2:0.2, while ERS 100 has a poly(ethyl acrylate, methyl methacrylate, trimethylammonioethyl methacrylate chloride) ratio of 1:2:0.1. The presence of trimethyl ammonio group in the salt form renders these polymers as inert/zwitterionic or neutral in nature and pH-independent fluid penetration and hence pH-independent release of drug from the matrices of these polymers. As the proportion of Eudragit increases in the formulation, the matrix tends to become harder, and the drug release was predominately governed by diffusion. At a lower proportion of ERL 100 and higher proportion of PEO 100 kDa, a relatively higher release was observed, this could be due to the hydrophilic nature of the PEO and also due to relative decrease in the hydrophobicity of the matrix with matrix erosion as the erosion as the primary mechanism of drug release.

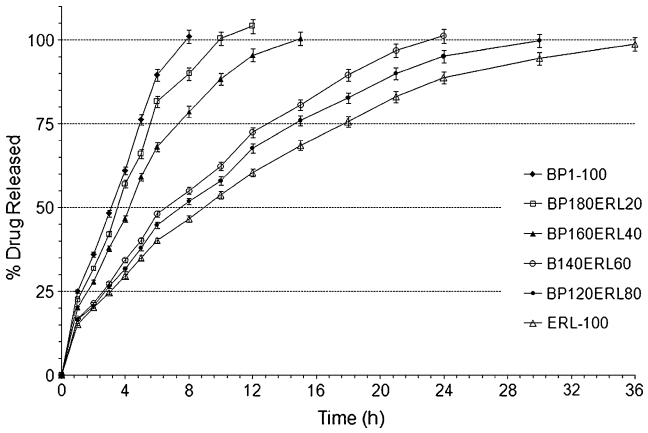

Fig. 5 In vitro drug release profile of polymer combination (PEO 100 kDa and ERL 100)-based brimonidine tartrate ocular insert formulations. Each data point represents the average of two batches in triplicate with standard deviation

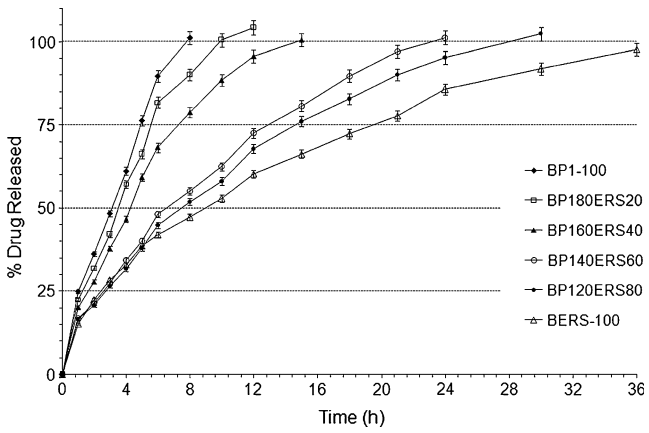

Fig. 6 In vitro drug release profile of polymer combination (PEO 100 kDa and ERS 100)-based brimonidine tartrate ocular insert formulations. Each data point represents the average of two batches in triplicate with standard deviation

PEO 400 kDa with Eudragits

The drug release from PEO 400 kDa and ERL 100-based ocular insert formulations is dependent on the relative proportion of both the polymers in the matrix. Results were depicted in Figs. 7 and 8. The drug release kinetics data upon fitting to Korsmeyer–Peppas model have been shown in Table 3. Formulation containing 100% *w/w* of PEO 400 kDa released the drug for 10 h ($t_{10\%}$ of 0.19 h and $t_{90\%}$ of 7.2 h), the release rate was found to be 0.36 $h^{-0.61}$, and the release exponent was 0.61. The different combinations of PEO 400 kDa and ERL 100 were investigated by varying the relative percentage of polymers. When the percentage of PEO 400 kDa was decreased to 80% w/w and ERL 100 percentage was increased to 20% *w/w*, the release rate was decreased significantly (0.32 $h^{-0.62}$), and the duration of BRT release was extended to 12 h ($t_{10\%}$ of 0.2 h and $t_{90\%}$ of 8.4 h; Table 3). Further decrease in PEO 400 kDa and relative increase in the percentage of ERL 100 resulted in drastic decrease in the release rate and increase in the duration of drug release. The ocular insert formulation with 100% *w/w* of ERL 100 retarded the release for beyond 24 h. The acceptable amount of initial drug release in the case of all the formulations could be due to release of surface-bound drug as well as increased porosity of the matrix due to the dissolution of hydrophilic polymer and the formation of micropores on the surface of the matrix, which also contributed for the release of drug in the later periods [21].

In the case of formulations with PEO 400 kDa and ERS 100, when the proportion of PEO 400 kDa was decreased to 80% and ERL proportion increased to 20% *w/w* (BP480ERL20), the *K* decreased to 0.32 $h^{-0.62}$, $t_{10\%}$ increased to 0.24 h and $t_{90\%}$ increased to 8.4 h. The formulation BP460ERL40 showed a controlled release of drug with a *K* value of $0.29^{-0.55}$, $t_{90\%}$ of 12.9 h and $t_{10\%}$ of 0.3 h. This is due to the decrease in the proportion of PEO 400 kDa and increase in the proportion of ERL. When the PEO 400 kDa proportion was further decreased to 20% with an increase in ERL proportion to 80%, the *K* decreased to 0.2 $h^{-0.51}$, $t_{10\%}$ increased to 0.4 h and $t_{90\%}$ increased to 26.4 h.

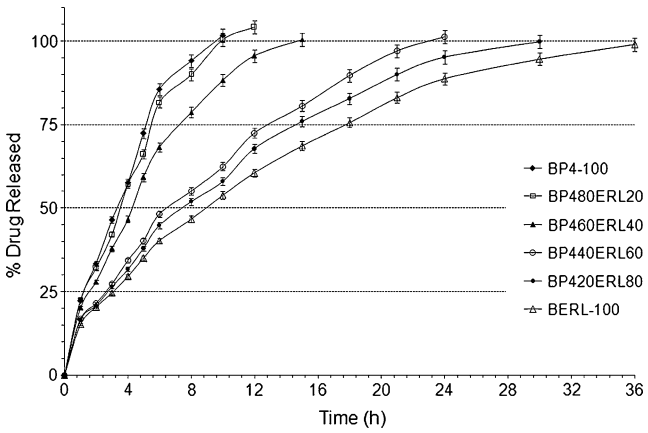

Fig. 7 In vitro drug release profile of polymer combination (PEO 400 kDa and ERL 100)-based brimonidine tartrate ocular insert formulations. Each data point represents the average of two batches in triplicate with standard deviation

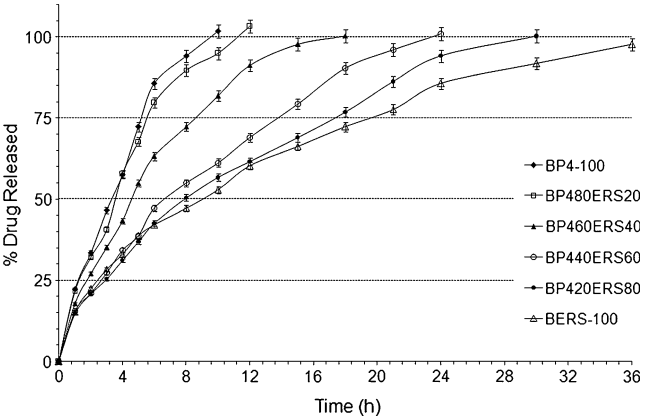

Fig. 8 In vitro drug release profile of polymer combination (PEO 400 kDa and ERL 100)-based brimonidine tartrate ocular insert formulations. Each data point represents the average of two batches in triplicate with standard deviation

Similar effects were observed in the case of formulations in combination of PEO 400 kDa and ERS 100. The release rate constant gradually decreased, and $t_{10\%}$ and $t_{90\%}$ were increased as the proportion of PEO 400 kDa was decreased and ERS was increased. As the proportion of PEO was decreased and Eudragit proportion was increased, the release mechanism slowly shifted towards diffusion controlled. This may be due to the decreased swelling and decreased erosion of the matrix in the presence of Eudragits.

Mucoadhesive strength determination

In the preliminary studies performed on designed ocular inserts prepared using combination of polymers using goat intestinal mucosal membrane showed that mucoadhesive strength of the ocular inserts was dependent on the relative proportion of PEO in the matrix.

Adequate mucoadhesion is required for the ocular inserts to be retained in the lower cul-de-sac on topical administration. Lower mucoadhesive strength to the ocular inserts can cause detachment of ocular inserts resulting in the blockade of vision and subsequent chances of formulation from falling off from the eye. Addition of Eudragits resulted in decrease in detachment force.

The formulations containing Eudragits alone did not show any mucoadhesive strength. The force of detachment for the ocular inserts containing two grades of PEO (PEO 100 kDa and 400 kDa) and Eudragits (ERL 100 and ERS 100) is shown in Fig. 9a (PEO 100 kDa and ERL 100), Fig. 9b (PEO 100 kDa and ERS 100 formulations), Fig. 10a (PEO

400 kDa and ERL 100), and Fig. 10b (PEO 400 kDa and ERS 100). Formulations with 100% *w/w* of PEO showed the highest force of detachment and caused a drastic increase in the detachment force of 0.83 N/cm^2 (PEO 100 kDa) and 1.02 N/cm^2 (PEO 400 kDa). Gradual decrease in PEO 100 kDa proportion and increase in ERL proportion resulted in decrease in force of detachment. Interestingly, with PEO 400 kDa-alone formulations, the force of detachment was found to be lesser than that of formulations with PEO 100 kDa alone, which could be due to the fact that as the number of chains in polymer increases with the increase in the molecular weight, the possibility of polymer–polymer interaction increases which subsequently leads to reduction in the number of penetration polymer chains per unit of mucosal volume [10, 22]. Ocular inserts prepared using Eudragit (ERL and ERS) showed no force of detachment.

Batch reproducibility and stability studies

Stability studies performed for the selected ocular insert formulations by storing at 25±2°C/60±5% RH and 40±2°C/75±5% RH showed that the ocular insert formulations were stable at both the storage conditions with no significant degradation observed even at accelerated conditions. The parameters like appearance, drug content, mucoadhesive strength, erosion pattern and in vitro drug release profiles remained unaltered for the entire duration of the study.

In vivo studies

Ocular irritation and tolerability studies

The results of ocular irritability and tolerability studies of selected suggested that all the formulations investigated were well tolerated with no signs of any irritation or toxicity. The scores were found to be as same that of marketed preparation, which shows the potential of the developed formulation as ocular delivery systems.

In vivo pharmacodynamic studies

The ocular insert formulations for in vivo studies were selected based on the in vitro performance such as physicochemical characteristics, mucoadhesion, in vitro release studies and stability studies. The criteria of selection were: (a) prolonged duration of release (up to 24 h), (b) adequate mucoadhesive strength, and (c) absence of any ocular irritability and toxicity.

The selected ocular insert formulations containing PEO 100 kDa (BP140ERS60 and BP140ERL60) and PEO 400 kDa (BP440ERS60 and BP440ERL60) prolonged BRT release in vitro for 24 h, and mucoadhesive strength was sufficiently high and was found to be stable. Upon ocular irritability and toxicity studies, these formulations were found to be non-irritant and free from any kind of ocular toxicity.

The glaucoma induction by alpha-chymotrypsin is primarily because of lysis of zonular material and trabecular meshwork which serves to drain the aqueous humour in and out of the eye, lysis of which results in accumulation of it and subsequent increase in IOP. This model has been found suitable for the studies involving comparison of effect of drugs on IOP reduction and can thus be extrapolated into human glaucoma [23, 24]. Two animals that developed severe topical inflammation were excluded from the study. The eye drop was administered only once to obtain relative comparison between single dose administration of both reference and test product. In the case of eye drop (2% *w/v* or 2 mg/ml), three drops of eye drop contain 0.33 mg of BRT. When administered 3–4 times a day, the actual dose administered will be 0.99 (~1 mg) to 1.33 mg per day. Therefore, ocular inserts were designed with 1 mg of BRT per insert for once daily administration.

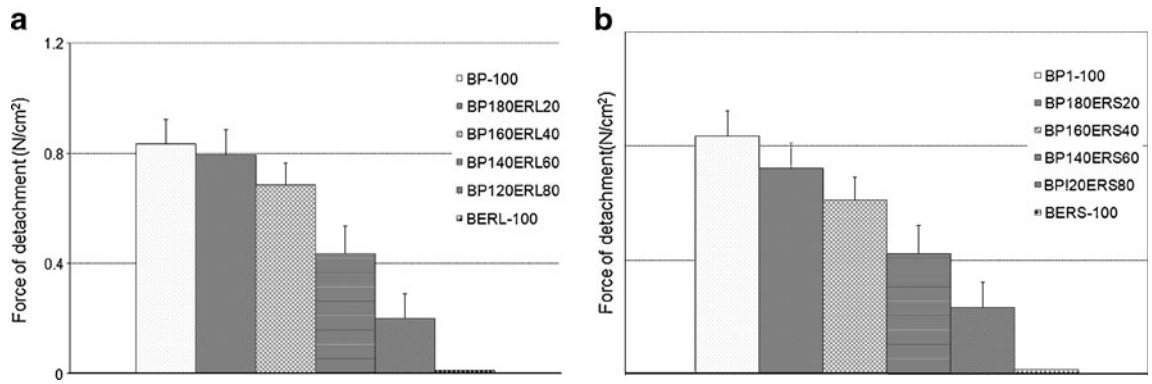

Fig. 9 Results of mucoadhesive strength determination studies for polymer combination (**a** PEO 400 kDa and ERL 100, **b** PEO 400 kDa and ERS 100)-based brimonidine tartrate ocular insert formulations. Each data point represents the average of two batches in triplicate with standard deviation

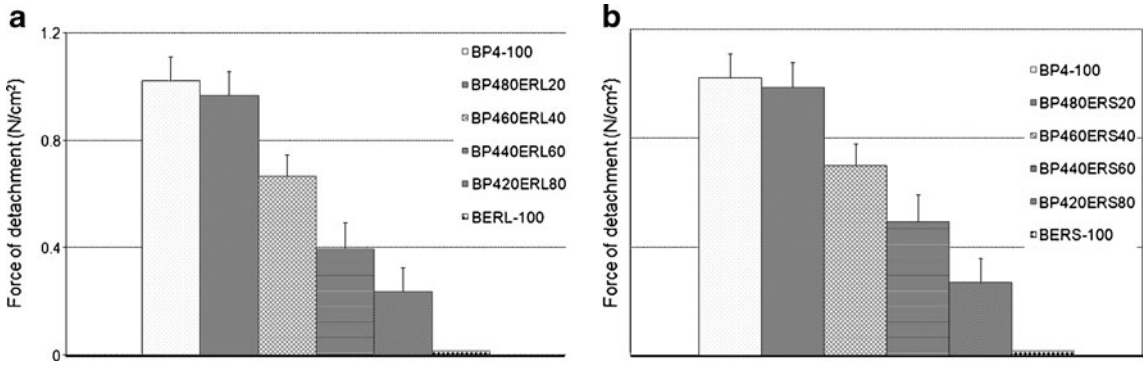

Fig. 10 Results of mucoadhesive strength determination studies for polymer combination (**a** PEO 100 kDa and ERL 100, **b** PEO 100 kDa and ERS 100)-based brimonidine tartrate ocular insert formulations. Each data point represents the average of two batches in triplicate with standard deviation

The ΔIOP time curve for PEO 100 kDa and ERL 100 or ERS 100-based ocular inserts has been shown in Fig. 11. As shown in Table 4, the I_{max} was found to be 7.8 and 7.94 mmHg for the selected formulations, while the t_{max} was 3 h for all the selected formulations. The $AUC_{(\Delta IOP\ vs.\ t)}$ for IOP reduction time curve was greatly increased for the ocular insert preparations in comparison to marketed eye drop preparation ($p<0.001$). It was found to be 93.14 h-mmHg for BP10ERS60 and 90.23 h-mmHg for BP1440ERL60. The AUC_{Rel} was found to be 2.4 and 2.3 respectively for BP10ERS60 and BP1440ERL60 ocular insert formulations. The duration of IOP reduction lasted for 24 h in comparison to 6–9 h for eye drops ($p<0.001$). Thus, it can be inferred that with single administration of ocular inserts, the IOP reduction can be obtained for 24 h.

Similarly for PEO 400 kDa and ERS 100 or ERL 100-based formulations, the AUC for the selected formulations was drastically enhanced with 92.35 h-mmHg for BP40ERS60 and 89.42 h-mmHg for BP440ERL60 ($p<0.01$, in comparison to eye drop). The duration of IOP reduction was observed for 24 h. The AUC_{Rel} was found to be 2.4 and 2.4 for BP40ERS60 and BP440ERL6, respectively. No difference in terms of IOP reduction was seen between formulations with ERS 100 and ERL 100 in combination with PEO 100 kDa and PEO 400 kDa. All the selected formulations were found to be retained in the cul-de-sac for the entire length of the study, suggesting that mucoadhesive strength of the formulations was sufficiently high for ocular administration.

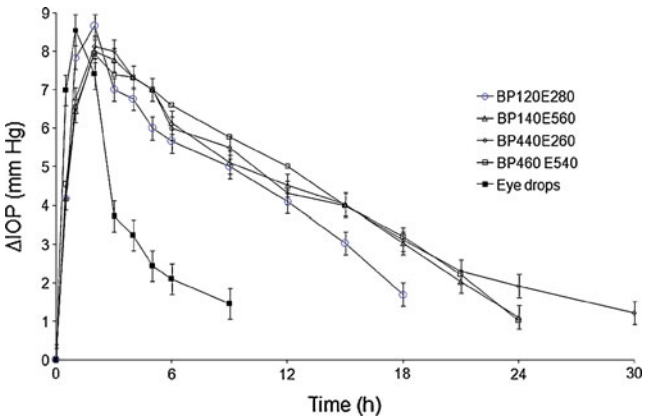

Fig. 11 Comparative IOP reduction profile for the selected brimonidine tartrate ocular insert formulations PEO 100 kDa with ERS 100 or ERL 100 and PEO 400 kDa with ERS 100 or ERL 100 in comparison to commercial eye drops (Iobrim® E/D) in glaucomatous rabbits. Each data point represents the average of three measurements per animal (number of animals=3) with standard deviation

Conclusions

In the present study, ocular inserts were developed by employing hydrophilic polymers such as PEO (PEO 100 kDa and PEO 400 kDa) and inert/zwitterionic polymers (ERL 100 and ERS 100) alone and in combination to attain prolonged release of drug while retaining sufficient mucoadhesive properties. The prepared ocular inserts showed good physicochemical properties like drug content, crushing strength and friability. Mucoadhesive strength was found to be dependent on the proportion of PEO in the formulations. In vitro drug release was found to extend up to 24 h to 36 h. A shift in the mechanism of drug release from non-Fickian anomalous (swelling and erosion) to diffusion controlled, when the proportion of Eudragits were increased and PEO proportion, was decreased. In the case of PEO alone formulations, the drug release was found to be dependent on erosion of the polymer, while in the case of Eudragit alone ocular inserts formulations, the drug release is dependent predominately on the diffusion. In vivo ocular irritation studies showed that all the selected formulations were devoid of ocular irritation for the entire duration of the study.

In vivo pharmacodynamic efficacy studies showed a drastic increase in the extent and duration of IOP reduction from ocular insert formulations in comparison to eye drop preparations. As the study was performed on glaucomatous

rabbits, where an elevation in IOP was brought about, the results can be extrapolated to humans, as this model is shown to be identical to human glaucoma [23, 24]. The duration of IOP-lowering effect was shown to be prolonged for 24 h; thus, better patient compliance can be achieved by reducing the frequency of BRT administration.

Though the results of ocular inserts having 1 mg drug were compared with 2–3 drops of eye drop preparation (0.33 mg of BRT), it can be inferred that single administration of ocular insert containing sufficient amount of drug to maintain IOP under control is much preferred over four-times-a-day-administered eye drops, as the latter would result in flip flop profile between each administration. Also, since the drug is released slowly from the ocular inserts in a controlled manner, the systemic absorption of drug and related side/toxic effects could be minimised significantly.

The prepared ocular inserts using combination of hydrophilic and inert/zwitterionic polymers have the potential to improve the clinical efficacy of antiglaucoma drugs in improving the therapeutic outcome. Longer acting (once a week or once a month) inserts can be designed using rate retarding polymer coat over the designed matrix inserts. However, the drug content needs to be increased per insert based on therapeutic requirement for such long-term treatment.

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Declaration of interest The authors also report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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