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Original Article

DEVELOPMENT OF POLOXAMER BASED THERMOSENSITIVE IN SITU OCULAR GEL OF BETAXOLOL HYDROCHLORIDE

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ABSTRACT

Objective: This work was conducted to develop and evaluate Poloxamer 407-HPMC K50M based *in situ* gelling formulation of Betaxolol Hydrochloride (0.25%w/v) for enhancing its ocular bioavailability and sustained topical drug delivery for the treatment of glaucoma.

Methods: The formulation were prepared by cold method and was investigated for their physicochemical properties like, pH, drug content, sol–gel transition temperature, gelling capacity, rheological properties and *in vitro* permeation across goat cornea was done and compared with a commercial eyedrop.

Results: It was found that physicochemical properties of Betaxolol *in situ* gels were affected by formulation compositions. Increment of poloxamer 407 content decreased sol–gel transition temperature and increases the viscosity of the formulations. In this study, HPMC K50 M affect sol–gel transition temperature and viscosity but it did not affect transparency and pH of the formulation.

Conclusion: In conclusion, the *in vitro* release studies indicated that the poloxamer-HPMC solution had the better ability to retard drug release than the commercial eyedrop over an 8 hr period.

Keywords: *In situ* gel, Ophthalmic delivery system, Betaxolol, Thermosensitive polymer.

INTRODUCTION

The drug delivery to the eye is one of the challenging tasks because the unique anatomy and physiology of the eye. It restricts drug absorption into the deep tissues leads to difficult to achieve an effective drug concentration at the target site. Topically administered drug from conventional dosage form is eliminated from the precorneal area due to precorneal factors and anatomical barriers. The Precorneal factors include blinking, tear film, tear turnover, induced lacrimation and nasolacrimal drainage [1-3]. Among 100% of administered dose, more than 50% drug lead to systemic absorption via nasolacrimal drainage. Some drugs have systemic side effects due to non corneal absorption. In such cases, it's important to make formulation changes that improve the oculoselectivity of a drug rather than only ocular drug absorption. It is known that increasing formulation viscosity has the potential to decrease drainage rate, thereby increasing precorneal residence time and prolonging the time for ocular absorption [4].

To increase ocular bioavailability and duration of drug action, various ophthalmic vehicles such as, suspensions, ointments, and polymeric inserts, have been investigated for topical application to the eye [4, 5]. However, they have not been used extensively because of some drawbacks, such as blurred vision with ointments, low patient compliance with inserts and dosage heterogeneity of suspensions [2, 6]. For enhancement of precorneal residence and improving bioavailability of ophthalmic drugs an alternative approach has been used which is *in situ* gelling systems that are instilled in a liquid form which converted into a gel form in cul-de-sac of eye [5, 7]. This *in situ* gel system consists of polymeric material which undergoes sol-gel transition is triggered by the physiological temperature [8], pH [4] or ionic composition [5, 9] of the lacrimal fluid.

The thermosensitive poloxamer 407 is block copolymer made of poly (ethylene oxide) and poly (propylene oxide) (PEO-PPO-PEO) that induced the sol-gel transition with increase in temperature [10, 11]. There gelation mechanism involves copolymer molecules are aggregates into micelles and micellization and gelation depends on different factors, such as temperature, polymer concentration and PEO block length. The polymer solution is a highly viscous gel at room temperature but becomes a liquid at refrigerator temperature [12, 13]. Poloxamer has evaluated for rheological characterisation and it show an improved contact in human volunteer [13]. The viscosity of poloxamer gel is increased by using cellulose derivatives as compared with poloxamer gel alone [14]. Betaxolol hydrochloride is cardioselective beta-blockers, preferentially inhibit β1 adrenoceptors. The β blocking agents produce ocular hypotensive effect by decreasing the production of aqueous humor by the ciliary body without producing substantial effect on aqueous humor outflow facility. Betaxolol hydrochloride provides relief in glaucoma and ocular hypertension in a patient those having history of cardiopulmonary disease [15]. Betaxolol hydrochloride also has neuroprotective action on the eye, due to its calcium and sodium channel blocking activities and slows down the changes seen in the retina after raised intraocular pressure induced ischemia/reperfusion [16]. The most frequent adverse reaction to Betaxolol is stinging upon instillation, which is minimized by an ocular suspension which is similarly effective at 2-fold reduced concentration (0.25%) as compared to ophthalmic solution (0.5%) [17].

The objective and novelty of a present work was to design of gel forming solution of Betaxolol hydrochloride in the view to improving bioavailability and reduce the systemic side effect along with sustained drug delivery of Betaxolol. Poloxamer-HPMC K50M was investigated as vehicle for a formulation of Betaxolol eye drop (0.25% w/v) which undergoes gelation when instilled into cul de sac of the eye. The present article also reports the transcorneal permeation of Betaxolol hydrochloride optimized formulation is comparison with marketed eyedrops (Iobet® 0.5%, FDC Pvt. Ltd) across the goat cornea.

MATERIALS AND METHODS

Materials

Poloxamer 407 was obtained from BASF Corp. (Mumbai, India); Betaxolol hydrochloride was obtained as a gift sample from FDC Pvt. Ltd. Mumbai (INDIA). Various grades of HPMC and Benzalkonium chlorides were obtained from Glenmark Pharmaceuticals Nasik. Commercial eyedrops (Iobet® 0.5%, FDC Pvt. Ltd) and all other chemicals used were commercially available products of analytical grade.

Methods

Preliminary drug-excipient compatibility studies

A compatibility study was carried out in order to establish, that there was no interaction between the drug and excipients used in the formulation. The polymers are poloxamer 407 and HPMC K50M was selected to check their preliminary compatibility with Betaxolol hydrochloride. The preliminary compatibility was carried out using a DSC (DSC-60, Shimadzu, Japan) and FTIR spectrometer (FTIR 8400S, Shimadzu, Japan).

The FTIR spectrum was recorded from 4500 cm-1 to 400 cm-1. Infrared spectra of pure drug, a binary mixture of drug with polymer and physical mixture of drug with the polymers were obtained. Thermal analysis of pure drug and physical mixture of drug with the polymers were performed using a DSC instrument. An accurately weighed amount of samples (2.0–5.0 mg) was transferred to an aluminum pan and then simply covered with an aluminum lid. A similar empty pan was used as a reference. Samples were scanned from 50 to 150 °C at a rate of 10 °C/min under nitrogen gas flow (20 ml/min). The instrument was previously calibrated by Indium standard.

Method of preparation

The gels were made on a weight basis using the modified cold method [18]. Poloxamer 407 was mixed with the Betaxolol hydrochloride, isotonic agent, and water. This solution stirred periodically until a homogeneous solution was obtained and refrigerated at 4 °C. The required amount of viscosity enhancing agent HPMC K50M was dissolved in hot water and cool to 4 °C, which is then transferred to drug-containing polymer solutions, stir and then samples were then transferred to vials and stored in refrigerator for overnight which were finally sterilized by autoclave at 121 °C at 15psi for 20 min.

Gelling capacity was determined by placing 100 µl of *in situ* gel formulation in a vial containing 2 ml of freshly prepared simulated tear fluid. It was equilibrated at 37 °C and then visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve [19].

Table 1: Combination of poloxamer and HPMC studied

Formulations	Poloxamer 407(%w/v)	HPMC K50M (%w/v)	Gelling Capacity
F1	15	0.5	--
F2	15	0.75	--
F3	15	1	+
F4	17.5	0.5	++
F5	17.5	0.75	++
F6	17.5	1	++
F7	20	0.5	+++
F8	20	0.75	+++
F9	20	1	+++

Notice: --, No gelation; +, Gel formed after a few minutes, dissolves rapidly; ++, Gelation immediate, remains for few hours; +++, Immediate gelation, remains for the extended period.

Evaluation of formulations

The prepared *in situ* gel formulation evaluated for transparency and pH at physiological condition (table 2). The pH of all formulations was measured using previously calibrated digital pH meter and pH range was found to be 6.40–7.00.

Measurement of Gelation temperature

A transparent beaker (25 ml) containing 10 mL of Betaxolol gelling forming solution was placed in a low-temperature water bath. A thermometer with an accuracy rating of 0.1 °C was immersed in the Betaxolol hydrochloride gelling solution. The solution was heated at the rate of 1 °C/1–2 min with constant stirring of 100 rpm (Magnetic stirrer, Model 2MLH, Remi, Mumbai). The temperature at which the magnetic bar completely stopped moving because of gelation was regarded as the phase transition temperature. Each sample was measured at least in triplicate [19].

Drug content

The drug content estimation was carried out by taking 1 ml of the prepared formulation and diluting it to 100 ml of distilled water. Aliquot of 5 ml was withdrawn & analyzed using UV-Visible spectrophotometer (UV 2450, Shimadzu, Japan) at 274 nm.

Rheological studies

Viscosity determinations of the prepared formulations were carried out by using a Brookfield viscometer (LVDV-II+, Brookfield Eng. Ltd., USA). The viscosity measurements of formulation F1 to F3 being in solution form were done by using small sample adaptor SC-31 spindle at 37±0.5 ^{0}C and angular velocity was increased gradually from 0.5 to 100 rpm with a waiting period of 60 seconds at each speed. The viscosity of formulation F4 to F9 being in gel form were done by using Helipath spindle T-96 at 37±0.5 ^{0}C. The angular velocity was increased from 0.3 to 60 rpm with a waiting period of 60 seconds at each speed.

***In vitro* transcorneal permeation studies**

Goat corneas were used to study the permeation of Betaxolol hydrochloride across the corneal membrane. Whole eyeballs of goat were procured from a slaughter house and transported to the laboratory in cold condition in normal saline maintained at 4 °C. The corneas were carefully removed along with a 5–6 mm of surrounding scleral tissue and washed with cold saline. The washed corneas were kept in cold freshly prepared solution of tear buffer of pH 7.4. Study was carried out in modified Franz diffusion cell consisted of upper and lower chambers.

The upper chamber served as a donor compartment in which 1 ml of Betaxolol gel forming solution (0.25%w/v) under study was placed. The upper and lower chambers were separated by goat cornea and lower chamber served as a receiver compartment which consist of simulated tear fluid. Precaution was taken that that the corneal membrane just touched the receptor medium surface. The whole system was maintained at 37±0.5 °C [20]. Aliquots of 0.5 ml were withdrawn at hourly intervals and replaced by an equal volume of receptor medium. The aliquots were suitably diluted with the receptor medium and subjected to the quantification of Betaxolol hydrochloride using by UV-Vis spectrophotometer at 274.2 nm [20, 21].

The *in vitro* drug permeation from the commercial eyedrop of Betaxolol hydrochloride formulations (Iobet 0.5% w/v) was carried out in the same manner as mentioned above using a modified Franz diffusion cell.

Kinetics analysis of drug release

The release data of the formulations of F1 to F9 over the whole time period were analyzed according to the treatment of zero-order, first order, Higuchi's, and Korsmeyer–Peppas equations that are proposed for drug release from semisolid vehicles containing dissolved drug and the model with the higher correlation coefficient was considered to be the best model.

Accelerated stability studies

Prepared *in situ* gelling formulations, F 4 of Betaxolol hydrochloride were subjected to accelerated stability studies at 40^0C±2^0C/75%±5% RH for a period of 90 days.

Samples were withdrawn after 90 days and were evaluated for parameters such as appearance, pH, Gelation studies, drug content and viscosity.

RESULTS AND DISCUSSION

Preliminary compatibility studies

FTIR spectroscopy (Non-thermal analysis)

FTIR spectra of drug and physical mixture of drug with polymer were shown in (fig. 1). All the principal peaks of Betaxolol hydrochloride were present in the infrared spectra of physical mixture of drug with polymer thus indicating compatibility between drug and the polymers. The spectrum confirmed that there is no significant change in chemical integrity of the drug.

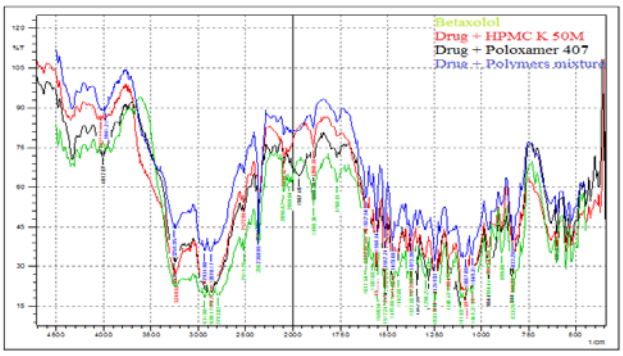

Fig. 1: Overlay spectrums of drug, binary mixture (drug+excipient 1:1) and physical mixture of drug with polymers

Differential scanning calorimetry (Thermal analysis)

DSC used in the pharmaceutical area to establish the identity and purity of solid state systems and detect interaction of the component, therefore, can be applied to the selection of suitable chemically compatible excipients. The thermogram of Betaxolol hydrochloride and its physical mixture with polymer are present in fig.2. The Betaxolol hydrochloride demonstrated a single, defined endotherm at 118.54 °C. In the physical mixture characteristic peak of Betaxolol hydrochloride appeared at 117.01 °C corresponding to its melting point and poloxamer show endothermic peak at 53 °C, however, characteristic of HPMC K50M was not found in fig.2. There was no appearance and disappearance of one or more new peak corresponding to these of the individual components thus indicating the absence of drug polymer interaction [22].

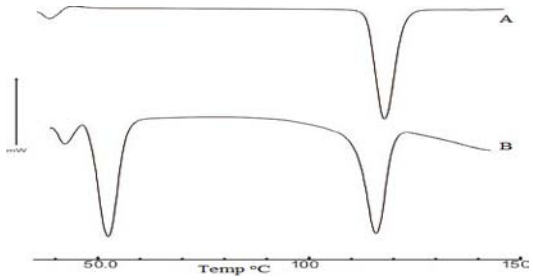

Fig. 2: DSC thermogram of drug (A) and physical mixture of drug with polymers (B)

Evaluation of formulations

Gelling capacity test and Phase transition temperature

Gelling capacity of the representative formulations were determined and results are shown in (table 1). The formulations containing 17.5% and 20% poloxamer formed gel, but those formulations contain 15% poloxamer doesn't form gel except F3. The transition temperature decreased with increasing polymer concentration (table 2). Formulation F3-F9 showed a sol-gel transition above 22^0C [23].

Table 2: Results of the evaluation of thermoresponsive Betaxolol hydrochloride ophthalmic *in situ* gels

Formulations	Transparency	Temp sol/gel	pH	Drug Content
F1	Transparent	--	6.79	99.44±2.42
F2	Transparent	--	7.00	100.05±3.84
F3	Transparent	42.0±0.4	6.56	99.15±1.41
F4	Transparent	36.1±0.2	6.85	101.58±1.48
F5	Transparent	37.9±0.1	6.60	96.76±1.39
F6	Transparent	30.6±0.3	6.53	98.84±1.23
F7	Transparent	28.3±0.1	6.61	101.51±1.90
F8	Transparent	23.7±0.4	6.40	97.39±1.58
F9	Transparent	22.1±0.5	6.41	95.63±2.31

Rheological studies

The formulations exhibited pseudoplastic rheology, as shown by shear thinning and a decrease in the viscosity with increased angular velocity (Fig.3 and 4). The viscosity was directly dependent on the polymeric content of the formulations. Viscosity increased with the increase in the concentration of Polymer. Increase in viscosity of ophthalmic solutions after instillation in an eye was a desired feature for the purpose of sustaining therapeutics actions of the Betaxolol hydrochloride by providing increased precorneal residence time [13].

The administration of ophthalmic preparations should have as little effect or irritation in the eye due to the pseudoplastic character of the *in situ* gel. Since the ocular shear rate is very high, ranging from 0.03 S–1 during inter-blinking periods to 4250–28,500 S–1 during blinking, formulation which shows pseudoplastic behavior with shear thinning property under high stress is often preferred [24].

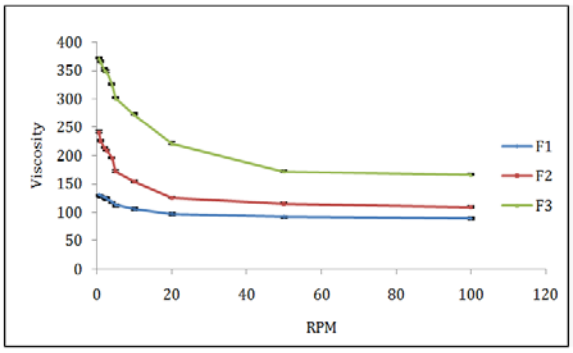

Fig. 3: Rheological profiles of the formulation F1to F3 (n= 3, error bars represent standard error).

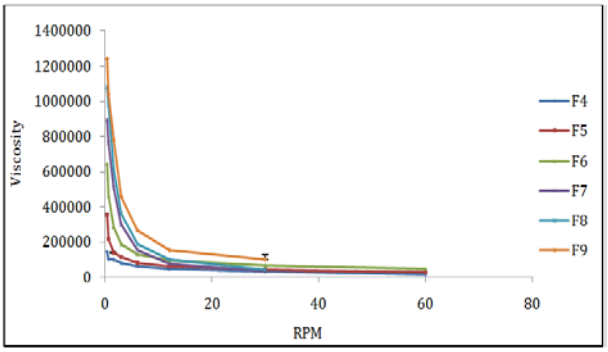

Fig. 4: Rheological profiles of the formulation F4 to F9 (n= 3, error bars represent standard error)

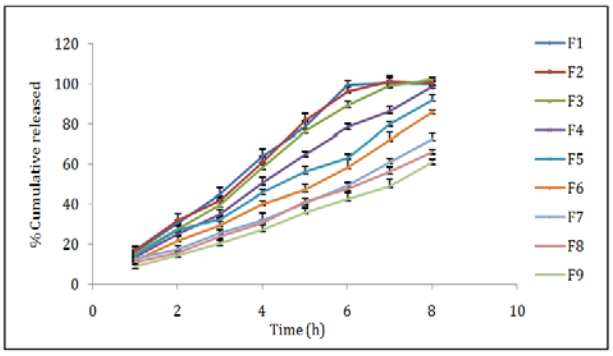

Fig. 5: *In vitro* release of Betaxolol hydrochloride from *in situ* gel formulations (n= 3, error bars represent standard error)

In vitro transcorneal permeation studies

The cumulative percent of Betaxolol hydrochloride released versus time profiles is shown in (Fig.5). For the formulation F1 to F3 contains 15%w/v poloxamer 407 almost all these formulation released above 85% of drug release within 6 hrs.

For the F4 to F6 containing 17.5% poloxamer 407 had shown the release above 85% in 8 h. The formulations F7 to F9 containing 20 % poloxamer 407 shown their release above 55% within 8 h which shows that release profile became very slow with increase in polymer concentration. These results showed that the formulations of F4 and F5 had relatively better sustained-release effect and can be used as an ophthalmic sustained release drug delivery system as they show above 90% of Betaxolol hydrochloride release in 8 h.

From this drug release profile, it demonstrated that the increase in Betaxolol release time with the increase in the viscosity imparted by high concentrations of poloxamer 407 and HPMC K50M. But the result also indicate that when the poloxamer 407 concentration level as 15% (F1-F3), 17.5% (F4-F6) and 20% (F7-F9) keep constant in group of three formulation and corresponding increasing the HPMC K50 M concentration by 0.5%, it was seen that those formulation containing poloxamer level constant the drug release retarded with the corresponding increase in HPMC K50M concentration by 0.5%. The linear relationships in conjunction with slow release rates suggest that the *in vitro* drug release from those polymeric vehicles at physiological conditions occurs primarily by diffusion.

In vitro release comparison between marketed product and *in situ* gel formulation (F4)

The comparative *in vitro* drug release profile (fig. 6) between the marketed conventional ophthalmic drops (Iobet® 0.5%, FDC Pvt. Ltd) and the formulation F4 showed 22.87 % and 13.51 % after initial 60 min respectively. At the end of 4 h. the drug release was found to be 99.24% from the marketed product and from the F4 50.74% indicating that the drug release was significantly prolonged by using the *in situ* gelling systems.

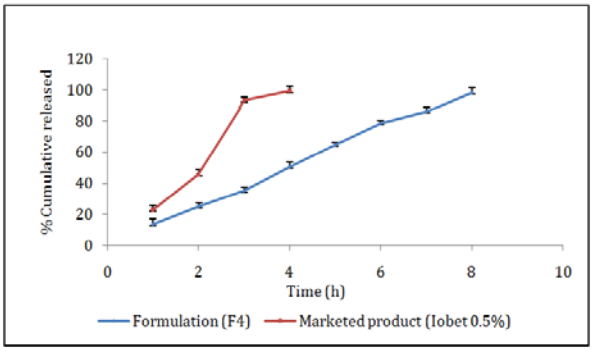

Fig. 6: Release profiles of drug from *in situ* gel formulations containing (F4) 0.25% w/v Betaxolol in comparison with 0.5%w/v Betaxolol commercial eye drop (n= 3, error bars represent standard error)

Release kinetics studies

Data obtained from release measurements was fitted to zero-order, firstorder, Higuchi's, and Korsmeyer–Peppas equations and used to interpret the release pattern from *in situ* gel system. It is found that the *in vitro* drug release was best explained by Korsmeyer-Peppas equation for the formulations F1to F4 and F6 and these formulations showed the highest linearity (r2) for Korsmeyer-Peppas equation. The formulations F5, F7 to F9 were showed the highest linearity (r2) for firstorder kinetics.

All the kinetic data are fitted to the Korsmeyer-Peppas equation $Qt/Q\infty = ktn$. Where $Qt/Q\infty$ is the fraction of drug released at time t; k is a constant related to structural and geometrical characteristics of formulation as release rate and 'n' is the release exponent indicative of the drug release mechanism. The values of release exponent varied from 0.8551 to 0.9869 which appeared to indicate an anomalous or non-Fickian drug diffusion [9].

Table 3: Correlation coefficients (r) and release exponent (n) for kinetic analysis of the release data of Betaxolol HCl calculated for various formulations

Formulation Code	Zero order	First order	Higuchi	Korsmeyer–peppas	
	r^2	r^2	r^2	r^2	N
F1	0.9470	0.8557	0.9671	0.9852	0.9438
F2	0.9525	0.9066	0.9648	0.9846	0.9194
F3	0.9751	0.7440	0.9770	0.9907	0.9807
F4	0.9956	0.7686	0.9799	0.9965	0.9869
F5	0.9907	0.8461	0.9580	0.9894	0.8894
F6	0.9898	0.8789	0.9504	0.9925	0.9341
F7	0.9841	0.9206	0.9339	0.9681	0.8551
F8	0.9951	0.9639	0.9609	0.9838	0.9047
F9	0.9967	0.9759	0.9651	0.9922	0.9313

Accelerated stability study

Selected sterilized formulations F4 were stored at 40^0C±2^0C/75%±5% RH for a period of 3 months. Formulations were evaluated after 3 months and it was found to be clear with no change in pH, drug content (96%–98%), viscosity, and gelling capacity. Stability data recorded over a 3 month period indicated that the formulation is stable under accelerated temperature conditions and normal laboratory conditions. The stability studies indicated that the formulation was physically and chemically stable with no significant change in any of the parameters evaluated.

CONCLUSION

It can be concluded from this study, that the bioavailability of Betaxolol improved due sustained release effect of poloxamer 407-HPMC K 50M vehicle. The overall results of this study indicate that the poloxamer-HPMC vehicle is an excellent drug carrier, well tolerated, and could be used for the development of a long-acting ophthalmic formulation of Betaxolol. *In vitro* transcorneal permeation, comparison was carried between commercial aqueous solution (Iobet 0.5%) and *in situ* gel forming formulation F4 (0.25%). It was found that F4 formulation retard drug release up to 8h as compare to marketed Betaxolol eye drops formulation. The formulations (F4) were liquid at 20 ºC and undergo rapid gelation at 37.1 ºC. Stability data recorded over a 3 month period under accelerated temperature condition indicates that formulation is stable. Moreover, this new formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer pre-corneal residence time at low dose along with sustain drug release. Also important is the ease of administration afforded and decreased frequency of administration resulting in better patient acceptance.

CONFLICT OF INTERESTS

Declared None

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