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In vitro and in vivo evaluation of Pluronic F127-based ocular delivery system for timolol maleate

A.H. El-Kamel

Department of Pharmaceutics, Faculty of Pharmacy, King Saud University,
Women Students-Medical Studies and Science Sections, PO Box 22452, Riyadh 11495, Saudi Arabia

Abstract

The purpose of this study was to develop Pluronic F127 (PF127) based formulations of timolol maleate (TM) aimed at enhancing its ocular bioavailability. The effect of isotonicity agents and PF127 concentrations on the rheological properties of the prepared formulations was examined. In an attempt to reduce the concentration of PF127 without compromising the in situ gelling capabilities, various viscosity enhancing agents were added to PF127 solution containing 0.5% TM. The viscosity and the ability of PF127 gels to deliver TM, in vitro, in absence and presence of various viscosity enhancing agents were also evaluated. At the used concentration, some of the examined isotonicity agents had effect on the viscosity of TM gel. However, the viscosity of gel increased as the PF127 concentrations increased. The viscosity of formulations containing thickening agents was in the order of PF–MC 3% > PF–HPMC 2% > PF–CMC 2.5% > PF127 15%. The slowest drug release was obtained from 15% PF127 formulations containing 3% methylcellulose. In vivo study showed that the ocular bioavailability of TM, measured in albino rabbits, increased by 2.5 and 2.4 fold for 25% PF127 gel formulation and 15% PF127 containing 3% methylcellulose, respectively, compared with 0.5% TM aqueous solution. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Poor bioavailability of ophthalmic solutions is caused partly by the rapid decrease of drug concentration in precorneal tear fluid. The rapid precorneal elimination of drugs given in eye drops is mainly due to conjunctival absorption and drainage of drug induced by lachrymation and normal tear turnover (Lee and Robinson, 1979).

Another reason for the low bioavailability is the slow diffusion of water-soluble drugs through the cornea (Robinson, 1989). Traditional dosage forms for delivery of drugs in the eye have been and remain, solutions. A large proportion of the topically applied drug is immediately diluted in the tear film and excess fluid spills over the lid margin and the remainder is rapidly drained into the nasolachrymal duct. A proportion of the drug is not available for therapeutic action since it binds to the surrounding extraorbital tissues. These processes lead to a typical corneal contact

E-mail address: amalelkamel@yahoo.com (A.H. El-Kamel).

time of about 1–2 min in humans, and an ocular bioavailability that is commonly less than 10% (Lee, 1993). Due to poor ocular bioavailability, many ophthalmic drugs are applied in high concentrations. This causes both ocular and systemic side-effects (Urtti et al., 1990).

Several new preparations have been developed for ophthalmic use not only to prolong the contact time of the vehicle at ocular surface, but at the same time slow down the elimination of the drug (Bourlais et al., 1998; Ding, 1998). Successful results were obtained with inserts (Ding, 1998) and collagen shields (Hill et al., 1993), although these preparations present some disadvantages, such as noncompliance, especially by elderly people and many patients lose the device sometimes without becoming aware of it. From the point of view of patient acceptability, a liquid dosage form is preferable.

This problem can be overcome by using in situ-forming gel ophthalmic drug delivery systems prepared from polymers that exhibit reversible phase transitions and pseudoplastic behavior to minimize interference with blinking. Such a system can be formulated as drug containing liquid suitable for administration by instillation into the eye, which upon exposure to physiological conditions will shift to the gel phase, thus increasing the precorneal residence of the delivery system and enhancing ocular bioavailability.

Pluronic F127 (PF127) consists of polyoxyethylene units (70%) and polyoxypropylene blocks (30%) (Lin and Sung, 2000). At a concentration of 15% or higher in aqueous solution PF127 is transformed from a low viscosity solution to a semisolid gel upon heating from 4 °C to temperature greater than 23 °C and this thermogelation is reversible upon cooling. The phenomenon of thermogelling is characterized by a sol–gel transition temperature. That is to say below this temperature, the sample is fluid allowing a comfortable and precise delivery, above this transition temperature, the solution becomes gel according to the increment of local temperature. The thermogelification results from the interaction between the different molecules of Pluronic. The increment of the temperature modifies the hydration spheres around the hydrophobic units

which in turn induces higher interactions between these different units (Dumortier et al., 1991). This made PF127 attractive in formulating thermoreversible gels for ophthalmic and controlled delivery of many drugs. PF127 gels containing various drugs have been used for treating patients with ocular conditions (Mengi and Deshpande, 1995; Desai and Blanchard, 1998).

Timolol maleate (TM) is the most commonly used drug that treats the open-angle glaucoma. As systemic absorption of TM may cause respiratory and cardiovascular side effects, it is important to minimize the systemic absorption and enhance ocular bioavailability of TM.

The overall objective of this study was to develop and evaluate PF127 gel forming solution to improve the ocular bioavailability and hence decrease the systemic absorption and side effects of TM. To achieve this objective, the rheological behavior of 0.5% TM gel containing various concentrations of PF127 was examined. Also the influence of addition of various tonicity agents on its viscosity was investigated. In an attempt to reduce the concentration of PF127 without compromising the in situ gelling capabilities as well as the overall rheological properties, various viscosity enhancing agents were added to PF127 solution containing 0.5% TM. The viscosity and the ability of PF127 gels to deliver TM in vitro in absence and presence of various viscosity enhancing agents were examined. The ocular bioavailability of TM from some selected formulae was also evaluated in albino rabbits.

2. Experimental

2.1. Materials

PF127 was obtained from BASF Corp., (Wyandotte, MI). TM was from Merck, USA. Methanol HPLC grade, hydroxypropylmethyl cellulose (HPMC, 80–120 cP), methyl cellulose (MC, 1.5% w/v solution 500–600 cP, degree of substitution is 1.9) and carboxymethylcellulose sodium (CMC Na, 1% w/v solution 1500 ± 400 cP, degree of substitution is 0.7–0.8) were from BDH chemicals Ltd. Poole, England. All other chemicals and solvents were reagent grade.

2.2. Methods

2.2.1. Preparation of PF127 timolol maleate formulations

The gels were made on a weight basis using the modified cold method (Schmolka, 1972). PF127 was mixed with the drug, isotonic agent and water and refrigerated at 4 °C and stirred periodically until a homogeneous solution was obtained. PF127 concentrations of 15, 20 and 25% w/w were prepared and denoted as PF-15, PF-20 and PF-25. To study the effect of various isotonicity agents on the rheological properties, 20% w/w gel was used.

Formulae containing 15% PF127 and viscosity enhancing agents, HPMC, MC and CMC Na were also prepared. These formulae were referred to as PF–HPMC, PF–MC and PF–CMC, respectively. In this case, the required amount of selected viscosity enhancing agent was dissolved in cold water except HPMC that was dissolved in hot water. The drug and isotonic agent were then added. An appropriate amount of PF127 was finally added slowly. The pH of all formulations was measured and was in the range of 6.8–7.4. The concentration of isotonicity agents that rendered the formulations isotonic with eye fluid was calculated by sodium chloride equivalent method (Martin, 1993).

2.2.2. Rheological studies

Rheological properties of gels were measured using Rotationsviskosimeter Rheotest type RV, Germany. The samples were thermostated at 34 ± 0.1 °C by circulating bath connected to the viscometer. The shear rate was increased from 0 to 14.56 s^{-1} in 10 min. The viscosity was determined from the flow curve obtained at different values of shear rate. The samples were equilibrated at 34 °C prior to each measurement. All measurements were made in triplicate.

2.2.3. In vitro release from PF127 gel formulations

A membraneless dissolution model was used for in vitro studies. The cold PF127 formulations (2 gm) were transferred into tared vials and mounted vertically in a water bath at 34 ± 0.1 °C and shaken at 20 rpm. Care was taken that the gel contained no air bubbles and that the surface was smooth. Artificial isotonic tear solution (10 ml) (Rozier et al., 1989) pre-equilibrated at the experimental temperature (34 °C), was used as the release medium.

Sample (1 ml) was drawn at various time intervals and replaced with 1 ml fresh artificial isotonic tear solution. The content of TM was determined by HPLC assay at 294 nm after suitable dilution. The release profile of TM was obtained by plotting the cumulative amount of drug released from each PF127 formulation against time. The dilution of the release medium due to replenishment following each aliquot withdrawal was taken into account in the calculation of the cumulative amount of TM released from the gel. Each experiment was performed in triplicate.

2.2.4. In vivo evaluation of PF127 formulations

New Zealand albino rabbits weighing 2–2.5 kg were used in this study. Fifty μl of the cold (4 °C) test solutions (0.5% TM aqueous solution, 0.5% TM–PF-25 or 0.5% TM–PF–MC) was instilled into the conjunctival sac of albino rabbits. For each formulation three animals were then killed at each of the following time intervals: 0.5, 1, 1.5, 2, 3 and 4 h post instillation. Aqueous humor was withdrawn with a 27-gauge, 1.3 cm needle attached to 1 ml disposable syringe inserted through the corneal–scleral junction and slightly upwards into the anterior chamber. The rabbits were starved for 24 h but had free access to water. All animals in the study conformed to the guidelines for animal experimentation in King Saud University.

The collected aqueous humor was centrifuged at 8000 rpm for 10 min. The aqueous humor samples (200 μl) were mixed with 1 M HCl (20 μl) and methanol (200 μl) containing benzocaine (1 μg ml^{-1}) as internal standard. The mixture was centrifuged at 8000 rpm for 15 min and 25 μl of supernatant was injected into the HPLC system.

2.2.5. HPLC assay of timolol maleate

The HPLC system (JASCO, UV-1575 detector, JASCO, Pu-1580 pump, Jasco Corporation, Tokyo, Japan, Shimadzu C-R6A Chromatopac

integrator, Tokyo, Japan) was used in the reversed-phase mode. Analysis was performed on a Novapack C_{18} packed column (300 mm length × 4.6 mm i.d.). The mobile phase was a mixture of methanol and 0.2% triethylamine HCl solution adjusted to pH 3 with phosphoric acid (45:55) (Ashton et al., 1991). The flow rate was 1 ml min^{-1}. UV detector was used at 294 nm and the detection limit of TM was 20 nM. The retention time of TM and benzocaine was 6.1 and 11.9 min, respectively.

3. Results and discussion

3.1. Rheological studies

3.1.1. Effect of isotonicity agents

Isotonic ophthalmic preparation was preferred by the majority of the patients. Mannitol, sorbitol, propylene glycol, glycerol and sodium chloride have been used to prepare isotonic vehicles for eyes and were used in this study as tonicity agents.

Table 1 shows the measured viscosity at various shear rates for 20% PF127 gel containing different isotonicity agents. The viscosity of PF127 gel in water was slightly lower than in presence of sodium chloride. This could be probably due to a lowering of critical micelle concentration (cmc) and critical micellar temperature (cm$_t$), facilitating closer packing of PF127 micelles and resulting in

gel formation at lower temperatures. This implies that, at any given temperature, a gel containing these salts consists of more closely packed micelles than a similar gel without salts (Pandit and Kisaka, 1996). Vadnere et al. (1984) reported that the effects of inorganic salts can be viewed in terms of reducing the water activity and as a result increasing the effective concentration of polymer in the system. A lowering of cmc by salts has been shown by Schott and Han (1976) for other non-ionic surfactants.

Glycerol and propylene glycol were reported to increase viscosity of PF127 in concentrations higher than 5% at 15 °C (Miller and Drabik, 1984). However, at 25 °C the change in rheological behavior of PF127 tends to be less marked as reported by Miller and Drabik (1984). In the present study, the concentration of propylene glycol and glycerol ranged from 1.5 to 2.5% which are considered to be low concentrations and hence did not give a marked effect on the viscosity of PF127 gel at 34 °C. Mannitol and sorbitol at a concentration of ~ 5 and 4.5%, respectively, slightly increased the viscosity of gel compared with that prepared without addition of isotonicity agents. This could be due to supplementation of hydrogen bonding involved in gel formation by hydroxyl groups of these isotonicity agents (BASF, 1989).

Analysis of variance for the results of rheological determinations indicated that there was statistical significant difference ($P \leq 0.05$) between the

Table 1
Rheological measurements on 20% PF127 formulations of TM at 34 °C containing various isotonicity agents ($n = 3$)

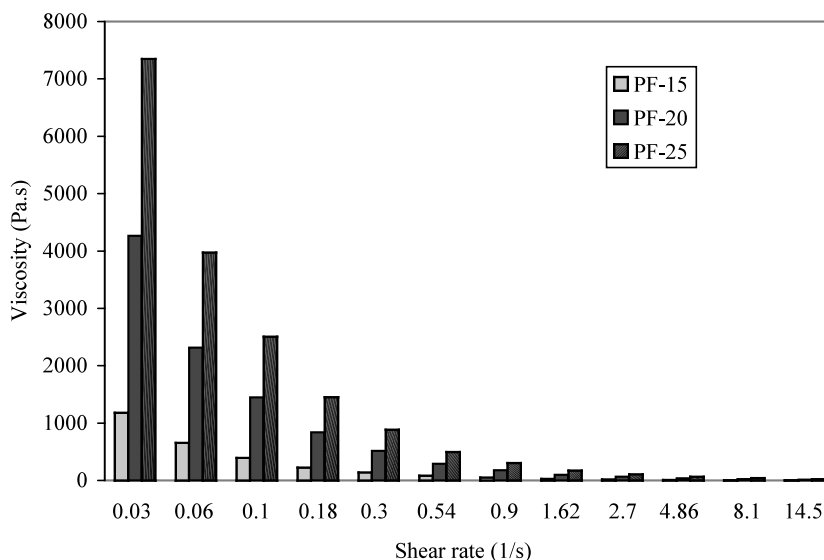| Shear rate (s^{-1})	Viscosity (Pa s)					
	Isotonicity agent					
	None (water)	Glycerol	Propylene glycol	Sorbitol	Mannitol	Sodium chloride
0.03	3356	3989	3991	4172	4353	4263
0.1	1208	1329	1389	1419	1510	1449
0.3	442.9	468.1	493.2	503.3	543.6	513.4
0.9	154.3	165	164.4	171.1	187.9	177.8
2.7	52.5	58.7	55.9	61.5	65.9	63.7
8.1	20.8	21.6	21.6	23.8	24.9	24.2
14.6	12.0	12.9	13	13.8	14.9	14.4

Fig. 1. Viscosity–shear rate bar chart of medicated gel containing various concentration of PF127 ($n = 3$).

viscosities of gel formulations prepared using various isotonicity agents. Duncan test was further performed and indicated the differences were only between viscosity of formulations containing sorbitol, mannitol or sodium chloride and that containing no isotonicity agent. Sodium chloride was used as isotonicity agent for subsequent studies.

3.1.2. Effect of Pluronic F127 concentration

PF127 gels containing 0.5% TM were prepared in water at concentrations of 15, 20 and 25%. All gel formulations exhibited pseudoplastic (shear thinning) flow behavior at 34 ± 0.1 °C (Fig. 1). The pseudoplastic behavior of PF127 formulations has been observed previously (Dumortier et al., 1991). The apparent viscosity of Pluronic as a function of concentration was examined to determine whether they adhered to the following equation:

$$\eta = \eta_0 e^{kc}$$

which describes apparent viscosity as a function of concentration (Chase, 1970). Values of 0.99, 0.1 and 0.5 for correlation coefficient, slope and intercept were obtained, respectively. These results

would suggest that logarithm of viscosity of the gel correlated reasonably well with PF127 concentration. Pluronics being nonionic, polyoxyethylene–polyoxypropylene–polyoxyethylene triblock copolymers, at 34 °C the copolymer molecules aggregate into micelles. It is generally accepted that micellization is due to the dehydration of the polymer blocks with temperature. It has been shown that gel formation is a result of micellar enlargements and packing and that the gel is more entangled at higher PF127 concentrations. As a result of these micelle entanglements, they cannot separate easily from each other, which accounts for the rigidity and high viscosity of gel containing high concentrations of Pluronics (Cabana et al., 1997; Jain et al., 1998).

3.1.3. Effect of additives

In an attempt to reduce PF127 concentration and to obtain reasonable viscosity for the prepared formulations, some viscosity enhancing agents were added to 15% PF127 gel containing 0.5% TM and their effect on the rheological behavior of the prepared gels was investigated. The additives used in this study were MC (1.5–3%), HPMC (1–2%) and CMC Na (1.5–2.5%). The concentrations of the additives used in this study

did not exceed the maximum concentrations that maintained the thermally reversible sol–gel characteristics of the PF127 based formulations.

The same additives have been used previously in ophthalmic formulations as wetting or viscosity-increasing or suspending agents (Desai and Blanchard, 1998).

Fig. 2 shows the relation between the viscosity and shear rate of 15% PF127 gel and 15% PF127 gel containing various concentrations of CMC Na, MC or HPMC at 34 ± 0.1 °C. A typical pseudoplastic flow was obtained. PF–CMC, PF–MC, PF–HPMC formulations exhibited higher viscosity than the control PF127 formulation (containing 15% PF127) and the viscosity increased as the concentration of additives increased.

Fig. 3 shows that the viscosity of PF–MC 3% (containing 15% PF127) was very comparable to that of 25% PF127 formulation containing no additives (PF-25). The rank order of increased viscosity was PF–MC 3% > PF–HPMC 2% > PF–CMC 2.5% > PF-15 (control). Block copolymer gels PF127 are thought to be formed by hydrogen bonding in aqueous systems, caused by the attraction of the Pluronic ether oxygen atom with protons of water. If the hydrogen bonding is supplemented by adding compounds with hydroxyl groups such as the examined cellulose derivatives, the desired gel strength may be achieved with reduced PF127 concentration (BASF, 1989). These results are in agreement with those of Desai and Blanchard (1998), who have also reported that the viscosity of Pluronic formulations containing MC and HPMC at a given shear stress was in the order: PF–MC > PF–HPMC > PF.

Formulations containing the highest concentrations of viscosity enhancing agents were chosen for further TM release study and comparison with the drug release from formulations containing 15 and 25% PF127.

3.2. In vitro release of TM from different formulations

3.2.1. Effect of PF127 concentration

The release of 0.5% TM from gel formulations

containing 15, 20 and 25% of PF127 was studied at 34 ± 0.1 °C (Fig. 4). The amount of TM released in vitro from the three formulations was
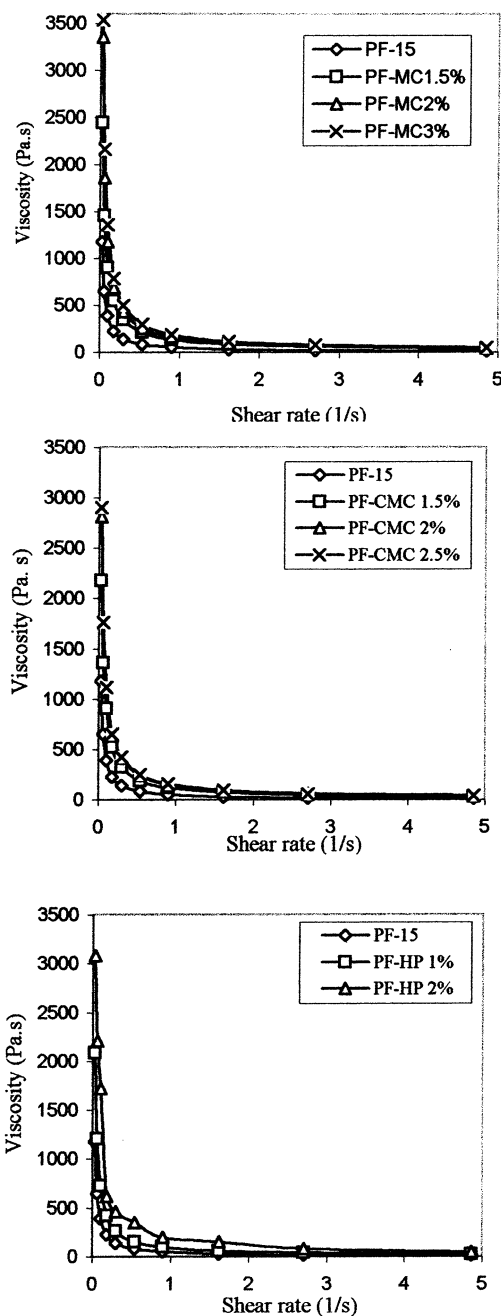

Fig. 2. Viscosity–shear rate profiles of medicated 15% PF127 containing different concentrations of various additives ($n = 3$).
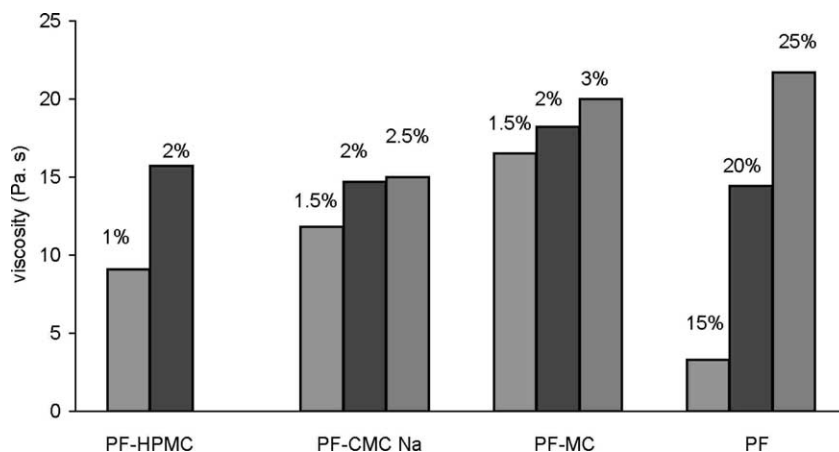
Fig. 3. Effect of concentration and type of additives on the viscosity of medicated PF127 gels (shear rate equals to 14.6 s^{-1}).

regressed against time using least square analysis. The slope of the regression line is a measure of the rate at which TM is released from PF127 gel formulations. The regression analysis indicated that as the concentration of PF127 increased the amount of the drug released decreased (slope: PF127 15% = 868 µg min^{-1}, PF127 20% = 810 µg min^{-1} and PF127 25% = 726 µg min^{-1}). These results indicate that the structure of the gel functioned as an increasingly resistant barrier to drug release as the concentration of PF127 increased. The mechanism for such enhanced resistance may be due to reduction in the number and dimension of water channels and to the increase in the number and size of micelles within the gel structure (Schmolka, 1991). The shorter intermicellar distance leads to greater numbers of cross-links between neighboring micelles leading to higher viscosity and lower rate of drug release (Bhardwaj and Blanchard, 1996; Alexandridis and Hatton, 1995). This assumption may be potentiated by the rheology study that indicates direct proportionality between gel concentration and viscosity. These results are in agreement with those reported by Chen-Chow and Frank (1981).

3.2.2. Effect of additives

The drug release from 15% PF127 containing 3% MC (PF–MC 3%), 2.5% CMC Na (PF–CMC 2.5%) and 2% HPMC (PF–HPMC 2%) was examined at 34 °C and compared with TM release

from the formula containing 15 and 25% PF127 without any additives (Fig. 5). The rank order of drug release was as follows: PF-15 > PF–CMC 2.5% ≥ PF–HPMC 2% > PF–MC 3% ≥ PF-25. In general, there was a reduction in TM release obtained upon addition of these viscosity-enhancing agents to 15% PF127 gel. The formula containing 15% PF and 3% MC (PF–MC 3%) exhibited comparable drug release to the formula containing 25% PF127 without additives. Similar results were obtained by Paavola et al. (1998), who reported that cellulose additives significantly prolonged ibuprofen release.
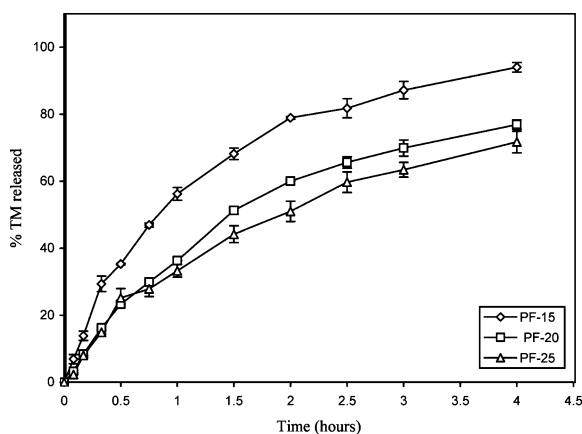

Fig. 4. Release profile of TM from gel formulations containing various concentrations of PF127 ($n = 3$, error bars represent standard error).
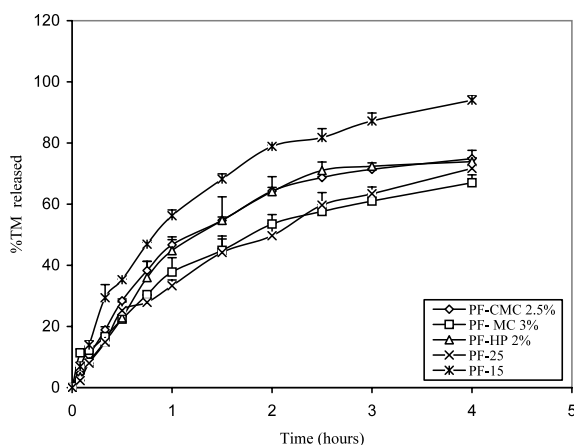
Fig. 5. Release profile of TM from gel formulations containing different additives ($n = 3$, error bars represent standard error).

MC, HPMC and CMC Na are of high molecular weight, dissolve in water and yield much more viscous solutions compared with 15% PF127 gel without additives. The viscosity of gels was in the order of PF–MC 3% > PF–HPMC 2% > PF–CMC 2.5% > PF 15%. PF–MC 3% yielded comparable viscosity to 25% PF127 gel formula (Fig. 3). The increased viscosity might have contribution to the decreased rate of drug release from these formulations compared with 15% PF127 gel containing 0.5% TM. The slowest rate of drug release was obtained from the formula containing MC. This could be due to the formation of micelle junction zones between methylcellulose and polyethyloxylene–polypropyloxylene block co-polymers (Pluronic) (Swarbrick and Boylan, 1992). Although the polymers differ in chemical structure, both have hydrophobic regions in their chains: the di- and trimethyl-D-glucose residues of methylcellulose and polypropyloxylene block of Pluronic. Another feature common to the two polymers is that their gels exhibit inverted thermal reversibility, that is, they gel with heating and melt with cooling. Both the inverted temperature behavior and the presence of hydrophobic regions in the polymers provide evidence for the formation of micelle junction zone. Water molecules structured around the hydrophobic regions of polymer chains in a sol become disordered with increases in temperature. Newly exposed hydrophobic regions

attract one another to form bonds, whereas hydrophilic areas rearrange to maximize their contact with the aqueous medium. The resulting structures are micelles, which continue to grow in size and number at higher temperatures, leading eventually to more rigid gel structure. Consequently, drug release is retarded.

3.3. In vivo study

Fig. 6 shows the level of TM in aqueous humor after instillation of 50 μl of 0.5% TM formulated as aqueous solution and as in situ gel formulations containing 25% PF127 or 15% PF127 with 3% MC (PF–MC 3%).

The aqueous humor content of timolol was significantly higher (ANOVA at $P \leq 0.05$), at all time points, after administration of TM in situ gel formulations than that obtained after instillation of 0.5% aqueous TM solution.

The maximum level of TM in aqueous humor C_{max} was 9.1, 8.7 and 4.5 μg ml^{-1} for PF-25, PF–MC 3% and TM aqueous solution, respectively. The time required to reach maximum concentration (T_{max}) for all formulations was 1 h.

Calculation of AUC (0–4 h) for the concentration/time profiles of various formulations showed that the ocular bioavailability increased in aqueous humor by 2.5 and 2.4 fold for PF-25%
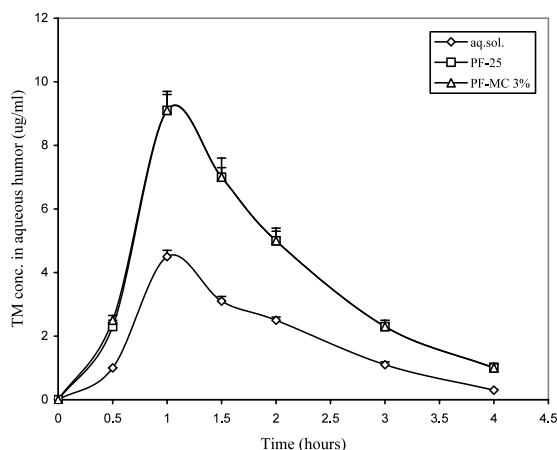

Fig. 6. Concentration of TM in aqueous humor after instillation of 0.5% TM aqueous solution, 0.5% TM–PF-25 and 0.5% TM–PF–MC-3% ($n = 6$, error bars represent standard error).

and PF–MC 3%, respectively, compared with 0.5% aqueous TM solution.

In conclusion PF127 formulations of TM can be used as liquid for administration by instillation into the eye, which up on exposure to eye temperature will shift to the gel phase. On the basis of in vitro and in vivo results, TM formulation containing 3% methylcellulose and low concentration of PF127 (15%) showed potential for use as delivery system with improved ocular bioavailability.

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