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FORMULATION AND EVALUATION OF SOME SELECTED TIMOLOL MALEATE OPHTHALMIC PREPARATIONS

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The purpose of this study was to prepare and evaluate certain Timolol maleate (TM) polymeric formulations including viscous solutions, hydrogels and in-situ gels aiming to improve its ocular bioavailability and decrease its side effects. In this study, Chitosan (CS), hydroxyehtylcellulose (HEC), hydroxypropylmethylcellulose (HPMC) and sodium carboxy-methylcellulose (NaCMC) were used to prepare TM viscous solutions and hydrogels. In-situ gels of Gelrite and Pluronic F-127 (Pl F-127) were prepared at different concentrations. Mucoadhesives namely CS or HEC were incorporated to Pl F-127 to produce mucoadhesive/in-situ gel. The prepared formulations were evaluated for their in-vitro drug release, viscosity, gelation temperature and mucoadhesive force. The selected TM formulations were tested for their effect on intraocular pressure (IOP) and systemic side effects based on blood pressure (BP), heart rate (HR) and respiration rate (RR). The results revealed that, TM in situ-gels F25 (containing Pl F-127 20% and CS 1.5% w/w) and F31(containing Gelrite 0.6% w/w) showed reasonable in-vitro results, and a marked IOP lowering activity without systemic side effects compared to TM marketed eye drops.

INTRODUCTION

Glaucoma, is a progressive optic neuropathy (neurofibroma), characterized by an increase in intraocular pressure (IOP), that if sufficiently high and persistent can damage the optic nerve head, decrease retinal sensitivity and steals sight without warning so called "the sneak thief of sight", till irreversible blindness. Therefore, all currently approved treatments of glaucoma and also anti-glaucoma therapies have focused on reduction or controlling the IOP either by decreasing aqueous humor production or increasing its outflow. Up till now, drugs that suppress aqueous humor production have proved to be the most successful[1&2].

Timolol maleate (TM), a non-selective β-blocker, had a "golden age" in reducing IOP, and still recommended by the European glaucoma society as the first drug of choice in the treatment of open-angle glaucoma because of its acceptable benefit-to-side effects ratio, longest record of safety, efficacy and economical low cost. Other antiglaucoma agents can be used as second line drugs[2&3].

However, topical delivery of conventional TM eye drops is extremely insufficient, because upon instillation of the ophthalmic solution rapid and excessive loss of the drug occurs by reflex tearing turnover, drug spillage and drainage to nasolacrymal duct and conjunctiva into systemic circulation. These factors lead to short precorneal residence time, poor bioavailability and serious systemic side effects including bradycardia, hypotension and bronchospasm. As a result, frequent instillation or using higher dose level of the drug is needed to achieve the desired therapeutic effect[4].

Since glaucoma is a chronic disease, and the drug may be used for decades, frequent instillation or using higher dose level may increase possibility of causing severe ocular and systemic side effects[5]. These problems can also be addressed by the use of other ophthalmic vehicles such as ointments,

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ocuserts and suspensions. However, these pharmaceutical preparations have not been extensively used because of some drawbacks such as blurred vision, low patient compliance and difficulties of application[6]. Therefore, various approaches have been focused on the use of viscolizers , bioadhesives and *in-situ* gel systems[7-9] to prolong precorneal residence time of the drug, improve patient compliance and bioavailability as well as to reduce systemic side effects.

The *in-situ* gelling systems can be easily instilled in a liquid form and gelled in the eye. These systems exhibit sol-gel phase transition due to change in specific physico-chemical parameters (Temperature, pH and ionic strength) in the cul-de-sac[7-9]. Two *in-situ* gel systems namely, thermosensitive and ion activated *in-situ* gels are considered as favorable delivery vehicles for ocular use.

A number of studies have been conducted on thermosensitive gels based on Pl F-127[8&9] which is changed from low viscosity solution at or below room temperature (25°C) to a semi-solid gel at the corneal surface (34°C). Moreover, many studies have been performed on ion-activated *in-situ* gels. Gellan gum (Gelrite^{R}), a polysaccharide which forms a clear gel on the ocular surface by crosslinking with mono or divalent cations present in the tear fluid. It has an excellent ocular tolerance, low irritating effect to ocular tissues compared to other *in-situ* gelling systems[10&11].

Although the poloxamer-based *in-situ* gel can be formed at physiological conditions without rapid precorneal elimination after administration, it exhibits a relatively short contact time when compared to gelrite, this is due to gradual dilution by lachrymal fluid. Therefore, addition of bio/mucoadhesive polymer to ophthalmic formula of poloxamer can reduce drainage from the precorneal surface i.e. improve the intimacy of contact and increase residence time. Mucoadhesive polymers such as hydroxyethylcellulose (HEC), hydroxypropyl methylcllulose (HPMC), sodium carboxymethylcellulose (NaCMC) can be added to *in-situ* gelling systems of Pl F-127[8&9]. Furthermore, Chitosan (CS), a ploycationic mucoadhesive biopolymer was evaluated as a potential component in ophthalmic gels, since, it exhibits favorable biological properties and slow drug elimination by lachrymal flow both by increasing solution viscosity and interacting with the negative charge of mucus[12-14].

The developed ophthalmic formulations of TM that has been reported were evaluated *in-vivo* for their ocular efficacy (IOP lowering activity, contact time and eye irritation)[8,9&11]. However, there was no or little attention on the systemic safety viz., heart rate (HR), blood pressure (BP) and respiration rate (RR) that is important to support the favorable benefit/risk ratio of the drug.

The objective of the present study was to prepare and evaluate certain ophthalmic polymeric liquid formulations of TM viz., viscous, bioadhesive and *in-situ* gels (gelrite ion-activated and Pl F-127 thermosensitive) delivery systems. Two mucoadhesives HEC and CS were adjuncted with Pl F-127 at different concentrations to prepare *in-situ*/mucoadhesive system.

The prepared formulations were subjected to:

1- *In-vitro* evaluations including viscosity, gelation temperature, bioadhesion strength and *in-vitro* drug release.
2- *In-vivo* evaluation of selected formulae were studied in albino rabbits for their effect on IOP lowering activity and systemic side effects on blood pressure (BP), heart rate (HR) and respiration rate (RR) and were compared to that of marketed conventional eye drops (Timolol Maleate USP 0.25% produced by Egyptian International for Pharmaceutical Industries Company (EIPICO).

EXPERIMENTAL

Materials

Timolol maleate, (TM); (Kindly supplied by Egyptian International for Pharmaceutical Industries Company (EIPICO), Cairo, Egypt), Chitosan, (CS); low M.Wt grade (Viscosity 100 cp, 98% degree of deacetylation) (Industrial Manufacturing Co., Japan), Hydroxyethylcellulose, (HEC); (Kolmar Company, California, USA), Sodium carboxy-methylcellulose, (NaCMC); (The General Chemical and Pharmaceutical Co., Ltd., England), Hydroxypropyl methylcllulose, (HPMC); (Kolmar Company, California,

USA), Pluronic F127, (PF-127); (Sigma Chem. Co, USA), Gelrite, (Gl); (Merck Sharp and Dohme Co., Germany), Procine stomach mucin (Sigma Aldrich Chem., Germany), semi-permeable cellophane membrane (No30/32, Fischer Sci. Co., London, England) and commercial eye drops under the name of Timolol Maleate USP 0.25% produced by Egyptian International for Pharmaceutical Industries Company (EIPICO) batch no. 1234567 Exp. Date 3/2013. All chemicals used were of pharmaceutical grade and were used as delivered.

Equipment

IR-Spectrophotometer, IR-470 (Schimadzu, Japan), Differential Scanning Calorimeter, DSC - 50 (Shcimadzu, Japan). Brookfield DV - III Ultra viscometer (RV model, USA), Water bath shaker (Gselschaft fűr, labor tachnik m.b.h. & CO, Germany), Double beam spectro-photometer (Schimadzu, UV - 150 - 02 Seisakusho, Ltd., Kyoto, Japan), Standardized Tonometer (Shiøtz, Germany) and Universal Oscillograph Cat. No.: 50 - 8622, Harvard Apparatus Limited, USA.

Methodology

I- Physico-chemical compatability studies

The IR & DSC studies were performed by comparing both IR spectra and DSC thermograms of TM alone and its physical mixtures (1:1 w/w) with the polymers selected in this study viz., CS, HEC, NaCMC, HPMC, Pl F-127 and gelrite.

A- Infra-red (IR) studies

IR spectroscopy was carried out at the range of 4000–800 cm^{-1} using KBr disc method. About 2 mg of the sample was mixed thoroughly with KBr (IR-grade) and compressed at a pressure of 6 tone/cm^2 using Schimadzu ssp–10A compression machine with an empty holder as a reference.

B- Differential scanning calorimetry (DSC)

The thermograms of samples were performed using Schimadzu medel DSC – 50. The instrument was calibrated with pure indium. The thermograms were obtained by heating 5 mg of the sample encapsulated in flat bottom pan at a scanning rate 10°C/min. from 30°C – 250°C under nitrogen gas stream at a flow rate of 40 ml/min. An empty pan was used as a reference and subjected to the same conditions. Transition temperature (°C) and heats of fusion of melting endotherms on the thermograms obtained were calculated using the DSC–T50 program, which directly integrates the melting endothermic and calculate the heat of fusion (ΔH, joule/g).

II- Preparation of TM formulations

TM viscous solutions and hydrogels

Hydrophilic polymers viz; CS, HEC, HPMC and NaCMC were selected to prepare TM viscous solutions (F1-F12) each was prepared at 1.5, 2 and 2.5% w/w viscolazer concentrations. The same polymers, except CS, were used to prepare TM hydrogels (F13-F21) but at higher concentrations. All formulae were prepared by dissolving the specified amount of the tested polymer in an isotonic phosphate buffer pH 7.4.

TM - Pl F-127 *in-situ* gels

In-situ gels of Pl F-127 with or without mucoadhesives (F22-F30) were prepared by dissolving the specified amount of the tested polymer in an isotonic phosphate buffer pH 7.4 containing 0.25% of TM during agitation. The solutions were kept in a refrigerator to enhance the dissolution[8&9]. *In-situ* gels of 20%w/w Pl F-127 with mucoadhesive polymers such as HEC or CS each at concentration of 1.5, 2 and 2.5% w/w were similarly prepared.

TM gelrite *in-situ* gel

Gelrite was dispersed in demineralized water. The solutions of gelrite (F31-F33) at different concentrations (0.6, 0.8 and 1% w/w) were prepared by heating the dispersions to 90°C for 20 min. while stirring. The solutions were allowed to cool at room temperature during stirring[10&11]. TM (0.25% w/w) was added to the gelrite solution and allowed to be dissolved. The compositions of all tested formulae are listed in table 1.

Table 1: Composition of different TM ophthalmic polymeric formulations.

Formulation	Ingredient (% w/w)						
	CS	HEC	NaCMC	HPMC	Pl F-127	Gelrite	Isotonic Phosphate Buffer pH 7.4
F1	1.5	-	-	-	-	-	98.25
F2	2	-	-	-	-	-	97.75
F3	2.5	-	-	-	-	-	97.25
F4	-	1.5	-	-	-	-	98.25
F5	-	2	-	-	-	-	97.75
F6	-	2.5	-	-	-	-	97.25
F7	-	-	1.5	-	-	-	98.25
F8	-	-	2	-	-	-	97.75
F9	-	-	2.5	-	-	-	97.25
F10	-	-	-	1.5	-	-	98.25
F11	-	-	-	2	-	-	97.75
F12	-	-	-	2.5	-	-	97.25
F13	-	5	-	-	-	-	94.75
F14	-	6	-	-	-	-	93.75
F15	-	7	-	-	-	-	92.75
F16	-	-	5	-	-	-	94.75
F17	-	-	6	-	-	-	93.75
F18	-	-	7	-	-	-	92.75
F19	-	-	-	3	-	-	96.75
F20	-	-	-	4	-	-	95.75
F21	-	-	-	5	-	-	94.75
F22	-	-	-	-	20	-	79.75
F23	-	-	-	-	25	-	74.75
F24	-	-	-	-	30	-	69.75
F25	1.5	-	-	-	20	-	78.25
F26	2	-	-	-	20	-	77.75
F27	2.5	-	-	-	20	-	77.25
F28	-	1.5	-	-	20	-	78.25
F29	-	2	-	-	20	-	77.75
F30	-	2.5	-	-	20	-	77.25
F31	-	-	-	-	-	0.6	99.15
F32	-	-	-	-	-	0.8	98.95
F33	-	-	-	-	-	1	98.75

The concentration of TM in all tested formulations was 0.25%.

III- Assessment of physical properties of TM ophthalmic polymeric formulations

1- Viscosity measurements

The viscosity of the tested formula was determined using Brookfield DV-III Ultra viscometer (RV model). The spindle used was no. 06 for viscous solutions and no. 95 for gels. The measurement of viscosity was carried out at spindle speed of 15 rpm. Each experiment was carried out in triplicate and the mean values were calculated.

2- Bioadhesive force of polymer gels

The mucoadhesive strength of the gel was determined by measuring the force required to detach the formulation from a mucin disc using our locally assymbelled device (Fig. 1) which is a modification of the reported methods[9&15]. The mucin disc (prepared by compression of 200 mg crude procin mucin at 2 tones using Hydraulic press) was horizontally glued to the upper stage of the modified device. The mucin disc was hydrated with few drops of isotonic phosphate buffer pH 7.4 prior to test. One gram

of the tested formula was placed on the lower movable stage of the balance which elevated till the surface of the sample become in contact with the hydrated mucin disc using a preload of 10 grams for 5 minutes to establish a perfect contact and formation of an adhesive bond. After completion of the preload time, water was allowed to drip (1 drop/sec) from a burette through an infusion tube into a preweighed plastic jar. When the mucin disc was detached from the tested sample, the drip of water was stopped. The volume of dripped water plus the weight of empty bag were considered as the weight required for detachment and taken as a measure of bioadhesion strength. Each experiment was carried out in triplicate and the mean values were calculated. The detachment force was determined using the following equation:

Detachment Force (dyne/cm^2**)** = m × (g)/A

Where;

m: is the weight of water in grams required for detachment.

g: is the acceleration gravity taken as 980 cm/sec^2.

A: is the surface area of mucin disc (area of the contact) which is equal to лr^2 (r is the radius of mucin disc).

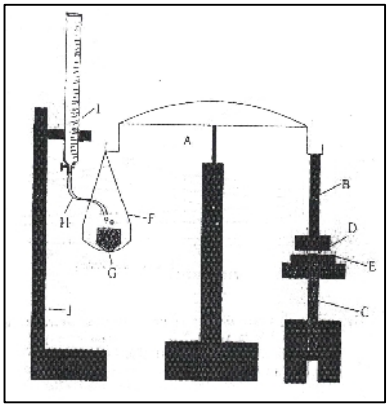

Fig. 1: Modified device for bioadhesion test.
(A) modified balance; (B) upper stage; (C) lower stage; (D) mucin disc; (E) gel preparation; (F) balance pan; (G) plastic jar; (H) IV infusion tube; (I) burette and (J) stand holder.

3- Sol-gel transition temperature (GT)

The sol-gel phase transition temperature (Gelation temperature) for all the prepared TM-Pl F-127 *in-situ* gels with or without mucoadhesive polymers viz; CS or HEC was measured by transferring 2 ml of refrigerated sample to a test tube sealed with a parafilm. The tube was placed in a thermostatically controlled water bath operated at 4°C. The temperature was raised gradually in increments of 3°C in the beginning of the experiment and then 1°C increments in the region of sol-gel transition temperature (25-34°C). The tested formulation was left to equilibrate for 10 minutes at each new setting. The maximum accepted gelation temperature was 34°C which represents the corneal surface temperature[16]. Each experiment was carried out in triplicate and the mean values were calculated.

IV- *In-vitro* release of TM

The release of TM from the tested formula was determined using cellophane membrane dialysis technique[4,7&11]. An accurately weighed 1 g of the tested formula was placed over a pre-soaked cellophane membrane. The loaded cellophane membrane was fixed to the lower end of the donor tube. The donor tube was placed in a beaker containing 100 ml of isotonic phosphate buffer pH 7.4 in such a way that the lower end of the tube containing the tested formula just below the surface of the diffusion media. The whole assembly was maintained at 37°C in a thermostatically controlled shaking water bath and was allowed to shake at 25 stroke/min.. An aliquot of 2 ml was withdrawn at a specified time intervals, diluted to 5 ml with isotonic phosphate buffer and replaced with an equal volume of fresh release medium. The samples were analyzed for TM spectrophotometrically at 295 nm (practically determined) using isotonic phosphate buffer as a blank. Each experiment was carried out in triplicate and the mean values were taken. The obtained release data were subjected to mathematical treatment accodrding to different kinetic models viz; zero and first order, Higuchi diffusion model[17] as well as Korsmeyer equation[18] and the highest regression coefficient was used to determine the release mechanism of TM from the tested formulae.

Higuchi diffusion equation

$$(M_t/M_\infty)^2 = K_H \cdot t$$

Where,

(M_t/M_∞)= the fractional amount of drug released at time t,

K_H= Higuchi constant and t= time in hours.

Korsmeyer-Peppas equation

$$M_t/M_\infty = kt^n$$

Where, M_t/M_∞ is the fractional amount of the drug released, at time t, k is the release rate constant and n is the diffusional exponent that characterizes the type of release mechanism. The values of n and k were estimated by linear regression of log (M_t/M_∞) versus log t.

V- *In-vivo* study

Five TM formulations were selected in this study. The selection was based on the physical properties regarding viscosity, bioadhesive strength and acceptable prolonged release. The formulations were tested for their IOP lowering activity and systemic side effects on BP, HR and RR of rabbits and compared with TM marketed eye drops.

Adult albino rabbits weighing 1.5-2 kg were used in the experiments. The rabbits were housed in a room with a controlled temperature of 25˚C, with a 12 hrs-light/12 hrs-dark cycle and free access to food and water[19]. The rabbits were divided into six groups each containing three rabbits for each formulation as follows:

Group I, received the commercial TM eye drops.

Group II, received the selected TM ophthalmic viscous solution F1.

Group III, received the selected TM ophthalmic *in-situ* gel F22.

Group IV, received the selected TM ophthalmic *in-situ* gel F25.

Group V, received the selected TM ophthalmic *in-situ* gel F31.

A single 50 µl/dose (equivalent to 125 µg/µl) of 0.25% TM preparations was instilled onto the corneal surface of the rabbit right eye. The contralateral eye (left eye) received an equivalent amount of isotonic phosphate buffer pH 7.4 and was used as control. Each animal was given a wash out of three days after each treatment.

1- Mean intra-ocular pressure

The IOP lowering activity of normalized rabbits was measured as a function of time using a standardized tonometer[19]. IOP was measured first immediately before drug instillation ($IOP_{zero\ time}$) then after 0.5 hour and every one hour intervals for 6 hours (IOP_{time}) following instillation. The ocular hypotensive activity is expressed as a change in IOP (Δ IOP) as follows[20]:

$$\Delta\ IOP = IOP_{\ 0} - IOP_{\ t}$$

2- Mean blood pressure, heart rate and respiration rate

Each animal was anaesthetized with intrapretoneal injection of urethane solution (25% w/v) in a dose of 6.4 mg/kg. After shaving the neck and canulating the trachea with polyethylene tube, the animal was ventilated with air room. Then it was prepared for I.V. injection of heparine saline (1000 U/kg) through a cannula placed in the right jugular vein. During the experiment the body temperature was maintained at 37°C. The BP and HR were measured after anesthesia from the canulated left common carotid artery which was canulated by a special cannula attached to blood pressure transductor and an amplifier of four channel oscillograph which is connected to two channel recorder. The BP, HR and RR were measured before and after instillation at 0.5, 1, 2, 3, 4, 5 and 6 hrs.

RESULTS AND DISCUSSION

I- Physico-chemical compatability studies

The compatibility of timolol maleate with the polymers selected in this study viz., CS, HEC, NaCMC, HPMC, Pl F-127 and gelrite was investigated by comparing both IR spectra and DSC thermograms of TM alone and its physical mixtures with the polymers (1:1 w/w).

A- IR spectra

Figure 2 shows the IR spectra of TM and TM/polymer physical mixture (1:1). Trace A, is the IR spectra of TM. In the N-H and O-H regions, TM showed a broad intense band extended from 3300 to 3025 cm^{-1}, which was due to the overlapped N-H and O-H vibrations. Three bands at 1693, 1223 and 1113 cm^{-1} corresponding to stretching C=N, C-OH and C-O-C, respectively. This is identical to the reported data[21]. Traces B-G represent the IR spectra of (1:1 w/w) physical mixtures of TM with the investigated polymers which gave the same characteristic stretching bands of TM without appearance of new peaks, dis-appearance and/or shift in the frequency of the bands. IR results revealed that TM is compatible with all the tested polymers.

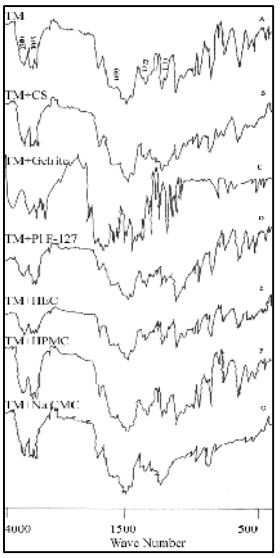

Fig. 2: IR spectra of: (A) TM and (B-G) TM/polymer physical mixture (1:1).

B- DSC thermograms

Figure 3 and table 2 shows the DSC thermograms of TM and TM/polymer physical mixture (1:1). Trace A, the thermogram of TM alone which showed an endothermic peak at 206°C. An additional endotherm with a peak temperature at 215°C is noted corresponding to compound decomposition. This is identical to the reported data[21].

Traces B-G represent the DSC thermograms of (1:1 w/w) physical mixture of TM with each of the investigated polymers. There was no appearance of new peaks, disappearance and/or shift in the characteristic endothermic or exothermic peaks of the drug. DSC studies proved that TM is compatible with all the investigated polymers.

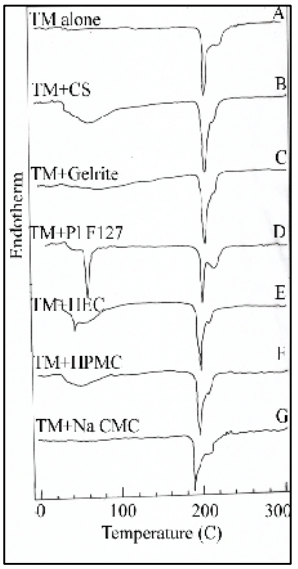

Fig. 3: DSC curves of thermograms: (A) TM and (B-G) TM/polymer physical mixture (1:1).

II- Physical properties of TM ophthalmic formulations

1- Viscosity measurements

The results of viscosity measurements of TM formulations (F1–F31) are presented in table 2. CS, HEC, NaCMC and HPMC at concentrations of 1.5, 2 and 2.5% w/w were used to increase the viscosity of TM solution (F1-F12). It is clear that addition of these polymers to 0.25% w/v TM solution resulted in an increase in the solution viscosity.

The viscosity of TM hydrogels containing the same cellulosic polymers used but at higher concentrations (F13-F21) are presented in table 2, and revealed that polymer type and its concentration affect the formulation viscosity.

With respect to *in-situ* gels, Pl F-127 gels (F22-F24) the viscosity was increased from 40 to 50×10^3 cp by increasing the concentration from 20 to 30% w/w. This can be explained by the fact that gel formation is a result of micellar entanglement at higher Pl F-127 concentration. As a result of these micelle entanglements, they can not separate easily from each other, which accounts for the rigidity and high viscosity of gel containing high concentration of Pl F-127[22].

The effect of addition of mucoadhesives such as CS (F25-F27) or HEC (F28-F30) on viscosity of Pl F-127 *in-situ* gel was studied. The results showed that the viscosities of formulae F25-F27 were ranging from 41.7 to 46.9×10^3 cp while the viscosity of formulae F28-F30 were 46.4 to 51.1×10^3 cp. It is clear that, the viscosity of *in-situ* gel F22 (containing 20% w/w Pl F-127) was 39 51.1×10^3 cp which is lower than in the presence of mucoadhesives. The viscosity increased as the concentration of the mucoadhesive polymer increased. This may be attributed to the fact that Pl F-127 thermosensitive gels are thought to be formed by hydrogen bonding in aqueous system, caused by the attraction of polymer ether oxygen atom with proton of water. The number of H-bonding is expected to increase by adding compounds with hydroxyl groups, thus leading to increasing the measured viscosity of the prepared formulation[9].

2- Bioadhesive force

Ocular mucoadhesion relies on the interaction of a polymer and mucin surface of the eye. The force of this interaction is taken as an important physico-chemical parameter for

ophthalmic gels, since it prevents the rapid drainage of the formulation and hence lengthens its precorneal residence time. In this investigation, table 2 shows that the bio-adhesive force of cellulosic hydrogels varied with polymer structure and concentration. Increasing polymer concentration increased the mucoadhesive force of the preparation. This can be explained by the increased sites of bond formation and the possibility for interactions with mucus membranes[9&15]. The rank order of the polymer bioadhesive force at 5%w/w polymer concentration was:
HEC > HPMC > NaCMC.

The cellulosic polymers, NaCMC and HPMC with neutral cellulose groups could bind weakly to oligosaccharide. Where-in, HEC is characterized by high bioadhesive forces (27.6-48.9×10^{-3} dyne/cm^2) due to high viscosity and molecular weight of the polymer. High molecular weight is important to maximize adhesion through entanglments and Van der Waal forces[9].

Pl F-127 *in-situ* gels (F22-F24) exhibited moderate adhesive properties (39-50.5×10^3 dyne/cm^2) that increased with increasing concentration from 20 to 30% w/w. This effect is attributed to the binding of hydrophilic oxide group to oligosaccharides chains[8&9].

Incorporation of the bioadhesive polymers CS (F25-F27) and HEC (F28-F30) to Pl F-127 *in-situ* gel enhanced the mucoadhesive ability of the *in-situ* gel under physiological conditions. This is based on the thermo-sensitive *in-situ* gelling property of Pl F-127 and the mucoadhesive force property of CS or HEC (Table 2). Also increasing the concentration of the bioadhesive polymer from 1.5 to 2.5% w/w in the formulation increases the bioadhesive effect from 41.7 to 46.9×10^3 dyne/cm^2 for CS and from 46.4 to 51.1×10^3 dyne/cm^2 for HEC. However, Felt *et al.*[13] stated that, a concentration as low as 0.5% of CS is sufficient to ensure an enhancement of the residence time of ophthalmic preparations. The effect of combining a bioadhesive polymer to Pl F-127 was reported aiming to improve drug bioavailability such as TM[8], Ciprofloxacin hydrochloride[9] and Puerarin[23].

Concerning gelrite *in-situ* gel (F31-F33), table 2 revealed that, the mucodhesive properties increased from 6.2–7.8×10^3 dyne/cm^2 with increasing the concentration of gelrite from 0.6 to 1% w/w. A result explained on the basis that, gelrite is a polysaccharide, rich in hydroxyl groups which are considered as the principle sources of mucoadhesion[10&11].

Table 2: Physical Properties of TM ophthalmic polymeric formulations.

Formula Code	Viscosity ×10^{-3} (cp) ± SD	Bioadhesive force×10^{-3} (dyne/Cm^2) ± SD	Gelation Temperature (^oC) ± SD
F1	0.05 ± 3.3	-	-
F2	0.10 ± 1.2	-	-
F3	0.13 ± 1.8	-	-
F4	0.60 ± 0.5	-	-
F5	1.13 ± 1.5	-	-
F6	1.67 ± 2.0	-	-
F7	1.20 ± 2.2	-	-
F8	4.00 ± 2.0	-	-
F9	13.3 ± 1.8	-	-
F10	0.80 ± 3.0	-	-
F11	1.40 ± 1.5	-	-
F12	3.10 ± 0.6	-	-
F13	30.0 ± 0.0	27.6 ± 0.7	-
F14	40.0 ± 2.4	30.7 ± 0.4	-
F15	49.0 ± 1.4	48.9 ± 0.04	-
F16	275 ± 3.0	27.1 ± 0.7	-
F17	300 ± 0.3	28.6 ± 0.4	-
F18	512 ± 0.0	39.0 ± 2.5	-
F19	24.0 ± 7.0	26.0 ± 3.7	-
F20	125 ± 3.6	27.0 ± 2.5	-
F21	265 ± 5.5	28.6 ± 0.4	-
F22	40.0 ± 0.3	39.0 ± 1.5	31.5 ± 0.0
F23	46.0 ± 1.8	42.6 ± 1.1	31 ± 0.2
F24	55.0 ± 0.8	50.5 ± 1.2	31 ± 0.1
F25	40.0 ± 0.0	41.7 ± 0.4	29 ± 0.0
F26	43.0 ± 0.0	44.8 ± 0.0	27 ± 0.0
F27	45.5 ± 0.9	46.9 ± 0.9	26 ± 0.3
F28	87.0 ± 0.8	46.4 ± 0.5	20 ± 0.3
F29	97.0 ± 2.5	49.0 ± 3.1	19 ± 0.2
F30	100 ± 1.0	51.1 ± 4.6	19 ± 0.1
F31	8.3 ± 0.0	6.2 ± 0.3	-
F32	12.5 ± 0.2	6.8 ± 0.8	-
F33	16.0 ± 1.2	7.8 ± 0.5	-

3- Sol-gel transition temperature (GT)

The sol-gel transition temperature of ophthalmic thermosensitive gel have been considered to be suitable for ocular delivery if they were in the range of 25-34°C. If the GT of the formulation is lower than 25°C, a gel may be formed at room temperature and if the gelation is higher than 34°C, a liquid dosage form still exists at corneal surface temperature,

resulting in the drainage of the formula from the eyes. Poloxamer solutions are known to exhibit thermoreversible gelation depending on the polymer content and other included components[9].

Results in table 2 reveal that TM *in-situ* forming gel using Pl F-127 at concentrations of 20, 25 and 30% (F22-F24) were transferred to gel between 31-31.5°C which are considered to be suitable for ophthalmic application.

TM *in-situ* gel F22 (containing Pl F-127 20%) was selected to study the effect of addition of mucoadhesive polymers CS and HEC on the gelation temperature. It is clear from table 2 that, the addition of these polymers resulted in lowering the gelation temperature of the *in-situ* gel. Also, increasing bioadhesive polymer concentration from 1.5 to 2.5% w/w produce a decrease in transition temperature. The reducing effect by using HEC (19–20°C) is greater than CS (26–29°C). The gelation lowering effect of mucoadhesives of such mucoadhesive polymers is explained by their ability to bind to polyoxyehylene chain present in the poloxamer molcules. This will promote dehydration, causing an increase in entanglement of adjacent molecules and extensively increasing intermolecular hydrogen bonding which will lead to gelation at low temperature. Similar results were reported by Abd El-Hady *et al.*[24] and Mansour *et al.*[9].

III- *In-vitro* release of TM

The *in-vitro* release profiles of TM from viscous solutions (F1-F12) showed a decrease in drug release rate with increasing the polymer concentration which is attributed to the increase in the formulation viscosity. At any concentration, the drug release can be ranked in the order: HEC> HPMC> NaCMC> CS(Fig.4).

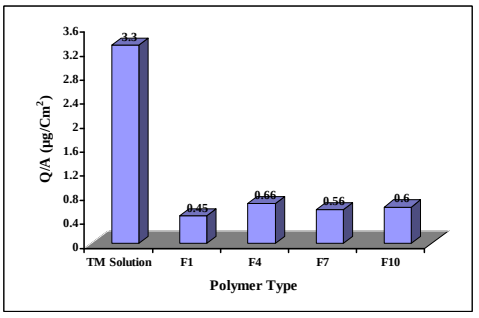

Fig. 4: Effect of polymer type on *in-vitro* release of TM from viscous solutions containing 1.5% w/w of each polymer into isotonic phosphate buffer pH 7.4 through a standard cellophane membrane.

The *in-vitro* release profiles of TM from the hydrogel formulations (F13–F21) are shown in figures 5&6 and the cumulative amount of drug released (Q/A µg/cm^2) after 4 hrs are shown in table 3. It is clear from the results that, the drug release from all formulations was much slower than that from aqueous solution of TM. The drug release characteristics are mainly dependent on the polymer type and concentration.

Generally, an increase in polymer concentration results in decreasing the amount of drug released. This may be explained by the increased tortousity of the polymer matrix which retards diffusion of the drug molecules through the polymer network by decreasing diffusion pathway. With respect to the release of TM from ophthalmic gels containing NaCMC, drug diffusion is promoted as the viscosity is decreased. Where, HPMC with its neutral groups has a weak binding force as compared to other polymers. So, the rate of drug release is controlled by polymer swelling, followed by drug diffusion through the swelled polymer[15]**.** The drug release was found to be correlated to viscosity and bioadhesive force.

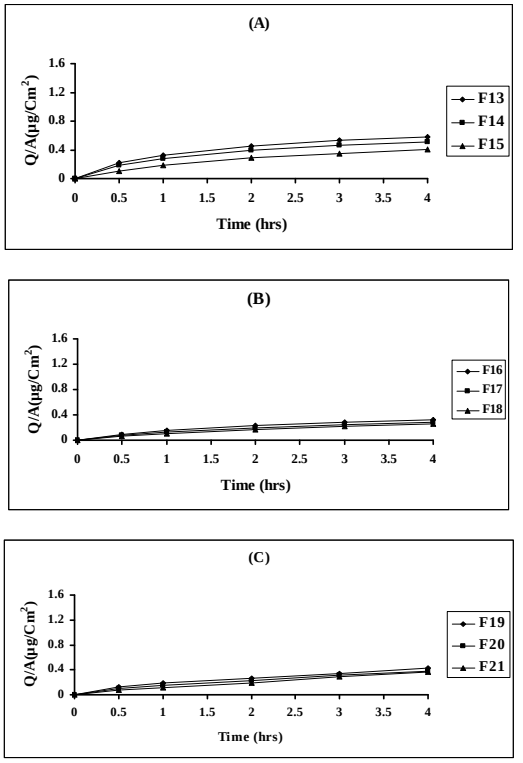

Fig. 5: The effect of different concentrations of (A) HEC, (B) NaCMC and (C) HPMC on *in-vitro* release of TM from hydrogels into isotonic phosphate buffer pH 7.4 through a standard cellophane membrane.

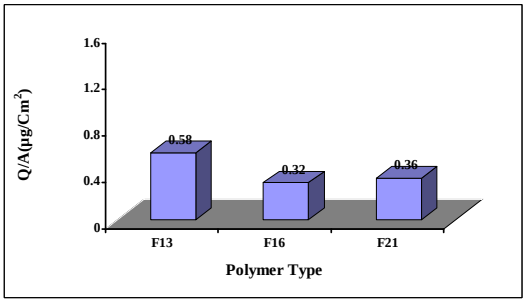

Fig. 6: Effect of polymer type on *in-vitro* release of TM from hydrogels containing 5% w/w of each polymer into isotonic phosphate buffer pH 7.4 through a standard cellophane membrane.

In-vitro release data of TM from Pl F-127 *in-situ* gel formulations (F22-F25) are shown in table 3 and figure 7. It is obvious that, as the polymer concentration increased from 20 to 30% w/w, the amount of the drug released was decreased, indicating that, the structure of the gel functioned as an increasingly resistant barrier to drug release as the concentration of the polymer increased. The mechanism for such enhanced resistance may be due to reduction in the number and dimension of water channels and to increase in number and size of micelles within the gel structure. This leads to greater numbers of cross-links between neighboring micelles resulting in higher viscosity and lower rate of drug release.

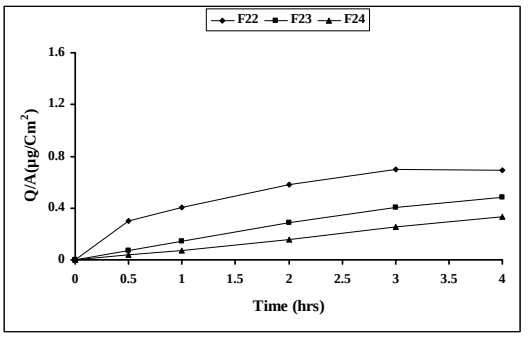

Fig. 7: The effect of different concentrations of Pl F-127 on *in-vitro* release of TM from *in-situ* gels into isotonic phosphate buffer pH 7.4 through a standard cellophane membrane.

The drug release from *in-situ* gel containing 20% w/w Pl F-127 and 1.5% CS or HEC (F25 & F28) were compared with that from the formula containing 20% w/w Pl F-127 alone (F22). It is obvious that the release of TM was reduced upon addition of bioadhesive polymers CS or HEC (Fig. 8). The retarding effect increased by increasing mucoadhesive polymer concentration, a result attributed to their ability to distort or squeeze the extra micellar aqueous channels of Pl F-127 through which the drug diffuses thereby delaying the release process. This is in good agreement with other investigators[8,9,22&25].

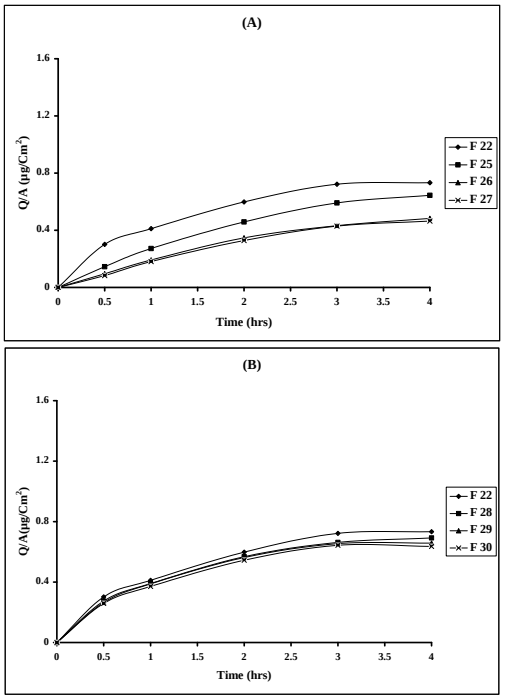

Fig. 8: Effect of addition of different of (A) CS and (B) HEC on the release of TM from 20% w/w ophthalmic Pl F-127 *in-situ* gel into isotonic phosphate buffer pH 7.4 through a standard cellophane membrane.

Concerning the release of TM from ion-activated *in-situ* gel of gelrite, it is evident from table 3 and figure 9 that increasing gelrite concentration from 0.6 to 1% w/w tends to decrease the drug release. This may be attributed to increased viscosity by increasing polymer concentration.

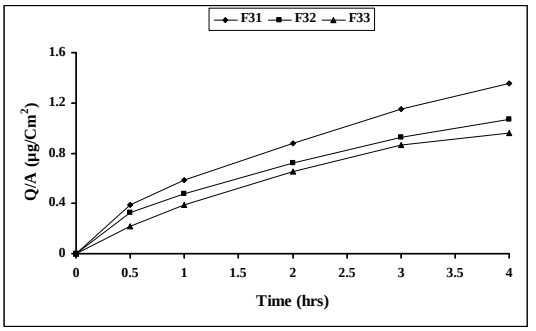

Fig. 9: Effect of different concentrations of gelrite on *in-vitro* release of TM from *in-situ* gels into isotonic phosphate buffer pH 7.4 through a standard cellophane membrane.

Kinentic release data of TM formulations have shown that by trying the three different models, the highest regression coefficient (r) was obtained with Higuchi diffusion model (r= 0.9055 to 0.9995) as shown in table 3. The release data were analyzed using the equation proposed by Korsmeyer *et al.*[18]. The results of this fitting are presented in table 3. The values of release exponents, however, indicate that the release mechanism changed with the type of the polymer used. For different formulations, the *n* values increased from 0.002 to 0.358 which indicates non-fickian diffusion for drug release.

Table 3: Release characteristics of TM from different ophthalmic polymeric formulations.

Formula Code	Q/A (µg/Cm^2) (after 4 hrs) ± SD	Linear Regression Analysis Using Correlation Coefficient (r) According to					
		Zero Order		First Order		Korsmeyer Model	
		r	K	R	K	R	Slope (n)
TM Soln.	3.30 ± 0.00	0.7434	0.084	0.5561	1.022	0.9790	2.30
F1	0.45±0.00	0.8628	0.096	0.5559	1.022	0.9055	0.216
F2	0.44±0.05	0.8621	0.082	0.5560	1.022	0.9689	0.228
F3	0.36±0.00	0.9316	0.150	0.5556	1.023	0.9652	0.193
F4	0.66±0.02	0.8891	0.134	0.5558	1.023	0.9942	0.338
F5	0.65±0.01	0.9259	0.138	0.5557	1.023	0.9789	0.312
F6	0.59±0.00	0.9081	0.116	0.5558	1.023	0.9921	0.311
F7	0.56±0.00	0.9213	0.115	0.5557	1.023	0.9612	0.260
F8	0.44±0.05	0.9368	0.099	0.5558	1.022	0.9545	0.252
F9	0.39±0.03	0.9429	0.134	0.5557	1.023	0.9590	0.214
F10	0.60±0.00	0.9816	0.080	0.5559	1.022	0.9938	0.298
F11	0.48±0.00	0.9805	0.067	0.5559	1.022	0.9887	0.171
F12	0.45±0.10	0.7434	0.084	0.5561	1.022	0.9918	0.140
F13	0.58±0.01	0.9157	0.148	0.5557	1.023	0.9927	0.338
F14	0.51±0.00	0.9370	0.117	0.5558	1.023	0.9963	0.262
F15	0.41±0.00	0.9694	0.096	0.5558	1.022	0.9963	0.209
F16	0.32±0.043	0.9819	0.106	0.5557	1.023	0.9941	0.002
F17	0.28±0.01	0.9857	0.097	0.5558	1.022	0.9886	0.203
F18	0.26±0.00	0.9855	0.065	0.5560	1.022	0.9904	0.136
F19	0.43±0.00	0.9289	0.131	0.5557	1.023	0.9966	0.297
F20	0.38±0.03	0.9574	0.108	0.5558	1.023	0.9995	0.237
F21	0.36±0.05	0.9687	0.092	0.5558	1.022	0.9969	0.188
F22	0.73±0.10	0.9113	0.160	0.5556	0.677	0.9886	0.365
F23	0.49±0.09	0.9700	0.124	0.5556	0.678	0.9947	0.256
F24	0.34±0.00	0.9464	0.085	0.5558	0.678	0.9991	0.169
F25	0.64±0.00	0.9157	0.148	0.5557	1.023	0.9947	0.358
F26	0.48±0.00	0.9370	0.117	0.5558	1.023	0.9984	0.282
F27	0.46±0.06	0.9694	0.096	0.5558	1.022	0.9963	0.209
F28	0.69±0.04	0.9819	0.106	0.5557	1.023	0.9811	0.203
F29	0.66±0.04	0.9857	0.097	0.5558	1.022	0.9898	0.209
F30	0.64±0.00	0.9855	0.065	0.5560	1.022	0.9904	0.136
F31	1.35±0.05	0.9289	0.131	0.5557	1.023	0.9856	0.300
F32	1.07±0.05	0.9574	0.108	0.5558	1.023	0.9995	0.237
F33	0.96±0.01	0.9687	0.092	0.5558	1.022	0.9979	0.189

IV- *In-vivo* study

The pharmacological effects of TM were evaluated by measuring the IOP lowering and systemic effects on BP, HR and RR.

Tables 4-6 and figure 10 show the time course of IOP lowering (mm Hg) in normotensive rabbits after a single dose instillation of each selected formulation and compared with TM marketed eye drops. The data were statistically interpreted in terms of area above the IOP lowering/time curve, duration of action, maximum response and time of maximum response which have been taken in consideration as parameters of drug activity (Table 5). Two-way ANOVA system was used to analyze the data between all pairs of rabbit groups.

From the results (table 4), it is obvious that the IOP lowering activity of marketed TM eye drops reached a maximum value of not more than 3.4 mm Hg after 1 hr of instillation. This effect was found to be markedly decreased and abolished completely during the time of experiment due to rapid elimination of eye drops by lachrymal flow and reflex tearing turnover. The effect of the prepared formulations was more significant and sustained for a period ranging from 0.5 to 6 hrs with different extent.

The maximum IOP lowering was observed among rabbits of group IV receiving the thermosensitive *in-situ*/mucoadhesive gel based on Pl F-127/CS (F25). The IOP lowering reached a value of 11 mm Hg after 3 hrs of administration and was sustained during the time of the experiment (6 hrs) and had the largest area above the intraocular pressure/ time curve (32.38 mm Hg.hr) as compared to other formulations (Tables 4&5).

Table 4: Effect of topically applied Timolol maleate polymeric formulations on the change in intraocular pressure in normotensive rabbits.

Time (hrs)	Δ IOP After Topical Application of TM (mm Hg) ± SD				
	Commercial Eye Drops	CS Viscous Solution	*In-situ* gel Pl F-127 (20% w/w)	*In-situ* gel (Pl F127 20% + CS 1.5%)	*In-situ* gel (gelrite 0.6% w/w)
0	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
0.5	0.0 ± 0.00	0.0 ± 0.00	3.4 ± 0.04	1.8 ± 0.00	3.4 ± 0.03
1	3.4 ± 0.30	1.8 ± 0.01	6.3 ± 0.07	4.9 ± 0.00	8.8 ± 1.20
2	3.4 ± 0.03	4.9 ± 0.05	8.8 ± 1.00	7.6 ± 0.03	6.3 ± 0.09
3	1.8 ± 0.01	3.4 ± 0.00	6.3 ± 0.00	11 ± 0.04	3.4 ± 0.09
4	0.0 ± 0.00	0.0 ± 0.00	1.8 ± 0.00	4.9 ± 0.03	3.4 ± 0.01
5	00. ± 0.00	0.0 ± 0.00	0.0 ± 0.00	3.4 ± 0.20	1.8 ± 0.00
6	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	1.8 ± 0.50	1.8 ± 0.30

Table 5: Values for the duration of action, area above intraocular pressure/time curve, maximum response and time of maximum response of TM in different polymeric formulations.

Formulations	Parameters of Activity			
	Duration of Action (hr.)	Area Above the Curve (mm Hg hr.)	Maximum Response (mm Hg hr.)	Time of maximum response (hr.)
Commercial Eye Drops	4	7.75	3.4 ± 0.30	1
CS Viscous Solution	4	9.65	4.9 ± 0.05	2
In-situ gel Pl F-127 (20% w/w)	5	23.33	8.8 ± 1.00	2
In-situ gel (Pl F127 20% + CS 1.5%)	6	32.38	11 ± 0.04	3
In-situ gel (gelrite 0.6% w/w)	6	24.10	8.8 ± 1.2	1

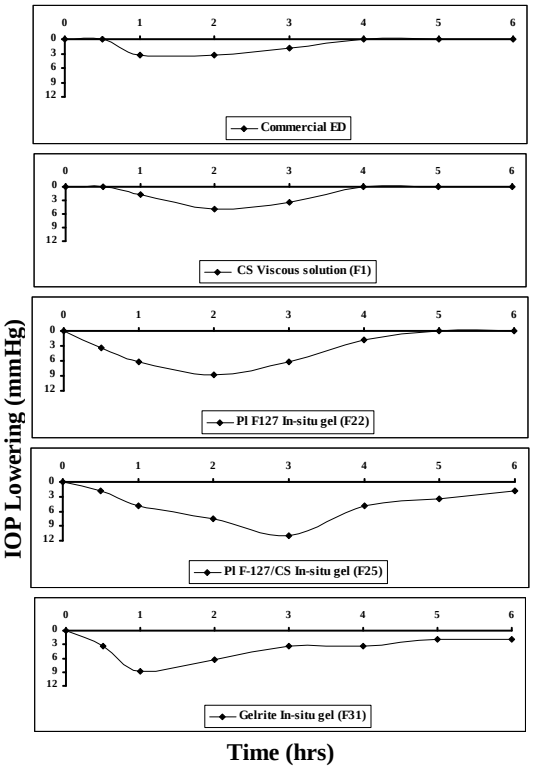

Fig. 10: IOP lowering (mmHg) in rabbit tested with 0.25% TM in solution and in different selected formulations.

It is noted that, the combined Pl F-127 and CS *in-situ*/mucoadhesive gel solution (F25) performed better in lowering and prolonging the IOP than other individual formulations did (F1 containing CS 1.5% w/w and F22 containing Pl F-127 20% w/w). Aggarwal and Kaur[26], developed TM niosomes coated with CS with an improved pharmaco-dynamics extended over a prolonged period and a limited systemic absorption compared to marketed formulations.This could be explained on the basis that, after instillation, the combined themosensitive *in-situ*/ mucoadhesive solution could insure in one hand, suitable gel strength, prevent rapid precorneal elimination and on the other hand attach to the ocular mucosal surface. Thus, prolonging contact time with the cornea, increases the bioavailability and reduce the systemic side effects. The prolonged precorneal residence time of ophthalmic formulations containing CS was attempted[12-14] and explained by the interaction between its positive charges with the negative charge of sialic acid residue of the mucus. Felt *et al.*[13] reported that, at least a three-fold increase of the corneal residence time is achieved in presence of CS when compared to conventional eye drops.

Gelrite solution, a novel ophthalmic vehicle, can be used to ocular mucosa as low viscosity salt free solution once in contact with mono or divalent cations of tear fluid, forms clear gel at the ocular surface. Group V receiving ion activated *in-situ* gel of 0.6% w/w gelrite solution (F31) exhibited maximum IOP lowering activity of 8.8 mm Hg after 1 hr and extended for 6 hrs. This increase in biovailability is due to the unique rapid ion-activated gelling property of gelrite in the presence of cations of tear fluid. Thus, the formulation resists the natural elimination process from the precorneal area, their residence time is prolonged and the amount of drug absorbed increased[27]. This result is emphasized by Carlfors *et al.*[28] who reported that, using hypotonic solution of gelrite, the gel formed remains in the human eye for 20 hrs. This is due to rapid sol-gel transition controlled by the osmolality of solution resulting in longer precorneal residence time super to those of other gels[28].

It is to be noted by reviewing the data in tables 4-6 that the *in-vitro* results are consistent with those of the IOP lowering effect of the formulations. Where, TM *in-situ*/mucoadhesive gel (F25) and gelrite *in-situ* gel (F31) with their prolonged drug release and enhanced mucoadhesive force showed the greater maximum IOP lowering activity and greater area above the curve compared to other formulations.

It is well known that, ocularly administered TM may cause severe cardiovascular and respiratory side effects. In this investigation, the systemic side effects including hypotension, bradycardia and bronchospasm of selected TM formulations before and after single dose instillation to rabbit eye were estimated and compared to marketed TM eye drops. The data in table 6 were statistically interpreted in terms of BP (mm Hg), HR (beat/min.) and RR (cycle/min.) which has been taken as parameters of drug safety. Two way ANOVA system was used to analyze the data between all pairs of rabbit groups.

An inspection of the data shows that, TM marketed eye drops exhibited a highly significant ($P < 0.0001$) bradycardia, hypotension and increased RR immediately after 0.5 hr of instillation and the effect extended for 6 hrs. These effects indicate the rapid drainage of

drug solution into naso-lacrymal duct to the systemic circulation. TM formulations F25 and F31 have non-signifincant side effects (P< 0.001) along the time of experiment when compared to TM eye drops. This may be due to reduced nasolacrymal drainage. These findings support the favorable benefit/risk ratio of ophthalmic TM *in-situ* gels versus conventional eye drops.

Table 6: Systemic effects of 0.25% TM selected ophthalmic formulations compared to commercial eye drops.

Formula	Time (hrs)	BP (mm Hg) ± SD		HR (Beat/min.) ± SD	RR (Cycle/min.) ± SD
		SBP	DBP		
Commercial ED	0	175.0 ± 5.0	155.0 ± 5.0	320 ± 34.6	280 ± 34.6
	0.5	140.0 ± 10.0	120.0 ± 2.9	260 ± 34.6	340 ± 34.6
	1	123.3 ± 5.8	103.3 ± 2.9	220 ± 69.3	340 ± 34.6
	2	120.0 ± 10.0	100.0 ± 2.9	200 ± 34.6	400 ± 34.6
	3	106.7 ± 11.5	86.7 ± 2.9	200 ± 34.6	340 ± 34.6
	4	100.0 ± 0.0	80.0 ± 5.0	200 ± 34.6	340 ± 34.6
	5	103.3 ± 5.8	83.3 ± 5.0	240 ± 0.0	320 ± 34.6
	6	106.7 ± 5.8	86.7 ± 5.0	240 ± 0.0	280 ± 34.6
Viscous Solution (F1)	0	175.0 ± 5.0	155.0 ± 5.0	320 ± 34.6	260 ± 34.6
	0.5	156.7 ± 5.8	136.7 ± 2.9	240 ± 0.0	300 ± 0.0
	1	150.0 ± 0.0	126.7 ± 7.6	220 ± 34.6	320 ± 34.6
	2	150.0 ± 0.0	130.0 ± 7.6	260 ± 34.6	320 ± 34.6
	3	150.0 ± 5.0	126.7 ± 5.8	320 ± 34.6	320 ± 34.6
	4	158.3 ± 2.9	138.3 ± 5.0	300 ± 0.0	320 ± 34.6
	5	171.7 ± 2.9	151.7 ± 5.0	300 ± 0.0	260 ± 34.6
	6	173.3 ± 2.9	153.3 ± 5.0	300 ± 0.0	260 ± 34.6
Pl F-127 *in-situ* gel (F22)	0	175.0 ± 5.0	155.0 ± 5.0	340 ± 34.6	280 ± 34.6
	0.5	171.7 ± 5.8	153.3 ± 2.9	300 ± 60.0	320 ± 34.6
	1	166.7 ± 5.8	153.3 ± 7.6	340 ± 34.6	300 ± 0.0
	2	168.3 ± 5.8	151.7 ± 7.6	340 ± 34.6	280 ± 34.6
	3	171.7 ± 2.9	153.3 ± 5.8	340 ± 34.6	280 ± 34.6
	4	175.0 ± 5.0	155.0 ± 5.0	340 ± 34.6	280 ± 34.6
	5	175.0 ± 5.0	155.0 ± 5.0	340 ± 34.6	280 ± 34.6
	6	175.0 ± 5.0	155.0 ± 5.0	340 ± 34.6	280 ± 34.6
Pl F127 + CS *in-situ* gel (F25)	0	175.0 ± 5.0	155.0 ± 5.0	300 ± 0.0	280 ± 34.6
	0.5	171.7 ± 5.8	151.7 ± 5.8	280 ± 34.6	300 ± 0.0
	1	170.0 ± 0.0	151.7 ± 2.9	300 ± 0.0	280 ± 34.6
	2	171.7 ± 2.9	153.3 ± 5.8	300 ± 0.0	280 ± 34.6
	3	173.3 ± 2.9	155.0 ± 5.0	300 ± 0.0	280 ± 34.6
	4	175.0 ± 5.0	155.0 ± 5.0	300 ± 0.0	280 ± 34.6
	5	175.0 ± 5.0	155.0 ± 5.0	300 ± 0.0	280 ± 34.6
	6	175.0 ± 5.0	155.0 ± 5.0	300 ± 0.0	280 ± 34.6
Gelrite *in-situ* gel (F31)	0	175.0 ± 5.0	155.0 ± 5.0	300 ± 0.0	280 ± 34.6
	0.5	171.7 ± 5.8	151.7 ± 5.8	280 ± 34.6	320 ± 34.6
	1	168.3 ± 2.9	148.3 ± 2.9	280 ± 34.6	300 ± 0.0
	2	168.3 ± 2.9	150.0 ± 8.7	300 ± 0.0	280 ± 34.6
	3	171.7 ± 2.9	153.3 ± 5.8	300 ± 0.0	280 ± 34.6
	4	173.3 ± 2.9	156.7 ± 2.9	300 ± 0.0	280 ± 34.6
	5	175.0 ± 5.0	156.7 ± 2.9	300 ± 0.0	280 ± 34.6
	6	175.0 ± 5.0	156.7 ± 2.9	300 ± 0.0	280 ± 34.6

Conclusion

It can be concluded that mucoadhesive thermosensetive gel F25 (composed of Pl F-127 20% w/w and CS 1.5% w/w) and ion-activated *in-situ* gel F31 (containing gelrite 0.6% w/w) showed acceptable *in-vitro* results that is supposed to be useful in preventing precorneal elimination and controlling release of TM. These results are evidenced by an improved IOP lowering activity without systemic side effects compared to TM marketed eye drops. Therefore, it is hopped the two formulations may be promising as ophthalmic *in-situ* gel to be used for further work.

REFRENCES

1- A. K. Singla, I. P. Kaur, A. Grag and D. Aggarwal, "Novel approches for topical delivery of acetazolamide", Pharm. Technology, 7, 24 (2002).

2- K. S. Rathore, R. K. Nema and S. S. Siodia, "Timolol maleate a gold standard drug in glaucoma used as ocular films and inserts: An overview", Int. J. Pharm., 3, 23 (2010).

3- M. Volotinen, J. Maenpaa, H. Kautiainen, A. Tolonen, J. Uusitalo, A. Ropo, H. Vapaatalo and E. Aine, "Ophthalmic timolol in a hydrogel vehicle leads to minor variation in timolol concentration in aqueous humor", Europ. J. of Pharm. Sci., 36, 292 (2009).

4- H. Lin and K. C. Sung, "Carbopol/ pluronic phase change solutions for ophthalmic drug delivery", J. Control. Release, 69, 379 (2000).

5- S. Paul, R. Mondol, S. Ranjit and S. Miti, "Anti-glaucomatic niosomal system: Recent trend in ocular drug delivery research", Int. J. Pharm., 2, 2, (2010).

6- V. H. L. Lee, "New directions in optimization of ocular drug delivery", J. Ocul. Pharmacol., 6, 157 (1999): Review.

7- B. Srividya, R. M. Cardoza and P. D. Amin, "Sustained ophthalmic delivery of ofloxacin from a pH trigged *In-situ* gelling system", J. Control. Release, 73, 205 (2001).

8- A. H. El-Kamel, "*In-vitro* and *In-vivo* evaluation of pluronic F-127 solutions based ocular delivery system for timolol maleate", Int. J. Pharm., 241, 47 (2002).

9- M. Mansour, S. Mansour, N. D. Mortada and S. S. Abd El-Hady, "Formulation, *in-vitro* and *in-vivo* evaluation of ocular poloxamer based ciprofloxacin HCl *in-situ* forming gel", Egypt. J. Biomed. Sci., 24 (2007).

10- I. D. Rupenthal, C. R. Creen and R. G. Alany, "Comparison of ion-activated *in-situ* gelling systems for ocular drug delivery. Part: Physicochemical characterization and *in-vitro* release", Int. J. Pharm., 3, 42 (2011).

11- A. Rozier, C. Mazuel, J. Grove and B. Plazonnet, "Gelrite: A novel ion-activated *in-situ* gelling polymer for ophthalmic vehicles. Effect on bioavailability of timolol", *ibid*., 57, 163 (1989).

12- G. Dicolo, Y. Zambito, S. Burgalassi and A. Serafini, "Effect of chitosan on *in-vitro* release and ocular delivery of ofloxacin from erodible inserts based on poly-ethyleneoxide", *ibid*., 248, 115 (2002).

13- O. Felt, P. Furrer, J. M. Mayer, B. Plazonnet, P. Buri and R. Gurny, "Topical use of chitosan in ophthalmology: Tolerence, assessment and evaluation of precorneal retention", *ibid*., 180, 185 (1999).

14- Y. Cao, C. Zhang, W. Shen, Z. Cheng, L. Yu and G. Ping, "Poly(N-isopropyl-acrylamide)-chitosan as thermosensitive *in-situ* gel forming drug delivery", J. Control. Release, 120, 186 (2007).

15- K. A. Khalid, A. M. Elsayed, S. El-Harras, A. M. Darwish and M. A. Ismail, "Development and evaluation of bromo-criptine mesylate buccal bioadhesive discs", Bull. Fac. Pharm., Cairo University, 46 (3), (2008).

16- J. C. Gilbert, J. L. Richardson, M. C. Davies, K. J. Palin and J. Hadgraft, "The effect of solutes and polymers on gelation properties of pluronic F-127 solutions for controlled drug delivery", J. Control. Release, 5, 113 (1987).

17- J. Varshosaz, M. Tabbakhian and Z. Salmani, "Designing of a thermosensitive chitosan/poloxamer *in-situ* gel for ocular delivery of ciprofloxacin", The Open Drug Delivery J., 2, 61 (2008).

18- R. Korsmeyer, R. Gurny, E. Doelker, E. Buri and N. Peppas, "Mechanisms of solute release from porous hydrophilic polymers", Int. J. Pharm., 15, 25 (1983).
19- A. S. Monem, M. Alif and M. W. Ismail, "Prolonged effect of liposomes encapsulating pilocarpine HCl in normal and glaucomatous rabbits", *ibid*., 198, 29 (2000).
20- K. I. P. Kaur, M. Singh and M. Kanwar, "Formulation and evaluation of ophthalmic preparation of acetozolamide", *ibid*., 199, 119 (2000).
21- A. A. Al-Badr and G. S. Brenner, "Timolol Maleate", In: K. Florey (Eds), "Analytical Profile of Drug Substances", Vol. 16, Academic Press, New York, USA, 1987, p. 641.
22- A. Cabana, A. Ait-Kadi and J. Juhasz, "Study of the gelation process of polyethylene oxide-propylene oxide-polyethylene oxide copolymer (poloxamer 407) aqueous solutions", J. Colloid Inter. Sci., 190, 307 (1997).
23- H. Qi, W. Chin, C. Huang, L. Li, C. Chin, W. Li and C. Wu, "Development of poloxamer analoges, carbopol *in-situ* gelling and mucoadhesive ophthalmic delivery system for puerarin", Int. J. Pharm., 337, 178 (2007).
24- S. S. Abd El-Hady, N. D. Mortada, G. A. S. Awad, N. M. Zaki, R. M. Zaki and R. A. Taha, "Development of *in-situ* gelling and mucoadhesive mebeverine HCl solution for rectal administration", Saudi Pharm. J., 11, 159 (2003).
25- A. Paavola, Y. Yliruusi and P. Rosenberg, "Controlled release and dura matter permeability of lidocaine and ibuprofen from injectable poloxamer based gels", J. Control. Release, 52, 169 (1998).
26- D. Aggarwal and I. P. Kaur, "Improved pharmacodynamics of timolol maleate from a mucoadhesive niosomal ophthalmic drug delivery system", Int. J. Pharm., 290, 155 (2005).
27- A. Rozier, C. Mazuel, J. Crove and P. Plazonnet, "Functionally testing of gellan gum, a polymeric excipient material for ophthalmic dosage forms" *ibid*., 153, 191 (1997).
28- J. Carlfors, K. Edsman, R. Petersson and K. Jornving, "Rheological evaluation of gelrite *in-situ* gel for ophthalmic use", European J. Pharm. Sci., 6, 113 (1998).

نشرة العلوم الصيدلية
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صياغة وتقييم بعض المستحضرات العينية المختارة لعقار ماليات التيمولول

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الهدف من هذه الدراسة هو تحضير وتقييم صيغ عينية لعقار ماليات التيمولول المستخدم في علاج ارتفاع ضغط العين. كما تهدف الدراسة ايضا إلى تعزيز الاتاحة الحيوية للعقار بالعين ودعم نسبة الفائدة لمخاطر العقار. تم تحضير صيغ عينية مختلفة مثل المحاليل عالية اللزوجة، الهلاميات المائية، هلام موضعي التكون لبوليمرات البولورونيك ف ١٢٧ والجليريت. جميع المستحضرات تم تقييمها في المعمل وتقييمها على عيون الأرانب. كما تمت دراسة التأثير الناتج عن اضافة تركيزات مختلفة من بوليمرات لها خاصية الالتصاق الحيوي بالعين مثل كيتوزان وهيدروكسي ايثيل سيليلوز. أظهرت النتائج أن الهلام موضعي التكون لعقار ماليات التيمولول رقم ف٢٥ وف٣١ لهما خصائص معملية مناسبة للاستخدام الموضعي على العين. كما وجد أن لهما تأثيرا أفضل في خفض ضغط العين مع تقليل الاثار الجانبية على ضغط الدم ومعدل ضربات القلب ومعدل التنفس وذلك مقارنة مع قطرات العين المتوفرة بالسوق المصري.