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Evaluation of two *in situ* gelling systems for ocular delivery of Moxifloxacin: *In vitro* and *in vivo* studies

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ABSTRACT

This work describes the formulation of ophthalmic delivery systems of Moxifloxacin (Mox), as a model drug of fourth fluoroquinolone generation. Seven formulations (P1-P7) based on the concept of temperature triggered in situ gelation using pluronic (PL), and nine formulations (C1-C9) based on pH triggered in situ gelation using carbopol 934 (CL), were prepared. The developed formulae were evaluated regarding their gelation temperature (for PL systems), gelling capacity (for CL systems), rheological characteristics, in vitro release behavior and mucoadhesion measurements. Among different formulae tested, P6 and C5 showed optimum gelation temperature of 33.9 ˚C after dilution with simulated tear fluid (STF) and immediate gellation that remains for few hours respectively. Although the measured mucoadhesion index was higher (7.325 Pa) for C5 compared to (1.947 Pa) for P6, higher amount of Mox was retained in the aqueous humor area over 8 h following instillation of P6 with significant 2.8 fold increase in the C_{max} and $AUC_{(0-\infty)}$ compared to C5. Therefore, PL in situ gelling system can be used to enhance the ocular bioavailability more readily than CL system.

Key words: Ophthalmic delivery systems, Moxifloxacin, in situ gelling systems, Carbopol, Pluronic.

INTRODUCTION

The eye is a unique organ that has virtually several natural mechanisms to defend itself against infection. However, predisposing factors such as injury, allergic hypersensitivity reactions, overuse of contact lenses may disrupt these defense mechanisms and permit bacteria to invade the ocular tissue resulting in ocular diseases **[1,2]**. Therefore, appropriate therapy must be initiated to control the infections and thereby minimize ocular morbidity **[3]**. Ocular diseases are

usually treated with topical administration of eye drop solution owing to their simplicity and good acceptance by patients. Unfortunately, the ocular residence time of this conventional eye drop solution is limited to a few minutes and only 1–10% of topically drug applied is absorbed due to rapid and extensive precorneal loss leading to poor bioavailability and therapeutic response **[4,5,6]**. Major progress to overcome these disadvantages has been made by the development of in situ-forming gels. These systems consist of polymers that exhibit sol-to-gel phase transitions as a result of specific physical/chemical change induced by the physiological environment in the cul-de-sac as pH, temperature or a specific ion **[7]**. The principle advantage of this formulation is the possibility of combining advantages of both solution and gel, such as accuracy and facility of administration of the former and prolonged residence time of the latter which in turn increase the bioavailability **[4,5]**.

Poloxamers, commercially available as Pluronic®, are triblock copolymers composed of polyethylene oxide (PEO) units and polypropylene oxide (PPO) units (PEO/PPO/PEO). It exhibits reverse thermal gelation under certain temperature and concentration **[4,5,7]**. Pluronic F68 (PF68), which is an analog of Pluronic F127 (PF127), was added to PF127 solution to increase its gelation temperature **[7,8,9]**. Carbopol® 934 is a synthetic polymer composed of 62% of carboxyl groups with approximate molecular weight (3×10^6) **[5]**. Carbopol shows a sol-to-gel phase transition in aqueous solution when the pH is raised above its pKa of about 5.5 and also it has mucoadhesive properties **[10]**. In order to reduce the polymer concentration and improve the gelling properties, viscosity enhancing agent has been used which is methylcellulose (MC). Also, it was incorporated into the formulations in order to enhance the flow behavior and to strengthen the gel formed even after the dilution with the tear fluid **[11]**. Moxifloxacin (Mox), fourth generation fluoroquinolone, has high potency against both Gram-positive, Gram-negative bacterial pathogens and its bactericidal activity is through inhibition of bacterial topoisomerase II (DNA gyrase) and topoisomerase IV enzymes which are critical in the maintenance, synthesis, and replication of DNA **[12,13].**

This study was aimed to develop two in situ-gelling Mox systems based on pluronic (PL) thermoreversible and carbopol (CL) pH triggered systems. In vitro evaluation (gelation temperature, gelling capacity, rheological behavior, in vitro release and mucoadhesion measurement) and in vivo evaluation (the amount of Mox retained in aqueous humor) were both performed on the two systems.

EXPERIMENTAL SECTION

2.1. Materials

Moxifloxacin hydrochloride (Mox), was kindly supplied by EVA pharma company (Cairo, Egypt). Pluronic® F-127 and Pluronic® F-68 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Carbopol 934NF was provided by Luna Pharma (Cairo, Egypt). Methylcellulose E461 (MC) was supplied by Carl Roth GmbH (Karlsruhe, Germany). Mucin from porcine stomach, Type III, (bound sialic acid 0.5-1.5%, partially purified powder) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Spectra/Por®3 dialysis membrane (Cellophane membrane of MWCO: 3500 Daltons) was obtained from Spectrum Laboratories Inc. (Rancho Dominguez, CA, USA). All other chemicals and solvents were of reagent grade and were obtained from standard commercial suppliers.

2.2. Preparation of Mox in-situ gelling systems

Table (1) and (2) shows the composition of in situ-gelling Mox systems based on pluronic (PL) and carbopol (CL), respectively. PL in situ-gels, were prepared using the cold method **[7,8]**. The

drug was dissolved in cold water to yield a final concentration of 0.5% w/v. The calculated amount of MC was dispersed in the drug solution and stirred until dissolved. Appropriate amount of PF127 and PF68 were added to the cold solution, refrigerated at 4°C and stirred periodically until a homogeneous solution was obtained. Distilled water was then added to make up the volume to the total amount. The pH of all formulations was adjusted to 7.4 ± 0.1 by 0.1 N NaOH.

CL in situ-gels, were prepared as follows. Initially, CL solutions were prepared by dispersing the required amount in a certain volume of distilled water with continuous stirring until completely dissolved. The desired amounts of MC were added to CL solutions while stirring until thoroughly mixed. The required amount of Mox to give a final drug concentration of 0.5% w/v was dissolved in distilled water. The drug solution was added to the polymeric solution then propylene glycol was added whilst stirring to prevent drug precipitation until a homogenous solution was obtained. The distilled water was then added to make the volume up to the total amount. The pH of the formulations was adjusted to 4.0 ± 0.1 by 0.1 N NaOH. All formulations were stored in a refrigerator (4-8°C) until further use.

2.3. Evaluation of formulations

2.3.1. Drug Content Uniformity

100 µl of each preparation was transferred to 100-ml volumetric flask and the final volume made up with simulated tear fluid (STF) then shake for 2-3min. The concentration of Mox in each formulation was determined spectrophotometrically (Shimadzu UV–1601 Double Beam, Kyoto, Japan) at λ 288 nm. The results were the mean value of 3 replicates. Freshly prepared STF composed of: sodium chloride, 0.670 g; sodium bicarbonate, 0.200 g; calcium chloride·2H2O, 0.008 g and purified water up to 100 g **[10].**

2.3.2. Measurement of Gelation temperature(GT)

Ten milliliters of prepared PL formulation were put into a transparent vial with a magnetic bar that was placed in a low-temperature water bath. A thermometer with accuracy of 0.1°C was immersed in the test formulation. The PL formulation was heated at the rate of 1°C/1–2 min with the continuous stirring of 100 rpm (Stirring Hot Plate MSH-420, BOECO, Hamburg, Germany). The GT was determined visually as the temperature at which the magnetic bar stopped moving due to gelation **[14]**. In order to evaluate the change in GT after administration, the test was repeated after diluting each system with STF in a ratio of 40:7. As the conventional commercial eyedropper delivers an average drop volume about 40 µl while available tear fluid is 7 µl **[7,14]**. The result of each sample was the mean of four replicate determinations.

2.3.3. Gelling capacity

The gelling capacity was determined for CL formulations by placing 100 µL of the prepared formulations into a vial containing 2 mL of STF freshly prepared. Gelation was assessed visually and noting the time for gelation and the time taken for the gel formed to dissolve **[7,15].**

2.3.4. Viscosity and rheological studies

The viscosity and rheological behavior of the prepared systems in centipoise (cp) was measured at various shear rates using Brookfield DV III viscometer fitted with CP-52 cone and plate spindle (Brookfield Engineering Inc., Stoughton, MA, USA). In order to evaluate the change in viscosity after administration, the measurements were repeated after increasing the temperature from 25 °C to 35 °C for PL formulations. While for CL formulations, measurements were repeated after increasing pH from pH 4 to pH 7.4 using 0.1 N NaOH.

2.3.5. In vitro release studies

This study was carried out using a USP Dissolution tester (Apparatus I, Hanson SR6, California, U.S.A.). A 1-mL volume of the formulation was accurately placed in glass cylindrical tubes (2.5 cm in diameter and 10 cm in length). Each tube is tightly covered with a Spectra/Por® (soaked overnight in STF) from one end and attached to the shafts of the USP dissolution tester apparatus, instead of the baskets, from the other end. The shafts were then lowered to the vessels of the dissolution apparatus containing 100 mL of STF. The release study was carried out at 35±1ºC, and the stirring shafts were rotated at a speed of 50 rpm. At predetermined time intervals, samples (3ml) were withdrawn and analyzed for Mox content spectrophotometrically at λ 288 nm against the sample withdrawn at respective time interval from plain Mox free system treated in a similar manner. Every withdrawal was followed by replacement with fresh medium to maintain a constant volume. The results were the mean value of 3 runs each representing one batch.

2.3.6. Mucoadhesion measurement

The mucoadhesive behavior was evaluated according to the method described by **(Hassan and Gallo, 1990)** based on the idea that the chemical interaction and entanglements between the polymer and glycoproteins in mucus causes a rheological synergism **[16]**.

Dried mucin was hydrated with STF by stirring for 3 hr at room temperature to yield a dispersion of 20% (w/w). Six grams of mucin dispersion were mixed for 15 min with 2 g of each test preparation before measurement such that the final concentration of mucin was 15% (w/w). The viscosity of mucin (15% w/w) was measured in absence (η_m) and presence of polymer solution (η_t) in order to evaluate the mucoadhesion properties of the tested polymer solution. Viscosity was measured at 35±1 °C at the shear rates D of 10, 20, 50 and 100 s^{-1}. The measurements were performed for 1 min after 3 min of applying the shear force to allow the shear force to be homogeneously distributed throughout the sample. The viscosity component of bioadhesion (η_b) was calculated from the following equation,

$$\eta_b=\eta_t-\eta_m-\eta_p. \quad (1)$$

Where (η_p) is the viscosity of corresponding polymer solution (the polymer solution diluted with STF). The mucoadhesion index M [cp] was calculated using the shear rate D [s^{-1}] and the viscosity component due to bioadhesion (η_b) [cp] according to the equation:

$$M=\eta_b*D. \quad (2)$$

Where D is the shear rate per second. (η_b) was calculated from Eq. (1). Since (η_b) may decrease with the increase in the applied shear rate D, it was decided to use a high value of D to eliminate weakly bioadhesive materials **[16].**

2.3.7. In vivo studies

Twelve male healthy Albino rabbits weighing between 2.0-2.5 kg were used in this study. All animals were healthy and free of clinically observable ocular abnormalities. The study performed was approved by the university protection for animals care and use committee and the protocol complied with “the Principles of Laboratory Animal Care” [NIH publication # 85-23, revised 1985]. The formulations used were freshly prepared without any preservative addition and were sterilized by autoclaving at 121°C, 15 p.s.i. for 20 min. The animals were randomly divided into two groups, each of six rabbits. The study was done to evaluate the concentration of drug in aqueous humor after 0.25, 0.5, 1, 4, 8, 12 and 24 hours following instillation of the selected in

situ gelling formula from each system. The rabbits were received a single dose (40 μL) of the tested preparations applied in the cul-de-sac of the right eyes. During the experiment, the rabbits were placed in restraining boxes where they could move their heads and eyes freely. At different times post-instillation the animals were anesthetized with intramuscular injections of ketamine hydrochloride 15 mg/kg, Xylazine 1.5 mg/kg **[17]**, (200μl) aqueous humor was withdrawn with the help of 26-gauge needle attached to 1 ml disposable syringe inserted through the corneal-scleral junction and slightly upwards into the anterior chamber **[11]**. The samples were collected and stored at -80°C until the spectrofluorimetry assay was carried out. The degree of drug penetration is expressed as the maximum Mox ocular concentration measured in μg per ml aqueous humor.

Spectrofluorimetric analysis

The Mox contents in aqueous humor were measured using spectrofluorimetric method depending on the native fluorescence of fluoroquinolones due to the high degree of conjugation found in the structure **[18]**. Fluorescence measurements were performed with a Shimadzu spectrofluorimeter Model RF-1501 (Kyoto, Japan) equipped with a Xenon lamp. All the measurements took place in a standard 10 mm pathlength quartz cell. The fluorescence intensity of Mox was measured at 520 nm using an excitation wavelength of 293 nm in the presence of 0.1M H_2SO_4 because at basic pH, the fluorescence was inhibited, whereas at acidic pH the fluorescence was enhanced **[19,20].**

Standard solutions

Blank aqueous humor samples were spiked with Mox stock solution (1 mg/mL) to give the range of 17-1666 ng/ml. To cover the fluorescence intensities of all samples measured, two calibration curves were constructed by plotting the fluorescence intensity versus Mox concentrations in aqueous humor. The standard solutions were in the range of 17-250 ng/ml and 333-1666 ng/ml measured at high and low sensitivity instrument condition respectively. During the assay of the samples, the intra and inter -day precision and accuracy of the analytical procedure were evaluated after replicate analysis (n = 9) of control aqueous humor samples spiked at three concentration levels for each standard calibration curve. The lower limit of quantification was 12.23 ng/mL and 324.65 ng/ml for high and low sensitivity standard curves, respectively. With a linear response across the full range of concentrations from 17 to 250 ng/mL (R^2 = 0.9998) and from 333 to 1666 ng/ml (R^2 = 0.9999).

Aqueous humor analysis

The samples were thawed at room temperature and 150 μl of each sample was extracted with acetonitrile in a ratio 1:5 and centrifuged at 5000 rpm for 30 minutes at 4°C. 300 μl from the supernatant was taken and evaporated to dryness. Residues were reconstituted with 1 ml 0.1M H_2SO_4 and its fluorescence intensity measured against plain Mox free aqueous humor treated in the same manner.

Pharmacokinetic analysis

The area under the curve, $\mathbf{AUC_{(0-\infty)}}$ (μg.h/mL) of Mox concentration in the aqueous humor was calculated using the trapezoidal rule. The maximum Mox concentration $\mathbf{C_{max}}$ **(μg/ml)** in the aqueous humor and the time at which $\mathbf{C_{max}}$ is achieved $\mathbf{T_{max}}$ **(hr)** were determined from actual data points.

RESULTS AND DISCUSSION

The composition and drug content of the various PL and CL based formulations are shown in Table (1) and (2), respectively. The drug content was found to be satisfactory.

In particular, the feasibility of the in situ gelling system as an ocular drug delivery should be a free flowing liquid with low viscosity at non-physiological condition to allow reproducible administration into the eye as drops; it should also undergo in situ sol-to-gel phase transition at physiological condition to form gel capable of enduring shear forces expected in the eye during and between blinking and facilitate sustained drug release **[10,21]**.

3.1. Measurement of Gelation temperature (GT)

The optimum ophthalmic thermoreversible in situ gels should have GT higher than ambient temperature (25°C) before mixed with STF and shift to gel at the conjunctival sac temperature (35°C) after mixed with STF **[7,14,22]**. Table (1) was shown that concentrated PF127 solution (25%) became a firm gel at temperature lower than room temperature (18.5 °C before STF dilution). That is a disadvantage which led to difficulty in preparation and administration as the solution must be stored in refrigerator **[14]**. GT of the mixed PL formulations (PF127/ PF68) increased as the PF127 concentration decreased before STF dilution. After STF dilution, the total concentration of the polymer lowered result in further increase in GT. According to the results, the formulation that presented an adequate GT contained 18% (w/v) PF127 and 5% (w/v) PF68, where the GT before STF dilution was 27.83 °C and that after STF dilution was 34.89 °C.

It is noted that the PPO that is hydrophobic has the GT lowered and the PEO that is hydrophilic has the GT increased. Therefore, a different PEO/PPO ratio will lead to a different GT **[22]**. The slight amount of PF68 can only change the PEO/PPO ratio, which causes the increase of gelation temperature. As the ratio of PEO and PPO is 7: 3 in PF127, whereas the ratio is 8: 2 in PF68, the proportion of the PEO will increase, which will lead to the increase of the gelation temperature **[22]**. However, the micellization of PF68 molecules can participate in the construction of the gel which may disturb the formation of the PF127 micelles, so the ability to endure the STF dilution will become weaker compared with PF127 only **[22]**. Moreover, PL gels have a drawback of weak mechanical strength that leads to rapid erosion (i.e. dissolution from the surface) **[5,23]**. In order to enhance the flow behavior and to strengthen the gel formed even after the dilution with the tear fluid, combination systems (P5 – P7) of 18% PF127 and 5% PF68 with various concentration of MC were tested. As depicted from results in Table 1, 0.5% and 1% MC added were suitable concentrations as their preparations present satisfactory attributes of gelation temperature and consistency.

3.2. Gelling capacity

The two main prerequisites of phase transition system are viscosity and gelling capacity (speed and extent of gelation) **[15]**. Moreover, the flow behavior of the formulation is an important parameter involved in utilization and in vivo performance as if it is too viscous it will lead to difficult instillation; on the contrary, if viscosity is too low it will increase drainage **[14]**. From visual and manual inspection we found that all formulae coded in Table (1) underwent transition into gel phase upon contact with STF except C1 could not form gel. However, it is clear that the nature of the gel formed depended upon the polymer concentration [**24]**. C2 and C4 formed weak gel that dissolved rapidly. The flow of C3 was liquid with very low viscosity while C7, C8 and C9 were difficult flow as gel formed during preparation. C5 and C6 had a satisfactory attributes of gelling capacity and consistency.

The observed phase transition was attributed to the increase of pH as the mutual repulsion of ionized carboxyl groups may produce more stretched CL backbone and also may form stable hydrogen bonds with water molecules through hydrophilic interactions. Moreover, the hydrophobic nature of CL backbone may form hydrophobic interchain aggregation and this cross-linking phenomenon may trigger the formation of more viscous gel **[10]**.

3.3. Viscosity and rheological studies

The viscosity of PL and CL formulations at non-physiological and physiological conditions was depicted in Fig. 1 and 2, respectively. Remarkable increase in the viscosity was observed as the temperature of PL formulations was increased to 35 °C, and the pH of CL formulations was raised to 7.4. This confirms the occurrence of phase transition process for both systems. It was clear also that the viscosity was directly dependent on the polymeric content of the formula in both systems, PL systems (P7 > P6 > P5) and CL systems (C9 > C8 > C7 > C6 > C5 > C3). Moreover, all formulations exhibited pseudoplastic property as evidenced by shear thinning and the decrease in viscosity with the increase in angular velocity. The pseudoplastic property is in favor of sustaining drainage of drugs from the conjunctival sac of the eye, without blinking difficulty for undergoing shear thinning **[25].** In addition, the range of ocular shear rates associated with normal blinking is extremely wide, ranging from 0.03 S^{-1} during inter-blinking periods to 4250-28500 S^{-1} during blinking. Therefore, viscoelastic fluids with a viscosity that is high under the conditions of low shear rate and low under the conditions of high shear rate are preferred **[15]**.

3.4. In vitro release studies

The cumulative amount of Mox release profiles of PL and CL formulations as a function of time are depicted in Fig.3. As it can be seen, all the formulations are able to retain the drug in its matrix network. Moreover, the release of the drug from these gels is continued within 48 h (time of the study). A slow diffusion rate was observed from all formulations tested which could be attributed to the occurrence of phase transition process. It was apparent that the release profiles of PL systems obtained were almost similar and exhibited an initial small burst of about 11.64%, 11.4% and 11.01% from P5, P6 and P7, respectively within the first hour followed by slow steady state drug release reaching 89.18%, 87.02% and 81.66% in 48 hours, respectively. The same case for CL systems, their release profiles within the first hour exhibited an initial small burst of about 13.55%, 12.92%, 11.61%, 11.39%, 10.6% and 8.66% from C3, C5, C6, C7, C8 and C9, respectively followed by slow steady state drug release reaching 92.1%, 91.22%, 91.08%, 89.15%, 88.27% and 85.56% in 48 hours, respectively.

The release data was kinetically analyzed using the empirical equation:
Log Q = Log k + n Log t **[26]** where, Q is the fraction of drug released in time *t*, *k* is a constant characteristic of the drug polymer interaction and *n* is an empirical parameter characterizing the release mechanism. Based on the diffusional exponent, PL and CL based formulations revealed n-values of (1>n>0.5), meaning non-Fickian or anomalous behavior that was obtained as a result of contribution from diffusion and polymer relaxation. Drug release was dependent on two simultaneous rate processes, water migration into the hydrogel and drug diffusion through continuously swelling hydrogel **[27,28,29].**

According to previous results obtained from the investigated formulations, P5 and P6 had nearly similar GT, flow behavior, rheological and release characteristics. Also, the same was in case of C5 and C6. However, increasing the polymer concentration would result in increasing the strength and adhesiveness of gels **[21,30]** therefore, P6 formula (1% MC and 18% PF127/5% PF68) was chosen as the candidate formula.

Moreover, C5 formula (0.2% CL and 1% MC) was chosen, as the less usage amount of CL, the less the potential stimulus to the eye will be **[25].** Therefore, the combinations that better fit the requirements for an acceptable ophthalmic delivery system from both systems were P6 and C5. These two formulations were chosen to be evaluated in vivo after in vitro mucoadhesion measurement.

3.5. Mucoadhesion measurement

The mucoadhesion is important parameter to be taken into consideration as the retention time of the formulation in the ocular area may be improved [**5]**. The (*ηb*) values of the two formulae P6 and C5 calculated at various shear rates are summarized in Table (3). The mucoadhesion index, (M), calculated at D = 100 s^{-1}were 7.325 and 1.947 (Pa) for C5 and P6, respectively. As it can be seen from results, the viscosity values of the mixture are higher than the sum of the corresponding values of separate components at all the shear rates investigated. These suggest positive interaction (synergism) between the polymer and mucin that expect an increase in the residence time as a result of its binding to the mucus layer coating the corneal and conjunctival epithelium **[5,31**]. The increased adhesiveness of the C5 than P6 may be attributed to the increased ability of CL polymer to interact with mucin greater than PL.

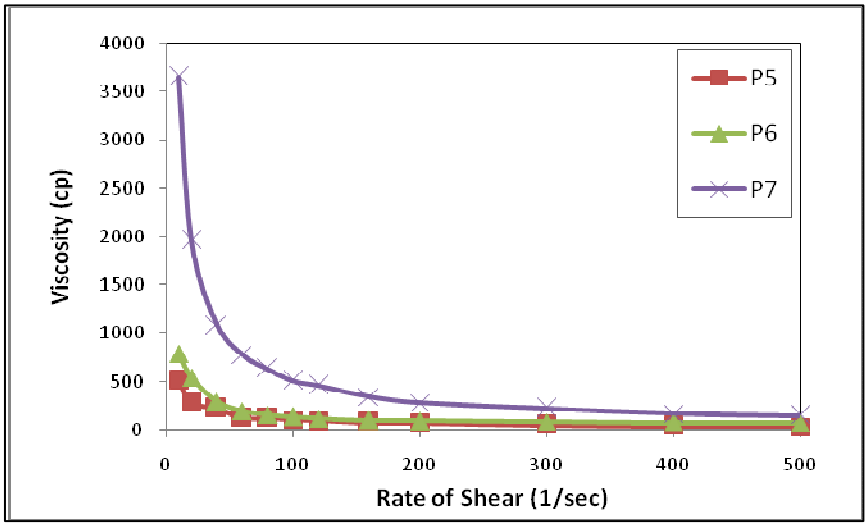
3.6. In vivo studies

The level of Mox in aqueous humor after topical instillation of P6 and C5 was shown in (Fig.4). The aqueous humor content of Mox was significantly higher ($P<0.05$) at all time points after administration of P6 than that obtained after instillation of C5. It was interesting to note that the aqueous humor level showed a maximum at 1 h post administration which decreased gradually afterwards since the higher concentration of the antibiotic is always desirable at early time of infection. The fold differences between P6 and C5 were 1.5, 4.52, 2.83 and 2.15 after 0.25, 0.5, 1 and 4 hr, respectively. More specifically, the intraocular Mox level attained in the aqueous humor following administration of P6 was fairly high for up to 8 hr in contrast to its intraocular level from C5 where it was cleared from the eye faster and its level went down below the limit of detection after 4 hr post-administration.

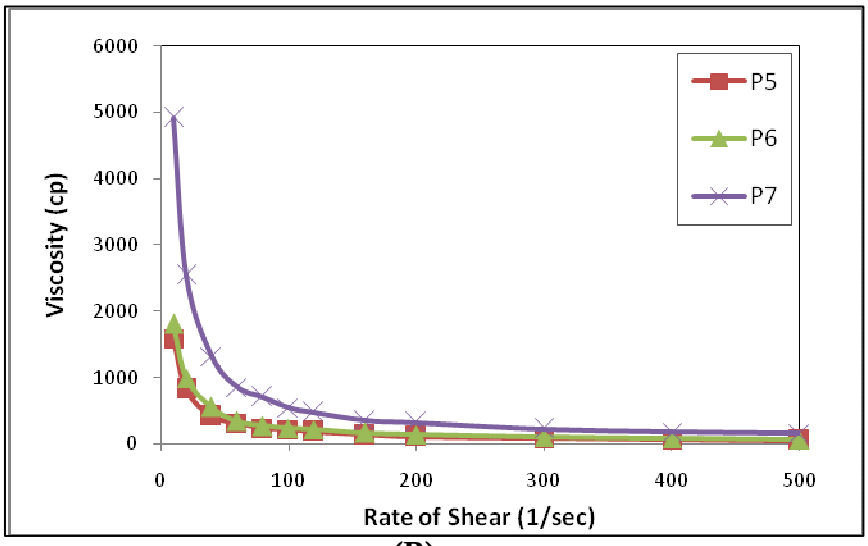
The ocular bioavailability of Mox was illustrated by the area under the curve (**AUC**), maximum Mox concentration $\mathbf{C_{max}}$ and the time at which the $\mathbf{C_{max}}$ is achieved $\mathbf{T_{max}}$ (Table 4). The $\mathbf{C_{max}}$ and $\mathbf{AUC_{(0-\infty)}}$ values in aqueous humor treated with P6 were 2.83 and 2.87 fold greater than those treated with C5, respectively. The results indicated that a greater amount of drug was attained in the aqueous humor for a prolonged period following instillation of P6 compared to C5.

However, the conditions during in vitro drug release studies were very different from those likely to be encountered in the eye. Due to the special configuration in the eyes, the ophthalmic gels will be continuously rinsed with tear fluid **[9]** and the shearing action during blinking the formulations will probably undergo faster dissolution **[15]**.

The difference between the two formulae may be due to physiological blinking frequency and lacrimation response upon topical instillation into the eye. Irritating eye drop will increase tear production and blinking frequency, also the gel will be diluted and will be transported from the eye more quickly **[32].** This is the case for C5 as it is acidic solution taken a time to be buffered by tear fluid to increase its pH. Therefore, P6 maintained for longer time with high Mox concentration than C5.

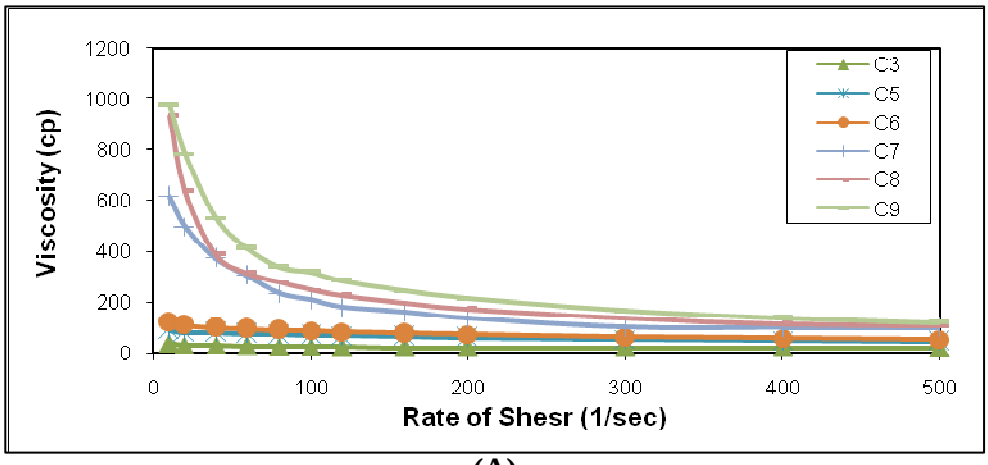


(A)

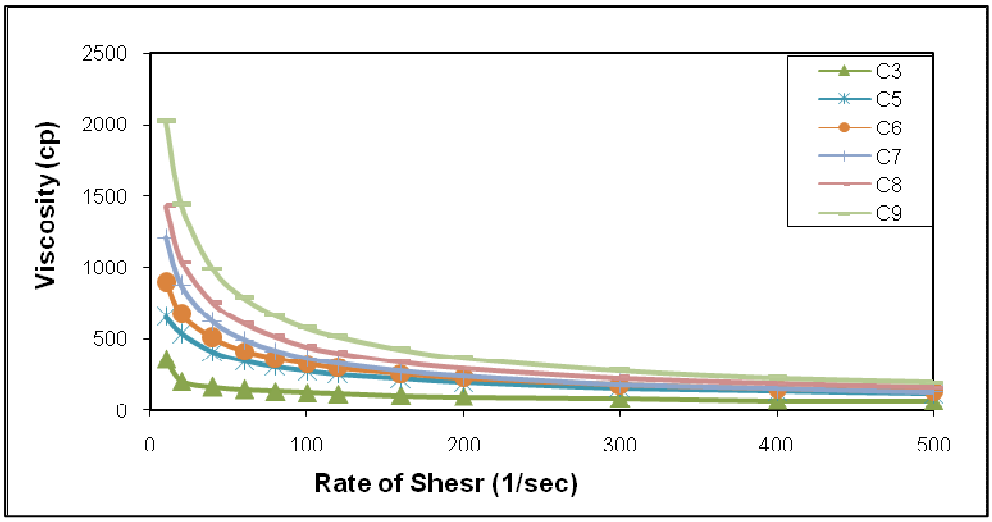


(B)

Fig.1. Rheology profiles of Pluronic formulation P5, P6 and P7 (A) at 25 ºC and (B) at 35 ºC. Remarkable increase in viscosity was observed as the temperature increased from 25 ºC to 35 ºC. This confirms the occurrence of phase transition process. The viscosity was directly dependent on the polymeric content of the formula (P7 > P6 > P5). All formulations exhibited pseudoplastic property as evidenced by shear thinning and the decrease in viscosity with the increase in angular velocity.



(A)



(B)

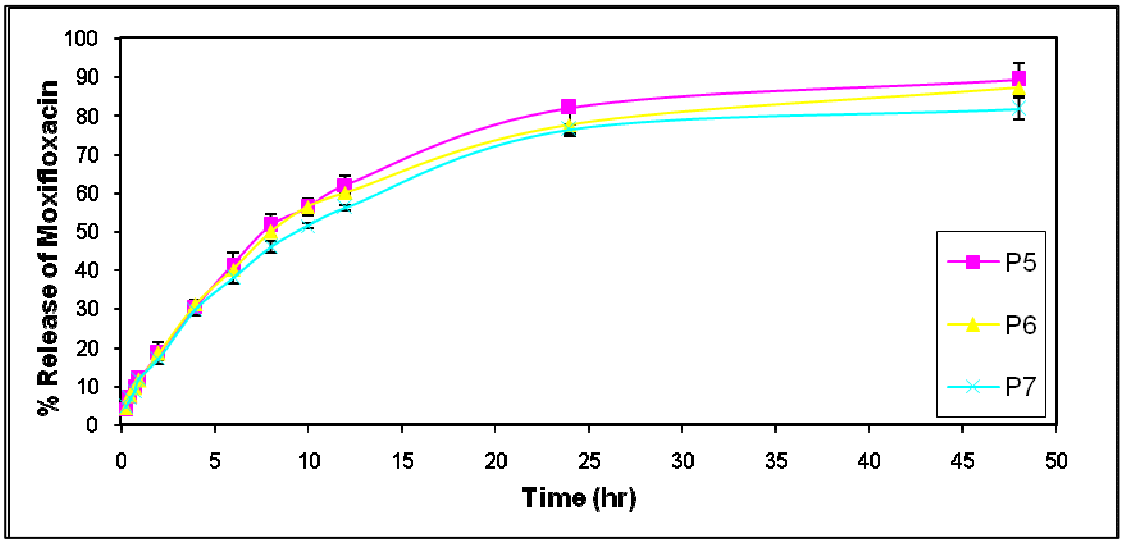
Fig.2. Rheology profiles of Carbopol formulation C3, C5, C6, C7, C8 and C9 (A) at pH 4 and (B) pH 7.4. Remarkable increase in viscosity was observed as the temperature increased from pH 4 to pH 7.4. This confirms the occurrence of phase transition process. The viscosity was directly dependent on the polymeric content of the formula (C9 > C8 > C7 > C6 > C5 > C3). All formulations exhibited pseudoplastic property as evidenced by shear thinning and the decrease in viscosity with the increase in angular velocity.

Table (1): Characterization of Pluronic in-situ gelling system of Moxifloxacin

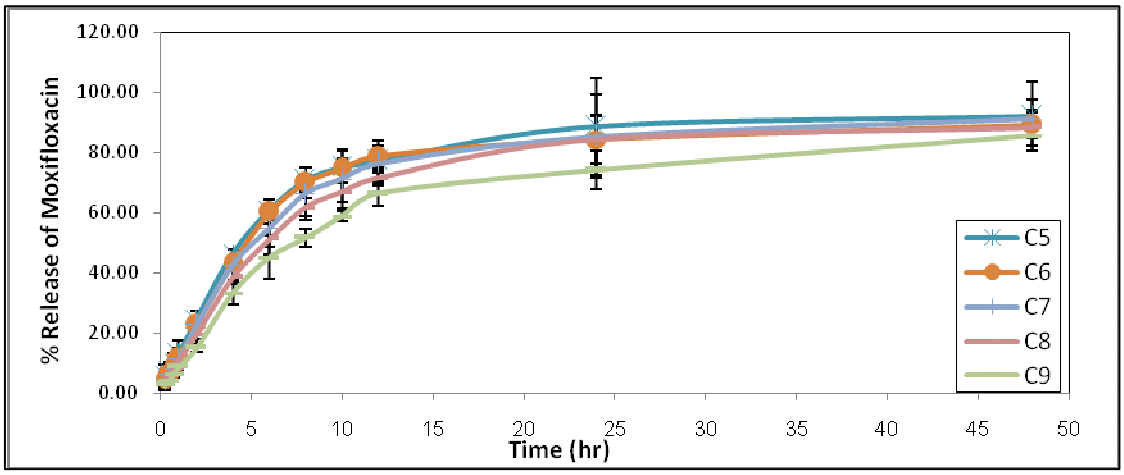
Formulation code	Concentration (% w/v)			Drug content (% w/v)[a]	GT (ºC)[a] before STF dilution	GT (ºC)[a] after STF dilution	Viscosity (cp)[b]
	PF127	PF68	MC				
P1	25	—	—	97.53 ± 1.12	18.5 ± 0.5	21.83 ± 0.29	—
P2	21	5	—	99.29 ± 0.61	24.17 ± 0.29	30.5 ± 0.41	—
P3	18	5	—	97.64 ± 0.9	27.83 ± 0.29	34.89 ± 0.85	—
P4	15	5	—	99.32 ± 1.05	36.9 ± 0.89	41 ± 1.08	—
P5	18	5	0.5	98.31 ± 1.70	27 ± 0.91	34.33 ± 0.29	228**
P6	18	5	1	100.21 ± 2.38	26.13 ± 0.25	33.88 ± 0.85	297**
P7	18	5	2	99.93 ± 0.93	24.38 ± 0.48	31.83 ± 0.76	1091***

[a] Each value represents the mean ± S.D. of three experiments.

*[b] the viscosity of samples evaluated at 20 rpm at non physiological condition (25 ˚C) — Not measured, * liquid, flow easy, ** liquid gel like, flow easy, *** gel formed, flow difficult.*



(A)



(B)

Fig.3. Cumulative amount of Moxifloxacin released as a function of time from Pluronic formulation P5, P6 and P7 (A) and Carbopol formulation C5, C6, C7, C8 and C9 (B) in STF at 35 ˚C. All measurements were conducted in triplicate and plotted as mean ± S.D. Their release profiles exhibited an initial small burst release within the first hour followed by slow steady state drug release. The release data was kinetically analyzed using Ritger–Peppas equation. The release mechanism was non-fickian behavior involving both diffusion and polymer relaxation (1>n>0.5).

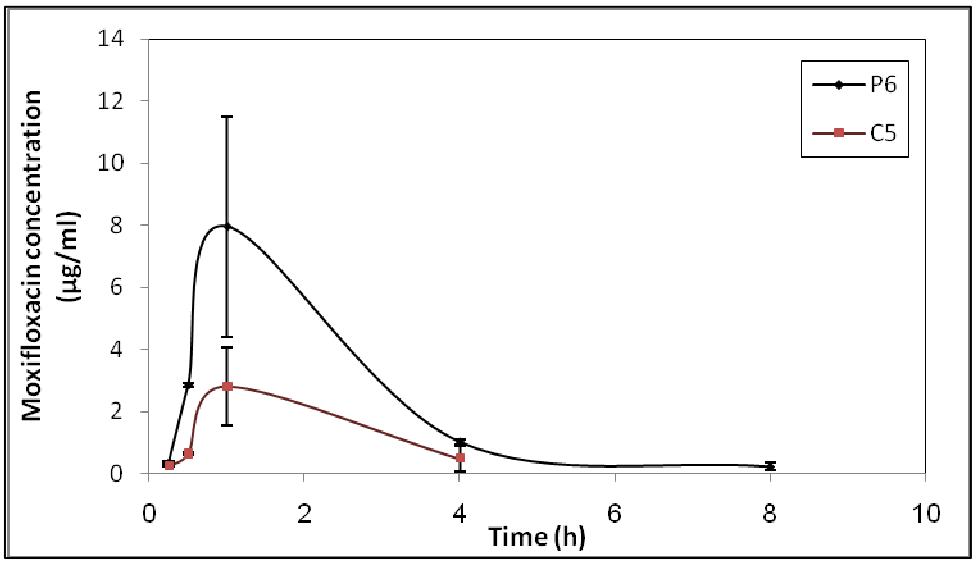


Fig.4. Moxifloxacin concentration attained in aqueous humor after application of P6 and C5. Mean ± S.D. of six determinations. Moxifloxacin concentration in aqueous humor was significantly higher (P<0.05) at all time points after administration of P6 than that obtained after instillation of C5. P6 maintained Moxifloxacin concentration for longer time up to 8 h.

Table (2): Characterization of Carbopol in-situ gelling system of Moxifloxacin

Formulation code	Concentration (% w/v)		Drug Content (% w/v)[a]	Gelling Capacity[b]	Viscosity (cp)[c]
	CL	MC			
C1	0.1	0.5	98.38 ± 0.26	--	–
C2	0.2	0.5	97.57 ± 0.78	+	–
C3	0.3	0.5	100.19 ± 3.78	++	29.8*
C4	0.1	1	98.61 ± 2.67	+	–
C5	0.2	1	98.13 ± 3.74	++	84.3**
C6	0.3	1	99.48 ± 1.15	++	99.2**
C7	0.1	2	99.59 ± 2.89	++	372***
C8	0.2	2	97.50 ± 1.91	+++	387***
C9	0.3	2	99.73 ± 0.83	+++	530***

[a] *Each value represents the mean ±S.D. of three experiments.*

[b] *--: No gelation, +: Gel formed after a few minutes, dissolves rapidly, ++: Immediate gelation, remains for few hours, +++: Immediate gelation, remains for extended period.*

[c] *the viscosity of samples evaluated at 20 rpm at non physiological condition (pH4), – Not measured, * Liquid, flow easy, ** liquid gel like, flow easy, *** gel formed, flow difficult.*

Table (3): The viscosity component ηb of C5 and P6 at various rate of shear

Rate of Shear	Viscosity component ηb (cp) *	
(1/sec)	C5	P6
10	317.5 ± 6.54	256.03 ± 4.61
20	223.87 ± 3.3	123.37 ± 6.36
50	131.90 ± 6.26	43.73 ± 4.08
100	73.25 ± 1.75	19.47 ± 4.36

* *Each value represents the mean ± S.D. of three experiments.*

Table (4): Pharmacokinetic parameters of Moxifloxacin in aqueous humor after instillation of P6 and C5

Ocular delivery system	C_{max} (µg/ml)*	T_{max} (h)*	$AUC_{(0-\infty)}$ (µg.h/ml)*
P6	7.9578 ± 5.2477	1	19.5280 ± 9.2928
C5	2.8063 ± 1.2519	1	6.8094 ± 3.5951

* *Each value represents the mean ± S.D. of six determinations.*

CONCLUSION

In this study we investigated the potential of in situ gelling systems triggered by temperature and pH for Mox delivery to ocular tissue. PL system compared to CL exhibited higher Mox level and prolonged residence time in the eye. Therefore, PL in situ gel system could improve ocular bioavailability than CL system.

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