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Designing of a Thermosensitive Chitosan/Poloxamer *In Situ* Gel for Ocular Delivery of Lomefloxacin HCl

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ABSTRACT

To increase the low bioavailability and short ocular residence time of lomefloxacin eye drops, aqueous solutions of drug in chitosan/ Pluronic (poloxamer) were prepared to identify suitable compositions with regard to gel forming properties and drug release behaviour. Mixtures of solutions of Pluronic (10-25% w/w) with chitosan (0.1-0.3% w/w) of different molecular weights (Mw) were prepared. Lomefloxacin release was determined using a membrane less dissolution model in artificial tear solution up to 8 hours and the samples were analyzed spectrophotometrically at 281nm. The rheological behaviour of solutions in response to dilution or temperature changes and also the phase change temperature (PCT) were determined using a brookfield's viscometer. Antimicrobial effect of the solutions was studied in nutrient agar using *Staphylococcus aureus* by using agar well diffusion method. The formulation consisted of 15% Pluronic and 0.1% low Mw chitosan, with the highest release efficiency (41.952 %) , is suggested as a suitable ophthalmic preparation for sustained release of lomefloxacin HCl. It was liquid in non-physiologic conditions (pH 4 and 25ºC) and transferred to the gel form upon physiologic conditions (pH 7.4 and 37ºC). The PCT of this *in situ* gel did not change upon dilution.

Keywords: Ocular drug delivery, *in situ* gels, chitosan, poloxamer, phase transition temperature.

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INTRODUCTION

In situ-forming systems are liquid aqueous solutions before administration, but gel under physiological conditions. There are several possible mechanisms that lead to *in situ* gelformation[1] solvent exchange, UV-irradiation, ionic cross linkage, pH change, and temperature modulation. These approaches, which do not require organic solvents, copolymerization agents, or an externally applied trigger for gelation, have gained increasing attention, such as a thermo sensitive approach for *in situ* hydrogel formation[2,3]. Several *in situ* gel forming systems have been developed to prolong the precorneal residence time of a drug and improve ocular bioavailability. Polymers are employed in such delivery systems to carry various drugs and they may demonstrate a transition from sol (liquid) to gel state once instilled in the cul-de-sac of the eye[4]. Examples of potential ophthalmic droppable gels reported in the literature include: I. gelling triggered by a change in pH: The viscosities increase when the pH is raised from its native value to the eye environment (pH 7.4) like cellulose acetophthalate (CAP) latex [5, 6], cross-linked polyacrylic acid derivatives such as carbomers and polycarbophil;II. gelling triggered by temperature change: Poloxamers or Pluronics[7,8], a class of block copolymers of poly(oxyethylene) and poly(oxypropylene), tetronics [9],ethyl(hydroxyethyl) cellulose [10], methyl cellulose and Smart Hydrogel™ exhibit thermoreversible gelation[11];III. gelling triggered by ionic strength change like: Gelrite [12-14] and alginate gel [15,16], in the presence of mono or divalent cations[17]. However, most of the systems require the use of high concentrations of polymers. For instance, it needs 25% (w/v) Pluronics and 30% (w/v) CAP to form stiff gel upon instillation in the eye. Carbopol is another gelling agent but as its concentration increases in the vehicle, its acidic nature may cause stimulation to the eye tissue. In order to reduce the total polymer content and improve the gelling properties, Joshi *et al.*[18] first used the combination of polymers in the delivery system. Kumar and other workers[4] developed an ocular drug delivery system based on a combination of Carbopol and methylcellulose or hydroxypropylmethylcellulose[19,20]. For both systems, it was found that a reduction in the carbopol concentration without compromising the *in situ* gelling properties as well as overall rheological behaviours can be achieved by adding a suitable viscosity-enhancing polymer [4, 19]. Gatifloxacin, useful in ocular infections, was successfully formulated as ion-activated *in situ* gel forming ophthalmic solutions (0.3%w/v) using alginate as a gelling agent in combination with HPMC E50Lv as a viscosity-enhancing agent [21]. Poloxamer (Pluronic®), a block copolymer that consists of polyethylene oxide (PEO) and polypropylene oxide (PPO) units, is known for exhibiting the phenomenon of reverse

thermal gelation under a certain concentration and temperature [22-24]. At a concentration of 18% (w/w) or higher in aqueous solution, poloxamer 407 (P407), is transformed from a low viscosity solution to a gel under the ambient temperature. But this lower concentration solution will lose the gelation ability after diluted by phosphate buffer. Therefore, poloxamer 188 (P188), was added to P407 solution as a regulatory substance and exhibited a good perspective to increase the gelling temperature (GT) of P407[25,26].Differentgel enhancing polymers has been used in combination with poloxamer: mono amine-terminated poloxamer with hyaluronic acid [27], the mixture of 0.3% carbopol and 14%Pluronic[28], linear poly(N-isopropylacrylamide-g-2-hydroxyethyl methacrylate) gel particles [29], and also poloxamer 407 and 188 (21 and 5%w/v respectively), with carbopol 1342P NF (0.1% - 0.2%) [30].Chitosan, a polysaccharide derived from naturally abundant chitin, is currently receiving a great deal of interest. Chenite *et al*. [31]developed a novel approach to produce thermosensitive neutral hydrogel based on chitosan/ polyolsalt combinations that could undergo sol-gel transition at a temperature close to 37 °C. Gupta *et al*. [32] also developed a clear, isotonic solution base on chitosan/ poloxamer for timolol maleate that converted into gel at temperatures above 35°C and pH 6.9-7.0. A significant higher drug transport across corneal membrane and increased ocular retention time was observed using the developed formulation. Lomefloxacin is a fluoroquinolone antibiotic that has demonstrated *in vitro* activity against *Staphylococcus* and *Bacillus* species and most gram negative organisms including *Pseudomonas* species. It has been suggested as a possible agent in the treatment and prevention of endophthalmitis [36].

MATERIALS AND METHOD

Pluronic (F127) obtained from (BASF Ltd. Mumbai) was used as received. Chitosan (Low MW 150000, Medium MW 400000 and High MW 600000) (Ozone international, Mumbai), Lomefloxacin HCl was supplied by Nakoda chemicals Ltd. Hyderabad.

Preparation of *In Situ* Gel Formulations

The chitosan solutions (0.1-0.3% w/w), were prepared by dispersing the required amount in acetic acid solution (2%w/v) with continuous stirring until completely dissolved. For preparation of Pluronic solutions (15-20% w/w), the required amount of polymer was dispersed in distilled, deionized water with continuous stirring for 1 h at room temperature. The partially dissolved Pluronic solutions were stored in the refrigerator (at 4ºC) until the entire polymer was completely dissolved (approximately 24 h). The chitosan / Pluronic solutions were prepared by dispersing the required amount of Pluronic in the desired concentration of chitosan with continuous stirring

for1 h. The partially dissolved solutions were then refrigerated until solutions were thoroughly mixed (approximately 24 h). The reported composition of chitosan/Pluronic mixture was the final concentration of chitosan and Pluronic content in the mixture. For preparation of lomefloxacin-containing polymer solutions, 0.3% of lomeofloxacin was added to the chitosan/Pluronic solutions with continuous stirring until thoroughly mixed. Benzalkonium chloride solution was added0.006% as preservative in all solutions. All the sample solutions were adjusted to pH 4.0 ± 0.1 or 7.4 ± 0.1 by 0.5 M sodium hydroxide solution, sterilized at 121ºC and 15 psi for 20min and then stored in the refrigerator prior to the evaluation of their rheological properties. Three formulation variable factors i.e., chitosan and Pluronic concentrations and also chitosan molecular weight each at three different levels were studied (Table 1) by a general full factorial design. Twenty seven formulations (3^3) were designed according to Table 2.

Table 1: **Variables and their Levels used in formulation of the *In Situ* Ophthalmic Gels of Lomefloxacin HCl**

Levels			
I	**II**	**III**	**Variables**
0.3%	0.2%	0.1%	Chitosan conc.
L	M	H	Chitosan molecular weight
25%	20%	15%	Pluronic conc.

Table 2: Composition of *in situ* ophthalmic gels of Lomefloxacin HCl

Code	Concentration (%)			
	P*	CH *	CM*	CL*
P1	15	0.1		
P1CH1	15	0.2		
P1CH2	15	0.3		
P1CH3	15		0.1	
P1CM1	15		0.2	
P1CM2	15		0.3	
P1CM3	15			0.1
P1CL1	15			0.2
P1CL2	15			0.3
P1CL3	15	0.1		
P2CH1	20	0.2		
P2CH2	20	0.3		
P2CH3	20		0.1	
P2CM1	20		0.2	
P2CM2	20		0.3	
P2CM3	20			0.1
P2CL1	20			0.2
P2CL2	20			0.3

P2CL3	20	0.1		
P3CH1	25	0.2		
P3CH2	25	0.3		
P3CH3	25		0.1	
P3CM1	25		0.2	
P3CM2	25		0.3	
P3CM3	25			0.1
P3CL1	25			0.2
P3CL2	25			0.3
P3CL3	25			

Evaluation of *in situ* gels

Measurement of Gelation Temperature (GT)

Ten milliliters of the sample solution and a magnetic bar were put into a transparent vial that was placed in a low temperature water bath. A thermometer with accuracy of 0.1 °C was immersed in the sample solution. The solution was heated at the rate of 2 °C/min with the continuous stirring. The temperature was determined as GT, at which the magnetic bar stopped moving due to gelation [38, 25] Each sample was measured at least in triplicate.

Rheological Studies

The viscosity of the different gel formulae was determined at 25°C using rotational Brookfield viscometer. The apparent viscosity was determined at shear rate 40 sec^{-1}. The flow index was determined by linear regression of the logarithmic form of the following equation:

$\tau = k \gamma n$…….Equation (1)

Where "τ" is the shear stress, "γ" is the shear rate, k is the consistency index, and n is the flow index. When the flow is Newtonian n=1, if n>1 or n<1, shear thickening or shear thinning is indicated, respectively. Evaluation was conducted in triplicate. The entire rheograms are shown in Figures (1-3).

Effect of Dilution on GT

The measurements were made at 15-37°C, the temperature in the conjunctival sac of the eye. The sol-gel transition temperature of poloxamer was determined from shearing stress measurements at 500 rpm, the temperature was increased4°C every 10 min. The GT was defined as the point where a sudden shift in shearing stress was observed. To mimic the properties in the eye, if all applied polymer solution (40µl) was immediately mixed with the phosphate buffer (7µl), which would be the worst case scenario, the polymer solution was mixed with phosphate buffer in a ratio of 40:7 [25]

***In Vitro* Release Studies**

The *in vitro* drug release from various polymer solutions was first carried out by filling 2 ml of lomefloxacin HCl containing polymer solution into small, circular plastic containers (2.5 cm i.d. and 1.5 cm in depth) in triplicate and placing each container in a 200 ml beaker. Care was taken to make sure that no air bubbles were inside the polymer solutions. The beaker was then filled with 200 ml phosphate buffer pH 7.4, temperature and stirring rate was maintained at 37°C and 20 rpm, respectively. Aliquots (5ml) were withdrawn from the release mediums at each sampling time and then replaced with fresh 37°C phosphate buffer solution. The samples were filtered through 0.45-mm syringe filters and subjected to spectrophotometric analysis (Systronic double beam spectrophotometer 2201) to determine the lomefloxacin HCl concentrations at λmax 281nm. Each time a blank gel was studied at the same conditions to omit the probable absorption of the base. Drug release kinetics was studied.

Antimicrobial Study

Weigh 5.5 gm of Macconkey's agar in 100ml distilled water. Sterilize the media, saline solution (0.9%NaCl), micropipettes, ear bud by autoclaving. After autoclaving put the media (5.5 gm in 100ml) in petri plates under the sterilized conditions. Allow the plates to solidify. Spread the *Staphylococcus aureus* suspension with the help of ear bud or glass spreader. Wells were prepared with the help of borer. Thermosensitive gel formulations were withdrawn by using micropipette (100-1000µg/ml) and poured in wells of plates. Then plates were incubated in 37^{o}C for 24 hrs. The zone of inhibition was measured by using scale (mm).

Accelerated Stabilities Studies

Stability is defined as the extent, to which a product retains with in specified limits and throughout its period of storage and use i.e., shelf life. Stability studies were carried out on optimized formulations according to International Conference on Harmonization (ICH) guidelines. A sufficient quantity of formulations in previously sterilized vials was stored in desiccators containing a saturated solution of sodium chloride, which gives a relative humidity of 75 %. The desiccators were placed in a hot air oven maintained at a temperature 40^{0}C, 0.5^{0}C and at room temperature. Samples were withdrawn at 7 days interval for 42 Days. The logarithms of percent drug remaining were calculated and plotted against time in days. It was also analyzed for visual appearance, clarity, and pH.

RESULTS AND DISCUSSION

Figure 1 (a-b) shows that the gels prepared with 15% Pluronic and 0.1% of different Mw of chitosan show Newtonian flow in non-physiologic state (pH 4 and 25ºC) while in physiological

conditions (pH 7.4 and 37ºC) they show psuedoplastic flow. Other concentrations of chitosan with the same percentage of Pluronic behaved similarly. Although 20% concentration of Pluronic with all combinations of chitosan from different mw show Newtonian flow in non physiologic state and psuedoplastic flow in physiological conditions Figure.2(a-b), but as Table 3 shows the phase change temperature (PCT) of these gels even before dilution is less than 37ºC which indicates that they are not applicable as eye drop. At higher concentration of Pluronic i.e., 25% in all combinations with different Mw of chitosan a pseudoplastic flow was observed both in physiologic and non-physiologic conditions (Figure. 3(a-b) that means this concentration is not also useful. An alteration (gelation) in the rheological behavior of the formulation, from a liquid to a semisolid (i.e. gel) happens. This would result in a change in the rheological behavior and an increase in the viscosity of the formulation at the thermogelation point. Hence, as a result of the increase in the viscosity, the resulting gel could remain in contact within the eye for a longer period of time and prolongs the precorneal residence time of a drug that improves ocular bioavailability of the drug. As Table 3 indicates Pluronic will lose the gelation ability after dilution by phosphate buffer (pH 7.4) since when it is not combined with chitosan its PCT will change significantly from 39ºC to 43ºC after dilution and its concentration is not enough any more for gelling. However, formulations prepared with a combination of a specific concentration of Pluronic with chitosan don't show significant difference between their PCT before and after dilution (for example P1CL1) while there is statistical significant difference between PCT of different concentrations of Pluronic after dilution. This means that PCT is more affected by the concentration of Pluronic. This table also shows that the highest GT relates to P1CL1, P1CM1 and P1CH1 while, the lowest ones are P3CL1, P1CM3 and P1CH3 and the best concentration from rheological behavior point of view and PCT before and after dilution of gels are P1CL1, P1CM1 and P1CH1. Figure 4 (a,b,c**)** shows the effect of different concentrations of chitosan and Figure.5 (a,b,c) the effect of different Mw of chitosan on lomefloxacin release from *in situ* gels containing 15% Pluronic F127. As these figures indicate increasing the concentration of chitosan from 0.1% to 0.3% and also its Mw from low to high Mw decreases the rate of drug release. Table 4 summarizes the release parameters i.e. % of drug release from *in situ* gels containing different concentrations and Mw of chitosan and 15% Pluronic F127. As this table shows the lowest mean release time is seen in gels containing 0.1% high Mw of chitosan and the highest one is related to the same type of chitosan but in 0.2% concentration. The greatest %drug release is seen in gels with 0.1% low Mw of chitosan and the least in gels containing 0.3% of high Mw of chitosan. Release kinetic models are shown in Table 4. From the results of release tests, it

showed that P1CL1 formulation with the highest drug release 93 % is the most suitable gel. It can extend the drug release in the eye and meanwhile shows acceptable flow properties (Newtonian flow before dilution by phosphate buffer and pseudoplastic flow after dilution). The results of release test are shown in Figures (6,7). As these profiles indicate increasing the chitosan concentration (Figure 6) or chitosan Mw (Figure **7**) in all gels reduces the release rate of lomefloxacin as the penetration rate of water decreases in higher viscosities of the gels. Lastly formulations were evaluated for the stability studies for 42 days. Results reveal that no changes were found in Visual appearance, Clarity and p^H. These formulations were also analyzed for % drug remaining. This study showed that there was no definite change observed in the intactness of the drug after accelerated study of 42 days.

Table 3: Comparison of phase change temperature (PCT) of *in situ* ophthalmic gels of Lomefloxacin HCl

Code	PCT* Before dilution (ºC)	PCT* After Dilution (ºC)
P1	39.0 ± 0.0	43.30 ± 1.0
P1CH1	36.0 ± 0.2	37.0 ± 0.5
P1CH2	33.5 ± 1.0	34.5 ± 1.4
P1CH3	34.0 ± 0.0	34.0 ± 0.8
P1CM1	36.5 ± 0.4	37.0 ± 0.5
P1CM2	33.5 ± 0.6	34.5 ± 0.8
P1CM3	33.0 ± 0.5	34.0 ± 0.5
P1CL1	37.0 ± 0.1	37.0 ± 0.0
P1CL2	34.0 ± 0.1	34.5 ± 0.2
P1CL3	33.5 ± 2.3	34.0 ± 1.1
P2	28.0 ± 1.8	33.0 ± 1.0
P2CH1	26.0 ± 0.1	26.5 ± 2.0
P2CH2	25.0 ± 0.2	26.0 ± 1.1
P2CH3	25.5 ± 0.0	27.0 ± 1.1
P2CM1	26.5 ± 0.0	26.5 ± 1.4
P2CM2	25.5 ± 0.2	26.0 ± 1.2
P2CM3	25.5 ± 1.0	27.0 ± 1.1
P2CL1	27.0 ± .1	26.5 ± 0.8
P2CL2	26.0 ± .5	26.0 ± 1.1
P2CL3	25.5 ± 0.3	21.0 ± 1.1
P3CH1	20.0 ± 0.2	21.0 ± 1.1
P3CH2	19.0 ± 0.6	20.0 ± 1.7
P3CH3	18.0 ± 0.2	19.0 ± 2.8
P3CM1	20.0 ± 0.5	21.0 ± 1.8
P3CM2	19.0 ± 0.0	20.0 ± 1.8
P3CM3	18.0 ± 0.7	19.0 ± 1.8
P3CL1	20.0 ± 0.7	21.0 ± 1.7
P3CL2	19.5 ± 0.3	20.0 ± 1.1

P3CL3	18.5 ± 0.1	19.0 ± 1.7

Note:- *PCT= phase change temperature

Table 4: Release parameters of *in situ* ophthalmic gels of lomefloxacin HCl

Code	% Release	n	k	Model fit
P1CH1	41.952	0.8943	0.348	Matrix
P1CH2	56.715	0.4101	5.9371	1st order
P1CH3	64.313	0.7849	0.9293	1st order
P1CM1	82.380	0.805	0.140	1st order
P1CM2	73.882	0.045	6.649	Matrix
P1CM3	72.210	1.077	5.123	Matrix
P1CL1	93.243	1.164	0.248	Hixcrow
P1CL2	65.086	1.053	0.484	1st order
P1CL3	63.311	0.936	0.284	1st order

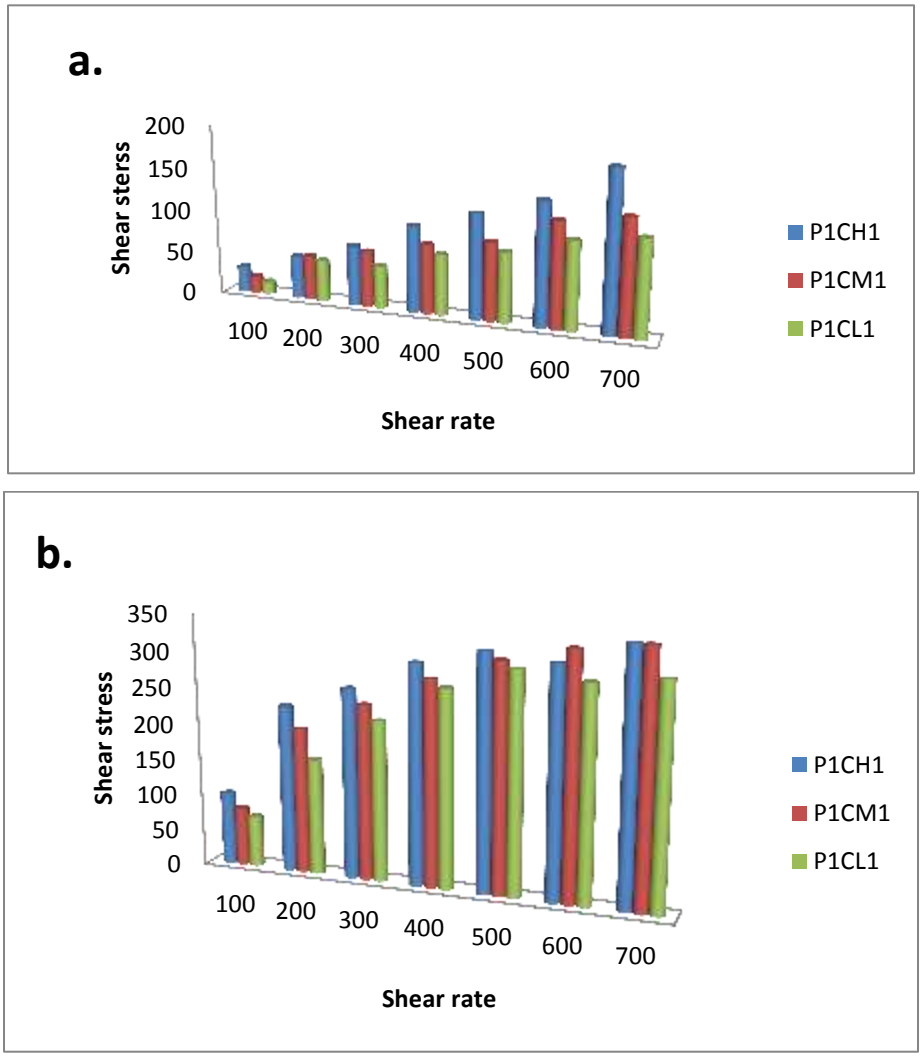


Figure 1: Rhegogram of *in situ* gels containing 15% Pluronic F127 and 0.1% chitosan with different molecular weights in (a) nonphysiologic conditions (pH 4 and 25ºC) and (b) physiologic conditions (pH 7.4 and 37ºC).

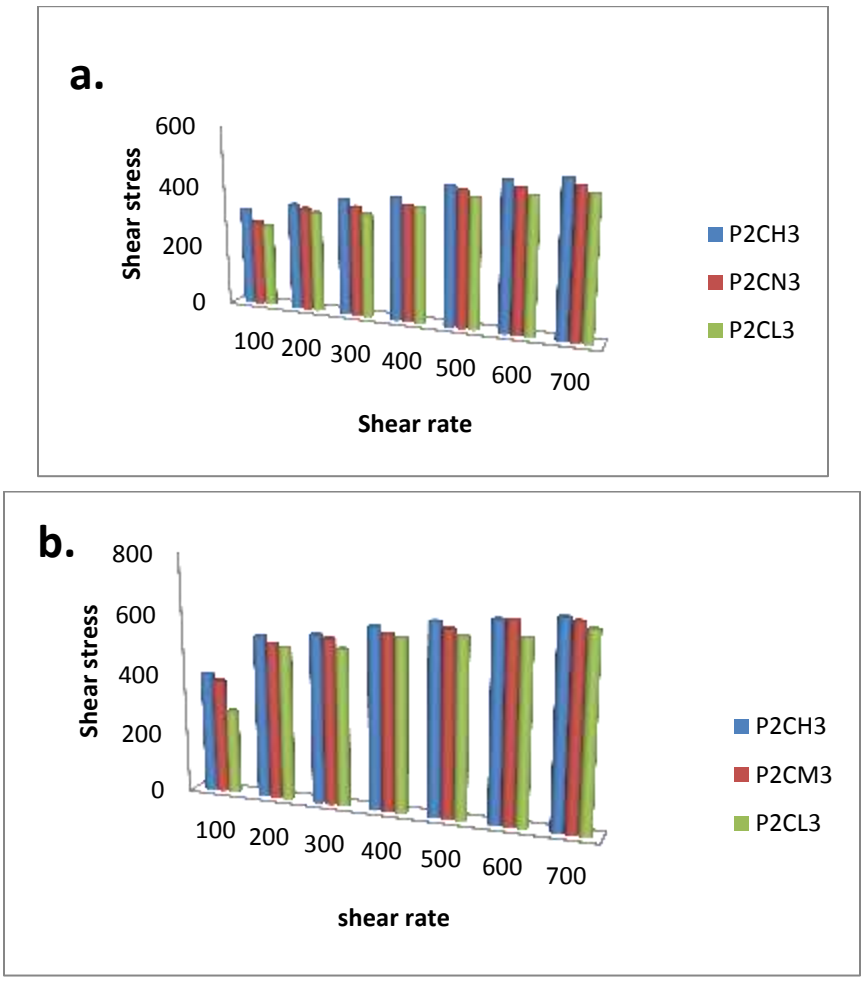
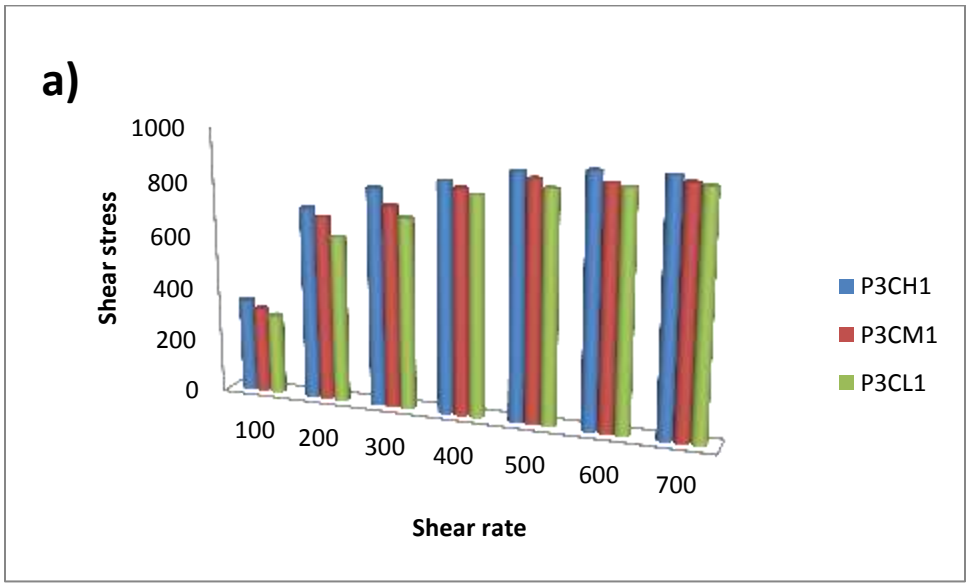


Figure 2: Rheogram of *in situ* gels containing 20% Pluronic F127 and 0.3% chitosan with different molecular weights in (a) nonphysiologic conditions (pH 4 and 25ºC) and (b) physiologic conditions (pH 7.4 and 37ºC).



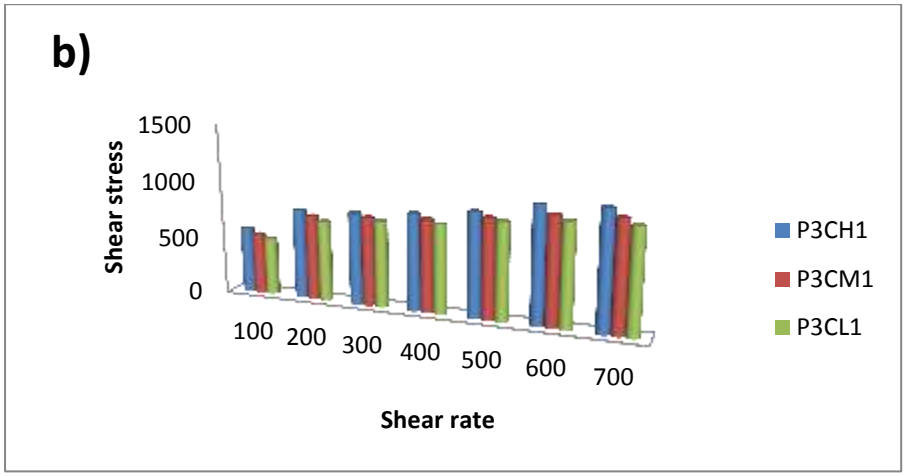
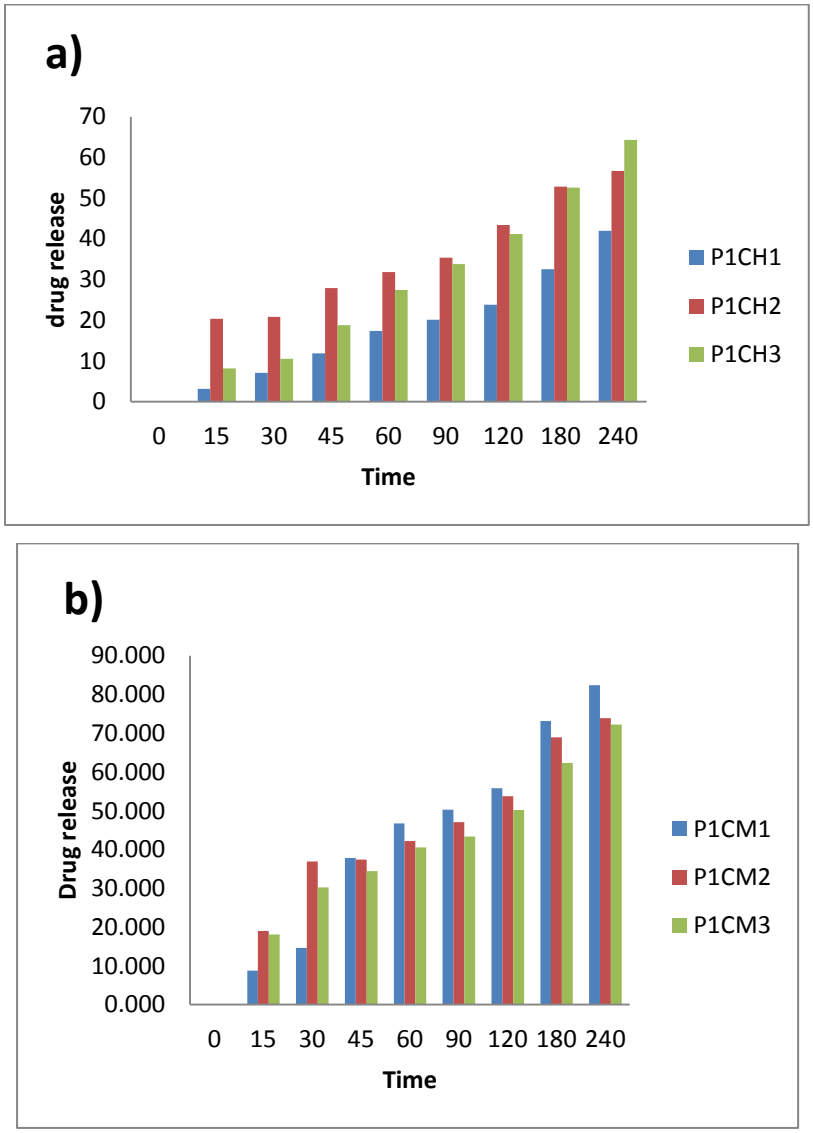


Figure 3: Rhegogram of *in situ* gels containing 25% Pluronic F127 and 0.1% chitosan with different molecular weights in (a) nonphysiologic conditions (pH 4 and 25ºC) and (b) physiologic conditions (pH 7.4 and 37ºC)



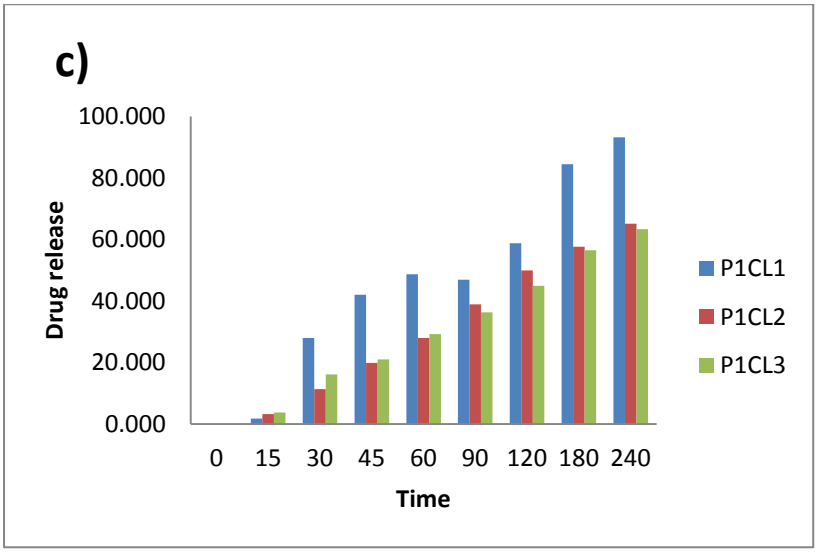
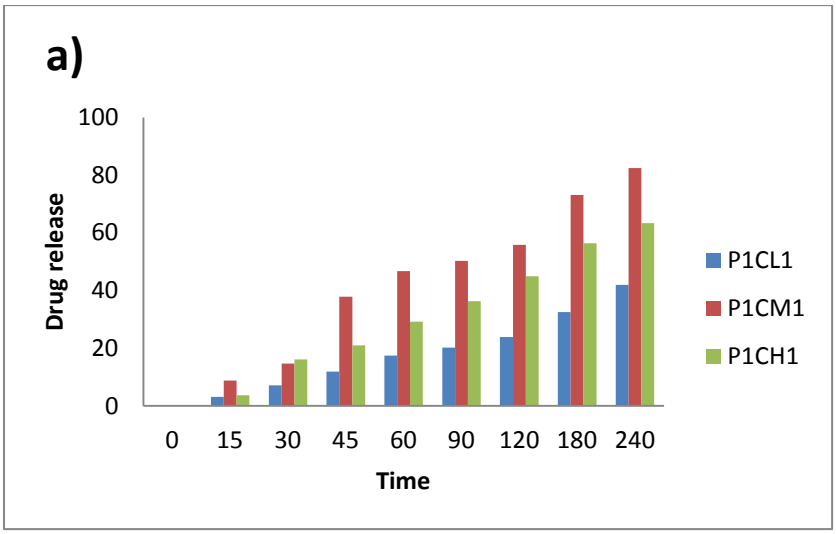
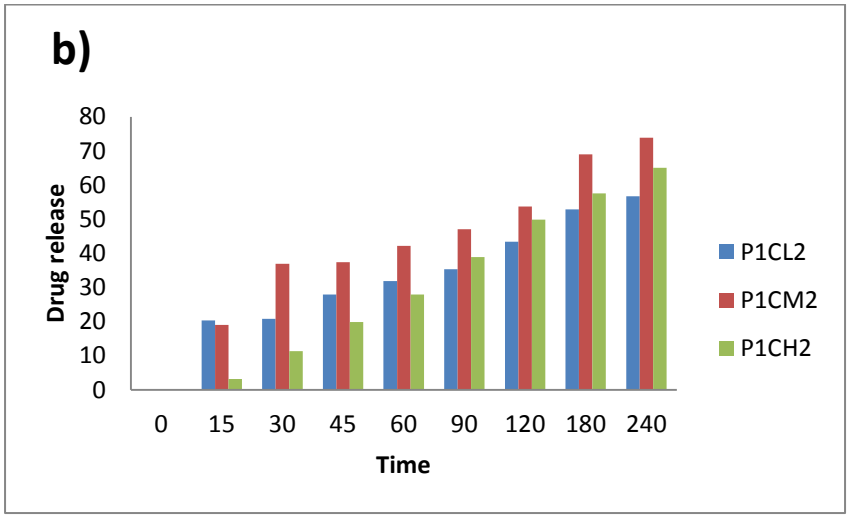


Figure 4: Effect of different concentrations of chitosan on lomefloxacin release from *in situ* gels containing 15% Pluronic F127 and (a)Low Mw, (b) Medium Mw and (c) High Mw of chitosan





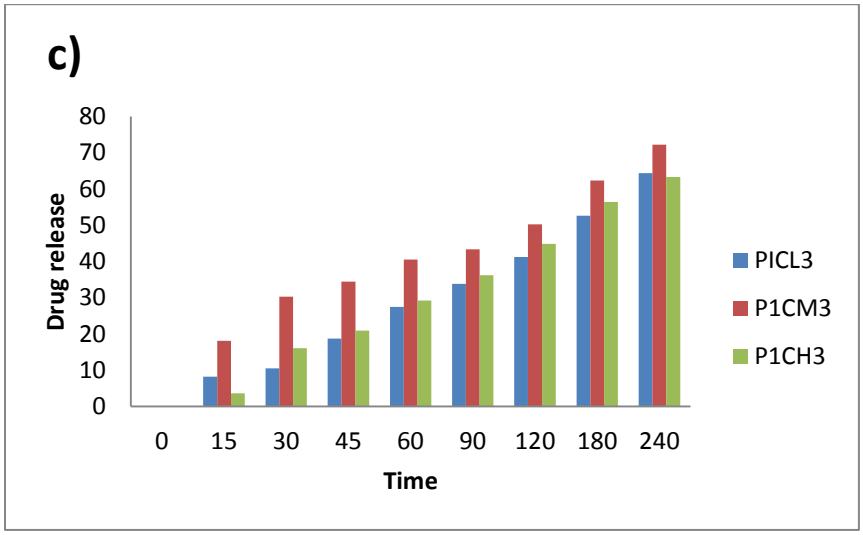


Figure 5: Effect of different Mw of chitosan on lomefloxacin release from *in situ* gels containing 15% Pluronic F127 and (a) 0.1%, (b) 0.2% and (c) 0.3% of chitosan . Results are represented as mean ± SD.

CONCLUSION

Lomefloxacin HCl (0.3% w/v%), a fluroquinolone and broad-spectrum antibacterial agent used in the treatment of ocular infections, was successfully formulated in thermo-responsive *In situ* gel-forming eye-drop using 15% Pluronic F127 as the gelling agent and 0.1% low molecular weight of chitosan as a viscosity enhancing agent. The formulation was liquid in non-physiologic conditions (pH 4 and 25ºC) and transferred to the gel form upon physiologic conditions (pH 7.4 and 37ºC). The PCT of *in situ* gel did not change upon dilution and it afforded sustained drug delivery over an 8 hr period. The developed formulation is a viable alternative to conventional eye drop by virtue of its ability to enhanced and longer antibacterial effect through its longer precorneal residence time and ability to sustain drug release.

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