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

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Abstract: To increase the low bioavailability and short ocular residence time of ciprofloxacin 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 behavior. Mixtures of solutions of Pluronic (10-25% w/w) with chitosan (0.1-0.3% w/w) of different molecular weights (Mw) were prepared. Ciprofloxacin release was determined using a membraneless dissolution model in artificial tear solution up to 8 hours and the samples were analyzed spectrophotometrically at 272.4nm. The rheological behavior of solutions in response to dilution or temperature changes and also the phase change temperature (PCT) were determined using a Cup & Bob viscometer. Antimicrobial effect of the solutions was studied in nutrient agar in comparison to marketed solutions of ciprofloxacin using *Pseudomonas aueroginosa* and *Staphylococcus aureus* by the agar diffusion test using the cup-plate technique. The formulation consisted of 15% Pluronic and 0.1% low Mw chitosan, with the highest release efficiency (46.61 ± 0.41%) and an acceptable mean release time (1.94 ± 0.27 hr), is suggested as a suitable ophthalmic preparation for sustained release of ciprofloxacin. This *in situ* gel released the drug by a Higuchi model and Fickian mechanism. 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 and the zone of inhibition of the growth of both studied bacteria was significantly greater for it than the marketed eye drop of ciprofloxacin.

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

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* gel formation [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 thermosensitive 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 behaviors 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 tempera-

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ture [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 lacrimal fluid. 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]. Different gel 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].

Sol-to-gel systems of ciprofloxacin hydrochloride were prepared utilizing the phase transition properties of hydroxy propyl methyl cellulose K15M and carbopol 934 [31]. This drug was also formulated in Gelrite gellan gum vehicle that gels in the presence of mono or divalent cations, present in the lacrimal fluid alone and in combinations with sodium alginate as the gelling agent [32]. Poloxamer / chondroitin 6-sulfate were also used to sustain the release of ciprofloxacin [33].

Chitosan, a polysaccharide derived from naturally abundant chitin, is currently receiving a great deal of interest. Chenite *et al*. [34] developed a novel approach to produce thermosensitive neutral hydrogel based on chitosan/polyol salt combinations that could undergo sol-gel transition at a temperature close to 37 °C. Gupta *et al*. [35] 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. General factorial design is a suitable way to study the effect of studied variables and all their possible interactions on the studied parameters. The general factorial design allows the formulator to have factors that each has a different number of levels. It will create an experiment that includes all possible combinations of studied factor levels. Ciprofloxacin 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].

Flouroquinolones and more specifically topical ciprofloxacin supplanted the other antibiotics and became the gold standard for treating ocular infections caused by *Pseudomona aeruginosa* [37]. The purpose of the present study is to develop a sustained release, ophthalmic delivery system of ciprofloxacin hydrochloride with more residence time in the eye. While designing this formulation, to find the effects of different molecular weights and concentrations of chitosan and poloxamer, a general factorial design was used in development of the thermosensitive *in situ* gels.

MATERIALS AND METHODS

Materials

Plorunic (F127) obtained from (Sigma, USA) was used as received. Chitosan (Low MW 150000, Medium MW 400000 and High MW 600000) (Fluka, Switzerland), Ciprofloxacin HCl was supplied by Arya Pharmaceutical Co. Ltd. (Iran). All other chemicals and solvents were reagent grade and from Merck chemical Company (Germany).

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 (15-25% 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 for 1 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 ciprofloxacin-containing polymer solutions, 0.3% of ciprofloxacin was added to the chitosan/Pluronic solutions with continuous stirring until thoroughly mixed. Benzalkonium chloride solution was added 0.006% 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 20 min 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** by Design Expert 6.0.10 program.

Table 1. Variables and their Levels Used in Production of the *In Situ* Ophthalmic Gels of Ciprofloxacin

Levels			Variable
III	II	I	
0.3%	0.2%	0.1%	Chitosan concentration
L	M	H	Chitosan molecular weight
25%	20%	15%	Pluronic concentration

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 of 500 rpm (Ruhromag, Germany). 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 rheological studies were carried out in a cup (measuring tube: No.#3, diameter: 15.18mm) and bob (measuring

Table 2. Composition of the *In Situ* Ophthalmic Gels of Ciprofloxacin All Containing 0.006% Benzalkonium Chloride and 0.3% Drug (P=Pluronic, CH=Chitosan High Mw, CM=Chitosan Medium Mw, CL=Chitosan Low Mw)

Code	Concentration (%)			
	P	CH	CM	CL
P_1	15			
P_1CH_1	15	0.1		
P_1CH_2	15	0.2		
P_1CH_3	15	0.3		
P_1CM_1	15		0.1	
P_1CM_2	15		0.2	
P_1CM_3	15		0.3	
P_1CL_1	15			0.1
P_1CL_2	15			0.2
P_1CL_3	15			0.3
P_2CH_1	20	0.1		
P_2CH_2	20	0.2		
P_2CH_3	20	0.3		
P_2CM_1	20		0.1	
P_2CM_2	20		0.2	
P_2CM_3	20		0.3	
P_2CL_1	20			0.1
P_2CL_2	20			0.2
P_2CL_3	20			0.3
P_3CH_1	25	0.1		
P_3CH_2	25	0.2		
P_3CH_3	25	0.3		
P_3CM_1	25		0.1	
P_3CM_2	25		0.2	
P_3CM_3	25		0.3	
P_3CL_1	25			0.1
P_3CL_2	25			0.2
P_3CL_3	25			0.3

bob: No.#3, diameter: 14mm) viscometer (Mettler, Model RM180, Germany). The viscosity and shear stress of the sample solutions were measured at various shear rates at 25°C and 37°C, respectively. The temperature was maintained within ± 0.1°C by a recirculating bath connected to the sample cup of viscometer. The 9 ml samples were equilibrated in the cup for 5 min to reach the running temperature prior to each measurement. The behavior of the gels was studied in two conditions: in the physiologic (37°C and pH 7.4) and non-physiologic (25°C and pH 4) conditions. An ideal gel should show a Newtonian flow in non-physiological condition while, pseudoplastic properties at physiological conditions [28]. In order to simulate the physiological disposition of gels more literally, the polymer solutions were diluted by simulated tear fluid (STF) in a ratio of 40:7 [25] and then adjusted to physiological pH value (7.4 ± 0.1) by adding the required amount of sodium hydroxide before the rheological studies were conducted at 37 ± 0.1 °C.

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 increased 4°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 (40 µl) was immediately mixed with the available tear fluid (7 µl), which would be the worst case scenario, the polymer solution was mixed with simulated tear fluid 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 ciprofloxacin-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 STF (pH 7.4), composition: NaCl 0.67 g, $NaHCO_3$ 0.20 g, $CaCl_2$, $2H_2O$ 0.008 g, and distilled, deionized water to 100 g) and placed in a circulating water bath equipped with stirring rods to stir the release medium. The temperature and stirring rate were 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 STF solution. The samples were filtered through 0.45-mm syringe filters and subjected to spectrophotometric analysis (Shimadzu, Model 1240 CE, Japan) to determine the ciprofloxacin concentrations in λ_{max} 272.4 nm. Each time a blank gel was studied at the same conditions to omit the probable absorption of the base.

Two dimensionless parameters were used to compare the release data; Mean release time (MRT) [39]. Release efficiency until 8 hr (RE_8%) [40].

$$MRT = \frac{\sum_1^n t\,mid \cdot \Delta C}{\sum_1^n \Delta C} \qquad \text{eq. 1}$$

$$RE8\% = \frac{\int_0^t y100 \cdot t}{100 \times t} \qquad \text{eq. 2}$$

In which *i* is the release sample number, *n* is the number of release sample time, t_{mid} is the time at the midpoint between *i* and *i-1* and ΔC is the additional concentration of drug released between *i* and *i-1* [39].

Drug release kinetics was studied by curve fitting method to different kinetic models of zero order, first order or Higuchi models:

(i) *Zero-order* release:

$$\frac{M_t}{M_\infty} = kt \qquad \text{eq. 3}$$

(ii) *First-order* release:

$$\ln\left(1 - \frac{M_t}{M_\infty}\right) = -kt \qquad \text{eq. 4}$$

The *Higuchi* square root of time model has been derived from Fick's first law of diffusion and is suited for the modeling of drug release from a homogeneous planar matrix, assuming that the matrix does not dissolve:

$$\left(\frac{M_t}{M_\infty}\right)^2 = kt \qquad \text{eq. 5}$$

The Korsmeyer-Peppas equation:

$$M_t/M_\infty = kt^n \qquad \text{eq. 6}$$

Was used to study the drug release mechanism by analyzing n as the diffusion exponent. According to this equation if $n \leq 0.45$ the Fickian mechanism, $0.5 \leq n \leq 0.8$ the Non-Fickian and if $0.8 \leq n \leq 1$ a zero-order mechanism is governing the drug release mechanism from the gels [41].

Antimicrobial Efficacy Studies

This test was carried out by the agar diffusion test using the cup-plate technique. Marketed eye-drop of ciprofloxacin (standard preparation) and the developed formulations, were poured into cups bored into sterile nutrient agar previously seeded with *Pseudomonas aueroginosa* ATCC 27853 (*P. aueroginosa*) and *Staphylococcus aureus* ATCC 25923 (*S. aureus*). After allowing diffusion of the solutions for 2 hr, the agar plates were incubated at 37 ± 0.5ºC for 24 hr. The zone of inhibition measured around each cup was compared with that of control. The entire operation except the incubation was carried out in a laminar flow unit. Each solution was tested in triplicate. Both positive and negative controls were maintained through the study.

Statistical Analysis

Differences in drug release parameters from *in situ* gels were statistically analyzed by two-way analysis of variance (ANOVA). Statistically significant differences between *in vitro* drug release of formulations were defined as $p<0.05$.

RESULTS

Figs. (**1-3**) shows the effect of 0.1% of different Mw of chitosan in combination with 15%, 20% and 30% of Pluronic in physiologic and non-physiologic conditions. As Figs. (**1**) and (**2**) indicate both 15% and 20% of Pluronic show Newtonian and pseudoplastic flow in non-physiologic and physiologic conditions respectively. However, when concentration of Pluronic is increased to 30% (Fig. **3**) the gels show a non-Newtonian flow in both non-physiologic and physiologic conditions which is inappropriate for instillation in the eye.

Figs. (**4**) and (**5**) show phase change temperature (PCT) of *in situ* gels containing 15% and 25% of Pluronic F127, different concentrations and different Mw of chitosan.

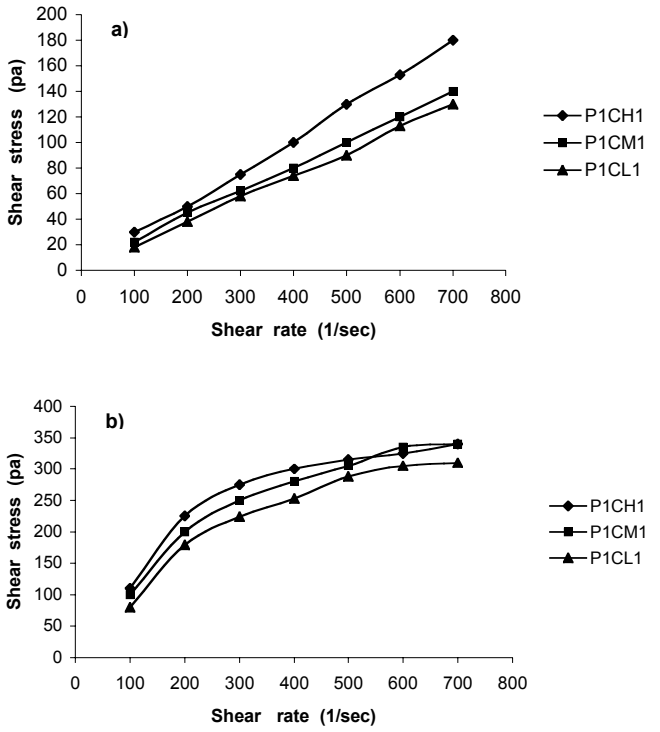

Fig. (1). Rhegogram of *in situ* gels containing 15% Pluronic F127 and 0.1% chitosan with different molecular weights in (**a**) non-physiologic conditions (pH 4 and 25ºC) and (**b**) physiologic conditions (pH 7.4 and 37ºC).

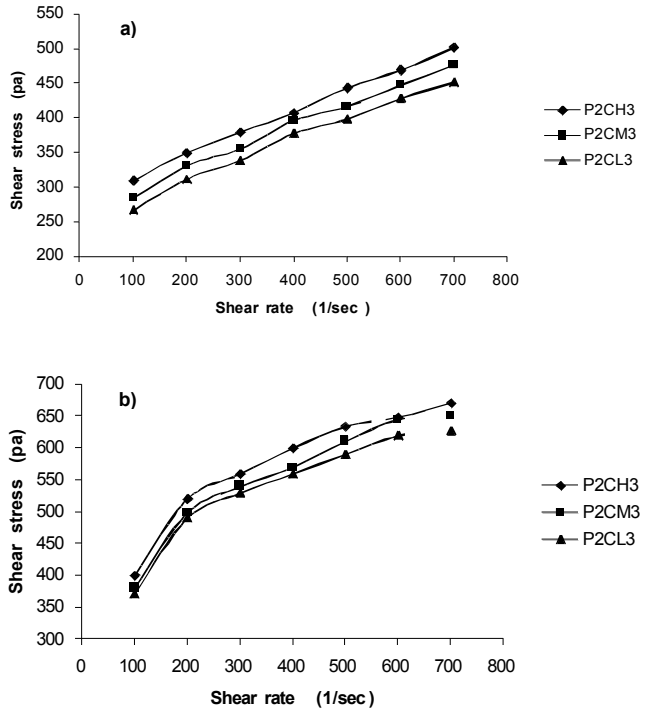

Fig. (2). Rhegogram of *in situ* gels containing 20% Pluronic F127 and 0.3% chitosan with different molecular weights in (**a**) non-physiologic conditions (pH 4 and 25ºC) and (**b**) physiologic conditions (pH 7.4 and 37ºC).

As these two figures indicate the gels with 25% Pluronic show sharper PCT around 20°C while those with 15% Pluronic have greater PCT at about 32-37°C. In Table **3** the

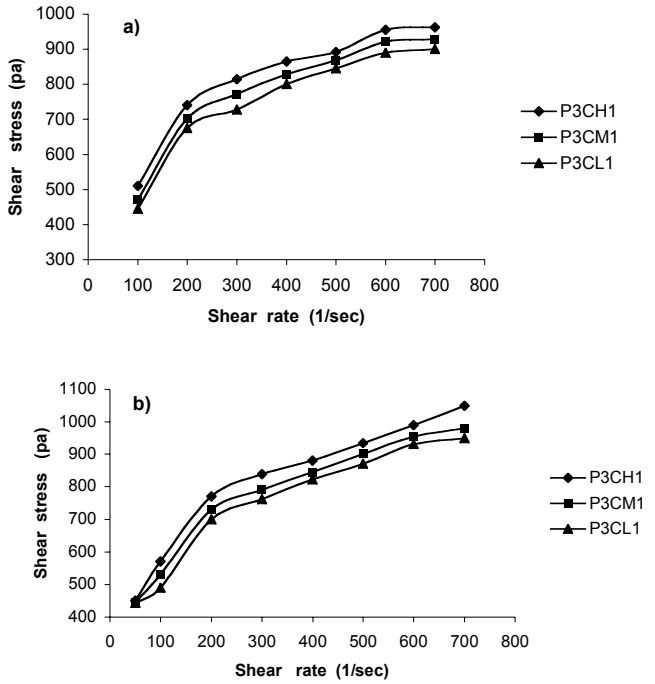

Fig. (3). Rhegogram of *in situ* gels containing 25% Pluronic F127 and 0.1% chitosan with different molecular weights in (**a**) non-physiologic conditions (pH 4 and 25ºC) and (**b**) physiologic conditions (pH 7.4 and 37ºC).

results of PCT before and after dilution of the gels with the artificial tear are compared. All of the formulations show higher PCT after dilution which indicates their increased viscosity by dilution in the eye. Fig. (**6**) shows the effect of different concentrations of chitosan and Fig. (**7**) the effect of different Mw of chitosan on ciprofloxacin release from *in situ* gles containing 1% 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., MRT and RE_8% 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 RE_8% 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 **5**. As this table indicates the correlation coefficient of release data fitted to Higuchi model in most cases is higher than other models and in all *in situ* gels the diffusion exponent of Peppas equation is less than 0.5 which indicates a Fickian mechanism is dominant and controls the drug release from the gels.

To study the simultaneous effect of the Mw and concentration of chitosan on the release parameters of ciprofloxacin from gels with 15% Pluronic three dimensional Figs. (**8**) and (**9**) were plotted.

From the results of release tests, and using MRT and RE in their maximum levels, the software of Design Expert

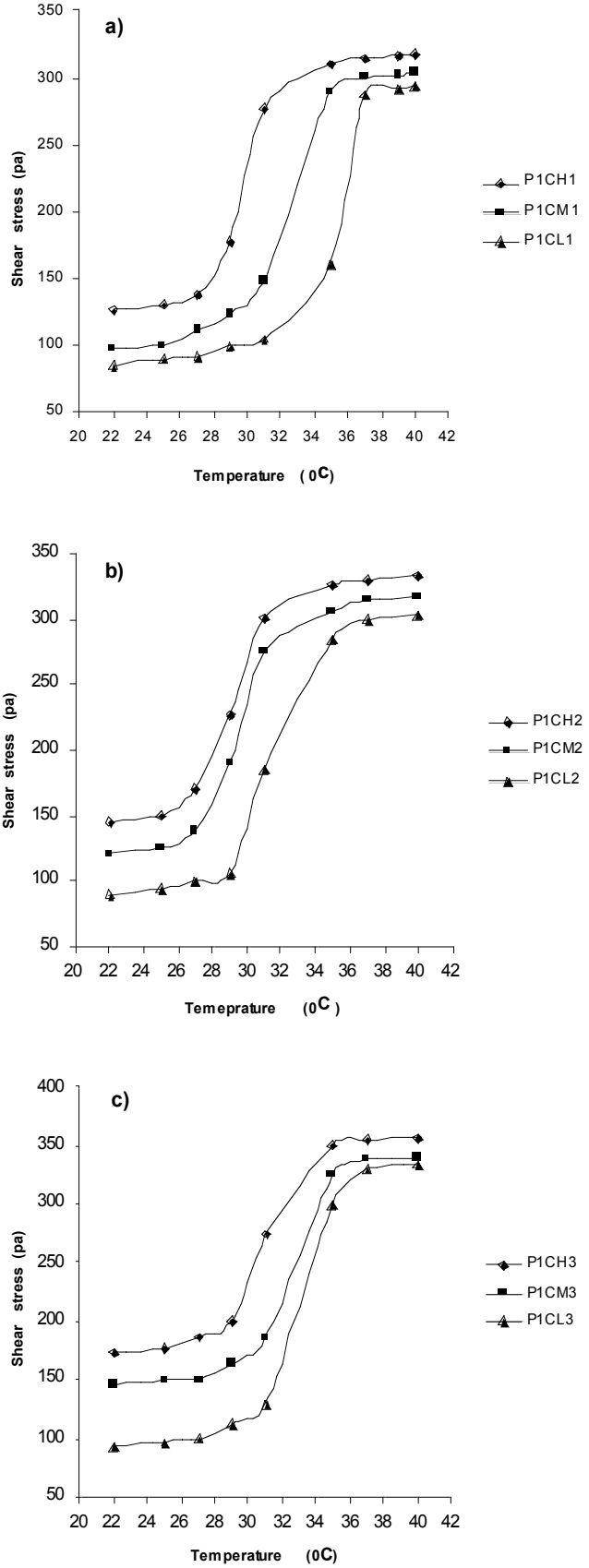

Fig. (4). Phase Change Temperature (PCT) of *in situ* gels containing 15% Pluronic F127 and a) 0.1%, b) 0.2% and c) 0.3% of different Mw of chitosan.

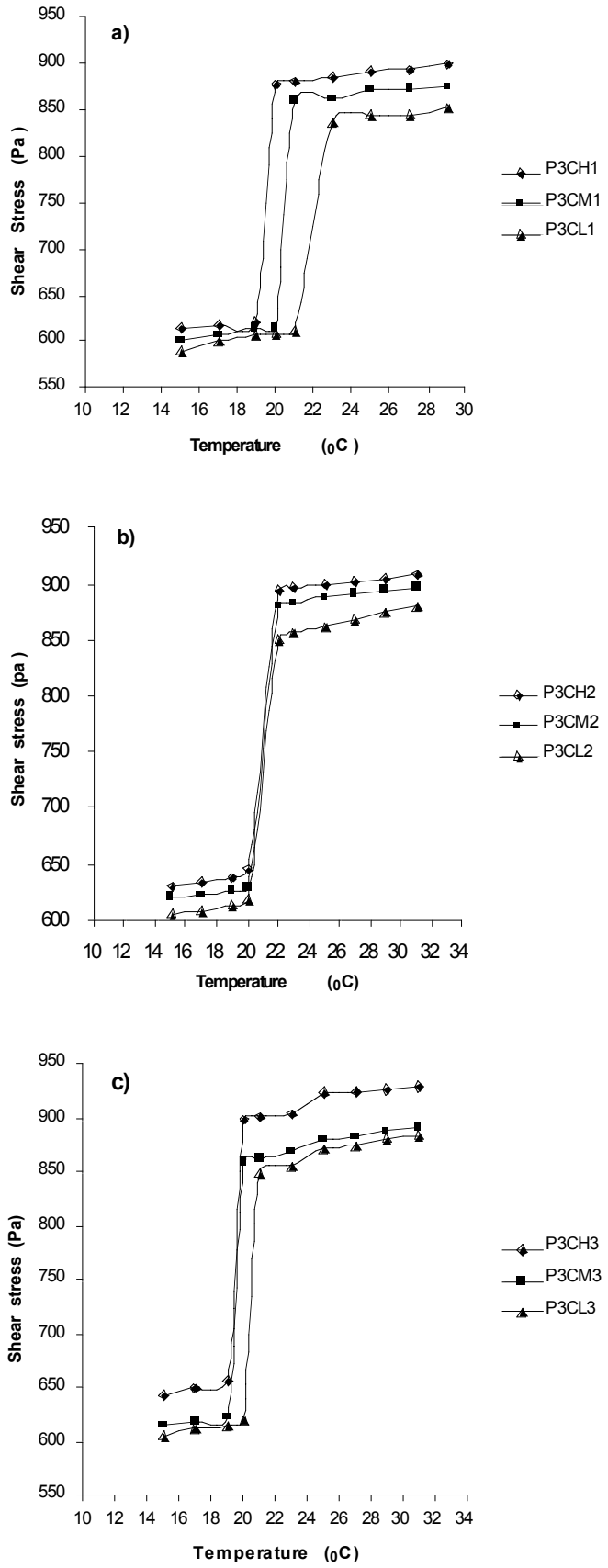

Fig. (5). Phase Change Temperature (PCT) of *in situ* gels containing 25% Pluronic F127 and (**a**) 0.1%, (**b**) 0.2% and (**c**) 0.3% of different Mw of chitosan.

predicted that P_1CL_1 formulation with the highest release efficiency (RE) and the greatest mean release time (MRT) is the most suitable gel with 0.733 desirability value. It can extend the drug release in the eye and meanwhile shows acceptable flow properties (Newtonian flow before dilution by the tear and pseudoplastic flow after dilution) was chosen for further studies from anti-microbial point of view. This effect was studied by comparing the zone of inhibition (ZOI) of the growth of *Pseudomonas aueroginosa* and *Staphylococcus aureus* in the marketed eye drop of ciprofloxacin and the designed *in situ* gel (Table **6**). As this table shows for both bacteria the ZOI is significantly greater in P_1CL_1 gel.

Table 3. Comparison of phase change temperature (PCT) of *in situ* ophthalmic gels of ciprofloxacin before and after dilution with simulated tear fluid. (n=3). Results are represented as mean ± SD.

Code	PCT Before Dilution (ºC)	PCT After Dilution (ºC)
P_1	39.0 ± 0.0	43.30 ± 1.0
P_1CH_1	36.0 ± 0.2	37.0 ± 0.5
P_1CH_2	33.5 ± 1.0	34.5 ± 1.4
P_1CH_3	34.0 ± 0.0	34.0 ± 0.8
P_1CM_1	36.5 ± 0.4	37.0 ± 0.5
P_1CM_2	33.5 ± 0.6	34.5 ± 0.8
P_1CM_3	33.0 ± 0.5	34.0 ± 0.5
P_1CL_1	37.0 ± 0.1	37.0 ± 0.0
P_1CL_2	34.0 ± 0.1	34.5 ± 0.2
P_1CL_3	33.5 ± 2.3	34.0 ± 1.1
P_2	28.0 ± 1.8	33.0 ± 1.0
P_2CH_1	26.0 ± 0.1	27.0 ± 1.7
P_2CH_2	25.0 ± 0.2	26.5 ± 2.0
P_2CH_3	25.5 ± 0.0	26.0 ± 1.1
P_2CM_1	26.5 ± 0.0	27.0 ± 1.1
P_2CM_2	25.5 ± 0.2	26.5 ± 1.4
P_2CM_3	25.5 ± 1.0	26.0 ± 1.2
P_2CL_1	27.0 ± .1	27.0 ± 1.1
P_2CL_2	26.0 ± .5	26.5 ± 0.8
P_2CL_3	25.5 ± 0.3	26.0 ± 1.1
P_3CH_1	20.0 ± 0.2	21.0 ± 1.1
P_3CH_2	19.0 ± 0.6	20.0 ± 1.7
P_3CH_3	18.0 ± 0.2	19.0 ± 2.8
P_3CM_1	20.0 ± 0.5	21.0 ± 1.8
P_3CM_2	19.0 ± 0.0	20.0 ± 1.8
P_3CM_3	18.0 ± 0.7	19.0 ± 1.8
P_3CL_1	20.0 ± 0.7	21.0 ± 1.7
P_3CL_2	19.5 ± 0.3	20.0 ± 1.1
P_3CL_3	18.5 ± 0.1	19.0 ± 1.7

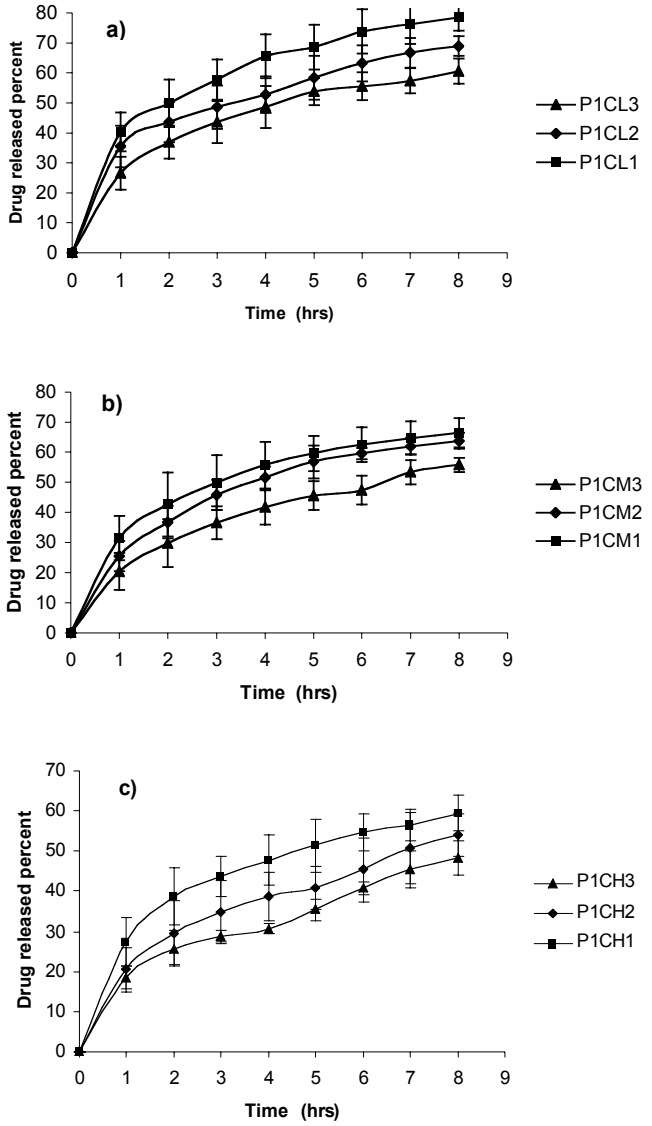

Fig. (6). Effect of different concentrations of chitosan on ciprofloxacin release from *in situ* gels containing 15% Pluronic F127 and (**a**) Low Mw, (**b**) Medium Mw and (**c**) High Mw of chitosan (n=3). Results are represented as mean ± SD.

Table 4. Ciprofloxacin Release Parameters from *In Situ* Ophthalmic Gels in Simulated Tear Fluid (MRT= Mean Release Time, RE_8%= Release Efficiency Up to 8 hr). (n=3). Results are Represented as Mean ± SD

Code	MRT (hr)	RE_8 (%)
P_1CH_1	1.94 ± 0.27	46.61 ± 0.41
P_1CH_2	2.61 ± 0.30	37.22 ± 4.18
P_1CH_3	2.19 ± 0.28	31.17 ± 2.85
P_1CM_1	2.28 ± 0.17	48.82 ± 7.68
P_1CM_2	2.29 ± 0.26	45.50 ± 2.04
P_1CM_3	2.59 ± 0.47	39.61 ± 6.04
P_1CL_1	2.30 ± 0.15	59.80 ± 1.37
P_1CL_2	2.53 ± 0.29	48.33 ± 5.84
P_1CL_3	2.16 ± 0.28	44.08 ± 4.96

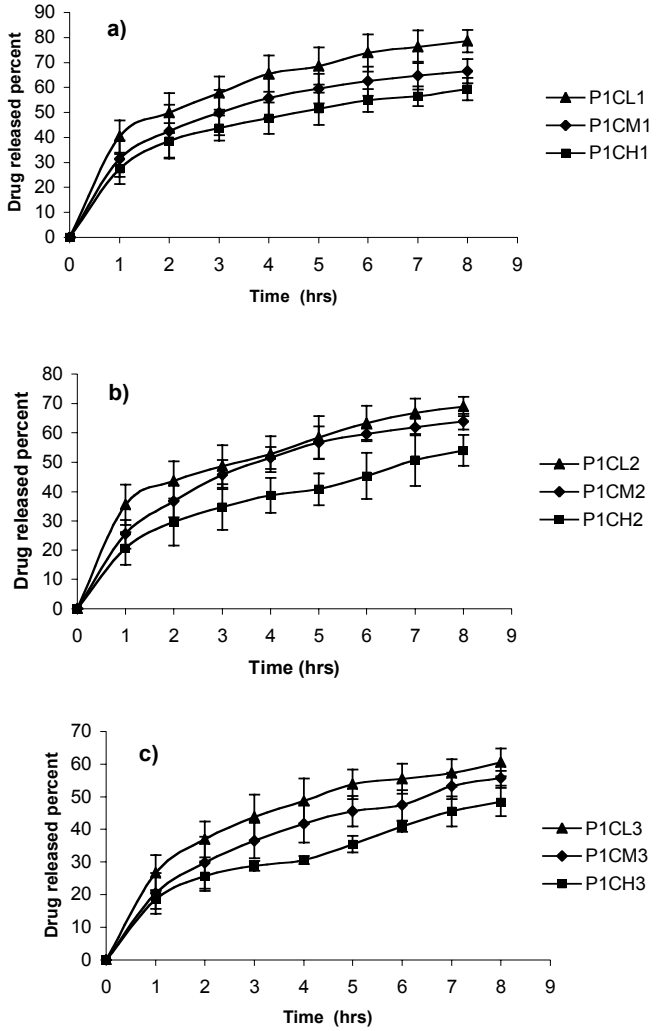

Fig. (7). Effect of different Mw of chitosan on ciprofloxacin release from *in situ* gels containing 15% Pluronic F127 and (**a**) 0.1%, (**b**) 0.2% and (**c**) 0.3% of chitosan (n=3). Results are represented as mean ± SD.

Table 5. Correlation Coefficient of Ciprofloxacin Release from *In Situ* Ophthalmic Gels by Different Kinetic Models Obtained from Curve Fitting Method. n Shows the Diffusion Exponent of Korsmeyer-Peppas Equation ($M_t/M_\infty = kt^n$). Results are Represented as Mean ± SD

Code	Zero Order	First Order	Higuchi Model	n
P_1CH_1	0.88 ± 0.10	0.93 ± 0.09	0.95 ± 0.067	0.43 ± 0.28
P_1CH_2	0.96 ± 0.03	0.98 ± 0.02	0.98 ± 0.01	0.44 ± 0.06
P_1CH_3	0.97 ± 0.02	0.97 ± 0.02	0.96 ± 0.03	0.40 ± 0.06
P_1CM_1	0.90 ± 0.07	0.94 ± 0.04	0.95 ± 0.03	0.49 ± 0.13
P_1CM_2	0.90 ± 0.05	0.94 ± 0.05	0.96 ± 0.03	0.49 ± 0.09
P_1CM_3	0.94 ± 0.04	0.97 ± 0.03	0.96 ± 0.05	0.36 ± 0.08
P_1CL_1	0.94 ± 0.05	0.98 ± 0.01	0.98 ± 0.00	0.38 ± 0.44
P_1CL_2	0.97 ± 0.01	0.97 ± 0.03	0.98 ± 0.07	0.34 ± 0.04
P_1CL_3	0.89 ± 0.05	0.94 ± 0.04	0.96 ± 0.03	0.33 ± 0.05

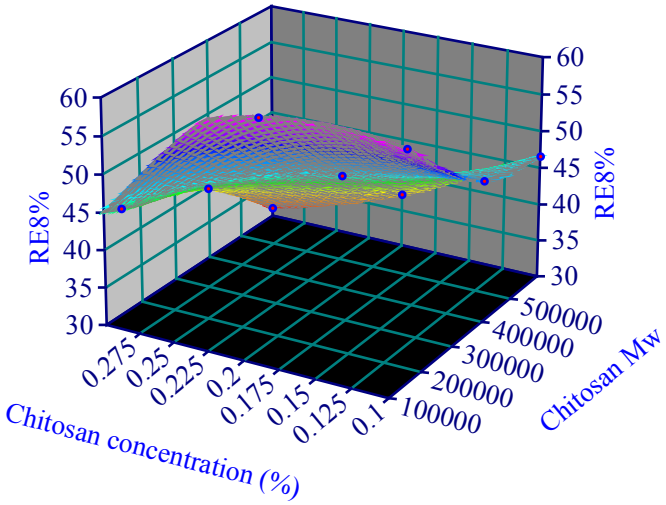

Fig. (8). Effect of different Mw and concentrations of chitosan on RE_8% of ciprofloxacin from *in situ* gels containing 15% Pluronic.

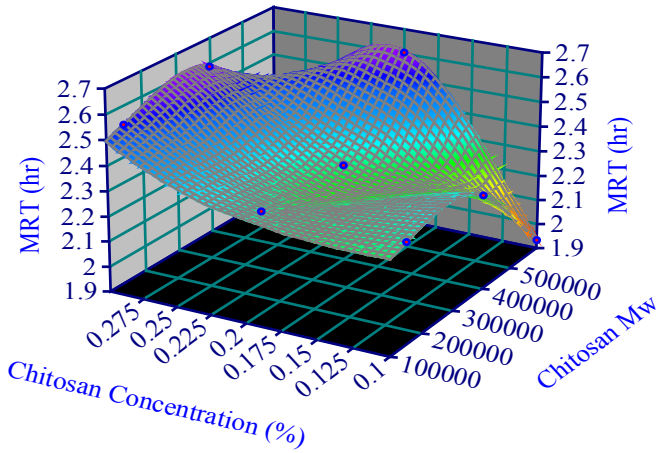

Fig. (9). Effect of different Mw and concentrations of chitosan on MRT of ciprofloxacin from *in situ* gels containing 15% Pluronic.

Table 6. Zone of Inhibition (ZOI) of *Pseudomonas auerogi-nosa* and *Staphylococcus aureus*

Formulation	ZOI (mm)	
	Pseudomonas auerginosa	***Staphylococcus aureus***
marketed eye drop	32.0 ± 1.2	31.0 ± 1.0
P_1CL_1	48.0 ± 6.1	48.0 ± 5.1

DISCUSSION

Fig. (**1**) 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 (Fig. **2**), but as Table **3** shows the phase change temperature (PCT) of these gels even before dilution with artificial tear 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 (Fig. **3**) 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. Fig. (**4**) and (**5**) typically shows the changes of shearing stress with temperature and behavior of gels in PCT. Fig. (**4a**) shows a rapid phase change around 37ºC for gel containing 15% Pluronic and 0.1% low Mw chitosan. However, Fig. (**5**) shows a rapid phase temperature occurs around 20ºC for all gels containing 25% of Pluronic and 0.2% of chitosan regardless of the Mw of chitosan (Fig. **5b**). For other mixtures of these two polymers lower PCTs after dilution (Fig. **5a** and **5c**) indicates that they are not suitable as their viscosity will increase and change to solid flow before use in the eye and can not be dripped.

As Table **3** indicates Pluronic will lose the gelation ability after dilution by lacrimal fluid since when it is not combined with chitosan its PCT will change significantly ($p<0.05$) 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 ($p>0.05$) between their PCT before and after dilution (for example P_1CL_1) while there is statistical significant difference ($p<0.05$) 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 P_1CL_1, P_1CM_1 and P_1CH_1 while, the lowest ones are P_3CL_1, P_1CM_3 and P_1CH_3 and the best concentration from rheological behavior point of view and PCT before and after dilution of gels are P_1CL_1, P_1CM_1 and P_1CH_1. Block copolymer gels of Pluronic F127 are thought to be formed by hydrogen bonding in aqueous systems, caused by the attraction of the Pluronic ether oxygen atom with protons of water [42]. If the hydrogen bonding is supplemented by adding compounds with hydroxyl such as cellulose derivatives or NH groups of chitosan, the desired gel strength may be achieved with reduced Pluronic concentration [42]. Yong *et al*. [43] showed GT of poloxamer solutions containing 18-25% of P407 alone or 30% of P188 was 13-25 °C and 48 °C, respectively. Their results indicated that P407 or P188 alone could not provide the suitable GT. In the cases of P407 and P188 mixtures, several formulations gelled at the physiological temperature. As the concentration of P407 increased, the mixtures needed smaller amounts of P188 to gel at the desirable GT. The w/w percentage ratios of P407/P188 with GT in the range of 30-36 °C were 9/25%, 12/20% and 15/15-15/20% [44].

The results of release test are shown in Figs. (**6**, **7**). As these profiles indicate increasing the chitosan concentration

(Fig. **6**) or chitosan Mw (Fig. **7**) in all gels reduces the release rate of ciprofloxacin as the penetration rate of water decreases in higher viscosities of the gels. All the drug release curves seem to follow a biphasic pattern, with a faster rate of drug release over the first 2h, followed by a slower and steadier rate of drug release for the remaining 6h that may be related to the drug trapped between the tortuous ways of the gels which takes longer time with a slower slop to be released. The greatest RE_8% is seen in P_1CL_1 gel and its MRT also shows a sustained release pattern (Table **4**). Fig. (**8**) shows that in concentrations of 0.1 and 0.3% of chitosan RE is almost constant in low and medium Mw and then in high Mw it suddenly reduces while in 0.2% of chitosan it will increases. However, Fig. (**9**) indicates that in all chitosan concentrations the highest MRT is seen in the low Mw and the least in high Mw. The amount of drug released was about 70% after 8h in most formulations. However, considering the MIC of ciprofloxacin for S. aureous and most other ocular infections is less than 1μg/ml [45], and as there is 0.3% ciprofloxacin in the gels, release of 70% of the drug after 8 hr seems enough for a dosage interval of 24hr.

Statistical analysis of data of Table **4** by a general factorial design showed that the effect of Mw of chitosan on the MRT and RE_8% is 55.01% and 10.08%, respectively, i.e., it affects more on the MRT. While the effect of chitosan concentration on these two parameters is 38.59% and 33.61%, respectively, i.e., chitosan concentration has similar effects on the release parameters. However, the effect of the interaction of these two variables on the MRT and RE is 6.4% and 56.31%, respectively that means RE is more affected by the interaction of the Mw and concentration of chitosan than MRT. These results indicate that the structure of the gel functioned as an increasingly resistant barrier to drug release as the concentration and Mw of chitosan increased. The mechanism for such enhanced resistance may be due to reduction in the number and dimension of water channels and to the increase in the number and size of micelles within the gel structure. The shorter intermicellar distance leads to greater numbers of cross-links between neighboring micelles leading to higher viscosity and lower rate of drug release [46, 47]. The concentration of Pluronic 127 also has an important effect on the viscosity of the gels. This assumption may be potentiated by the rheology studies that indicate direct proportionality between gel concentration and viscosity [48]. The slowest rate of drug release was obtained from the formula containing 0.3% high Mw of chitosan. This could be due to the formation of micelle junction zones between chitosan and polyethyloxylene-polypropyloxylene block copolymers (Pluronic) similar to the formation of these physical cross-links between Pluronic and methylcellulose mentioned by [49]. Chitosan is a copolymer consisting of 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose units linked with β-(1→4) bonds. Although the polymers differ in chemical structure, both have hydrophobic regions in their chains: the D-glucose residues of chitosan and polypropyloxylene block of Pluronic. Water molecules structured around the hydrophobic regions of polymer chains in a sol become disordered with increases in temperature. Newly exposed hydrophobic regions attract one another to form bonds, whereas hydrophilic areas rearrange to maximize their contact with the aqueous medium. The resulting structures are micelles, which continue to grow in size and number at higher temperatures, leading eventually to more rigid gel structure [42]. Consequently, drug release is retarded. The curve fitting method to release profiles indicated no significant difference between first order and Higuchi models (Table **5**) but analysis of release data by Peppas model showed that all gels release the drug by Fickian or diffusion mechanism (Table **5**).

Table **6** shows the zone of inhibition (ZOI) values for P_1CL_1 gel were higher than the standard preparation of ciprofloxacin eye drop for both tested micro-organisms. The higher ZOI values obtained for P_1CL_1 gel in comparison with standard eye-drop, may be attributed to the slow and prolonged diffusion of the drug from the polymeric gel due to its higher viscosity.

CONCLUSIONS

Ciprofloxacin HCl (0.3% w/v%), a fluroquinolon and broad-spectrum antibacterial agent used in the treatment of ocular infections, was successfully formulated in thermoresponsive *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 percorneal residence time and ability to sustain drug release.

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