

## Preparation and Evaluation of Chloramphenicol as Thermosensitive Ocular *in-situ* Gel

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### Abstract

The purpose of this study was to develop poloxamer-based in-situ gel of chloramphenicol aiming to increase bioavailability and prolong corneal contact time, controlling drug release, and enhancing ocular bioavailability. The in-situ gel was prepared using different concentrations of poloxamer 407 combined with hydroxypropyl methyl cellulose (HPMC) or carbapol 940 to achieve gelation temperature about physiological temperature and improve rheological behavior and gelling properties of poloxamer gel. The prepared formulations were evaluated for their appearance, pH, and sol-gel transition temperature. The formulations F<sub>2</sub>, F<sub>3</sub>, and F<sub>5</sub> have a gelation temperature within the accepted range 35-37<sup>0</sup>C and were evaluated for their isotonicity, rheological studies, ocular irritation test, sterility and release studies. The selected formulations (F<sub>2</sub>, F<sub>3</sub>, and F<sub>5</sub>) isotonic, pseudoplastic, non irritant, pass sterility test, and the in vitro release demonstrated a diffusion-erosion controlled release of chloramphenicol over a period of 4 hr., 6 hr., and 6 hr. respectively.

**Key words:** Chloramphenicol, in-situ gel, ocular dosage form, poloxamer.

### تحضير وتقييم خارج الجسم للكلورامفينيكول كهلام موضعي عيني متحسس للحرارة علي كاظم علي الله\* و شيماء نزار عبد الحميد<sup>١،\*\*</sup>

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### الخلاصة

الهدف من هذه الدراسة هو لتطوير هلام موضعي من مادة الكلورامفينيكول مع بوليمرات البوليبيكسامر تهدف الى اطالة زمن التماس مع القرنية والسيطرة على تحرر الدواء اضافة الى تحسين التوافر الحيوي من العين. لقد تم تحضير الهلام الموضعي من استعمال تراكيز مختلفة من بوليمرات البوليبيكسامر ٤٠٧ مع مادة الهيدروكسي بروبيل ميثيل سليولوز او الكاربابول ٩٤٠ للحصول على درجة حرارة تكوين المادة الهلامية على مقربة من الدرجة الفزيولوجية للجسم ولتحسين طريقة جريان السوائل وخواص تكون المادة الهلامية لهلام البوليبيكسامر. الصيغ الدوائية المحضرة تم تقييم شكلها الخارجي ودرجة الحامضية ودرجة حرارة انتقال المادة من سائل الى هلام. الصيغ الدوائية المحضرة (F<sub>2</sub>, F<sub>3</sub>, F<sub>5</sub>) لديها درجة حرارة تكوين المادة الهلامية ضمن الحدود المقبولة (٣٥-٣٧) درجة مئوية وتم تقييم الضغط التناضحي لها. كما تم دراسة طريقة جريان السوائل واختبار تحدد العين اضافة الى اختبار درجة تطهرها من الجراثيم (اخبار العقامية). ودراسة تحرر الدواء. الصيغ الدوائية المحضرة المختارة (F<sub>2</sub>, F<sub>3</sub>, F<sub>5</sub>) كانت متساوية الضغط التناضحي وشبه طيعة وغير مخرشة ومعقمة كما ان تحرر الدواء خارج الجسم اظهر نفوذية وتآكل لتحرر مسيطر عليه لدواء الكلورامفينيكول على فترة طولها (٤) ساعات و (٦) ساعات و (٦) ساعات بالتتابع. الكلمات المفتاحية: كلورامفينيكول ، هلام موضعي ، صيغة دوائية عينية ، البوليبيكسامر .

### Introduction

The conventional liquid ophthalmic delivery systems exhibit short pre corneal residence time and poor bioavailability due to rapid elimination induced by lachrymal flow, blinking, normal tear turnover, and solution drainage by gravity. As a result, frequent instillation of solution is needed in order to achieve the desired therapeutic effect<sup>(1)</sup>.

One of the major disadvantages of eye drops is the pulsatile drug level, with a transient period of overdose followed by extended period of subtherapeutic levels before next dose is administered. This means that the infectious

agent will be exposed by low concentration of the antibiotic leading to bacterial resistance<sup>(2)</sup>.

Various ophthalmic vehicles, such as inserts, ointments, suspensions, and aqueous gels, have been developed to lengthen the residence time of instilled dose and enhance ophthalmic bioavailability. These ocular drug delivery systems, however, have not been used extensively because of some drawbacks, such as blurred vision from ointment or low patient compliance from inserts<sup>(3)</sup>.

Several in situ gelling systems have been developed to prolong the precorneal residence

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time of a drug, improve patient compliance and consequently enhance ocular bioavailability. These systems exhibit sol-to-gel phase transitions due to a change in a specific physicochemical parameter (e.g.: pH, temperature, and ions) in the cul-de-sac<sup>(4)</sup>.

The productive absorption of most ophthalmic drugs results from diffusional process across the corneal membrane. The efficiency of the absorption process is a function of the rate and extent at which the transport processes occur. The flux of any drug molecule across a biological membrane depends on the physicochemical properties of the permeating molecule and its interaction with the membrane. The absorption process is also a function of the physiological mechanism of pre-corneal fluid drainage or turnover<sup>(5)</sup>.

Chloramphenicol is an antibiotic used for serious infections in which the benefit of the drug outweighs its uncommon but serious hematological toxicity such as infections caused by haemophilus influenza resistant to other antibiotics, Meningitis in patients in whom penicillin can not be used, typhoid fever, and bacterial conjunctivitis. It inhibits bacterial protein synthesis by binding to 50S subunit of the bacterial ribosome<sup>(6)</sup>. It is slightly soluble in H<sub>2</sub>O with melting point 149-153°C and Mwt 323<sup>(6)</sup>.

## Materials and Method

### Materials

Chloramphenicol powder from Fluka Biochemika (Switzerland), Hypromellose USP (Metolose 90 SH- 4000SR)(HPMC4000) Ex05097, Soyabean-Casein digest medium and Carbopol 940 from Himedia-Mumbai (India), Poloxamer (pluronic F127) (Sybronic BF127) from Actico, Monosodium dihydrogen

phosphate from Laboratory Rasayan sdfine. Chemical limited Mumbai India, Disodium hydrogen phosphate, Phosphoric Acid, Hydrochloric Acid and Sodium Chloride from Riedel-De Haenagseele Hannover (Germany), Sodium bicarbonate from SDI (Iraq), Calcium Chloride dehydrate and Fluid thioglycolate medium from Merck (Germany).

### Methods

#### Preparation of *in-situ* gel

Ten formulas were prepared as shown in table (1) using different ratios of poloxamer 407(pluronic F127) (10%, 15%) as gelling agent in combination with carbapol 940 (0.02%, 0.04%) or HPMC (0.5%, 1.5%) using as viscosifying agents.

Thermoreversible gels were prepared using cold technique. First of all the aqueous dispersions of selected concentrations of carbapol 940 (0.02%, 0.04%) for formulas (F<sub>2</sub>, F<sub>3</sub>, F<sub>7</sub>, and F<sub>8</sub>) and HPMC for formulas (F<sub>4</sub>, F<sub>5</sub>, F<sub>9</sub>, and F<sub>10</sub>), and pluronic F127 for formulas (F<sub>1</sub> and F<sub>6</sub>) in phosphate buffer pH5.9 were prepared. The pluronic/carbapol combination and the pluronic/HPMC combination were prepared by dispersing the pluronic in the desired concentration of respective polymer solutions. Then the partially dissolved solutions were refrigerated until thoroughly mixed (approximately 24 hrs).

An appropriate amount of drug dissolved in phosphate buffer pH5.9, then benzalkonium chloride 0.01% added with continuous stirring until uniform drug solution was obtained. The drug solution was finally added to polymer solution with continuous stirring. The developed formulations were filled in amber glass containers<sup>(7)</sup>.

**Table (1): Composition of different formulas *in-situ* gelling systems of chloramphenicol.**

No.	Formulation code	Chloramphenicol % w/v	Pluronic F127 % w/v	Carbopol 940% w/v	HPMC% w/v	Benzalkonium % chloride w/v	Formulation code	phosphate buffer pH 5.9
1.	F <sub>1</sub>	0.5	10	-	-	0.01	F <sub>1</sub>	Q.S to 100ml
2.	F <sub>2</sub>	0.5	10	0.02	-	0.01	F <sub>2</sub>	Q.S to 100ml
3.	F <sub>3</sub>	0.5	10	0.04	-	0.01	F <sub>3</sub>	Q.S to 100ml
4.	F <sub>4</sub>	0.5	10	-	0.5	0.01	F <sub>4</sub>	Q.S to 100ml
5.	F <sub>5</sub>	0.5	10	-	1.5	0.01	F <sub>5</sub>	Q.S to 100ml
6.	F <sub>6</sub>	0.5	15	-	-	0.01	F <sub>6</sub>	Q.S to 100ml
7.	F <sub>7</sub>	0.5	15	0.02	-	0.01	F <sub>7</sub>	Q.S to 100ml
8.	F <sub>8</sub>	0.5	15	0.04	-	0.01	F <sub>8</sub>	Q.S to 100ml
9.	F <sub>9</sub>	0.5	15	-	0.5	0.01	F <sub>9</sub>	Q.S to 100ml
10.	F <sub>10</sub>	0.5	15	-	1.5	0.01	F <sub>10</sub>	Q.S to 100ml

### Compatibility study

A thin layer chromatography test was done using methanol: chloroform 60:40 as the mobile phase and aluminum paper silica gel as the stationary phase. Spots were made for the prepared formula on silica gel to discover any incompatibility among ingredients.

**Sterilization:** The formulations were sterilized by filtration by passage through a sterile membrane filter of nominal pore size of 0.22  $\mu\text{m}$  (Millipore type).<sup>(8)</sup>

### Measurement of Sol-Gel transition temperature

The sol-to-gel phase transition temperature (gelation temperature) measured for all the prepared formulations according to the technique described by vadnere and Gilbert<sup>(9)</sup>. An aliquot of 2ml refrigerated tested formulation was transferred to a test tube and sealed with a parafilm. The tube was maintained in a thermostatically controlled water bath at 4°C. the temperature of the water bath was increased gradually in increment of 3°C in the beginning of the experiment and then 1°C increment in the region of sol-gel transition temperature, the tested formulation was left to equilibrate for 10 min at each new setting<sup>(10)</sup>. The gelation is considered to be occurred when the meniscus of the formula would no longer move upon tilting through angle 90°<sup>(11)</sup>.

### Isotonicity evaluation

Isotonicity is an important characteristic of the ophthalmic preparation. Isotonicity has to be maintained to prevent tissue damage or irritation of the eye. The three selected ophthalmic preparations are subjected to isotonicity testing by using osmometer apparatus<sup>(12)</sup>.

### Rheological studies

The three selected formulation were subjected for rheological studies. Viscosity of the instilled formulation is an important factor in determining residence time of drug in the eye. The solutions were allowed to gel at physiological temperature and then the viscosity determination was carried out by using viscometer with spindle 2. The angular velocity increased gradually 6, 12, 30, 60 and the viscosity of the formulation is measured<sup>(13)</sup>.

### In-vitro release studies

The *in-vitro* drug release from the selected formulation was studied through cellophane membrane using a modified USP XXIII dissolution testing apparatus (Figure 1). The dissolution medium used was artificial tear fluid freshly prepared (pH 7.4)<sup>(14)</sup>. Cellophane membrane, previously soaked overnight in the dissolution medium, was tied

to one end of a specifically designed glass cylinder (open at both ends and of 2.5 cm diameter). A 1-ml volume of the formulation was accurately pipette into this assembly. The cylinder was attached to the metallic driveshaft and suspended in 200ml of dissolution medium maintained at  $37 \pm 0.5$  °C so that the membrane just touched the receptor medium surface. The dissolution medium was stirred at 50 rpm. Aliquots, each of 5ml volume, were withdrawn at half an hour intervals and replaced by an equal volume of fresh dissolution medium<sup>(15)</sup>. The samples were filtered through 0.45-mm syringe filters and subjected to spectrophotometric analysis at 278nm<sup>(16)</sup>.

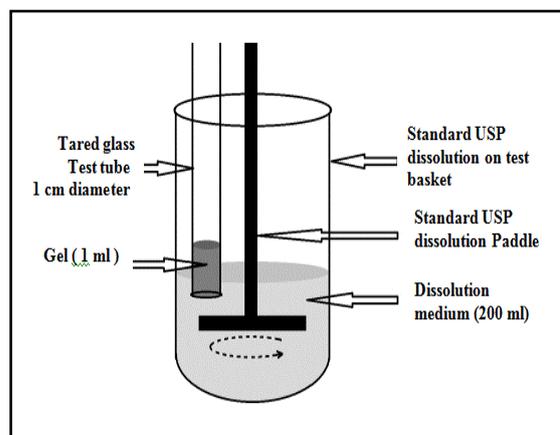


Figure (1): Modified USP XXIII in vitro dissolution testing apparatus

### Sterility

2ml of the prepared formula was withdrawn with a sterile syringe then, aseptically transferred to thioglycolate medium (20ml) and soyabean- casein digest medium (20ml) separately. The liquid mixed with the media. The inoculated media incubated 14 days at 30-35°C in case of fluid thioglycolate medium and 20-25°C in case of soyabean-casein digest medium<sup>(17)</sup>.

### Ocular irritancy test

The draize irritancy test was designed for the ocular irritation potential of the ophthalmic product prior to marketing. According to the draize test, the amount of substance applied to the eye is normally 100  $\mu\text{l}$  placed into the lower cul-de-sac with observation of the various criteria made at a designed required time interval of 1hr, 24 hrs, 48hrs, 72 hrs, and 1 week after administration. Three rabbits (male) weighing 1.5 to 2kg are used for the study. The sterile formulation is instilled twice a day for a period of 7 days, and a cross-over study is carried out (a 3day washing period with saline was carried out before the cross-over study). Rabbits are

observed periodically for redness, swelling, and watering of the eye<sup>(18, 19)</sup>.

### Statistical Analysis

The results of the experiments are given as a mean of triplicate samples and were analyzed according to the one way analysis of variance (ANOVA) at the level of ( $P < 0.05$ ).

### Results and Discussion

#### Compatibility study

The thin layer chromatography test showed that only 5 compounds appeared; these are Chloramphenicol, pluronic F127, Carbapol, HPMC and Benzalkonium chloride. This indicates that no chemical interaction takes place since there is no new compound appears at the silica gel layer.

#### Measurement of the Sol-Gel transition temperature

Thermoreversible polymers have been considered to be suitable for ocular delivery of chloramphenicol if they are liquid at room temperature (20-25°C) and undergo gelation with the increase in temperature in cul-de-sac (35-37°C)<sup>(20)</sup>.

Poloxamer solutions are known to exhibit thermoreversible gelation depending on the polymer grade, concentration, and other included formulation components. At a certain concentration of the polymer and temperature, poloxamer molecules in aqueous solution will self-assemble to form spherical micelles with a dehydrated PPO (poly propylene oxide) core surrounded by hydrated swollen PEO (poly ethylene oxide) chains, packing and entanglements of micelles with increase of temperature are said to be possible mechanisms of the gelation of poloxamer solutions.<sup>(21)</sup>

The micelles would occupy a high fraction volume of solution, and come into contact and entangle with each other resulting in a three-dimension network structure and forming stiff gel. Therefore, the product containing more effective concentration of pluronic F127 would contain more number of micelles. They would need lower energy to promote sol-gel transition and could perform sol-gel transition at lower temperature than products containing less F127 content.<sup>(22)</sup>

Significant decrease in gelation temperature occurs when carbopol added to poloxamer ( $p < 0.05$ ). This could be explained by the interactions between the polymers. One presumption is the formation of a three dimensional network between carboxyl groups of carbopol and ether groups of poloxamer due to hydrogen bonds which will lead to gelation at lower temperature.<sup>(11)</sup>

In addition to that, carbopol molecules become associated with cavities between polymolecular poloxamer micelles and chains of carbopol and this would block the interaction between poloxamer chains which would also induce the lower gelation temperature<sup>(23)</sup>.

Drainage of ophthalmic formulations from the precorneal surface would be considerably reduced by addition of mucoadhesive polymers such as HPMC which allow attachment of the formulae to the corneal mucin and decrease the gelation temperature of the in situ forming gels. Table (2) shows the sol-gel transition temperature of the prepared formulations and only three formulations (F<sub>2</sub>, F<sub>3</sub>, and F<sub>5</sub>) have gelation temperature within the acceptable range (35-37°C) therefore subjected for further studies.

**Table (2): pH Values and Sol-Gel Transition Temperatures of the Prepared In-Situ Gel Formulations & Osmolarity Values for the Selected Formulations (F<sub>2</sub>, F<sub>3</sub>, and F<sub>5</sub>).**

Formulation	pH after formulation	Sol-gel transition temp. °C	Osmol-arity osmol
F <sub>1</sub>	6.2	40	-
F <sub>2</sub>	5.9	36	0.298
F <sub>3</sub>	5.9	36	0.321
F <sub>4</sub>	6.2	38	-
F <sub>5</sub>	6.2	37	0.310
F <sub>6</sub>	6.2	32.5	-
F <sub>7</sub>	6	26	-
F <sub>8</sub>	5.9	24	-
F <sub>9</sub>	6.2	26	-
F <sub>10</sub>	6.3	24	-

#### Isotonicity evaluation

Table (2) show the osmolality for the selected formulations (F<sub>2</sub>, F<sub>3</sub>, and F<sub>5</sub>) as measured by osmometer. All values within the acceptable range (0.6-2%). No significant difference between them.

#### Rheological studies

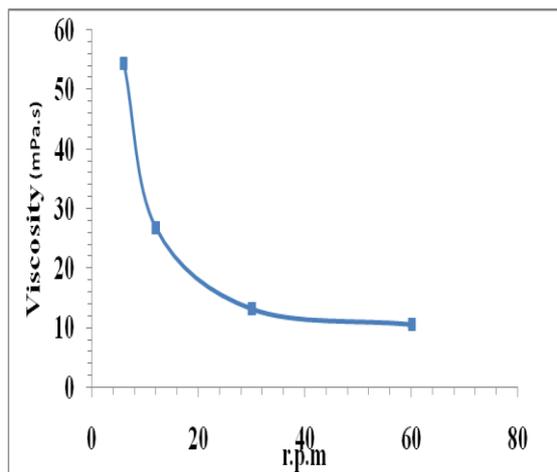
Table (3) showed the viscosity values obtained for formulations F<sub>2</sub>, F<sub>3</sub>, and F<sub>5</sub> at different angular velocity. The formulations exhibited pseudoplastic rheology, as evidenced by shear thinning and decrease in viscosity with increased angular velocity that can be observed in the figures (2, 3, 4).

The viscosity was directly dependent on the polymeric content. The administration of ophthalmic preparations should influence as little as possible the pseudoplastic character of the pre-corneal film<sup>(24)</sup>. Since the ocular shear rate is very high ranging from 0.03 S<sup>-1</sup> during inter-blinking periods to 4250-28500 S<sup>-1</sup> during blinking, viscoelastic fluids with a

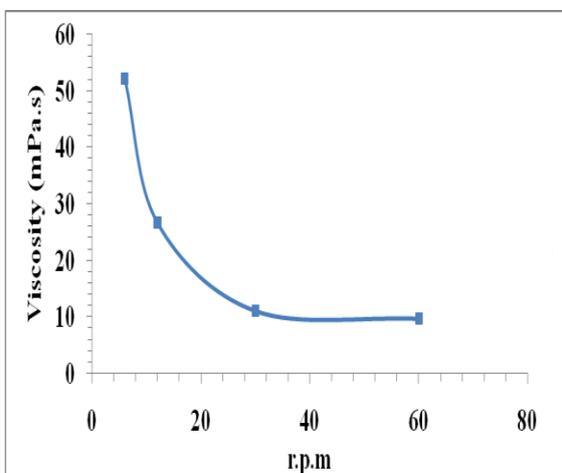
viscosity that is high under the low shear rate conditions and low under the high shear rate conditions are preferred for ophthalmic drug delivery<sup>(29)</sup>.

**Table (3): Viscosity Values for the Selected Formulations (F<sub>2</sub>, F<sub>3</sub>, and F<sub>5</sub>).**

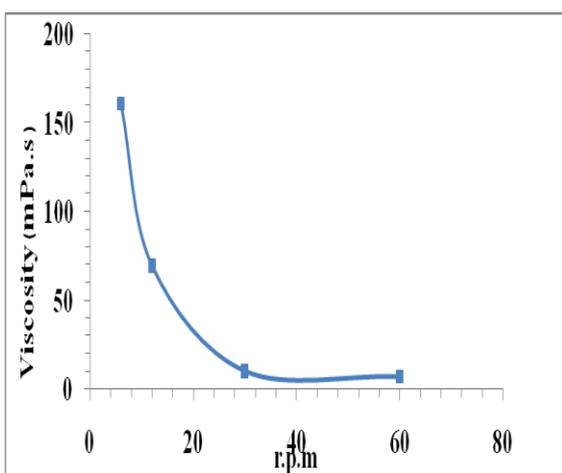
Angular velocity (rpm)	Viscosity		
	F <sub>2</sub>	F <sub>3</sub>	F <sub>5</sub>
6	52.1	160.6	54.4
12	26.6	69.6	26.7
30	11	10.4	13.1
60	9.62	7.4	10.5



**Figure (4): Viscosity versus rpm at 37<sup>o</sup>C for F<sub>5</sub>.**



**Figure (2): Viscosity versus rpm at 37<sup>o</sup>C for F<sub>2</sub>.**



**Figure (3): Viscosity versus rpm at 37<sup>o</sup>C for F<sub>3</sub>.**

**In - vitro Release Studies**

The *in-vitro* release studies were carried out for the selected formulations (F<sub>2</sub>, F<sub>3</sub>, and F<sub>5</sub>) using simulated tear fluids (STF pH 7.4) as the dissolution medium. The standard curve and release profiles are shown in figures (5, 6, 7 and 8) respectively. It is obvious that F<sub>3</sub> sustain the release of chloramphenicol more than F<sub>5</sub> and the latter (F<sub>5</sub>) sustain the release more than F<sub>2</sub>, this may be potentiated by the rheological studies where the rate of drug release decrease when the viscosity increase<sup>(25)</sup>. In formulation F<sub>5</sub>, the retarding effect of the HPMC polymer could be attributed to its ability to increase the overall product viscosity.<sup>(26)</sup> Also it has ability to distort or squeeze the extra-micelle aqueous channels of poloxamer micelles through which the drug diffuses, thereby delaying the release process<sup>(27)</sup>. F<sub>3</sub> contain more carbopol 940 content than F<sub>2</sub> therefore sustain the release more than F<sub>2</sub>. This is probably that STF (pH 7.4) led to ionization of carboxyl groups in carbopol molecules and thus repulsion of these ions. Then, carbopol would be in an extended configuration and form strong three-dimensional networks, therefore, F<sub>3</sub> possess stronger gel structure and this will cause more retardation for drug release<sup>(28)</sup>. Drug release data were fitted to different kinetic models like zero-order, first-order, higuchi and korsmeyer-peppas to ascertain the kinetic modeling of the drug release<sup>(29)</sup>.

To confirm the diffusion mechanism, the data were fit into korsmeyer's equation.<sup>(30)</sup> The exponent n gives information about the release mechanism=0.45 (indicates fickian diffusion-controlled drug release), 0.45 < n < 0.89 indicates anomalous (non-fickian transport), and n=0.89 ,indicates (a case II

relaxational release transport, non-fickian, zero-order release).<sup>(31)</sup>

The kinetic values obtained for the selected formulations (F<sub>2</sub>, F<sub>3</sub>, and F<sub>5</sub>) are indicated in table (4). From this table, it is obvious that F<sub>2</sub> and F<sub>5</sub> follows Higuchi kinetic and the drug releases by diffusion and erosion, while F<sub>3</sub> follow Peppas Korsmeyer kinetic and the drug releases by diffusion and erosion. Significant difference between the release of (F<sub>2</sub>, F<sub>5</sub>) and the release of F<sub>3</sub> (p<0.05).

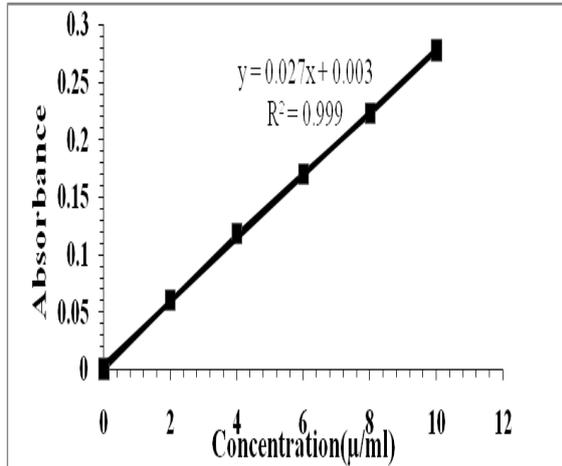


Figure (5): Calibration curve of chloramphenicol in artificial tears (pH=7.4)

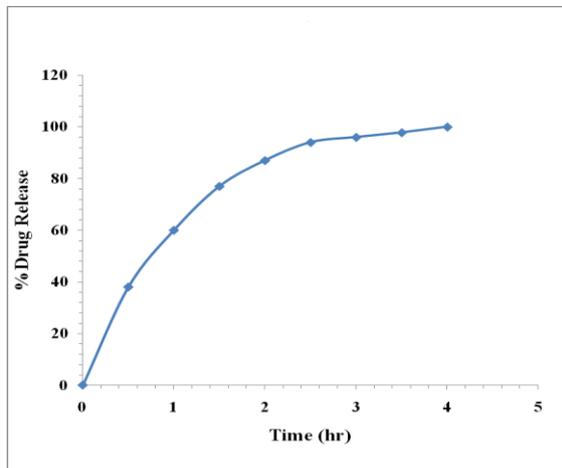


Figure (6): The release profile of formula 2 in simulated tear fluids at 37°C.

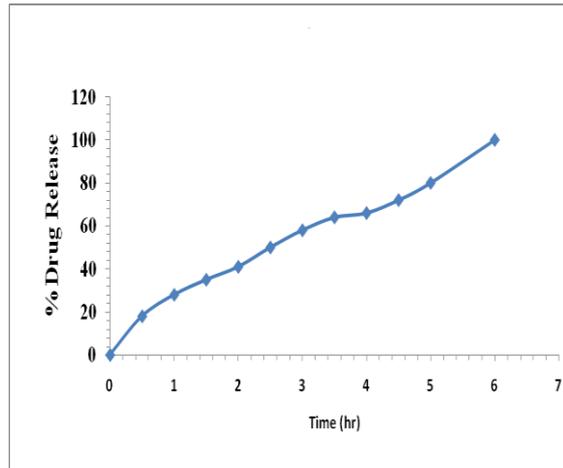


Figure (7): The release profile of formula 3 in simulated tear fluids at 37°C.

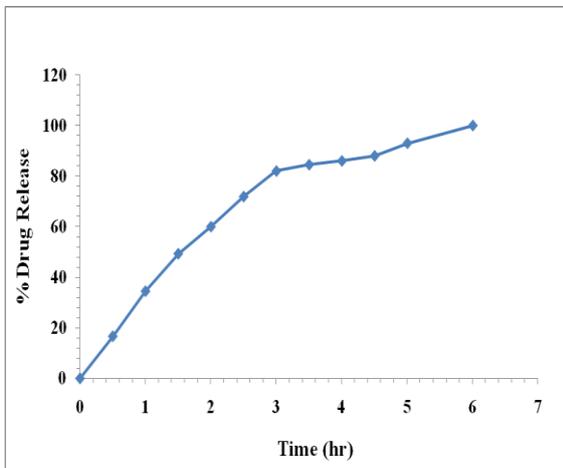


Figure (8): The release profile of formula 5 in simulated tear fluids at 37°C

**Sterility test**

No appearance of turbidity and no evidence of microbial growth when incubated for 14 days at 30-35 °C in case of fluid thioglycolate medium and 20-25°C in case of soyabean casein digest medium.

Table (4): Drug release kinetics of F<sub>2</sub>, F<sub>3</sub>, and F<sub>5</sub>.

Formula No	Zero order		First order		Higuchi		Peppas korsmeyer	
	R <sup>2</sup>	m	R <sup>2</sup>	m	R <sup>2</sup>	m	R <sup>2</sup>	M
F <sub>2</sub>	0.586	32.03	0.37	0.688	0.9598	55.67	0.944	0.4638
F <sub>3</sub>	0.94	17.25	0.43	0.458	0.945	39.31	0.992	0.665
F <sub>5</sub>	0.774	20.89	0.54	0.48	0.9656	42.225	0.95	0.7

**Ocular irritancy studies**

The results of the ocular irritation studies indicated that formulations (F<sub>2</sub>, F<sub>3</sub>, and F<sub>5</sub>) were non-irritant with excellent ocular tolerance. No significant ocular damage or abnormal clinical signs to the cornea, iris or conjunctiva were visible ( $P \geq 0.05$ ). No signs of redness, watering of the eye and swelling were observed throughout the study with the three formulations.

**Conclusion**

The use of polymeric in-situ gels for controlled release of chloramphenicol provides a number of advantages over conventional dosage form:

- 1- Sustained and prolonged release of chloramphenicol.
- 2- Good stability and biocompatibility characteristics make the in situ gel dosage forms very reliable.
- 3- Use of biodegradable and water soluble polymers for the in situ gel formulations can make them more acceptable and excellent drug delivery systems.

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