International Journal for Pharmaceutical Research Scholars (IJPRS)
ISSN No: 2277 - 7873
RESEARCH ARTICLE **V-1, I-2, 2012**

Temperature and pH Triggered Insitu Hydrogel of Doxycycline for the Treatment of Chlamydial Conjunctivitis

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Manuscript No: IJPRS/V1/I2/00027, Received On: 03/04/2012, Accepted On: 05/04/2012

ABSTRACT

The present investigation describes the formulation and characterization of ophthalmic in situ hydrogel for sustained delivery of doxycycline (DOX) that is frequently used to treat chlymydial conjunctivitis. Insitu hydrogel were prepared using thermo-reversible gelling polymer, Pluronic F 127 (PF127) and PH sensitive and viscosity enhancer polymer Carbopol 940 (CP940). Because of high concentration (20 to 25%w/v) of PF127 polymer required for insitu gelation causes irritation to the eye. So, to reduce this concentration, an attempt was made to combine the PF127 with CP940 showing a pH triggered sol-gel transition by pH of tear fluid. Different batches were prepared of varying concentrations of CP940 (0.1-0.3%) with PF127 (12%-18%) using DOX 2%w/v. Pluronic F68 (PF68) with 2% and 4% concentration were mixed to obtain a hydrogel with an appropriate gelation temperature. The formulations were optimized by the viscosity measurement and in vitro gelation study. Selected formulations were evaluated for in vitro drug release study using Franz diffusion cell and indicated sustain drug release over a period of 10 h. Stability testing at 80C and 400C and effect of sterilization and were on drug content, pH and clarity were also evaluated. The prepared formulation could reduce not only the concentration of individual polymers but also the side effects without compromising the in vitro gelling capacity. This formulation of Doxycycline insitu hydrogel represents potentially effective ophthalmic delivery system for the treatment of chlaymydial conjunctivitis.

KEYWORDS

Doxycycline, Pluronic F127, Carbopol 940, insitu gelation, simulated tear fluid, Chlamydial conjunctivitis

INTRODUCTION

Doxycycline (DOX) is a member of the tetracycline antibiotics group derived from oxytetracycline, which is frequently used to treat Chlamydial conjuctivitis, chronic prostatitis sinusitis, syphilis, pelvic inflammatory disease, acne and rosace and in the treatment and prophylaxis of Bacillus anthracis and malaria.[1,2]

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DOX was shown to be effective against several other pathogens causing a number of diseases and, since it is at sub-antimicrobial doses an inhibitor of matrix metalloproteinases.[3] DOX can be proposed for a number of purposes. Doxycycline is an inexpensive, FDA approved antibiotic that likely promotes wound healing by reducing inflammation and protease activity.[4] DOX is available as hyclate, calcium, and monohydrate salts.[5,6] DOX is well absorbed after oral administration, and bioavailability is 90–100% in humans. DOX is lipid-soluble and penetrates body tissues and fluids better than tetracycline HCl or oxytetracycline, including

distribution to the cerebrospinal fluids, prostate, and eye. The blood ocular barriers, which include the blood–aqueous and blood–retina barriers protect the eye, but prevent drug distribution to the anterior and posterior chambers, limiting ocular bioavailability.[7,8] Drug diffusion into the eyes from the systemic circulation is slow and inefficient.

Most drugs applied to the eye surface as solutions have ocular bioavailability in the range of about10% with most of the drug being cleared by local systemic absorption.[8,9] Solutions are in contact with the eye surface very short period of time as the tear film quickly washes them away. The contact time, local drug concentration and thereby duration of action can be prolonged by designing topical formulations with higher viscosities.[10] Such as viscous solutions, ointments, gels, or polymeric inserts, have been used. The corneal contact time has been increased to varying degrees by these vehicles, but because of blurred vision (i.e. ointments) or lack of patient compliance (i.e. inserts), they have not been widely accepted. So to improve the patient compliance and get sustained drug release gel system that are instilled as drops into the eye and undergo a sol-gel transition in the cul-de-sac is ideal.[11, 12]

Several polymers, demonstrating phase transition due to changes in their microenvironment, were investigated. However, most of these vehicles are characterized by a high polymer concentration (25% poloxamer, 30% CAP) which is not well tolerated by the eye.13 PF127 is a polyethylene oxide–polypropylene oxide copolymer well recognized for gel-forming properties in a range of temperatures close to body temperature. It is also widely used due to physicochemical characteristics and safety.[14]

This work was meant to provide a proof-of-principle on the possibility to obtain a sustained release of DOX over a period of 10 hrs aimed at the improvement of the treatment. According to the literature addressing the use of thermogels as controlled release delivery systems and several strategies conceive the use of polysaccharides and some copolymers as gel-forming system used with poloxamer to achieve sustained delivery.[13] In the present work, two strategies were followed: (1) use of copolymer systems such as poly(oxy-ethylene)–poly(oxy-propylene) copolymers, which provide a thermo gel behavior and (2) use of pH sensitive polymer CP940 that goes to rapid neutralization by pH of tear fluid and provides sustained release of DOX.[15,16]

MATERIAL AND METHOD

PF127 and PF68 were obtained from BASF Co. (Ludwigshafen, Germany). Carbopol 940 was supplied from Corel Pharma, Ahmedabad. Gift sample of Doxycycline monohydrate (purity 98.5%) was kindly provided by Yancheng Suhai Pharmaceutical Co. (Jiangsu, China). Other ingredients used were of analytical grade.

Preparation of Insitu Hydrogel

Prepare the solution of DOX (2%) in citro phosphate buffer pH 5.0, disperse the PF127 into drug solution and agitated uniformly at room temperature. Prepare aqueous dispersion of selected concentration of CP940 (0.1%, 0.2%, 0.3%) in citro phosphate buffer pH 5.0. Add the dispersion containing DOX and PF127 to desired concentration of CP940 polymer solution. The partially dissolved solutions were stored in the refrigerator until the entire polymers were completely dissolved (approximately 24 h).[17]

Measurement of Gelation Temperature

The gelation temperature was measured using magnetic stirrer with constant heating arrangement. The temperature at which magnetic stirrer stopped rotating due to gelation was observed visually and selected as gelation temperature.[18]

In vitro Gelling Efficiency

The *in vitro* gelling efficiency was determined by placing a drop of the system in a test-tube containing 2 ml of simulated tear fluid (STF) freshly prepared and equilibrated at 37°C. The visual assessment of gel formation was carried

out simultaneously the time required for gelation as well as time taken for the formed gel to dissolve was also noted. The viscosity of the systems was measured using Brookfield LVDV-E rheometer at 12 rpm for the purposes of comparative evaluation. The flow behavior of vehicles was determined by various signs obtained by visual inspection. The flow behavior with the "+" sign indicates the vehicle is in the liquid form and is very easy to flow which shows mild gelation after a few minutes and the gel dissolves rapidly. The "++" sign indicates that the vehicle is in the liquid-gel like form and flows less readily, which shows gelation immediate the gel remains for 1 h. The flow behavior with the "+++" sign indicates that the sample is in the gel form and is very difficult to flow which also shows immediate gelation and the gel remains for the extended period of time. The flow behavior with the "++++" sign indicates that the vehicle is a strong gel and cannot flow at 25°C and 5.0 pH.[19]

In vitro Release Study

Doxycycline release from the prepared gels was determined in simulated tear fluid (STF)21 (2.18g sodium bicarbonate, 6.78 g sodium chloride, 0.032 g anhydrous calcium chloride and 1.38 g potassium chloride in 1 L of water; 290 mosm/L, pH 7.8). Approximately 3.0 g poloxamer solution was poured into a weighing bottle (Φ22 mm X 50 mm) preheated to 33.50C to transform the solution into a gel. STF (1.5 ml) was then poured gently down the wall of the bottle onto the top of the gel and the vessel shaken horizontally at a constant rate in a water bath at 33.50C. At various times, the solution was decanted off and collected and the vessel wiped dry and weighed. An aliquot of fresh STF was then added on top of the gel and the vessel again shaken at 33.50C. The steps were repeated until the weight of the remaining gel changed by no more than 10% of the initial weight in that incubation period.[20] All solutions were combined, immediately filtered through a 0.45 mm membrane filter, suitably diluted and analyzed for DOX by Shimadzu 1800 double beam UV-visible spectrophotometer at 351 nm. Each experiment was performed in triplicate.

Release Mechanism

In order to determine the release mechanism, the suitability of two equations, the zero-order model and Higuchi model were tested with respect to the release data. These are described by the following equations.[21,22]

Zero-order model: $Mt = M0 + K0t$

Higuchi model: $Mt = M0 + KHt0.5$

Where, Mt is the amount of drug dissolved at time t, M0 is the initial amount of drug in the sample and KH and K0 are the Higuchi rate constant and zero-order release constant, respectively.

Effect of Sterilization

The selected formulation was filled in 10 ml capacity amber glass vials, closed with grey butyl rubber closure and sealed with aluminum cap. The vial was subjected to terminal sterilization by autoclaving at 121 °C and 15 psi for 20 min. The formulation was evaluated for drug content, viscosity, clarity, and pH before and after the terminal sterilization.18

Stability Studies

Doxycycline hydrogels were stored at 400C for 10 days to evaluate stability at elevated temperature. Drug content was determined initially and after 5 and 10 days. Other samples stored at 80C for 30 days to assess stability in the cold were sampled initially and after 10, 20 and 30 days.

RESULTS AND DISCUSSION

Effect of Polymers Concentration and Composition on Gelation Temperature and Gelling Capacity

The gelation temperature is the temperature at which a thermally sensitive solution changes to semisolid. The gelation temperature of a gel for ophthalmic drug delivery should be around 33.5 ^{0}C after dilution with STF (gel:STF ratio of 40:7, v/v). An ideal in situ forming gel should be free flowing at a low temperature, transform

into a semisolid after contacting the ocular surface, and remain in the gel form under conditions of maximum lachrymal fluid dilution.

Table: 1 Effects of PF127 and CP940

Formulation Code	PF127 (wt%)	CP940 (wt%)	Gelation temperature (^{0}C)	Gelation Capacity*
F1	12	0.1	35.91±0.21	+
F2	14	0.1	32.18±0.65	+
F3	16	0.1	28.96±0.50	++
F4	18	0.1	24.91±0.11	+++
F5	12	0.2	35.96±0.45	+
F6	14	0.2	32.48±0.90	+++
F7	16	0.2	29.10±0.28	+++
F8	18	0.2	25.08±0.85	++++
F9	12	0.3	34.74±0.14	++
F10	14	0.3	32.56±0.40	+++
F11	16	0.3	28.92±0.52	++++
F12	18	0.3	24.82±0.31	++++

*+ Mild gelation after a few minutes

++ Gelation immediate remains for not more than 1hr.

+++ Gelation immediate remains for extended period.

concentration on the gelation temperature and gelling capacity

The result shows that gelation temperature increased with decreasing PF127 concentration (Table 1). Poloxamers are composed of polyethylene oxide (PEO) and polypropylene oxide (PPO) units. As temperature increases, dehydration of PPO leads to formation of a micelle core while hydration of PEO causes it to expand and form an outer skin. As the temperature continues to rise, the micelles arrange themselves in sequence to form a hydrogel. Typically, the gelation temperature depends on the PEO:PPO ratio in the polymer solution.

There was no significant effect of concentration of CP940 on gelation temperature of thermogel. The gelling capacity was increase with increase in concentration of PF127 and CP940. To obtain an appropriate gelation temperature PF68 was included in the formulation (Table 2) because it increases the ratio of PEO leading the micelles to become less entangled thereby raising the critical micelles temperature. The results show that the gelation temperature increases with increasing PF68 concentration (Table 2).

Table: 2 Effects of PF68 concentration on the gelation temperature

Formulation Code	PF127 (wt%)	PF68 (wt%)	Gelation temperature (^{0}C)
PF1	14	2	34.21±0.21
PF2	15	2	33.54±0.65
PF3	14	4	35.06±0.50
PF4	15	4	36.91±0.11

Concentration of CP 940 is 0.3% for all batches

In vitro drug release study

Batch F6 and F10 were selected for in vitro drug release since they have good gelling capacity and gelation temperature 29.10±0.28^{0}C and 32.56±0.40^{0}C respectively. The result shows that drug release rate decreases with increases the concentration of CP940 (fig.1). The all prepared solutions were clear, so for further study 0.3% concentration of CP940 was selected. The result shows that drug release rate decreases with increases the concentration of PF127 (fig. 2).

Batch PF2 shows the sustained release of DOX upto 10 hrs with 33.54±0.65^{0}C gelation temperature which shows the ideal value for ophthalmic drug delivery.

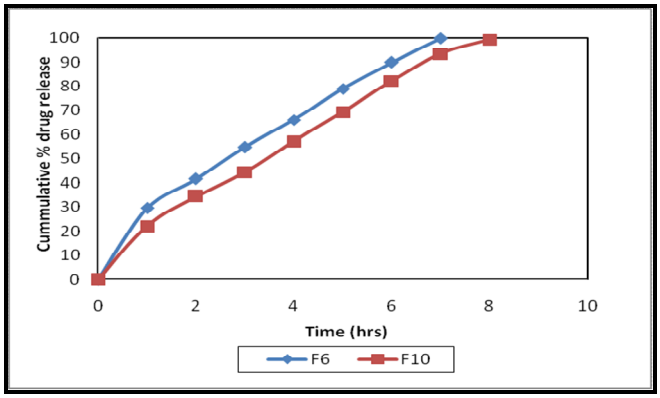

Figure: 1 Effect of concentration of CP940 on DOX release from hydrogel in STF

According to the results in Table 2, a formulation containing 15 % PF127, 0.3% CP940 and 2% PF68 was chosen since it has a gelation temperature of 33.5^{0}C when diluted with STF in a gel:STF ratio of 40:7 (v/v).

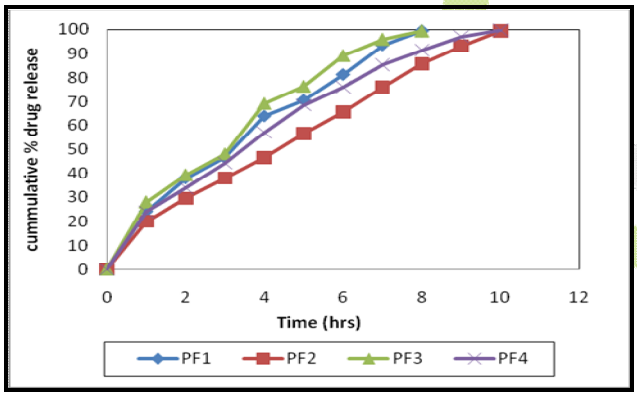

Figure: 2 Effect of concentration of PF127 on DOX release from hydrogel in STF

Release Mechanism

The drug release profiles were subjected to zero order and Higuchi equation to assess the release mechanism ruling DOX liberation from such systems. The results of fitting the release data to these models are shown in Table 3. Release appears to fit the Zero order and Higuchi equation suggesting release occur by diffusion mechanism and also by corrosion of poloxamer hydrogel.

Effect of sterilization

Before the sterilization process the drug content, pH and viscosity (at 12 rpm) value of optimized batch was 98.5%, 5.02 and 857 cps respectively and after the sterilization process the value was 98.1%, 4.98 and 857 cps. The result shows that the autoclaving exerted insignificant effect on the drug content, pH and viscosity of the optimized formulations. However, haziness was observed in formulations after autoclaving due to gelation of Pluronic at elevated temperature. But it was found to disappear and the original clarity was regained after overnight storage at ambient conditions.

Table: 3 Kinetics models fitting for DOX insitu hydrogel

Formulation	Zero order equation		Higuchi equation	
	K_0	R^2	K_h	R^2
PF1	11.93	0.977	36.28	0.972
PF2	7.637	0.990	32.74	0.958
PF3	11.96	0.959	37.37	0.974
PF4	13.17	0.961	34.01	0.981

Stability study

Results of stability studies are summarized in Tables 4. Doxycycline degraded at 40^{0}C accompanied by a colour change from yellow to brown and also the change in pH and drug content was appeared. There was no significant change in color, pH and drug content was appeared for *insitu* hydrogel at 8^{0}C.

CONCLUSION

The prepared insitu hydrogel remains as a free flowing liquid at room temperature and changes into a hydrogel when it comes into contact with STF at temperature above its gelation temperature. In this study combining of PF127 (15%) as a thermosensitive polymer with CP940 (0.3%) as a pH sensitive and viscosity enhancer polymer and PF68 (2%) to obtained gelation temperature 33.5^{0}C gives sustained DOX

release upto10 hrs and we can also reduce the concentration of PF127 from 25% to 15% and also able to reduce the individual polymer concentration. So the prepared ophthalmic insitu hydrogel is suitable to instill into eyes because of low viscosity. Prepared insitu hydrogel can be applied as effective ophthalmic delivery system for doxycycline for the treatment of chlaymidial conjunctivitis. This formulation should be further evaluated by suitable *invivo* model to confirm its therapeutic efficacy.

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