AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Formulation and Evaluation of Polyox /HPMC Based *In Situ* Gel Formulation for Levofloxacin hemihydrate Ophthalmic Delivery System.

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ABSTRACT

The aim of the present work was to formulate and evaluate in situ gelling system of levofloxacin hemihydrate. Levofloxacin hemihydrate is an antibacterial agent which exhibits rapid pre-corneal elimination and poor ocular bioavailability when administered in the form of conventional ophthalmic solutions like eye drop. To overcome this, an attempt has been made to formulate pH induced in situ gelling system of levofloxacin to provide sustained release of drug. Polymeric carriers that undergo sol-to-gel transition upon change in pH. The levofloxacin hemihydrate in situ gelling system formulated by using polyox in combination with hydroxyl propyl methyl cellulose (HPMC K4M) which acted as viscosity enhancing agent. The developed formulation was stable, non-irritant and provided sustained release over 8-hour period and can be a viable alternative to conventional eye drops. The formulations were found to be non-irritating.

Keywords: Ophthalmic drug delivery system, pH induced, in situ gel, Levofloxacin hemihydrate.

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Received 27July 2014, Accepted13 August2014

Please cite this article in press as: Sonawane SD *et al*., Formulation and Evaluation of Polyox /HPMC Based in SITU Gel Formulation for Levofloxacinhemihydrate Ophthalmic Delivery System. American Journal of PharmTech Research 2014.

INTRODUCTION

Conventional ophthalmic delivery systems like eye drops result in poor ocular drug bioavailability due to drainage of the instilled formulation in eye or the relative impermeability of the corneal epithelial membrane tear dynamics and nasolacrimal drainage. Most of the topically applied drug formulations are washed off from the eye by different mechanisms like lacrimation, tear dilution and the residence time of most conventional ocular solutions ranges between 5 and 25 minutes. Very small amount of topically applied drug is absorbed and major part of drug absorbed systemically results in systemic side effects[1-3]. A significant increase in the pre-corneal residence time of drug formulation and better bioavailability can be achieved by using delivery system based on the concept of in situ gel formation. These in situ gelling systems consist of polymer that undergoes sol-to-gel phase transitions by change in specific physicochemical stimulilike pH, Temperature, ionic strength in the environment, cul-de-sac in the case of eye. In case of pH sensitive hydrogels, the pH-sensitive polymers contain acidic or basic groups that either accept or release protons in response to changes in environmental pH. The polymers with a large number of ionisable groups are known as polyelectrolytes. Swelling of hydrogel increases as the external pH increases in the case of weakly acidic (anionic) groups but decreases when polymer contains weakly basic (cationic) groups. Another mechanism of in situ hydrogel is ion induced gelation. In this, polymers may undergo phase transition in presence of various ions. Temperature sensitive hydrogels are probably the most commonly studied class of environment sensitive polymer systems in drug delivery research. The use of biomaterial whose transitions from sol-gel is triggered by increase in temperature is an attractive way to approach in situ formation. The fluoroquinolones represent an expanding class of broad-spectrum antibacterial agents which cover a host of gram-negative and anaerobic species responsible for ocular infections. These antibacterial agents have gained popularity in the ophthalmology field since they have been shown to be equivalent to combination therapy in the treatment of many ocular infections. Fluoroquinolones are also effective against a variety of gram-positive organisms, including streptococcal and staphylococcal species however; resistance is emerging among some of these organisms. The classification and mechanism of action of fluoroquinolones are given below.

Mechanism of Action for Fluoroquinolones[4, 5, 6]

Fluoroquinolones act by inhibiting two enzymes involved in bacterial DNA synthesis, both of which are DNA topoisomerases that human cells lack and that are essential for bacterial DNA

replication, thereby enabling these agents to be both specific and bactericide. DNA topoisomerases are responsible for separating strands of duplex bacterial DNA and inserting another strand of DNA through the break, then resealing the originally separated strands. DNA gyrase introduces negative superhelical twists in the bacterial DNA double helix ahead of the replication fork thereby catalysing the separation of daughter chromosomes.

Commonly used Fluoroquinolones in Ophthalmic Delivery

Antibiotic generation	Example	Use
1st Generation	Nalidixic acid	Have limited activity against gram negative & gram positive organism.
2nd Generation	Oxolinic acid Cinoxacin Pipemic acid	Improvement in gram negative coverage including antipseudomonal activity Shows limited activity against gram positive organism.
3rd Generation	Norfloxacin Ciprofloxacin Leavofloxacin Ofloxacin	Having antipseudomonal activity against gram negative bacilli
4th Generation	Ciprofloxacin Moxifloxacin	Having dual mechanism of action in gram positive bacteria in addition reducing efflux from the bacterial cell

This activity is essential for initiation of DNA replication and it allows binding of initiation proteins. Topoisomerase IV is responsible for decantation i.e removing the interlinking of daughter chromosomes thereby allowing segregation into two daughter cells at the end of around of replication. Fluoroquinolones interact with the enzyme-bound DNA complex (DNA gyrase with bacterial DNA or topoisomerase IV with bacterial DNA) to create conformational changes that result in the inhibition of normal enzyme activity. As a result, the new drug-enzyme-DNA complex blocks progression of the replication fork, thereby inhibiting normal bacterial DNA synthesis and ultimately resulting in rapid bacterial cell death. Older fluoroquinolones exhibit a relatively consistent pattern with respect to specificity of enzyme inhibition in different types of bacteria. The newer fourth generation fluoroquinolones like moxifloxacin, gatifloxacin have a dual-binding mechanism of action, inhibiting both DNA gyrase and topoisomerase IV, in gram-positive species[7].

MATERIALS AND METHOD

Levofloxacin hemihydrate was kindly supplied as a gift sample from Neuland Laboratories Limited, Hyderabad, Andhra Pradesh. Polyox was obtained as a gift sample from Dow. Hydroxyl propyl methyl cellulose (HPMC K4M) was gift sample from Colorcon Asia Pvt. Ltd. All chemicals were used of analytical grade. For the preparation of Polyox containing in situ gel,

a viscofying agent, HPMC K4M was first added to 75 ml of citrophosphate buffer pH 6 and allowed to hydrate. After that predetermined quantity of bezalkonium chloride was added to the solution. Then polyox was sprinkled over this solution and allowed to hydrate overnight. Levofloxacin was dissolved in 25 ml of buffer solution separately and then added to polymer solution under constant stirring[8]. Table 1 shows the composition of all formulation. Formulations were tested for ocular irritation study (Protocol approval no. : MCP/IAEC/130/2014).

Table 1: Different compositions of Levofloxacin hemihydrate in situ gel formulation.

Sr. No	Ingredients	Concentration in %w/v			
		FR1	FR2	FR3	FR4
1	Levofloxacin hemihydrate	0.5	0.5	0.5	0.5
2	Polyox	0.6	0.9	1.2	1.8
3	HPMC K4M	0.5	0.5	0.5	0.5
4	Benzalkonium Chloride	0.02	0.02	0.02	0.02
5	Citrophosphate buffer	100 ml	100 ml	100 ml	100 ml

Evaluation Parameters

Appearance

Clarity is one of the most important characteristic features of ophthalmic preparations. All developed formulations were evaluated for clarity by visual observation against a black and white background.

pH

pH is one of the most important parameter involved in the ophthalmic formulation. The two areas of critical importance are the effect of pH on solubility and stability. The pH of ophthalmic formulation should be such that the formulation will be stable at that pH and at the same time there would be no irritation to the patient upon administration of the formulation. Ophthalmic formulations should have pH range in between 6 to 7.4. The developed formulations were evaluated for pH by using Metler Toledo India Systronics digital pH meter.

Drug Content

Uniform distribution of active ingredient is important for achieving dose uniformity. The drug content was determined by diluting 1 ml of the formulation to 50 ml with phosphate buffer solution pH 7.4. Aliquot of 5 ml was withdrawn and further diluted to 50 ml with PBS. Levofloxacin concentration was then determined at 288 nm by using UV-Vis spectrophotometer.

Rheological studies

Viscosity of instilled formulation is an important factor in determining residence time of drug in the eye. The developed formulations were poured into the small sample adaptor of the Brookfield Synchrolectric viscometer and the angular velocity increased gradually from 10 to 100 rpm. The hierarchy of the angular velocity was reversed. The average of the two readings was used to calculate the viscosity.

***In vitro* release studies**

The *in vitro* release from the formulations was studied using cellophane membrane. Dissolution medium used was freshly prepared (pH 7.4) artificial tear fluid. 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 5 cm diameter). A 1-ml volume of the formulation was accurately pipetted into this assembly. The cylinder was attached to the metallic driven shaft and suspended in 50 ml of dissolution medium maintained at 37^0C so that the membrane just touched the receptor medium surface. The dissolution medium was stirred at 50 rpm using magnetic stirrer. Aliquots, each of 1-ml volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with the receptor medium and analysed by UV-vis. spectrophotometer at 288 nm.

Ocular irritation studies[9]

Ocular irritation studies were performed on male albino rabbits weighing 1-2kg.The modified Draize technique was designed for the ocular irritation potential of the ophthalmic product. According to Draize test, the eye drops (100µl) was normally placed in the lower cul-de-sac and irritancy was tested at the time interval of 1hr, 24hrs, 48hrs, 72hrs, and 1 week after administration. The rabbits were observed 18 periodically for redness, swelling and watering of the eye.

Stability studies[10]

Stability is defined as the extent to which a product retains, within specified limits and throughout its period of storage and use (i.e. its shelf life), the same properties and characteristics that it possessed at the time of its manufacture. Stability testing is performed to ensure that drug products retain their fitness for use until the end of their expiration dates. All the formulations were subjected to stability studies at accelerated condition i.e. 40^0C for a period of one month. The samples were withdrawn after 30 days and were evaluated for drug content, visual appearance and clarity.

RESULTS AND DISCUSSION

Appearance

Clarity of all the formulations was found to be satisfactory. There was no effect of terminal sterilization by autoclaving on the clarity and other physicochemical properties of the formulations. The haziness that was observed after autoclaving due to precipitation of HPMC at elevated temperature but it disappears and the original clarity was regained after overnight standing.The details are given in table2.

pH

The pH of the formulations was kept in the range of 6.7 - 7.4. The formulations were liquid at room temperature and at the pH formulated. Terminal sterilization by autoclaving had no effect on the pH. The details are given in table 2.

Drug Content

Results show that the percent drug content for all the formulations were well within limit and it shows uniform distribution of drug. The details are given in table 2**.**

Table 2: Result of Evaluation Parameter

Formulation	Appearance	pH	Gelling capacity	% Drug content
FR1	Transparent	7.20	++	100.30
FR2	Transparent	7.10	++	99.98
FR3	Transparent	6.98	+++	99.86
FR4	Transparent	7.12	+++	98.12

Rheological studies

Viscosity of the instilled formulation is an important in order to determine the residence time of the formulation in eye. It was determined by using Brookfield viscometer. Viscosity was measured at different angular velocities. Details are given in table 3and figure 1.

***In vitro* release studies**

All the formulations were subjected to in vitro release study and it was observed that formulation FR1, FR2shows comparatively very fast release and the FR4 shows incomplete drug release from formulation. Whereas FR3 found to produce prolong, sustained and complete release from the formulation. Table 4 and Figure2 give information about the release profile of the formulation.

Table 3: Rheological study of in situ gel formulation.

Angular velocity (rpm)	FR1 (CPS)	FR2 (CPS)	FR3 (CPS)	FR4 (CPS)
10	350	458	589	720
20	319	431	548	684
30	280	410	506	625

40	248	389	481	586
50	229	360	438	559
60	200	346	410	519
70	175	294	367	473
80	158	245	329	420
90	136	212	290	360
100	120	178	230	290

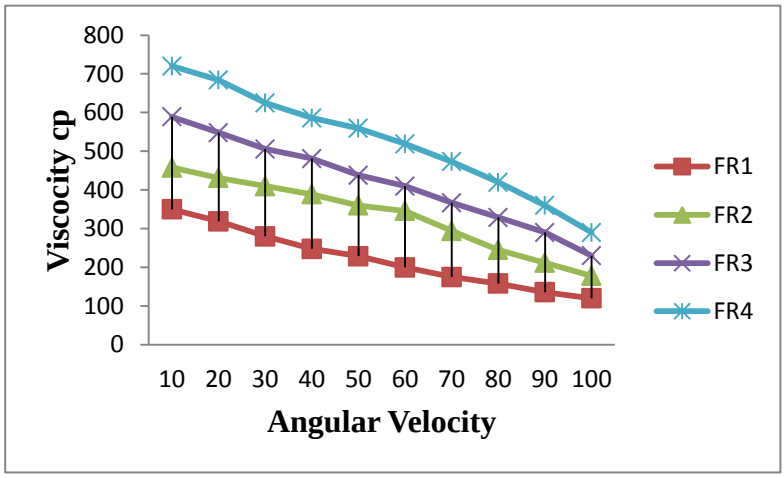

Figure 1: Rheological study of prepared in situ gel formulation.

***In vitro* release studies**

All the formulations were subjected to in vitro release study and it was observed that formulation FR1, FR2shows comparatively very fast release and the FR4 shows incomplete drug release from formulation. Whereas FR3 found to produce prolong, sustained and complete release from the formulation. Table 4 and Figure2 give information about the release profile of the formulation.

Table 4: % Cumulative drug release of in situ gel formulation.

Time in hrs.	FR1 (%)	FR2(%)	FR3(%)	FR4(%)
	0	0	0	0
1	28.3	23.478	15.11	10.34
2	45.89	41.304	25.66	17.76
3	53.65	48.695	38.45	26.09
4	68.98	64.608	49.52	34.23
5	77.32	74.347	60.73	49.34
6	89.9	84.521	74.09	60.88
7	98.55	92.347	89.39	74.12
8	100	99.89	99.78	84.09

Ocular irritation studies[9]

The pH of all the formulation was kept in the range of 6 to 7.4 and they found non irritating for eye. Even there was no abnormality or redness was observed during testing.

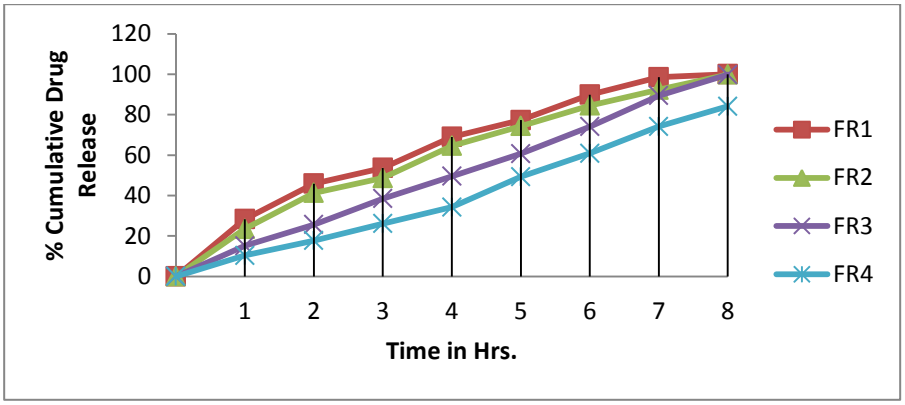

Figure 2: % Cumulative drug release from different formulation.

Stability studies[10]

All the formulations were stable at 40^0C for a period of one month. Results are given in table 5.

Table 5: Comparative data of % drug content and pH of the formulation over stability at accelerated condition.

Formulation	Visual appearance		Clarity		pH		Drug Content (In %)	
	Initial	30 days	Initial	30 days	Initial	After 30 days	Initial	After 30 days
FR3	Transparent	Transparent	Clear	Clear	7.20	7.19	99. 68	99.76

CONCLUSION

Levofloxacin is an antibiotic used for treatment of ocular conjunctivitis was successfully formulated using polyox pH triggered and HPMC K4M as viscosity enhancing agent. In the present work FR3 formulation found to produce sustained the drug release for 8 hours and it was also compatible with eye and it is non irritating. It also ensures the reduced dosing frequency thus improving patient compliance.

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