Journal of Applied Pharmaceutical Science Vol. 4 (01), pp. 101-104, January, 2014
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2014.40117
ISSN 2231-3354 

pH-induced in situ gelling system of an anti-infective drug for sustained ocular delivery

Abdul Malik PH* and S Satyananda

Department of pharmaceutics, Karpagam University, Pollachi Main Road, Coimbatore, Tamilnadu-641021, India.

ARTICLE INFO

Article history:
Received on: 18/10/2013
Revised on: 20/12/2013
Accepted on: 14/01/2014
Available online: 30/01/2014

Key words:
pH induced in situ gel, Gatifloxacin, Carbopol 940, HPMC E50LV, HPMC E4M.

ABSTRACT

Recently, in situ gel formation has been extensively studied to enhance ocular bioavailability and the duration of drug activity. Poor ocular bioavailability of drugs (<1%) from conventional eye drops is mainly due to the precorneal loss factors that include rapid tear turnover**,** nonproductive absorption, transient residence time in the cul-de-sac, and the relative impermeability of the drugs to corneal epithelial membrane. These problems may overcome by the use of in situ gel-forming systems that are instilled as drops into the eye and undergoes sol-gel transition in the cul-de-sac. In this study, in situ gelling system of Gatifloxacin were prepared using polymers carbopol 940 (0.1% to 0.5% w/v) and HPMC E4M (0.2% to 0.6% w/v). The developed formulation was characterized for various in vitro parameters such as clarity, temperature, pH, tonicity, sterility, rheological behavior, drug release profile, transcorneal permeation profile, and ocular irritation. Developed formulation was clear isotonic solution, converted into gel at temperatures above 35 °C and pH 6.9–7.0. The results demonstrated that the carbopol/ HPMC mixture can be used as an in situ gelling vehicle to enhance the ocular bioavailability of Gatifloxacin. The developed system is a viable alternative to conventional eye drops for the treatment of Bacterial conjunctivitis and various other ocular infections.

INTRODUCTION

Most commonly available ophthalmic preparation is eye drops and ointments. But the preparations when instilled into the cul-de sac are rapidly drained away from the ocular cavity due to tear flow and lachrymal nasal drainage. Only a small amount is available for its therapeutic effect, which results in frequent dosing.

When a drug solution is dropped into the eye, effective tear drainage and blinking results in 10-fold reduction of drug concentration in 4-20 minutes. The limited permeability of the cornea contributes to low absorption of ocular drugs. Due to tear drainage, most of the administered dose is absorbed via the nasal-lachrymal duct to the GI tract, leading to side effect the rapid elimination of the administered eye drops often results in short duration of the therapeutic regimen necessary (Srividya *et al*., 2001; Miyazaki *et al*., 2001). Ocular therapy could be significantly improved if the pre-corneal residence time of drugs could be increased.

Several new preparations have been developed for ophthalmic use, not only prolong the contact time of the vehicle at ocular surface, but also to slow down the elimination of the drugs.

This problem can be overcome by using *In-situ* gel forming ophthalmic drug delivery systems, prepared from polymers that exhibit reversible phase transition and pseudo-plastic behavior to minimize interference with blinking. Such system can be formulated as liquid dosage form suitable for administration by instillation in to the eye, which upon exposure to the eye, shift to the gel phase depends upon physiological pH condition of eye (Geeta *et al.*, 2007).

Treatment of bacterial conjunctivitis requires frequent administration of antibiotics as eye drops. This associated with transient peaks of high drug concentration in the eye, which in turn results in undesirable side effects. These short comings may be overcome by the development of “In situ gelling system”, which have the potential to enhance bioavailability and to reduce the side effects of potent new drugs . In this study, In situ gelling system of Gatifloxacin were prepared using polymers carbopol 940 (0.1% to 0.5% w/v) and HPMC E4M (0.2% to 0.6% w/v) (Charoo *et al*., 2002).

* *Corresponding Author*
Abdul Malik PH,Department of pharmaceutics, Karpagam University, Pollachi Main Road, Coimbatore, Tamilnadu-641021, India.

MATERIALS AND METHODS

Gatifloxacin was obtained from Sun pharmaceutical pvt ltd (Mumbai, India). Carbopol 940, HPMC E50LV and HPMC E4M were a gift sample from Color cone Asia Ltd, Verna, Goa. All other chemicals and solvents were of analytical grade.

MEDICATED FORMULATION

For antibacterial activity Gatifloxacin is prescribed as 0.3% to 0.4% w/v solution. Hence, a final drug concentration of 0.3% was used in formulation. The buffer salts were dissolved in 50 ml of purified water; HPMC (E50LV / E4M) was added to hydrate. Carbopol 940 was sprinkled over this solution and allowed to hydrate overnight.

The solution was stirred with an overhead stirrer. Gatifloxacin was dissolved in small quantity of water. Benzalkonium Chloride (Preservative) and Sodium chloride (Isotonocity adjusting agent) was added to this solution; the drug solution was added to the polymer solution under constant stirring until a uniform solution was obtained. Purified water was then added to make up the volume to 100 ml. Formulation pH was adjusted to pH 5 with the help of 0.5 M Sodium Hydroxide. This solution was filtered through 0.2µm filter paper. The optimized formulations were sterilized in an autoclave at 121º C and 15 psi for 15 minutes.

EVALUATION OF FORMULATION

Visual Appearance and Clarity

Visual appearance and Clarity was done under fluorescent light against a white and black back ground for presence of any particulate matter

pH

The pH of the prepared in-situ gelling system after addition of all the ingredients was measured using pH meter.

Drug Content Analysis

Drug content analysis of prepared in-situ gelling systems was carried out using Spectrophotometric method. The assay of these formulations was carried out by pipetting 0.1 ml of all four optimized formulations, and it was diluted up to 100 ml of Simulated Tear Fluid (pH 7.4). The absorbance was measured at 287.5 nm using UV-Visible spectrophotometer.

In-Vitro Gellation

The Gelling capacity of the formulations containing different ratio of Carbopol 940 and HPMC (E50LV / E4M) was evaluated. It was performed by placing a drop of polymeric solution in vials containing 1 ml of Simulated Tear Fluid, freshly prepared and equilibrated at 34°C, and visually assessed the gel formed and time for gelation as well as time taken for the gel formed to dissolve.

Interaction studies

Prepared in-situ gel formulations were tested for the intactness of drug in the various formulations by comparing with pure drug. These were done to ensure that, the therapeutically active drug has not undergone any change after it has been subjected to processing steps during preparation of in-situ gelling systems. These studies were performed by taking IR spectra using KBr method.

Sterility Testing

Sterility testing were intended for detecting the presence of viable form of microorganisms and were performed for Aerobic and Anaerobic bacteria and fungi by using Fluid Thioglycolate Medium and Soyaban Casein Digest medium respectively, as per the Indian Pharmacopoeia.

Rheological Studies

Rheological properties of the prepared in-situ gelling systems under the different Shear rates (2, 4, 6, 10, 20, and 30 rpm) were measured at nonphysiological (pH 5 and 25°C) and physiological condition (pH 7.4 and 34°C), respectively. The hierarchy of the shear rate was reversed, and the average of two reading was used to calculate the viscosity.

In-Vitro Release Studies

The in-vitro release of Gatifloxacin as pure drug well as from the prepared formulations was studied through cellophane membrane using diffusion cell. The cellophane membrane was soaked overnight in the receptor medium (Simulated Tear Fluid, pH 7.4). It was tied to one end of a glass diffusion cell. 100 ml of receptor medium was taken in the 200 ml beaker. The diffusion cell was filled with 2 ml of the formulation and suspended in 100 ml of receptor containing beaker by assuring that the membrane was just touched the receptor medium surface. The whole assembly was transferred on magnetic stirrer and was maintained at 34°C ± 1°C and 22 rpm. The drug samples (1 ml) were withdrawn at the interval of 30 minutes from receptor medium and replaced by equal volumes of the receptor medium. The samples were diluted with appropriate receptor medium and analyzed by a UV-Visible spectrophotometer at 287.5 nm using receptor medium as a blank

Ocular Irritancy Studies

In developing a novel ophthalmic delivery system, an injury to the eye was taken into consideration. Since, eye being a sensitive, most delicate and yet most valuable of the sense organs, the injuries to the Cornea, Conjunctiva, and Iris were measured according to Draize test. According to the Draize test, the amount of the test substance applied to the eye is normally 100 µl placed into the lower cul-de-sac with observation of the various criteria made at a designated required time interval of 1hr, 24 hrs, 48 hrs, 72 hrs, and 1 week after administration. A total four albino rabbits (male) weighing 1.5-2 kg was used for the present study. The

sterile formulations were instilled twice a day for a period of 7 days. Rabbits were observed periodically for redness, swelling and watering of the eye. The evaluation was made according to the Draize test protocol.

Stabilities Studies

Stability studies were carried out on optimized formulation according to ICH guidelines. Stability is defined as the extent, to which a product retains with in specified limits and through out its period of storage and use i.e., shelf life. Stability studies were carried out on optimized formulations according to International Conference on Harmonization (ICH) guidelines. A sufficient quantity of formulations in previously sterilized vials was stored in desiccato+-rs containing a saturated solution of sodium chloride, which gives a relative humidity of 75±5 %. The desiccators were placed in a hot air oven maintained at a temperature 40°C±0.5°C and at room temperature. Samples were withdrawn at 7 days interval for 42 Days. The logarithms of percent drug remaining were calculated and plotted against time in days.

RESULTS AND DISCUSSION

In the present work, the in-situ gelling systems were prepared by pH triggering method with the help of gelling agent Carbopol 940 and viscosity increasing agent, HPMC E50LV & HPMC E4M. Firstly the formulation containing Carbopol 940 (0.1-0.5 %) alone was prepared. As observed during practical work that Carbopol solution with lower concentration of it, viscosity of gels formed after gellation at pH 7.4 was very low where as at higher concentration, it was very high. One more problem was found that, as Carbopol concentration increase, the pH of the formulation became acidic. So, to decrease the concentration of Carbopol 940 without compromising gelling capacity of it, it was decided to use the viscosity increasing agent, HPMC. Different viscosity grades of HPMC (E50LV / E4M) were used in combination with Carbopol 940 to optimize the concentration of them for desire viscosity and gelling capacity. From the prepared formulation, best 4 formulations were selected on the basis of Viscosity and Gelling capacity. Drug was incorporated in these optimized formulations with other excipients. The formulations are tabulated below.

Table. 1: Contents of Formulations.

Ingredients	Concentrations (% w/v)			
	A	B	C	D
Gatifloxacin	0.3	0.3	0.3	0.3
Carbopol 940	-	-	-	-
HPMC E4M	-	-	-	-
SodiumChloride	0.9	0.9	0.9	0.9
Benzalkonium Chloride	0.01	0.01	0.01	0.01
Acetate Buffer (pH 5) up to	100	100		100

All formulations exhibited pseudo plastic viscosity and the viscosity was directly dependent on the polymeric content of the formulations. No change in the viscosity of the formulations was observed after autoclaving. Optimized in-situ gels were subjected for preliminary evaluation such as, Visual appearance, Clarity, pH and Drug content. All formulations were found transparent and clear. pH of the formulations was within 5 ± 0.5. Drug content was found within 99.38 % to 99.71%. In all optimized in-situ gelling systems. The formulations were both liquid at room temperature and when refrigerated. To evaluate the Rheological behaviour, viscosity of the formulations before and after addition of STF was evaluated using Brook Field viscometer. It shows that as viscosity of all formulations decreases, the shear rate also increases which shows the character of pseudoplastic fluid.

In-vitro release of Gatifloxacin from the selected formulations was studied through diffusion cell using cellophane membrane for 8 hours. It was compared with the pure drug as well as marketed eye drop. Results reveals that all formulations exhibited sustained release of the drug (above 84 %) from the Carbopol 940 and HPMC E50LV/E4M network over 8 hours. Further all formulations were subjected for sterility testing using nutrient agar media and incubated for 7 days under daily observation. This study shows that the formulations does not have any microbial contamination, and was sterile. Antimicrobial efficacy study carried out by using Staphylococcus Aureus, Pseudomonas Aeruginosa and E.coli as test microorganisms. After incubation up to 24 hours, it was found that all formulations were effective as antimicrobial action.

Lastly, formulations were evaluated for the stability studies (at RT and 34ºC, 75±5 % RH) for 42 days. Results reveal that no changes were found in Visual appearance, Clarity and pH. These formulations were also analysed for % drug remaining. This study shows that no definite changes were observed in the intactness of the drug after accelerated study of 42 days. The release percentage at regular intervals were represented as follows.

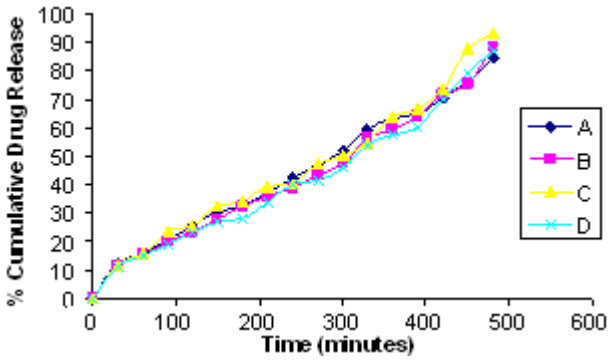

Fig. 1: In-Vitro Release Profile of In-Situ Gel Formulations.

REFERENCES

Geeta MP, Madhubhai MP. Recent Advances and Challenges in Ocular Drug Delivery Systems. Pharma Times, 2007; 39(1): 21-25.

Shymala BP, Lakshmi K, Harish CG. Topical ocular drug elivery. Indian J Pharm Sci, 2005; 67(3): 404-408.

Kumar S, Haglund BO, Himmelstein KJ. In Situ Forming Gels for Ophthalmic Drug Delivery. J Ocu Pharmacology, 1994; 10(1): 47-56.

Cohen S, Lobel E, Trevgoda A, Peled YA. Novel In Situ Forming Ophthalmic Drug Delivery System from Alginates Undergoing Gellation in the Eye. Journal of Controlled Release, 1997; 44(3): 201-208.

Ding S. Recent Developments in Ophthalmic Drug Delivery. Pharm Sci Tech Today, 1998; 1(8): 328-335.

Dimitrova E, Bogdanova SV, Mitcheva M, Tanev I, Minkov E. Development of Model Aqueous Ophthalmic Solution of Indomethacin. Drug Development and Industrial Pharmacy 2000; 26(12): 1297-1301.

Sechoy O, Tissie G, Sebastian C, Maurin F, Driat J, Trinquand CA. New Long Acting Ophthalmic Formulation of Carteolol containing Alginic Acid. Int J Pharm 2000; 207: 109-116.

Lin H and Sung K. C. Carbopol / Pluronic Phase change Solution for Ophthalmic Drug Delivery. J Cont Rel, 2000; 69(3): 379-388.

Srividya B, Cardoza RM Amin PD. Sustained Ophthalmic Delivery of Ofloxacin from a pH Triggered in Situ Gelling System. J Cont Rel, 2001; 73(2): 205-211.

Miyazaki S, Suzuki S, Kawasaki N, Endo K, Takahasi A, Attwood D. In Situ Gelling Xyloglucan Formulation for Sustained Release Ocular Delivery of Pilocarpine HCL. Int J Pharm, 2001; 229(1): 29-36.

Charoo NA, Kohil K, Ali A. Preparation of In Situ Forming Ophthalmic Gels of Ciprofloxacin HCL for the Treatment of Conjunctivitis: In Vitro and In Vivo Studies. J Pharml Sci, 2002; 92(2): 407-413.

Roizer A, Manzuel C, Grove J, Planzonnet B. In Situ Gelling Polymer for Ophthalmic Vehicles Effect on Bioavailability of Timolol. Int J Pharm, 1989; 57(2): 162-168.

Lindell K, Engstrom S. In Vitro Release of Timolol from An In Situ Gelling Polymer System. Int J Pharm, 1993; 95(3): 219-228.

Kumar S, Himmelstein K. Modification of In Situ Gelling Behavior of Carbopol Solutions by HPMC. J Pharm Sci, 1995; 84(3): 344-348.

How to cite this article:

Abdul Malik PH and S Satyananda. pH-Induced in Situ Gelling System of an Anti-Infective Drug for Sustained Ocular Delivery. J App Pharm Sci, 2014; 4 (01): 101-104.