ejpmr, 2022, 9(9), 196-200

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

FORMULATION AND EVALUATION OF PH TRIGGERED INSITU GELLING SYSTEM CONTAINING LEVOFLOXACIN

Harshitha V. Y.*, Somashekar C. N. and Navaneethan S.

*Department of Pharmaceutics, Bharathi College of Pharmacy, Bharathinagar-571422, Maddur Taluk, Mandya District, Karnataka, India.

***Corresponding Author: Harshitha V. Y.**

Department of Pharmaceutics, Bharathi College of Pharmacy, Bharathinagar-571422, Maddur Taluk, Mandya District, Karnataka, India.

Article Received on 30/06/2022 **Article Revised on 20/07/2022** **Article Accepted on 10/08/2022**

ABSTRACT

Topical administration of a drug in the conjunctival cul-de-sac is the treatment of choice for diseases of the anterior segment of the eye. The Development of ophthalmic drug delivery systems has always been challenging because of the drawbacks of this route, like non-productive absorption, drainage, induced lacrimation, tear turnover, and impermeability of drugs to the cornea. New approaches have been investigated to deliver drugs to the eye utilizing polymeric delivery of ophthalmic drugs to the pre-and intraocular tissues. They have attempted to increase the bioavailability and the duration of therapeutic action of ocular drugs. Specific new approaches to increase the ocular bioavailability and duration of the drug action and reduce the undesirable side effects are using drug carriers that regulate pre-corneal drug loss and improve the corneal contact time. Many of these systems prolong ocular bioavailability but do not control drug penetration through the cornea. Consequently, the drug concentration at the site of action might remain inadequate. Therefore, developing a safer, more productive, and more acceptable ocular therapeutic system is necessary. they can improve the ocular bioavailability of the drugs by prolonging their residence time in the cul-de-sac and increasing their corneal permeability. Various new dosage forms include in-situ gel, collagen shield, etc. Conventional ocular drug delivery systems like eye drops, ointments, and suspensions have varied disadvantages of lachrymation, and blurred vision. The developed formulation was therapeutically efficacious, stable, non-bother and provided sustained unleash of the drug. This review tries to debate the newer Developments and techniques for this drug delivery together with physiological factors, physicochemical factors and formulation factors to be thought about within the Development of an unchanged drug delivery system.

KEYWORDS: Opthalmic Fomulations, pH Triggering systems, Gellan Gum, etc.

INTRODUCTION

The 'in situ gel' system has emerged as one of the best novel drug delivery systems. The in-situ gelling system helps for the sustained and controlled release of the drugs and improves patient compliance and comfort through its unique characteristic feature of the 'Sol to Gel' transition.[1]

The effective dose administered may be altered by increasing the retention time of medication in the eye by using in situ gel-forming systems. Ophthalmic drug delivery is an exciting and highly challenging endeavour. The eye's anatomy, physiology, and biochemistry render this organ exquisitely impervious to foreign substances. The challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage.

The attempt to develop in-situ gel systems started in the past few years. There are a large number of inventions and many patents in the field of the in-situ gelling system. There are several advantages to in-situ gel forming polymeric delivery systems, such as ease of administration and reduced frequency of administration, improved patient compliance and comfort, and enhanced pre-corneal retention time. One of the essential advantages of in-situ gel formulations is drugs that are directly given for local action in the eye to treat allergic conditions. In-situ gel formation is characterized by proportionately using a polymer or mixture of polymers. When the formulation is instilled into the eyes, the gel may form due to one or more combinations of different stimuli like pH change, temperature modulation, solvent exchange and ion exchange mechanism. This formed gel will lead to de333livering the drug in the proposed manner.[2]

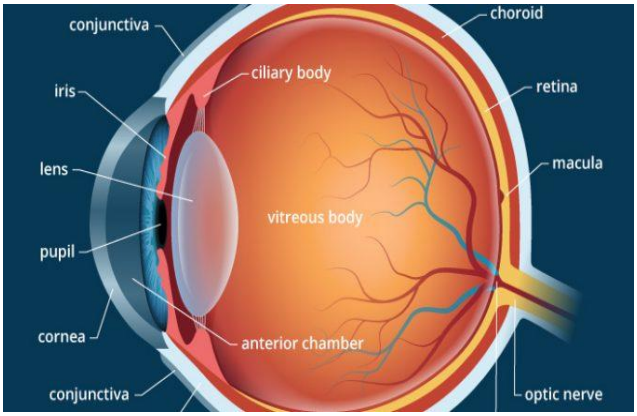


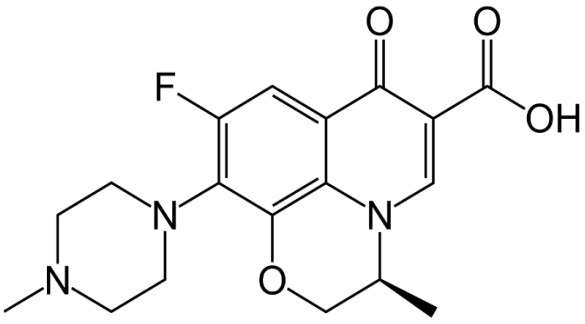
Figure 1: Anatomy of Human Eye.

GELLAN GUM

Gellan gum is a polysaccharide that can be used to induce ion-sensitive hydrogels. It is a linear anionic heteropolysaccharide made up of a tetrasaccharide repeating unit of glucose, glucuronic acid and rhamnose in the ratio of 2:1:1. Gellan comprises hydroxyl and carboxylic functional groups, which may interact with other polymers it via hydrogen bonding and electrostatic attractions. A low-acetyl gellan gum is commonly available in the market as Gelrite®, which undergoes gelation in the presence of mono- or divalent cations. The electrolytes of the tear fluid, especially Na+, Mg2+ and Ca2+ cations, are known to induce gel formation of the polymer upon instillation as a liquid solution into the cul-de-sac. Incorporating optimal quantities of calcium gluconate into gellan formulations leads to forming gellan calcium gluconate-simulated tear fluid (STF) gels with a significantly higher] strength than when gellan alone was mixed with STF. It undergoes gelation by both temperature-sensitive or cations-induced mechanisms. The possible mechanism of gelation includes forming double-helical junction zones followed by aggregation of the double-helical segments to create a three-dimensional network by hydrogen bonding with water and complexation with cations.[3]

LEVOFLOXACIN

Molecular formula: $C_{18}H_{20}FN_3O_4.1/2H_2O$



$1/2H_2o$

Figure 1: Structure of Levofloxacin.

Mechanism of Action

Levofloxacin is a bactericidal antibiotic of the fluoroquinolone drug class that directly inhibits bacterial DNA synthesis by inhibiting the type II topoisomerase enzyme, topoisomerase IV. Topoisomerase IV is necessary to separate DNA that has been replicated before bacteria cell division. The process is stopped with the DNA not being separated, and the bacterium cannot divide. DNA-gyrase and Levofloxacin promote the breakage of DNA strands by inhibiting DNA-gyrase in susceptible organisms, inhibiting the relaxation of supercoiled DNA. Both the mechanisms kill the bacterium and result in the death of bacteria.

METHOD

COLD METHOD: The buffer salts were dissolved in 50ml of purified water, HPMC E-50lv was added and allowed to hydrate, and carbopol was sprinkled over this solution and hydrated overnight. The solution was stirred with an overhead stirrer, and Levofloxacin was dissolved in a small quantity of water. Benzalkonium chloride (BKC) was added to this solution, and to add the drug solution to the polymer solution. Add purified water to make up the volume of 100ml of this solution and filter through 0-2mm filter paper. When the drug solution and polymer solution were mixed, immediate precipitation of carbopol occurred due to a decrease in pH brought about by carbopol. Therefore the drug was incorporated in a sufficient quantity of 0.5M NaOH and then added to the polymer solution to get a clear solution of drug and polymer.[4]

PREFORMULATIONS STUDIES[5]

a) **Identification of drug:** The identification of levofloxacin was done by UV, DSC, and FTIR and confirmed as per monographs.

b) **Solubility analysis:** Solubility analysis of levofloxacin was carried out in various solvents and phosphate buffers. As a result, 10mg of levofloxacin was dissolved in water, pH 1.2, pH 4.8, pH 6.0, pH 7.4

c) **Melting point determination:** The capillary method has been used to determination of the melting point of levofloxacin. A small amount of compound was placed in a thin-walled capillary tube of about 10-15cm long and 1mm inside diameter and closed at one end. The capillary containing the sample and a thermometer is then suspended in an oil bath containing liquid paraffin. So, they can be heated slowly and evenly. The temperature range over which the sample is observed to melt is taken as the melting point

d) **Fourier transform infra- red spectroscopy:** The FT-IR of levofloxacin has been recorded with a nature of interacting forces that can be evaluated using the potassium bromide pellet method.

e) **Thermal analysis by DSC:** DSC can be conducted for in situ forming polymeric systems to quantify the hydrogel's water percentage. Differential scanning calorimetry is used to observe if there are any changes in thermograms as compared with the pure ingredients used, thus indicating the interactions.

EVALUATIONS[6,7,8]

1. **Drug content:** It is determined by taking 1ml of the formulation and diluting it to 100ml with distilled water. 5 ml was withdrawn and further diluted to 25 ml with distilled water. Concentration was determined at 200-400mm by using UV visible spectroscopy.
2. **pH of the gels:** To evaluate the prepared in situ gel formulations for pH measurement using a pH meter.
3. **Gelling capacity**: The gelling capacity was measured by the visual method. 2ml sample was placed in a vial containing 5ml of freshly prepared artificial tear fluid and was visually assessed for gel formation. The time taken for gel formation is noted.
4. **Visual appearance and clarity:** Visual appearance and clarity of prepared in situ formulations are checked for the presence of any particulate matter under a fluorescent light against a white and black background.
5. **In vitro drug release study:** In vitro drug release study is done by using Franz diffusion cell. In the receptor compartment, freshly prepared artificial tear fluid (ATF) is placed. The dialysis membrane is placed in between receptor and donor compartments. The whole assembly is kept on the thermostatically controlled magnetic stirrer to simulate in vivo conditions, and the temperature of the medium is maintained at 37degree celsius ± 0.5degree celsius. Medium is continuously stirred at 20 rpm. 1ml of the formulation is placed in the donor compartment. Sample (0.5ml) is withdrawn at a predetermined time interval, and the ATF replaces same. Samples are analyzed either on a UV spectrophotometer or HPLC.
6. **Sterility testing:** To carry out sterility testing, the formulation should be incubated at 300-350 degrees Celsius for not less than 14 days in fluid thioglycollate media. Incubation of formulation at 200-250 degrees Celsius in soya bean casein digest medium. Thioglycolate medium is used to find the growth of bacteria, whereas the soya bean casein medium is for fungi in the formulation.
7. **Accelerated stability studies:** Place formulation in an amber colour vial and seal it with aluminium foil for accelerated stability studies at 40± 20 c and relative humidity 75±5% as mentioned in ICH, and place the vial for stability studies after every month sample is analyzed for clarity, pH, gelling capacity, drug content, rheological evaluation and in vitro dissolution.

RESULTS

1. FORMULATION

Table 1: Formulation of In Situ gel.

Ingredients (grams)	F1	F2	F3	F4
Levofloxacin	0.5	0.5	0.5	0.5
Carbopol 940	0.1	0.2	0.3	0.4
HPMC E50LV	1.5	1.5	1.5	1.5
Sodium alginate	-	-	-	-
Benzylalkonium chloride	0.02	0.02	0.02	0.02
Gellan gum	0.1	0.2	0.3	0.4
Sodium chloride	0.9	0.9	0.9	0.9
Distilled water (q.s)	100	100	100	100

Melting point determination

Table 2: Melting point of Levofloxacin.

Reported	Method	Observed
224-227 ℃	Thiel's tube method	224 ℃
	DSC	227℃

2. FTIR OF LEVOFLOXACIN

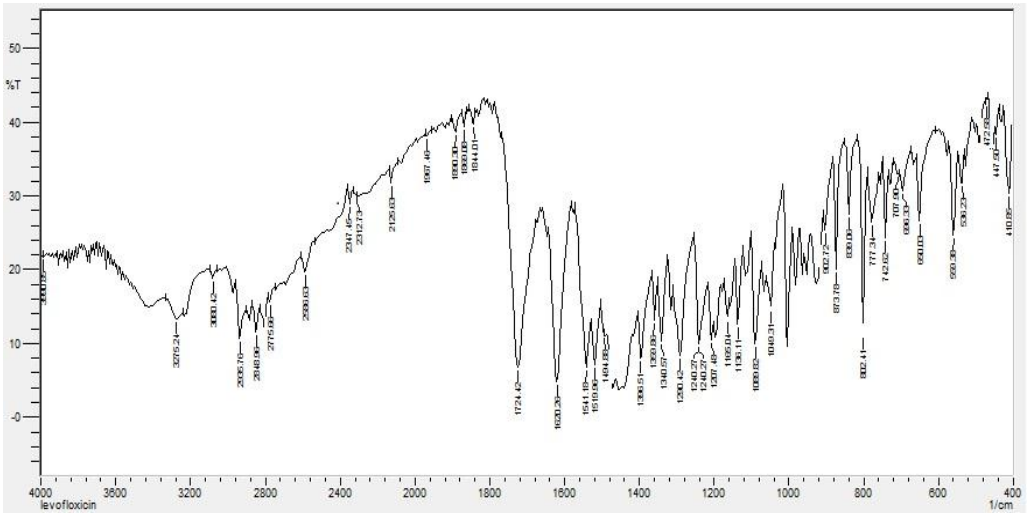


Figure 2: FTIR of Levofloxacin.

3. DSC THERMOGRAPH OF LEVOFLOXACIN

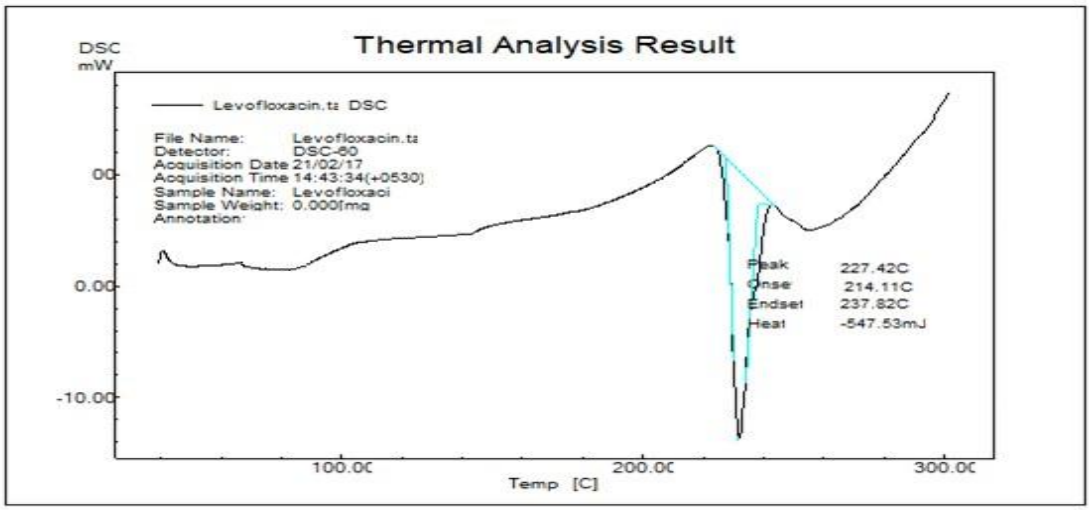


Figure 3: DSC of Levofloxacin.

4. pH of the Gel

Table 3: pH of the In Situ gel.

Formulation code	pH
F1	6.18 0.01
F2	6.24 0.04
F3	6.86 0.03
F4	6.55 0.03

5. Gelling Capacity of Gel

Table 4: Gelling Capacity of In Situ gel.

Formulations	Gelling Capacity
F1	+++
F2	+++
F3	+++
F4	+++

6. Visual Appearance

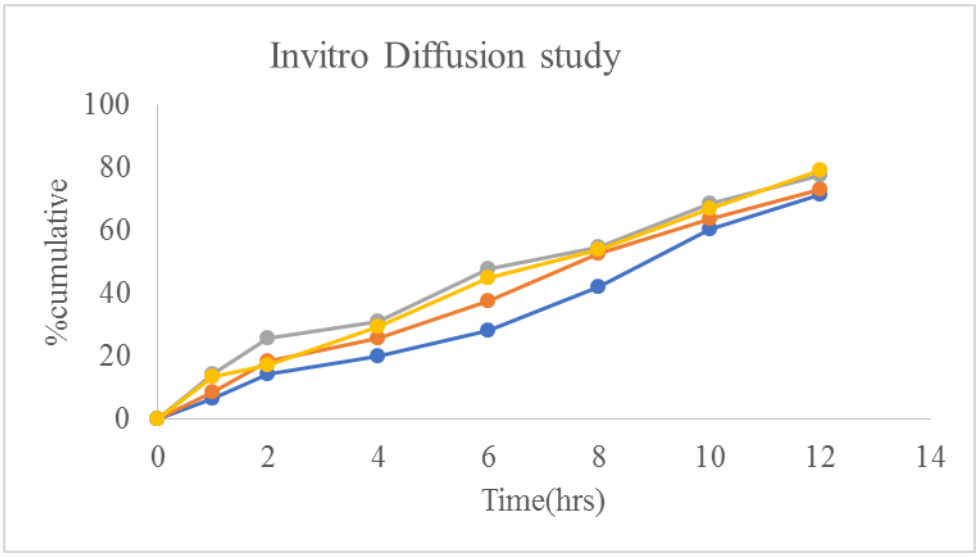
Table 5: Visual Appearance of In Situ gel.

Formulations code	Visual Apperance
F1	Transparent
F2	Transparent
F3	Transparent
F4	Transparent

7. In vitro Diffusion

Table 6: In vitro Diffusion of In Situ gel.

TIME	F1	F2	F3	F4
0	0	0	0	0
1	6.548431	8.45839	14.46112	13.36971
2	14.18827	18.28104	25.64802	17.18963
4	20.191	25.92087	31.10505	29.46794
6	28.37653	37.65348	47.74898	44.74761
8	42.0191	52.6603	54.84311	54.02456
10	60.30014	63.8472	68.48568	66.84857
12	71.48704	73.12415	77.48977	79.39973



Graph 1: In vitro Diffusion study of In Site gel.

CONCLUSION AND DISCUSSION

Several disadvantages related to conventional dosage form polymeric in-situ gels are developed to prolong the drug's release from the formulation by forming gels. This provides other advantages over conventional dosage forms, such as good stability, biocompatibility, and biodegradable polymers, making the ocular in-situ gelling system more preferable for treating ocular diseases.

- This research aimed to develop an ophthalmic in situ drug delivery system of levofloxacin to improve its poor ocular bioavailability.
- The ocular in situ levofloxacin gel was prepared using carbopol 940 and HPMC (E50LV) by pH-triggered in situ gelling technique.
- The broad-spectrum antibacterial agent used in treating ocular infections like conjunctivitis was successfully formulated in situ gelling system using 0.5%W/V of levofloxacin.
- The formulated in situ gelling systems were characterized for appearance, colour, pH, gelling capacity, rheological character, and in vitro release in simulated tear fluid.
- The viscosity results revealed that F-4 showed better pseudo-plastic behaviour in the highest concentration of Carbopol 940 (0.4%) than in other formulations.
- The in vitro release studies revealed that the increase in polymer concentration retards the drug release and the decrease in polymer concentration increases the drug release.
- The pH-triggered in situ gelling system afforded controlled drug release over a 12-h period.
- The pH-triggered in situ gel of levofloxacin has controlled drug release than conventional ophthalmic solutions of levofloxacin, and it is a viable alternative to conventional eye drops by its ability to retard drug release.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the contributions to our Guide Mr.Somshekhar C N and We would like to thank our principal, and proffessors for providing us suitable environment for this work. Special thanks to teaching and non-teaching staff of M. Pharmcy department.

REFERENCE

1. Mohanty D, Bakshi V, Simharaju N, Haque MA, Sahoo CK. A review on in situ gel: a novel drug delivery system. Int. J. Pharm. Sci. Rev. Res, 2018; 50(1): 175-81.
2. Mandal S, Thimmasetty MK, Prabhushankar GL, Geetha MS. Formulation and evaluation of an in situ gel-forming ophthalmic formulation of moxifloxacin hydrochloride. International journal of pharmaceutical investigation, 2012 Apr; 2(2): 78.
3. Wu Y, Liu Y, Li X, Kebebe D, Zhang B, Ren J, Lu J, Li J, Du S, Liu Z. Research progress of in-situ gelling ophthalmic drug delivery system. Asian journal of pharmaceutical sciences, 2019 Jan 1; 14(1): 1-5.
4. Mohan EC, Kandukuri JM, Allenki V. Preparation and evaluation of in-situ-gels for ocular drug delivery. J Pharm Res, 2009 Jun 2; 2(6): 1089-94.
5. Kashyap N, Viswanad B, Sharma G, Bhardwaj V, Ramarao P, Kumar MNV. Design and evaluation Of biodegradable, biosensitive in situ gelling Systems for pulsatile delivery of insulin. Biomaterials, 2007; 28: 2051-60.
6. Preetha JP, Karthika K, Rekha NR, Elshafie K. Formulation and evaluation of in situ ophthalmic gels of diclofenac sodium. Journal of Chemical and Pharmaceutical Research, 2010; 2(3): 528-35.
7. Majeed A, Khan NA. Ocular in situ gel: An overview. Journal of Drug Delivery and Therapeutics, 2019 Jan 15; 9(1): 337-47.
8. Rukari TG, Jadhav AS, Londhe R, Phalke PL. A review on ophthalmic in situ gels. Am. J. Pharm. Tech. Res, 2019; 9: 159-70.