Design and Characterization of Flucytosine Loaded Bioadhesive In Situ Ophthalmic Gel for Improved Bioavailability

Ram Garg*, Vikas Kumar, Vandana Sharma

Arya College of Pharmacy, Kukas, Jaipur (RJ)- 302001, India

Article Information

Received 25 July 2019

Received in revised form 26 Dec 2019

Accepted 27 Dec 2019

Keywords:

Flucytosine,
In Situ Ophthalmic Gel,
Bioavailability

Corresponding Author:

E-mail : pawanrgcp@gmail.com

Mob.: +917376007276

DOI: [10.20510/ukjpb/7/i6/1581754222](https://doi.org/10.20510/ukjpb/7/i6/1581754222)

Abstract

Flucytosine is a semisynthetic macrolide antibiotic used for the treatment of trachoma caused by *Chlamydia trachomatis*, a gram negative bacteria. The study was planned to formulate in situ ocular gel of Flucytosine to prolong the pre-corneal residence time and gives the better bioavailability as compare to conventional dosage form. The in situ gel of Flucytosine was prepared by mixing with polymer of xanthan gum, HPMC and sodium alginate in different ratio. The six different formulations were prepared and evaluated for Clarity, visual appearance, pH, gelling capacity, drug content, in vitro drug release, kinetic model and ocular irritancy. The best formulation was subjected for stability study. The results of evaluation parameters were satisfactorily for all formulations, while F4 demonstrated maximum drug release at 8 hr and follow zero order drug release. The findings of ocular irritant indicates the F4 were non irritant and safe to use. The outcomes of stability study indicate that the F4 gel stored at room temperature and accelerated temperature were found to be comparatively stable. The study concluded that the in situ ocular gel of Flucytosine will be substitute for conventional eye drops in future.

1 Introduction

Ophthalmic ointments guarantee unrivaled drug bioavailability by expanding the contact time, limiting the dilution by tears, and opposing nasolacrimal drainage. Significant impediment of ointment, giving obscured vision, because of this it could be utilized either evening or for treatment outwardly and edges of the eyelids. Suspension as ophthalmic delivery systems relies on the assumption that particles may persist in conjunctival sac. Precorneal drug loss can be minimal, for example, hindering waste by utilizing dispersion controlled, nonerodible polymeric insert. The significant detriment of inserts is the lack of of patient acknowledgment inferable from difficult administration. The improvement of more up to date, increasingly sensitive diagnostic techniques and therapeutic agents render criticalness to the advancement of progressively fruitful ocular delivery systems. The primitive ophthalmic solution, suspension, and ointment dosage forms are unmistakably no longer adequate to battle these ailments, and flow innovative wor current research and

development efforts to configuration better therapeutic systems are the essential focal point of this exploration work.

In this way, these might be overwhelmed by fabricating the drug as a formulation that undergoes instantaneous in situ gel formation upon ophthalmic administration. They experience gelation after instillation because of physical-chemical changes occurring in the eye. It increases the pre-corneal residence time and better bioavailability of drug can be achieved by accomplished in situ gel¹⁻³.

Flucytosine is a semisynthetic macrolide antibiotic utilized for the treatment of trachoma caused by *Chlamydia trachomatis*, a gram negative bacteria. As indicated by WHO, there are around 84,000,000 individuals who had been influenced by trachoma. It is accounted for that the existent oral dosage forms of Flucytosine are suitable to treat the ocular infections for example, conjunctivitis and others brought about by delicate pathogens. But when Flucytosine is taken orally to cure ocular infection, it needs at least 1.0 g Flucytosine per dose to ensure the drug content in aqueous humor, tear fluid and conjunctival coat reach

the minimal inhibitory concentration⁴⁻⁶. Subsequently not exclusively will it squander a lot however it can expedite symptoms as a result of high drug level in each tissue. Therefore, a topical ophthalmic formulation of Flucytosine with a low dosing frequency would be considerably more helpful for patients and in this way guarantee better consistence, along these lines diminishing the risk of selection of resistant bacteria. An US patent on topical treatment of ocular infections relates to a sustained release ophthalmic composition comprised of an aqueous suspension of the azalide antibiotic and a polymer suspending agent⁷. However ocular pharmacokinetic results demonstrated that only a viscous solution of Flucytosine can maintain a precorneal concentration. However, profoundly highly viscous solutions and gels lack accuracy and ease of administration. Lachrymation and hazy vision related with preformed hydrogels might be dodged by utilizing in situ activated gel forming systems which are viscous liquids that upon exposure to physiological conditions change into a gel or solid phase in the cul de sac upon its instillation into the eye. In situ gelation approach joins favorable circumstances of the two arrangements and gels, such as accuracy and ease of administration of the former and prolonged precorneal retention of the latter⁸.

The aim of study was to develop in situ gel of Flucytosine by using blend of different polymers. *In situ* gel solution increases the residence time and also sustain the release mechanism of the drug.

2 Material and Methods

2.1 Preparation of formulation of in situ ocular gel of Flucytosine (pH triggered system and ion activated system)

The polymeric solution was prepared by dispersing required quantity of sodium alginate as main polymer and HPMC K4M, Xanthan gum as co-polymers in water using a magnetic stirrer until the polymers completely dissolve. Aqueous solution of Flucytosine was added in to the polymeric solution with continuous stirring. Buffering and osmolality agents were added to the resulting solution along with benzalkonium chloride. The pH of the solution was adjusted to 6.5 using 0.1 N NaOH/0.1 N HCl. The in situ gel formulations are depicted in Table 1.

2.2 Evaluations

2.2.1 Clarity and visual appearance

The clarity and visual appearance of the formulations before and after gelling was determined by visual examination of the formulations under light alternatively against white and black backgrounds.

2.2.2 pH

The pH of each of prepared ophthalmic formulations was determined by using pH meter (equip-tronics). The pH meter was calibrated before each use with standard pH 4, 7 and 9.2 buffer solutions.

Table 1: Quantity of raw materials for preparation of *in situ* ocular gel

Ingredient	F1	F2	F3	F4	F5	F6
Flucytosine (mg)	400	400	400	400	400	400
Sodium alginate (mg)	500	750	1000	500	750	1000
HPMC K4M (mg)	1200	1000	800	600	400	-
Xanthan gum (mg)	-	400	600	800	1000	1200
Benzalkonium chloride (%w/v)	0.02	0.02	0.02	0.02	0.02	0.02
Sodium Chloride (mg)	750	750	750	750	750	750
Distilled water	qs	qs	qs	qs	qs	qs

2.2.3 Gelling capacity

The gelling capacity of the prepared formulation was determined by placing a drop of the formulation in a vial containing 2 ml of freshly prepared simulated tear fluid and visually observed. The time taken for its gelling was noted.

2.2.4 Drug Content

The drug content estimation was carried out by diluting 1 ml of prepared formulation in 100 ml of distilled water and analyzed using UV-visible spectrophotometer (Shimadzu UV-1700 PC, Shimadzu Corporation, Japan) at 285 nm (Zhidong et al., 2006; Indu et al., 2000;).

2.2.5 In-vitro dissolution study

The *in vitro* release of Flucytosine from the prepared formulations was studied using a modified diffusion testing apparatus. The freshly prepared simulated tear fluid (pH 7.4) was used as a diffusion medium. Semi permeable membrane, previously soaked in the diffusion medium for overnight, was tied to one end of a specially designed glass cylinder (open at both ends) having inner diameter of 3.4 cm. Two milliliter of formulation was accurately pipette into the glass cylinder known as donor chamber. The cylinder was suspended in a beaker (Acceptor chamber) containing 100 ml of diffusion medium so that the membrane just touches the surface of the medium. Acceptor chamber was maintained at a temperature of $37 \pm 2^\circ\text{C}$ with a stirring rate of 50 rpm using magnetic stirrer. About 1 ml of sample was withdrawn at a time interval of 1 hour and replaced with an equal volume of fresh diffusion medium. The aliquots were diluted with the diffusion medium and analyzed at 285 nm using UV spectrophotometer.

2.2.6 Kinetic modeling

The formulations were exposed to determine the kinetics of drug release. The *in vitro* drug release data were analyzed by fitting them into different kinetic models namely zero-order, first-order, Higuchi and Korsmeyer-Peppas in order to investigate the release mechanism of Gatifloxacin from the formulation.

2.3 Ocular irritancy

Ocular irritation study was performed on optimized formulation in four albino rabbits (male), each weighing about 2 to 3 kg, and 0.1 ml of the optimized sterile Flucytosine formulation was instilled in to cul-de-sac twice a day for a period of 14 days. The rabbits was monitored periodically for redness, swelling, watering of the eye⁹⁻¹².

2.4 Stability study of ocular gel

Stability studies was carry out as per ICH guidelines. The stability studies of *in situ* gel of Flucytosine was stored at 25 °C ± 2 °C / 60% RH ± 5% and 40 °C ± 2 °C / 75% RH ± 5% for 3 months. The gel was analyzed for psychochemical parameter like color change, Clarity, visual appearance, gelling capacity, drug content, and pH for three months¹³.

3 Results and Discussions

3.1 Evaluation of *in situ* ocular gel

The six different *in situ* ocular gel of Flucytosine (F1 to F6) were prepared, and subjected for various evaluation. The visual appearance of *in situ* gel of Flucytosine were observed and was transparent (Table 2). This suggested that the drug were homogeneously distributed in to the formulation.

The clarity of *in situ* ocular gel of Flucytosine was transparent, demonstrated uniform distribution of drug in dosage form. The pH of the *in situ* ocular gels were established between 6.59±0.17 to 7.12±0.06, and these values were good for eye (Table 2). The pH of the prepared *in situ* ocular gels were nearer to eyes pH.

The percentage of drug content for F1 to F6 ranged between 95.23±0.32% to 98.62±0.43% (Table 2). The F2 have minimum drug while F4 displayed maximum drug. The findings demonstrated the gelling capacity of *in situ* ocular gel of Flucytosine was acceptable and retain higher time in cornea (Table 2).

Table 2: Analysis of Visual appearance, clarity, pH, drug content and gelling capacity of formulations

Formulations	Visual appearance	Clarity	pH	Drug content (%)	Gelling capacity
F1	Transparent	Clear	7.02±0.18	97.14±0.68	++
F2	Transparent	Clear	6.72±0.23	95.23±0.32	++
F3	Transparent	Clear	6.81±0.08	96.73±0.67	++
F4	Transparent	Clear	7.12±0.06	98.62±0.43	++
F5	Transparent	Clear	6.59±0.17	97.59±0.54	++
F6	Transparent	Clear	6.67±0.32	98.02±0.15	++

Values are mean ± S.D

The *in vitro* releases of drug from Flucytosine *in situ* gel are illustrated in Fig 1. The F1, F2, F5 and F6 release maximum drug at 6 h, while F4 and F6 release maximum drug at 8 h. The formulation F4 has maximum drug release as compared to other formulations at 8 hrs. The sustained release of F4 was due to higher concentration of Sodium alginate and xanthan gum among the developed formulations.

The formulations of Flucytosine *in situ* gel were subjected to four model fitting analysis. The findings demonstrated that the formulations follow the zero order kinetics as the co-efficient of regression (R^2) was more near to unity as compared to the regression value of first order and higuchi order. The Korsmeyer-Peppas equation indicates the n values obtained were between 0.6966 and 0.7397 for all formulations. These suggested the drug release follow the pattern of anomalous kinetics (non-Fickian) and super case-II transport.

3.2 Ocular irritation

The F4 was selected best formulation and studied for ocular irritant study in rabbit. The eyes of each rabbits were examined at particular time interval after instillation F4. There was no redness, continuous blinking, swelling or watering of eyes. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctiva were visible. The result of ocular irritation studies indicates that F4 containing all ingredients are non-irritant to rabbit eye.

3.3 Stability study of the ocular gel

Stability studies for F4 gel was performed for three months, at room temperature (25 ± 2°C) and at 40°C / RH 75%. The F4 gel was evaluated for physical properties like color change, Clarity, visual appearance, gelling capacity, drug content, and pH to determine the drug compatibility with polymer. The prepared F4 gel was subjected for drug release after every one month interval.

The findings demonstrated that the F4 gel stored at room temperature and accelerated temperature were found to be comparatively stable.

Fig 1: *In vitro* dissolution profile of various formulation of in situ ocular gel of Flucytosine

4 Conclusion

The prepared in situ ocular gel of Flucytosine by mixing with polymer of sodium alginate, HPMC and xanthan gum were acceptable. The F4 demonstrated worthy consequence as compared to other formulations. Further, F4 was free from ocular irritant and stable at room and accelerated temperature. The study suggested that the in situ ocular gel of Flucytosine will be substitute for conventional eye drops in future.

5 Conflicts of Interest

None

6 Author's Contributions

RG, VK and VS were equally participated in the conduction of experiment and preparation of manuscript. All authors read and approved the final manuscript.

7 References

1. Mandal S, Thimmasetty MK, Prabhushankar GL, Geetha MS. Formulation and evaluation of an in situ gel-forming ophthalmic formulation of moxifloxacin hydrochloride. *Int J Pharma Investig*. 2012; 2: 78-82.
2. Karolewicz B, Gajda M, Pluta J. Ophthalmic Drug Dosage Forms: Characterisation and Research Methods. *The Scientific World Journal*. 2014; 1-14.

3. Folk A, Cotoraci, Balta, Suci M, Herman, H, Boldura OM, Dinescu S, Paiusan L, Ardelean A, Hermenean A. Evaluation of Hepatotoxicity with Treatment Doses of Flucytosine and Amphotericin B for Invasive Fungal Infections. *BioMed Research International*. 2016; 1-9.
4. Al-Bazzaz FY, Al-Kotaji M. Ophthalmic In-Situ Sustained Gel of Ciprofloxacin, Preparation and Evaluation Study. *International Journal of Applied Pharmaceutics*. 2018; 10(4): 153-161.
5. Vermes A, HJ Guchelaar, J Dankert. Flucytosine: a review of its pharmacology, clinical indications, pharmacokinetics, toxicity and drug interactions. *Journal of Antimicrobial Chemotherapy*. 2000; 46: 171-179.
6. M Klepser. The value of amphotericin B in the treatment of invasive fungal infections. *Journal of Critical Care*. 2011; 26: 225.
7. Dawson CR, Bowman LM. Topical treatment or prevention of ocular infections, United States Patent. 2003.
8. Vijaya C, Goud KS, Vijaya C, Goud KS. Ion-activated In Situ Gelling Ophthalmic Delivery Systems of Azithromycin. *Indian J Pharm Sci*. 2011; 73(6):615-620.
9. Almeida H, Amaral MH, Lobão P, Lobo JMS. In situ gelling systems: a strategy to improve the bioavailability of ophthalmic pharmaceutical formulations. *Drug Discovery Today* 2014;19:400-12.
10. Patel N, Thakkar V, Metalia V, Baldaniya L, Gandhi T, Gohel M. Formulation and development of ophthalmic in situ gel for the treatment ocular inflammation and infection using application of quality by design concept. *Drug Dev Ind Pharm*. 2016; 42:1406-23.
11. Paradkar MU, Parmar M. Formulation development and evaluation of natamycin niosomal in-situ gel for ophthalmic drug delivery. *J Drug Delivery Sci Technol*. 2017; 39:113-22.
12. Kala S, Gurudiwan P, Juyal D. Formulation and Evaluation of Besifloxacin Loaded In Situ Gel For Ophthalmic Delivery. *UKJPB*. 2018; 6(2): 36-40.
13. Doijad RC, Manvi FV, Rao VSNM, Alase P. Sustained ophthalmic delivery of gatifloxacin from In-situ gelling system. *Indian J Pharma Sci*. 2006; 8: 814-818.