In vivo evaluation of an *in situ* gelling system for ocular drug delivery

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Abstract

The use of in situ gels as an eye drug delivery mechanism has been thoroughly investigated to increase bioavailability and efficacy. The purpose of the current study was to use an animal model to assess the effectiveness of prepared in situ gel. In vivo irritation and pharmacokinetics of the improved formulation (G7) were investigated in rabbits. All the physicochemical characteristics of G7 were within acceptable bounds. The results of the investigation on ocular irritation show that the chosen formulation is secure and non-irritating when administered intravenously. As shown by higher Cmax Cmax (834±47 µg/ml) and AUC (2451± 94 µg h/ml) when compared with commercial eye drops (Cmax: 503±38 µg/ml and AUC: 876±54 µg h/ml), in-vivo pharmacokinetics data shows a substantial enhancement of fluconazole bioavailability (p 0.0001) from G7.

Keywords: Fluconazole, *in-situ* gel, *in-vivo*, sodium alginate, sustained release

Introduction

The earliest mention of the novel idea of creating a gel in situ (in situ delivery) dates back to the early 1980s. Latin words that mean "in position" are literally translated as "*in-situ*" [1] Low-viscosity solutions that undergo phase transition in the conjunctival cul-de-sac to form viscoelastic gels as a result of conformational changes in polymers in response to changes in a particular physicochemical parameter, such as ionic strength, pH, or temperature, are known as in situ gel-forming systems [2]. Given their capacity to delay medication release, gel dosage forms are used successfully as drug delivery systems [3].

Ocular dosage forms are made to be injected into the eye, given inside the eye, or applied close to the eye. The best ophthalmic drug delivery must be able to maintain drug release while

hanging about the front of the eye for a long time [4]. *In-situ* gels helps to provide drugs a prolonged release, extend their duration in the body, and boost their bioavailability [5].

In situ formulations increase the drug's ocular bioavailability and the precorneal resident time [6]. The *in-situ* formulation had an excellent viscosity, contained the medication, and released it slowly [7]. A continuous lacrimal discharge in the eye results in inadequate bioavailability for traditional liquid ophthalmic preparations [8].

Changes in temperature, the electric field, and light are examples of physical stimuli. Changes in pH and ion activation from biological fluid are examples of chemical stimuli [9]. Changes in glucose level are examples of biochemical stimuli. An antifungal medication called fluconazole is used to treat a number of illnesses brought on by yeast or fungi. By preventing the cytochrome P450-mediated 14 alpha-lanosterol demethylation, a crucial step in fungal ergosterol biosynthesis, fluconazole binds to and inhibits ergosterol synthesis [10]. It is possible that the antifungal activity is caused by the buildup of 14 alpha methyl sterols, which is correlated with the eventual loss of ergosterol in the fungal cell wall [11]. On the market, fluconazole is offered as a pill, oral suspension, and powder for infusion [12].

Materials and methods

Fluconazole (purity of 99.99%), carbopol 940P (Torrent Pharma, Ahmedabad, India) were received as gift. Hydroxypropyl methylcellulose (HPMC), and methyl cellulose (MC) were obtained from Colorcon Lim- ited, Dartford, England. Calcium chloride, mannitol and methyl paraben were purchased com-mercially from CDH Ltd., Mumbai, India.

Formulation of *in situ* gels

In situ gel formulations created by ion activation (sodium alginate) and pH triggering (carbopol 940P) were evaluated for their potential sol to gel phase transition. As agents to increase viscosity, HPMC was utilised. For in situ gel formulations, the polymer concentrations were chosen based on a literature search. The table below lists the makeup of the various formulation batches (G1–G9):

Table No:1 Composition of developed In Situ gel formulations

Ingredients	**Formulation code**								
	G1	G2	G3	G4	G5	G6	G7	G8	G9
Sodium alginate (% w/v)	-	-	0.5	0.5	0.3	0.3	0.3	0.3	0.3
Carbopol 940P (% w/v)	-	-	-	-	0.1	0.2	0.3	0.4	0.5
HPMC (% w/v)	-	-	-	-	0.4	0.4	0.4	0.4	0.4
Methyl cellulose (% w/v)	-	0.5	-	0.5	-	0.5	0.5	0.5	0.5

Phosphate buffer pH 7.4 (ml)	100	100	100	100	100	100	100	100	100
Acetate buffer pH 6.5 (ml)	100	100	100	100	-	-	-	-	-
Boric acid buffer pH 4.7 (ml)	-	-	100	-	100	-	-	100	100

Ocular irritation

Albino rabbits were used to test G7's ocular irritability. (2–3 kg). The animals were kept in their natural habitat with unrestricted movement. Before the trial began, the animals spent a week becoming used to the lab setting. All of the study's animals received free access to food and water. The Committee for the Purpose of Control and Supervision of tests on Animals (CPCSEA), Ministry of Fisheries, Animal Husbandry, and Dairy, India, issued tight standards that were adhered to when conducting the tests. Throughout the experiment, University's institutional animal ethics committee procedure for animal care was folowed. The Draize approach was used as the basis for the *in-vivo* ocular irritation experiment [13-17]. A single instillation of 60 µl was used in the left eye of each rabbit, with the right untreated eye serving as the control. Two tests every day for 21 days were conducted on the sterile formulation. The symptoms of sensitive reactions in the rabbits were redness, swelling, cloudiness, edema, hemorrhage, discharge, and blindness.

***In vivo* pharmacokinetics**

To evaluate the ocular bioavailability between G7 and commercial fluconazole ophthalmic drops (0.5% w/v), the amount of fluconazole that diffused into the aqueous humour of rabbit eyes following ophthalmic administration was measured. Two groups (n = 6) of New Zealand Albino rabbits (2-3 kg) were used for the in vivo pharmacokinetic studies. The experiment was carried out in accordance with the institutional animal ethics committee's approved protocol at Swami Vivekanad Subharti University. The lower cul-de-sac of one rabbit's eye received a single topical instillation of G7 (60 µl of 0.5% w/v medication) in the first group, whereas the second group of rabbits received commercial eye drops with a similar concentration and volume [18-20].

The untreated eye was used as the control in each instance. All rabbits had their eyes briefly closed for two minutes to increase the drug's interaction with the corneal membrane. Individual animals were anaesthetized by intramuscular injection of xylazine and ketamine prior to aqueous humour removal. Using a 29-gauge insulin syringe needle, samples of aqueous humour (20 µl) were taken, mixed with acetonitrile, and promptly stored at -80 C until further research. The samples were centrifuged for 10 minutes at 5000 rpm, and the organic layer's drug concentration was determined by HPLC [21-24].

Statistical analysis

One-way ANOVA (SPSS 23, Chicago, IL, USA) was used to analyze experimental data statistically. Statistics classifies a difference in values as statistically significant when p 0.05.

Results and discussion

Ocular irritation

To determine the final eye irritation score, the individual rabbits' eye irritation scores were summed to produce a total irritation score, which was then divided by the total number of rabbits utilized for the ocular irritancy test. The computed eye irritation score for the control group was 0.28 whereas it was 0.68 for the G7 group, showing good ocular tolerance like marketed formulation. Additionally, G7 injection did not result in significant lachrymation, edema, or redness in the eyes. There were no abnormal clinical symptoms in the various eye areas (cornea, iris, or conjunctiva) or signs of ocular injury. As a result, this study concludes that administering G7 topically is secure and non-irritating.

In vivo pharmacokinetic study

Based on how much fluconazole permeated into the aqueous humour of rabbit eyes from the first (G7) and second group, ocular bioavailability was calculated. Through non-compartment model analysis, a number of pharmacokinetic parameters, including tmax, Cmax, and AUC, were calculated from the graph between aqueous humour concentrations (μg/ml) and time (h). The pharmacokinetic characteristics for G7 and control in rabbit aqueous humour are clearly different, as shown in Fig. 1. Fluconazole levels in the aqueous humour increased after 1 hour (724.31± 57.06 μg/ml and 653.09± 72.44 μg/ml in G7 and control, respectively).

However, by 2 hours, the drug level was higher (p 0.0001) in G7, but the fluconazole level in traditional eye drops rapidly decreased. (Fig 1). These findings suggest that ophthalmic drops were only briefly held in the ocular cavity due to the significant pre-corneal medication loss caused by nasolacrimal discharge and tear turnover. Furthermore, tmax for G7 was 2 h as opposed to 1 h for control. Comparing G7 to commercial eye drops (Cmax: 503±38 μg/ml and AUC: 876±54 μg h/ml), however, revealed higher Cmax (834±47 μg/ml) and bigger AUC (2451± 94 μg h/ml) (p 0.0001).

Thus, it can be inferred from the information at hand that designing and creating an *in-situ* gel system considerably increased fluconazole's ability to permeate the eye. This finding concurs with *ex-vivo* permeation studies, which showed that the flux was larger in G7. In comparison to commercial eye drops, the ocular residence time of G7 shows a prolonged duration of activity. The average medication concentration found in Fig. 1 in the aqueous humour was higher than the fluconazole therapeutic response minimum effective concentration required for several bacteria causing eye infections.

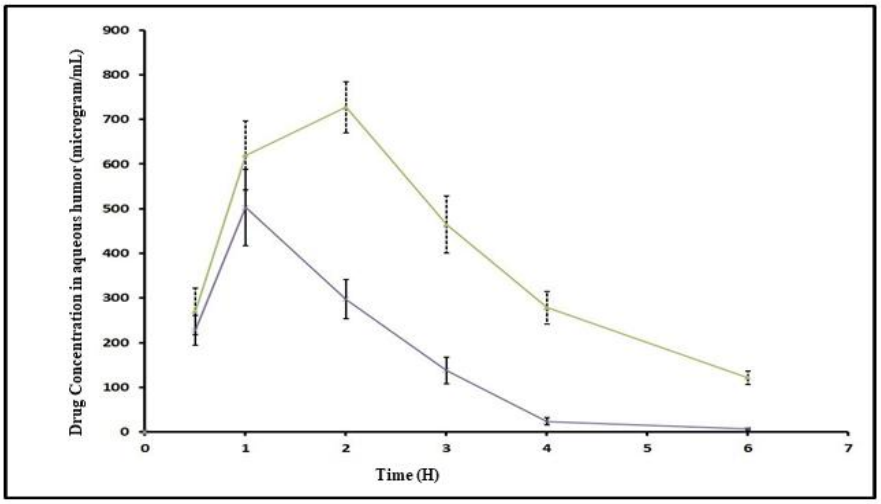

Fig No:1 Comparison of mean fluconazole concentration in the aqueous humor following topical installation of optimized in situ gel (G7) and control (commercial eye drops) in rabbits. The data represents average ± SD of six trials.

Conclusion

In this study, ion activated in situ gel containing fluconazole was optimized utilizing an experimental design using a simplex lattice DoE. It was successful to create an *in-situ* gel utilizing sodium alginate and HPMC. Under accelerated stability conditions, the *in-situ* gel prolonged drug release by up to 12 hours and maintained stability for up to 6 months. This innovative ophthalmic in situ gelling technology has the intrinsic ability to increase ocular bioavailability through sustained drug release, increased ocular permeability, and prolonged residence time, making it a workable alternative to ophthalmic drops. Better patient compliance may result from additional advantages such as non-irritability, improved miscibility with lachrymal fluids, ease of instillation, reduced frequency of application, and total dose of fluconazole. Due to this, a topical in situ gel system was created. (G7)

References

1. Abdul Hasan Sathali A, Mohanambal E, Arun K. Formulation and evaluation of pH triggered in situ gelling system of levofloxacin. Indian J Pharm Educ Res 2011;45:58-64.

2. Rathore KS, Nema RK. http://www.articlesbase.com/visionarticles/ ophthalmic-nanoparticles-drug-deliverysystems-1273176.html. [Last accessed on 2010 May 27].

3. Kumar S, Haglund BO, Himmelstein KJ. In situ-forming gels for ophthalmic drug delivery. J Ocul Pharmacol 1994;10:47-56.

4. Nanjawade BK, Manvi FV, Manjappa AS. In situ-forming hydrogels for sustained ophthalmic drug delivery. J Control Release 2007;122:119-34.

5. Nirmal HB, Bakliwal SR, Pawar SP. In situ gel: New trends in controlled and sustained drug delivery system. Int J Pharm Tech Res 2010;2:1398-408.

6. Kaur IP, Singh M, Kanwar M. Formulation and evaluation of ophthalmic preparations of

acetazolamide. Int J Pharm 2000;199:119-27.

7. Gokulgandhi MR, Parikh JR, Megha Barot M, Modi DM. A pH triggered in situ gel forming ophthalmic drug delivery system for tropicamide. Drug Deliv Technol 2007;5:44-9.

8. Lavanya B, Indira S, Srinivas P. Formulation and evaluation of ocular niosomal in situ gels of linezolid. Int J Pharm Sci Res 2014;4:1367-75.

9. Nagalakshmi S, Seshank, Ramaswamy R, Shanmuganathan S. Formulation and evaluation of stimuli sensitive pH triggered in-situ gelling system of fluconazole in ocular drug delivery. Int J Pharm Sci Res 2014;5:1339-44.

10. Nanjawade BK, Manjappa AS, Murthy RS, Pol YD. A novel pH triggered in situ gel for sustained ophthalmic delivery of ketorlac tromithamine. Asian J Pharm Sci 2009;4:189-99.

11. Moorthi C, Krishnan K, Manavalan R, Kathiresan K. Preparation and characterization of curcumin-piperine dual drug loaded nanoparticles. Asian Pac J Trop Biomed 2012;2:841-8

12. Abraham S, Furtado S, Bharath S, Basavaraj BV, Deveswaran R, Madhavan V. Sustained ophthalmic delivery of ofloxacin from an ionactivated in situ gelling system. Pak J Pharm Sci 2009;22:175-9.

13. Padma J, Preetha K, Karthika, Rekha NR, Elshafie K. Formulation and evaluation of in situ ophthalmic gels of diclofenac sodium. J Chem Pharm Res 2010;2:528-35.

14. Srividya B, Cardoza RM, Amin PD. Sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system. J Control Release 2001;73:205-11.

15. Kumar SR, Himmestein KJ. Modification of in situ gelling behavior of carbopol solutions by hydroxyl propyl methyl cellulose. J Pharm Sci 1995;84:344-8.

16. Nagesh C, Patil M, Chandrashekhara S, Sutar R. A novel in situ gel for sustained ophthalmic delivery of ciprofloxacin hydrochloride and dexamethasone - Design and characterization. Der Pharmacia Lettre 2012;4:821-7.

17. Darwhekar G, Jain P, Jain DK, Agrawal G. Development and optimization of dorzolamide hydrochloride and timolol maleate in situ gel for glaucoma treatment. Asian J Pharm Anal 2011;1:93-7.

18. Pandey A, Mali PY, Sachdeva D, Patel DK, Ramesh R. Development and optimization of levobunolol hydrochloride in-situ gel for glaucoma treatment. Int J Pharm Biol Arch 2010;1:134-9.

19. Mohan EC, Kandukuri JM, Allenki V. Preparation and evaluation of in-situ-gels for ocular drug delivery. J Pharm Res 2009;2: 1089-94.

20. Carlfors J, Edsman K, Petersson R, Jörnving K. Rheological evaluation of gelrite in situ gels for ophthalmic use. Eur J Pharm Sci 1998;6:113-9.

21. Wamorkar V, Varma MM, Manjunath SY. Formulation and evaluation of stomach specific in-situ gel of metoclopramide using natural, bio-degradable polymers. Int J Res Pharm Biomed Sci 2011;2:193-201.

22. Cohen S, Lobel E, Trevgoda A, Peled Y. A novel in situ-forming ophthalmic drug delivery system from alginates undergoing gelation in the eye. J Control Rel 1997;44:201-8.

23. OECD. Acute Eye Irritation/Corrosion Guideline for Testing of Chemicals No. 405. Organisation for Economic Cooperation and Development, Paris. 2012.

24. Ruel-Gariépy E, Leroux JC. In situ-forming hydrogels--review of temperature-sensitive systems. Eur J Pharm Biopharm 2004;58:409-26