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FORMULATION, DEVELOPMENT AND EVALUATION OF OFLOXACIN AND DEXAMETHASONE IN-SITU GEL

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ABSTRACT

In situ gels have been extensively explored as ocular drug delivery system to enhance bioavailability and efficacy. The objective of present study was to design, formulate and evaluate *in situ* gel to enhance the ocular penetration and therapeutic performance of ofloxacin and dexamethasone in ophthalmic delivery. formulation development of ofloxacin and dexamethasone ocular *in situ* gelling system using thermo reversible gelling polymer pluronic F127.Because of high concentration (20-25%) of this polymer required for *In situ* gelation causes irritation to the eye. So, to reduce this concentration an attempt was made to combine the pluronic F127 with other polymers like HPMC and carbopol. Viscosity of formulation was determined before and after gelation by using Brookfield's viscometer in the small volume adaptor and the angular velocity was determined at 10rpm. The comparative study of viscosity was done at 10 rpm. F3, show comparatively better viscosity and good consistency gel. The *In vitro* drug release data of the optimized formulation was subjected to goodness of fit test by linear regression analysis according to zero order and first order kinetic equation in order to determine the mechanism of drug release. When the regression coefficient values of were compared, it was observed that 'r' values of Higuchi was maximum hence indicating drug release from formulations was found to follow Higuchi release kinetics.

Keywords: Ofloxacin, Dexamethasone, In-Situ gel, Formulation development, Evaluation.

INTRODUCTION

Ophthalmic*In-situ* gels are viscous polymer-based liquids that exhibit sol-to-gel phase transition on the ocular surface due to change in a specific physicochemical parameter like ionic strength, pH or temperature.Gel dosage forms are successfully used as drug delivery systems considering their ability to prolong the drug release. To prolong the precorneal resident time and improve ocular bioavailability of the drug various polymers system were studied as *in situ* gelling vehicle for ophthalmic drug delivery system. The *In situ* formulation exhibited well, viscosity, drug content and sustained drug release. Conventional liquid ophthalmic formulations demonstrate low bioavailability because of a constant

lacrimal drainage in the eye. The normal drainage of an instilled drug dose commences immediately upon instillation and is essentially completed within 5 min. typically ophthalmic bioavailability of only 1–10% are achieved due to the short precorneal residence time of ophthalmic solutions[1-2].

Each system has its own advantages and drawbacks. The choice of a particular hydro gel depends on its intrinsic properties and envisaged therapeutic use. This research includes temperature and pH, induced in situ-forming polymeric systems used to achieve prolonged contact time of drugs with the cornea and increase their bioavailability. Ophthalmic drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. The conventional ocular drug delivery systems like solutions, suspensions, and ointments show drawbacks such as increased precorneal elimination, high variability in efficiency, and blurred vision respectively[3].

Rapid and efficient drainage by the nasolacrimal apparatus, noncorneal absorption, and the relative impermeability of the cornea to both hydrophilic and hydrophobic molecules, all account for such poor ocular bioavailability[4-5]. A significant challenge to the formulator is to circumvent (bypass) the protective barriers of the eye without causing permanent tissue damage. Thus, to increase the ocular bioavailability of drug, we need to increase the ocular residence time of the drug. Several in situ gelling systems have been developed to prolong the precorneal residence time of a drug, improve patient compliance, and consequently enhance ocular bioavailability[6].In situ forming gels are formulations, conveniently dropped in the eye as a solution, where they undergo transition into a gel.

The present work describes the formulation development of ofloxacin and dexamethasone ocular *in situ* gelling system using thermo reversible gelling polymer pluronic F127.Because of high concentration (20-25%) of this polymer required for *In situ* gelation causes irritation to the eye. So, to reduce this concentration an attempt was made to combine the pluronic F127 with other polymers like HPMC and carbopol.Thistype of combination system reduces not only the concentration of individual polymers but also the side effects without compromising the in vitro gelling capacity as well as overall rheology of the system.This research helps in increase the bioavailability of the drug prolong the drug action, increase the retention time of formulation in the eye reduce the dose frequency provide comfort, and better compliance to the patient.

MATERIALS AND METHODS

Materials:

Ofloxacin was obtained as a gift sample from Micro Labs Ltd., Bangalore, India. Dexamethasone (DXM) was obtained as gift samples from MSN labs, Hyderabad. Carbopol 934 and Carbopol 940 was obtained from Sigma Aldrich, Mumbai. Benzalkonium chloride from Merck Ltd, Mumbai, Sodium chloride from Loba chemicals, Hydroxypropyl methylcellulose (HPMC-15cps) from Central Drug House, Mumbai,

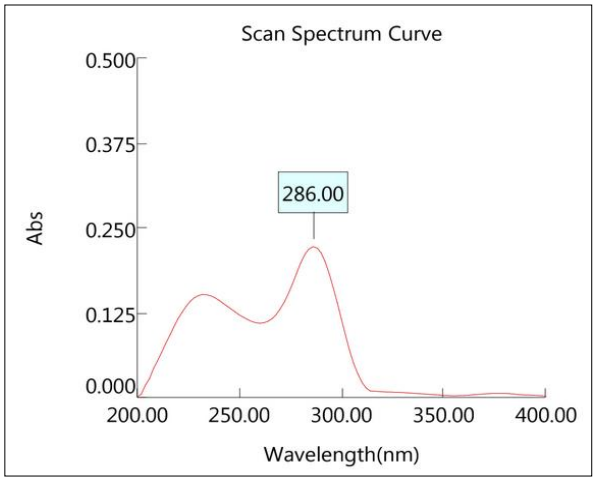
India. All other chemicals and solvents were of analytical grade and used as received. Distilled water was prepared in laboratory using all glass distillation apparatus.

Methods:

Determination of λ_{max} of Ofloxacin and Dexamethasone

The λ_{max} of Ofloxacin and Dexamethasone was determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer.

Accurately weighed 10 mg of drug was dissolved in 10 ml of simulated tear fluid (pH 7.4) in 10 ml of volumetric flask. The resulted solution 1000µg/ml and from this solution 0.1 ml pipette out and transfer into 10 ml volumetric flask and volume make up with simulated tear fluid (7.4) solution prepare suitable dilution to make it to a concentration of 10µg/ml for Dexamethasone. The spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Labindia-3000+).



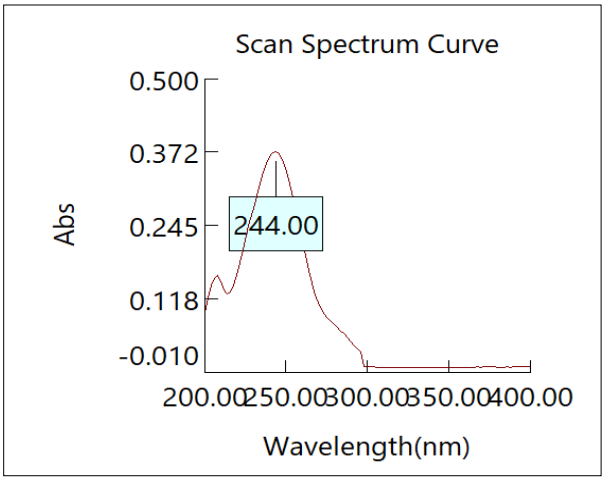


Figure 1: Determination of λ_{max} of Ofloxacin Figure 2: Determination of λ_{max} of Dexamethasone

Preparation of in-situ Gels of Ofloxacin and Dexamethasone:

Aqueous solutions of varying concentrations of different polymers and drug were prepared and evaluated for gelling capacity and viscosity in order to identify the compositions suitable for use as in situ gelling systems. All ingredients were weighed accurately and the formulations were prepared by dispersing different grades of polymers carbopol (934 and 940) solutions in different concentrations (0.1% w/v, 0.3% w/v, and 0.5% w/v) and HPMC (K4M) solutions in different concentrations (0.5% w/v 1.0% and 1.5% w/v) in distilled water with continuous stirring until completely dispersed and allowed to hydrate overnight. Ofloxacin (0.1%) and Dexamethasone (0.3%) was dissolved in 0.5 ml of ethanol and then added to the above polymeric solutions under constant stirring to obtain a uniform solution[7].

Table 1: Composition of in-situ gelling systems by pH sensitive systems

Ingredient (% w/v)	F1	F2	F3	F4	F5	F6
Ofloxacin	0.1	0.1	0.1	0.1	0.1	0.1
Dexamethasone	0.3	0.3	0.3	0.3	0.3	0.3
Carbopol 934	0.1	0.3	0.5	-	-	-
Carbopol 940	-	-	-	0.1	0.3	0.5
HPMC	0.5	1.0	1.5	0.5	1.0	1.5
Benzalkonium chloride	0.02	0.02	0.02	0.02	0.02	0.02
Water	qs	qs	qs	qs	qs	qs

Evaluations of formulations

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[8].

Drug content

The assay of drug Ofloxacin and Dexamethasone was performed by UV method. The calculation was based on calibration curve method using simultaneous equation method[9].

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 5 to 7.4. The developed formulations were evaluated for pH by using calibrated digital pH meter[10].

For *In situ* gel pH 5.0 should be optimum because both the drug is stable at pH 3.5-5.0. Lowering the pH from 5.0 can causes irritation to eye and on raise the above 5 will result in gelation of formulation due to presence of carbopol.

***In-Situ* gelling capacity**

In situ gelling capacity determined by visual inspection. The formulation has been exposed to the physiological conditions of temperature and pH. Simulated tear fluid (STF) was prepared and warm up to 37^0C. Formulations were introduce into STF in a ratio of 1:2 Change in consistency of Formulations were visually inspected[11].

Composition of Simulated Tear Fluid (STF)

Sodium chloride: 0.670 gm

Sodium bicarbonate: 0.2 gm

Calcium chloride dihydrate: 8 mg

Water upto: 100ml

pH was adjusted by 0.5 N NaOH to 7.4

Gelling capacity of all formulations are depicted as + (gels after five minutes and dissolves rapidly), ++ (gelation immediate, remains for few hours) and +++ (gelation immediate, remains for extended period upto 8 hours).

Viscosity study

At pH 5.0 and temperature less than 16^0C the developed formulations were in liquid state and show low viscosity. For viscosity studies the pH of formulations were raised from pH 5.0 to pH 7.4 and the temperature was raised to 37^0C. pH was raised to 7.4 by the addition of 0.5M NaOH[12].

The resulting gel studied for viscosity on Brookfield Synchrolectric Viscometer using Spindle No.7 at 50 RPM for comparative study. The angular viscosity was measured by gradually increase the RPM from 10 to 70.

Sterility testing

The test for sterility is applied to pharmacopoeial articles that are required according to the Pharmacopoeia to be sterile. However, a satisfactory result only indicates that no contaminating viable micro-organisms have been found in the sample examined in the conditions of the test. The test must be carried out under aseptic conditions designed to avoid accidental contamination of the product during testing. For achieving these conditions, a grade A laminar airflow cabinet or an isolator is recommended. The test environment has to be adapted to the way in which the tests are performed. Precautions taken for this purpose should not adversely affect any micro-organisms, which are to be revealed in the tests. The working conditions in which the tests are carried out should be monitored regularly by appropriate sampling of the air and surfaces of the working area and by carrying out control tests[13].

Culture Media

The following culture media have been found to be suitable for the test. Fluid thioglycollate medium is primarily intended for the culture of anaerobic bacteria; however, it will also detect aerobic bacteria. Soyabean-casein digest medium is suitable or the culture of both fungi and aerobic bacteria.

Table 2: Composition of fluid thioglycollate medium

Ingredients	Quantity(gm)
L-Cystine	0.5 g
Sodium chloride	2.5 g

Dextrose monohydrate/anhydrous	5.5 g/5.0 g
Granular agar	0.75 g
Yeast extract (water-soluble)	5.0 g
Pancreatic digest of casein	15.0 g
Sodium thioglycollate or	0.5 g
Thioglycollic acid	0.3 ml
Resazurin sodium solution (0.1 per cent), freshly prepared	1.0ml
Distilled water to	1000 ml
pH of the medium after sterilization	7.1 ± 0.2

I.P. recommends two methods viz.

Method A: Membrane filtration.

Method B: Direct inoculation

Method B is recommended for clear aqueous preparation

Method B – Direct Inoculation

Quantities of Sample to be used

The quantity of the substance or preparation under examination to be used for inoculation in the culture media varies according to the quantity in each container. The sample was used was not less than 200 mg.

Method of Test

For aqueous solutions: Remove the liquid from the test containers with a sterile pipette or with a sterile syringe or a needle. Transfer the quantity of the preparation under examination into the culture medium so that the volume of the preparation under examination is not more than 10 per cent of the volume of the medium, unless otherwise prescribed. If the preparation under examination has antimicrobial activity, carry out the test after neutralising this with a suitable neutralizing substance or by dilution in a sufficient quantity of culture medium. When it is necessary to use a large volume of the product it may be preferable to use a concentrated culture medium prepared in such a way that it takes account of the subsequent dilution. Where appropriate, the concentrated medium may be added directly to the product in its container. Incubate the inoculated media for not less than 14 days. Observe the cultures several times during the incubation period. Observe the containers of media periodically during the 14 days of incubation. If the test specimen is positive before 14 days of incubation, further incubation is not

necessary. For products terminally sterilized by a validated moist heat process, incubate the test specimen for not less than 7 days.

In-vitro drug release

In-vitro drug diffusion study

The *in vitro* release of drugs from the formulations was studied through cellophane membrane. The dissolution medium used was artificial tear fluid freshly prepared (pH 7.4). 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 driveshaft and suspended in 50 ml of dissolution medium maintained at 37±1°C so that the membrane just touched the receptor medium surface. The dissolution medium was stirred at 50 rpm using magnetic stirrer. Methodology Aliquots, each of 1-ml volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium[14-15].

RESULTS AND DISCUSSION

The eye is a complex and unique part of the human organs that has been considered as the window to the human soul. Broadly, the human eye is divided into two segments that are anterior and posterior segments. The specific disease conditions of the eye are associated with each of these broad segments. For instance, conjunctivitis, glaucoma, blepharitis, and cataract are some of the diseases that affect the anterior segment of the eye, while diabetic retinopathy and age-related macular degeneration are known to affect the posterior segment.Ocular disposition and elimination of a therapeutic agent is dependent upon its physicochemical properties as well as the relevant ocular anatomy and physiology. A successful design of a drug delivery system, therefore, requires an integrated knowledge of the drug molecule and the constraints offered by the ocular route of administration.

The various approaches that have been attempted to increase the bioavailability and the duration of the therapeutic action of ocular drugs can be divided into two categories. The first one is based on the use of sustained drug delivery systems, which provide the controlled and continuous delivery of ophthalmic drugs. The second involves maximizing corneal drug absorption and minimizing precorneal drug loss.Ideal ophthalmic drug delivery must be able to sustain the drug release and to remain in the vicinity of frontof the eye for prolong period of time.Use of biodegradable and water soluble polymers for the in situ gel formulations can make them more acceptable and excellent drug delivery systems.

Ophthalmic *in situ* gelling system of Ofloxacin and Dexamethasone was successfully formulated using polymeric combination of gelling agents Carbopol 934, 940 as, temperature sensitive and pH-sensitive respectively along with HPMC as viscosity enhancing agent.The clarity of the prepared formulations was

found satisfactory but precipitate observed in formulation during storage table 3. The pH of all formulations was found 5.0 table 5. The drug content of the prepared formulations was within the acceptable range, and ensures dose uniformity. The formulation F3 showed maximum drug content table 3.*In-situ* gelling capacity determined by visual inspection table 6. The formulation has been exposed to the physiological conditions of temperature and pH. Simulated tear fluid (STF) was prepared and warm up to 37°C. Solution was introduced into STF in a ratio of 1:2 changes in consistency of solution visually inspected. Formulation F4, F5 and F6 show poor gelling capacity in simulated physiological conditions of pH and temperature because of comparatively more concentration of carbopol 940.

Viscosity of formulation was determined before and after gelation by using Brookfield's viscometer in the small volume adaptor and the angular velocity was determined at 10rpm. The comparative study of viscosity was done at 10 rpm. F3, show comparatively better viscosity and good consistency gel table 7.For sterility testing formulations were diluted ten times by sterile distilled water. From this dilution remove quantity and placed in culture media, this quantity should be equivalent to more than 200 mg of the formulation. Petri dishes then placed in incubation chamber for 7 days and observed for microbial growth table 8. The *In vitro* drug release data of the optimized formulation was subjected to goodness of fit test by linear regression analysis according to zero order and first order kinetic equation in order to determine the mechanism of drug release. When the regression coefficient values of were compared, it was observed that 'r' values of Higuchi was maximum hence indicating drug release from formulations was found to follow Higuchi release kinetics table 9, 10 and 11.

Table 3: Clarity test of *in-situ* gel formulations

Formulation code	Clarity
F1	Clear
F2	Clear
F3	Clear
F4	Clear
F5	Clear
F6	Clear

Table 4: Drug content analysis

Formulation	Drug Content (%)*	
	Ofloxacin	**Dexamethasone**
F1	98.45	97.85
F2	98.12	98.15
F3	99.25	99.65
F4	98.74	98.74
F5	97.65	98.65
F6	97.24	98.12

Table 5: pH determination

Formulation	pH	Adjust to
F1	4.8	5.0±0.1
F2	4.7	5.0±0.1
F3	4.3	5.0±0.1
F4	4.7	5.0±0.1
F5	4.4	5.0±0.1
F6	4.7	5.0±0.1

Table 6: *In-situ* gelling capacity of *In-situ* gel formations

Formulation code	*In-situ* gelling capacity
F1	"+++"
F2	"+++"
F3	"+++"
F4	"++"
F5	"++"
F6	"+"

"+" gelation after five minutes and dissolves rapidly, "++" gelation immediate, remains for few hours, "+++" gelation immediate, remains for extended period 8 hours

Table 7: Comparative viscosity* of *in-situ* formulation

Formulation code	Viscosity of solution (in cps)	Viscosity after galation
F1	898	2465
F2	965	2645
F3	1050	2878
F4	987	2312
F5	1020	2545
F6	1140	2910

*Spindle no.7, rpm 10

Table 8: Sterility testing of formulations

Formulation code	Observation
F1	No growth observed
F2	No growth observed
F3	No growth observed
F4	No growth observed
F5	No growth observed
F6	No growth observed

Table 9: *In-vitro* drug release profile of Ofloxacin from *in-situ* Formulation F3

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative*% Drug Released	Log Cumulative % Drug Released	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	12.25	1.088	87.75	1.943
1	1	0	26.65	1.426	73.35	1.865
1.5	1.225	0.176	39.98	1.602	60.02	1.778
2	1.414	0.301	49.54	1.695	50.46	1.703
2.5	1.581	0.398	65.45	1.816	34.55	1.538
3	1.732	0.477	79.98	1.903	20.02	1.301
4	2	0.602	88.87	1.949	11.13	1.046
5	2.236	0.699	98.12	1.992	1.88	0.274

Table 10: *In-vitro* drug release profile of Dexamethasone from *in-situ*Formulation F3

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative*% Drug Released	Log Cumulative % Drug Released	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	15.32	1.185	84.68	1.928
1	1	0	33.12	1.520	66.88	1.825
1.5	1.225	0.176	45.65	1.659	54.35	1.735
2	1.414	0.301	58.89	1.770	41.11	1.614
2.5	1.581	0.398	69.98	1.845	30.02	1.477
3	1.732	0.477	82.12	1.914	17.88	1.252
4	2	0.602	91.16	1.960	8.84	0.946
5	2.236	0.699	98.55	1.994	1.45	0.161

Table 11: Comparative study of regression coefficient for selection of optimize Formulation F3

r^2	Zero order	First order	Higuchi	Peppas
Ofloxacin	0.949	0.925	0.983	0.701
Dexamethasone	0.931	0.934	0.984	0.718

Conclusion

In conclusion, evaluation of *in-situ* gel is determined to ensure that the prepared preparation meets the standard and is safe. In the chemical evaluation *in-situ* gel determined the diffusion of the active substance of a compound by measuring its concentration. In microbiology evaluation determine if the preparations is contaminated or not, also be effective and safe.

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