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Research article

Formulation and evaluation of *in -situ* ocular drug delivery system of gentamicin sulphate

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ABSTRACT

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 pre-corneal elimination, high variability in efficiency and blurred vision respectively. Each system has its own advantages and disadvantages.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 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.

In-Situ gel were evaluated for clarity, pH measurement, gelling capacity, drug content estimation, rheological study, in vitro diffusion study and ICH stability studies. The developed formulations exhibited sustained release of drug over a period of 8 hours thus increasing residence time of the drug and optimized formulations also found satisfactorily stable, thus these in situ gelling systems may be a valuable alternative to the conventional systems.

Keywords: Ophthalmic in situ gel, Gentamicin Sulphate, PluronicF127, Carbopol.

INTRODUCTION

Ocular drug delivery has remained as one of the most challenging task for pharmaceutical scientists. The unique structure of the eye restricts the entry of drug molecules at the required site of action. Drug delivery to the eye can be broadly classified into anterior and posterior segments. Conventional systems like eye drops, suspensions and ointments cannot be considered optimal in the treatment of vision threatening ocular diseases however, more than 90% of the marketed ophthalmic formulations are in the form of eye drops. These formulations mainly target the anterior segment eye diseases. Most of the topically applied drugs are washed off from the eye by various mechanisms (lachrymal drainage, tear dilution and tear turnover) resulting in low ocular bioavailability of drugs. Moreover, human cornea comprising of epithelium, substantia propria and endothelium also restricts the ocular entry of drug molecules. As a result of these factors less than 5% of administered drug enters the eye. The conventional drug delivery systems like solutions, suspensions and ointments are no longer sufficient to fulfill the present day requirements of providing a constant rate delivery and prolonged time. One of the main reason for that is poor residence time of drug at the site of action, which results into poor bioavaibility [1].

Advantages of In-situ ocular drug delivery systems

Increased accurate dosing. To overcome the side effects of pulsed dosing produced by conventional systems.

To provide sustained and controlled drug delivery.

To increase the ocular bioavailability of drug by increasing the corneal contact time. This can be achieved by effective adherence to corneal surface.

To provide targeting within the ocular globe so as to prevent the loss to other ocular tissues.

To circumvent the protective barriers like drainage, lacrimation and conjunctival absorption.

To provide comfort, better compliance to the patient and to improve therapeutic performance of drug.

To provide better housing of delivery system [2, 3].

Limitation of conventional dosage form

The conventional liquid ophthalmic formulation is eliminated from the precorneal area immediately upon instillation because of lacrimal secretion and nasolacrimal drainage.

Only a small fraction of the drug being ocularly absorbed. Only 10% drug Concentrations available at the site of actions.

In vivo resident experiments showed the drug resident time and the total resident amount in rabbit's conjunctiveal sac was 2. L6 to 5.0 folds less in conventional than in situ gel.

Some conventional ophthalmic preparation such as gels, ointment, and viscous preparation were reported to blurred vision.

These preparations have no bioadhesive property [4].

Formulation Approaches of In-Situ gel drug delivery

In situ formation based on physiological stimuli

Thermally trigged system

pH triggered systems

In situ formation based on physical mechanism

Swelling

Diffusion

.In situ formation based on chemical reactions

Ionic cross linking

Enzymatic cross-linking

Photo-polymerisation [5].

MATERIAL AND METHOD

Data Set

Procurement of Material and purpose

A gift sample of Gentamicin from Dr. Reddy's laboratory (Active drug) and other excipients and reagent collect from local vendors. Sulphate Pluronic F127 (Gelling agent) Carbopol 934 (Gelling agent) HPMC 15cps (Thichning agent) EDTA (Stabilizer) Benzalkonium Chloride (Preservative) NaCl (Tonicity Adjustment) Poly ethylene glycol (Anti foaming agent) [6].

Methodology of formulations

For the preparation of Pluronic F127 based ocular *In-situ* gel all the ingredients were sieved from sieve no 44.

Then solution of 0.3% of gentamicin sulphate was prepared in acetate buffer 5.0 I.P.

The solution was cooled in a ice bath and pluronic F127 was added slowly with continuous stirring.

Then the resulting solution was kept in a refrigerator under 4^0C for 24h. This storage was help in dissolving the Pluronic F 127 completely.

After 24h carbopol 934 and HPMC 15cps were added slowly along with other exepients with continuous stirring. The stirring should be continued to 2-3 hours for proper mixing and avoid slug formation. The resulting formulation kept on probe sonicator to remove air bubble. All formulations were stored in LDPE (Low Density Polyethelene) bottles for further use. All the containers stored in refrigerator [7].

Table 1: Composition of Different formulations of In-situ gel

Ingredient (%)	Formulations								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Gentamicin Sulphate (w/v)	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%
Pluronic F127	18	16	14	18	16	14	18	16	14
Carbopol 934	0.2	0.2	0.2	0.3	0.3	0.3	0.4	0.4	0.4
HPMC 15cps	1.0	1.0	1.0	0.75	0.75	0.75	0.5	0.5	0.5
EDTA	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Benzalkonium Chloride	0.013 %	0.013 %	0.013 %	0.013 %	0.013 %	0.013%	0.013 %	0.013%	0.013%
NaCl	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Poly ethylene glycol	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Acetate Buffer (pH 5.0)	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml

Evaluations of in-situ gel

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. Result shown in table no. 2. Drug content. The assay of drug Gentamicin was performed by colorimetric method. The method was based on the ninhydrin reaction with primary and secondary amines present in the gentamicin. This reaction produces a purple color, Result shown in table no. 3 [8].

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, Result shown in table no. 4.

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 370C. Formulations were introduce into STF in a ratio of 1:2 Change in consistency of Formulations were visually inspected.

Viscosity study

At pH 5.0 and temperature less than 160C 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 370C. pH was raised to 7.4 by the addition of 0.5M NaOH.

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. The test for sterility is applied to pharmacopoeial articles that are required according to the Pharmacopoeia to be sterile.

The microbiological assay of an antibiotic is based upon a comparison of the inhibition of growth of micro-organisms by measured concentrations of the antibiotics under examination with that produced by known concentrations of a standard preparation of the antibiotic having a known activity [9].

Eye irritation test

Accidental contact with hazardous chemicals frequently causes eye injury and visual impairment. United States and international regulatory agencies currently use the Draize rabbit eye test (Draize et al. 1944) to identify potential ocular hazards associated with chemicals.

LVET (low volume eye test)

Primarily by applying 10 µL (instead of 100 µL) of a test substance directly on the cornea (instead of the conjunctival sac). Scoring of corneal, iridal, and conjunctival lesions in the LVET is identical to that of the Draize rabbit eye test.

Draize eye irritation test

100 milligrams of a concentrated solution are dripped into the eyes of six to nine conscious albino rabbits

The damage to the rabbits' eyes is recorded at specific intervals over an average period of 72 hours, with the test sometimes lasting 7-18 days.

In-vitro Drug diffusion study

The in vitro release of Gentamicin Sulphate 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.

Stability studies

Stability is defined as the extent to which a product retains, within specified limits and throughout its period of storage and use (i.e. its shelf life), the same properties and characteristics that it possessed at the time of its manufacture. Stability testing is performed to ensure that drug products retain their fitness for use until the end of their expiration dates. All the five formulations were subjected to stability studies at ambient humidity [10].

RESULTS

Table 2: Clarity test

Formulation	Clarity
F1	Clear
F2	Clear
F3	Turbid
F4	Clear
F5	Clear
F6	Clear
F7	Precipitate observed
F8	Clear
F9	Clear

Table 3: Drug Content

Formulation	Drug Content (%)
F1	98.22
F2	99.14
F4	97.22
F5	98.65
F8	95.51

Table 4: pH Determination

Formulation	pH	Adjust to
F1	4.1	5.0 ±0.1
F2	4.3	5.0 ±0.1
F3	4.2	5.0 ±0.1
F4	3.9	5.0 ±0.1
F5	3.8	5.0 ±0.1
F6	4.1	5.0 ±0.1
F7	3.9	5.0 ±0.1
F8	4.0	5.0 ±0.1
F9	4.2	5.0 ±0.1

CONCLUSION AND DISCUSSION

Present work was a satisfactory preliminary study in developing *in situ* gelling system of Gentamicin Sulphate. The formulation development was started with 9 formulation but formulation F3, F6 and F9 wasn't show good gelling capacity in simulated tear fluid (STF), formulation F7 had some stability problem during storage, F4 possessed irritation in eye irritation study, so we can conclude that **F5** might be the best formulation in term of *In Situ* gelation, viscosity, eye irritancy, stability than the other formulation.

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