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FORMULATION AND EVALUATION OF AN OPHTHALMIC *IN-SITU* GEL OF GENTAMICIN SULPHATE

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ABSTRACT

The aim of the present study is to prepare and evaluate an ophthalmic *in-situ* gel formulation of gentamicin sulphate. The different gelling solutions were made with Pluronic F127 in combination with different polymers such as Carbopol 934 and HPMC, which acted as a viscosity-enhancing agent. Suitable concentrations of buffering agent were used for pH adjustment. All the formulations were sterilized in an autoclave at 121°C for 15 minutes. The formulations were evaluated for clarity, pH measurement, gelling capacity, drug content, rheological study, sterility testing, *in vitro* drug release study, antibacterial activity, ocular irritation study and stability study. The developed formulations exhibited sustained release of drug from formulation over a period of 8 hours. The prepared formulations were tested for eye irritation on albino rabbit (male). The formulations were found to be non-irritating. Thus, the *in situ* gelling systems may be used as an alternative to the conventional systems.

KEYWORDS: Gentamicin sulphate, *in situ* gel, *in vitro* drug release, rheological study.

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.[1] Gel dosage forms are successfully used as drug delivery systems considering their ability to prolong the drug release.[2] To prolong the precorneal residence time and improve ocular bioavailability of the drug various polymers system were studied as *in situ* gelling vehicle for ophthalmic drug delivery system.[3] The *in situ* formulation exhibited well, viscosity, drug content and sustained drug release.[4]

Conventional liquid ophthalmic formulations demonstrate low bioavailability because of a constant lachrymal 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.[3]

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. 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.[5] The present study aimed to prepare and evaluate ophthalmic *in-situ* gel formulations of gentamicin sulphate. The different gelling solutions were made with pluronic F127 in combination with different polymers such as carbopol 934 and HPMC, which acted as a viscosity-enhancing agent.

MATERIALS AND METHODS

MATERIALS

Gentamicin sulphate was procured from Kee Pharma Ltd., New Delhi. PluronicF127 was obtained from Sigma Aldrich, Mumbai. Benzalkonium chloride from Merck Ltd, Mumbai, Sodium chloride from Loba chemicals, Hydroxypropyl methylcellulose (HPMC-E15) and carbopol- 934P 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

Selection of Vehicle

The solubility of gentamicin sulphate was tested in various buffers such as acetate buffer I.P. (pH 5.0 and 6.0), citrophosphate buffer B.P. (pH 6.0 and 6.2) and phosphate buffer USP (pH 7.2 and 7.4) in order to select a suitable vehicle. Solutions of gentamicin sulphate in the above buffers were prepared to test its solubility at the dosage level desired (0.3% w/v). Based on visual appearance and solubility at the dosage level desired (0.3% w/v), acetate buffer pH 5.0 was chosen for further studies as it gave clear solutions.

Preparation of in-situ gel[6]

For the preparation of Pluronic F127 based ocular *in-situ* gel all the ingredients were sieved from sieve no 44. Solution of 0.3% of gentamicin sulphate was prepared in acetate buffer I.P. pH 5.0. The solution was cooled in an 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 helped in dissolving the pluronic F127 completely. After 24h carbopol 934 and HPMC 15cps were added slowly along with other excipients with continuous stirring for 2-3 hours for proper mixing and avoiding slug formation. Buffering and osmolality agents were added to the resulting solution along with benzalkonium chloride. The pH of the solution was adjusted using 0.5 N NaOH.

The resulting formulation was kept on probe sonicator to remove air bubble. All formulations were stored in LDPE (Low Density Polyethylene) bottles for further use. All the containers were stored in refrigerator. Composition of different formulations of in-situ gel is given in Table 1.

Table 1 Composition of Different Formulations of *In-Situ* Gel

S. No.	Ingredient (%)	Formulations								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1.	Gentamicin Sulphate (w/v)	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%
2.	Pluronic F127	18	16	14	18	16	14	18	16	14
3.	Carbopol 934	0.2	0.2	0.2	0.3	0.3	0.3	0.4	0.4	0.4
4.	HPMC 15cps	1.0	1.0	1.0	0.75	0.75	0.75	0.5	0.5	0.5
5.	EDTA	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
6.	Benzalkonium Chloride	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%
7.	NaCl	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
8.	Poly ethylene glycol	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
9.	Acetate Buffer (pH 5.0)	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml

Physical parameters[7]**:** The formulated in-situ gel solution was tested for clarity, pH, gelling capacity and drug content.

Clarity: Clarity is one of the most important characteristic features of ophthalmic preparations. Formulations were evaluated for clarity by visual observation against a black and white background.

Drug content[9]

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 produced a purple color.

pH

The pH of ophthalmic formulation should be such that the formulation is stable at that pH and at the same time is non-irritant to the patient upon administration. 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.

***In-situ* gelling capacity**

In situ gelling capacity was determined by visual inspection by placing a drop of formulation in a vial containing 2ml of freshly prepared simulated tear fluid (STF) equilibrated at 37^0C. The time taken for gelation was noted.

Rheological studies

At pH 5.0 and temperature less than 16^0C, the developed formulations were in liquid state and showed 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. The pH was raised to 7.4 by the addition of 0.5M NaOH. The resulting gel was studied for viscosity on Brookfield Synchrolectric Viscometer using Spindle No.7 at 50 rpm for comparative study. The angular viscosity was measured by gradually increasing the rpm from 10 to 70.

Sterility testing[10] [12]

The sterility testing was performed with the help of two media namely, Fluid thioglycollate medium and Soya Bean-Casein Digest medium (IP) and investigated the presence or absence of aerobic, anaerobic bacteria and fungi in the formulated ophthalmic in situ gels.

Antimicrobial activity

Antimicrobial activity was determined by agar diffusion test employing cup plate technique. The test microorganism used in the present study was *Staphylococcus epidermidis* (ATCC 12228).

Ocular irritation test[11]: Ocular irritancy was determined using two tests: LVET (low volume eye test) and Draize eye irritation test. The LVET differs from the Draize rabbit 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. Rabbits were monitored periodically for redness, inflammation, watering of the eyes.

***In-vitro* drug release study[6][8]:** The *in vitro* release of drug from the formulations was studied through cellophane membrane. The dissolution medium used was freshly prepared artificial tear fluid (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). About 1 ml 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. Aliquots (each of 1ml) were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium.

Stability studies: All the five formulations were subjected to stability studies at ambient humidity conditions at 2ºC to 8ºC, ambient temperature and 40ºC for a period of one month. The samples were withdrawn after 7, 15 and 30 days and were evaluated for the drug content, pH, clarity, viscosity and in-situ gelling capacity.

RESULTS AND DISCUSSION

Physical parameters: The formulated in-situ gel solution was tested for clarity, pH, gelling capacity and drug content. Only those formulations were selected for further studies which were clear. For in situ gel of Gentamicin sulphate pH 5.0 should be optimum because Gentamicin sulphate is stable at pH 3.5-5.0. Lowering the pH from 5.0 can causes irritation to eye and increasing it above 5 resulted in gelation of formulation due to the presence of carbopol. The pH of formulations was decreased because of acidic groups of carbopol so the pH was adjusted to 5.0 by using 0.5N NaOH in all the formulations.

The drug content of only those formulations was determined which passed the clarity test. Simulated tear fluid (STF) was prepared and warm up to 37^0C. Solution was introduced into STF in a ratio of 1:2. Changes in consistency of solution were visually inspected.

Formulation F3, F6 and F9 showed poor gelling capacity in simulated physiological conditions of pH and temperature because of comparatively less concentration of pluronic F127 in F3, F6 and F9 respectively and due to low concentration of carbopol in F1. Formulations F4 and F5 showed better gelling capacity. The results are depicted in table 2.

Table 2: Physical Parameters of Gel Formulations

Formulation code	Clarity	pH	Drug Content (%)	*In situ* gelling capacity
F1	Clear	5.0	98.22	"++"
F2	Clear	5.0	99.14	"++"
F3	Turbid	5.1	nd	"+"
F4	Clear	5.0	97.22	"+++"
F5	Clear	5.0	98.65	"+++"
F6	Clear	5.1	nd	"+"
F7	Precipitate formation	5.1	nd	"++"
F8	Clear	5.0	95.51	"++"
F9	Turbid	5.1	nd	"+"

"+" represents gelation after five minutes which dissolves rapidly

"++" represents immediate gelation, remains for few hours

"+++" represents immediate gelation, remains for extended period 8 hours

nd- not determined.

Rheological studies: Viscosity of formulation was determined before and after gelation by using Brookfield's viscometer in the small volume adaptor and the angular velocity was increased gradually from 10, 20, 40, 50, 60 and 70 rpm. The comparative study of viscosity was done at 50 rpm. F4, F5, and F7 show comparatively better viscosity and good consistency gel (Table 3). A comparison of angular viscosities of the formulations is shown in figure 1.

Table 3: Comparative viscosity* of *in situ* gel formulations

Formulation code	% of Pluronic F 127	Viscosity of solution (in cps)	Viscosity after gelation
F1	18	987	2423
F2	16	881	2205
F3	14	811	2150
F4	18	1053	2671
F5	16	933	3056
F6	14	613	2330
F7	18	741	2685
F8	16	771	2535
F9	14	668	2831

*Spindle no.7, at 50 rpm

Angular viscosity of formulations

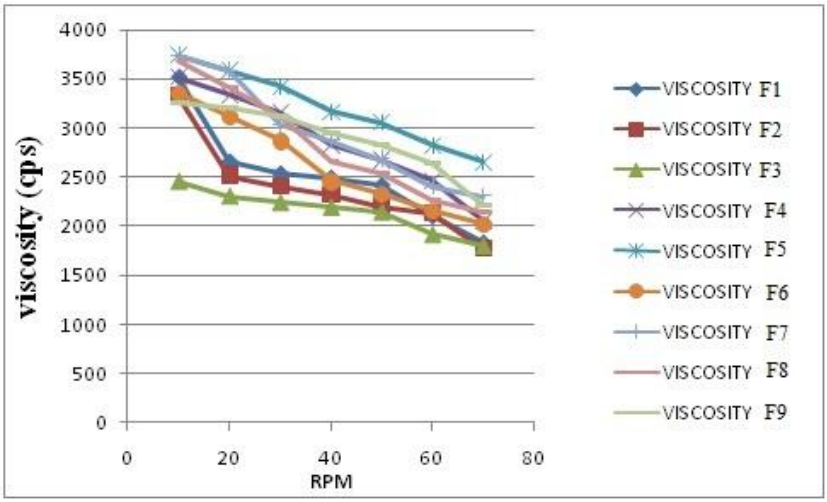

Fig. 1: Comparison of angular viscosities of formulations

Sterility testing

The formulation was inoculated separately with Fluid thioglycollate medium and Soya Bean-Casein Digest medium (IP). Direct inoculation method is recommended for clear aqueous preparations as per IP.[12] All the formulations were terminally sterilized by autoclaving. For sterility testing formulations were diluted ten times by sterile distilled water. Petri dishes then placed in incubation chamber for 7 days and observed for microbial growth. No turbidity or microbial growth was observed in formulations F1 to F9.

Antimicrobial activity

The Petri dishes with addition of specific micro-organism (*Staphylococcus epidermidis*, ATCC 12228) were incubated 24 hours at 33 ± 2°C. After the incubation period, the growth inhibition zones were measured and the results were expressed as the arithmetic mean of three measurements for each sample. Antimicrobial activity was valued on the basis of the diameter of the growth inhibition zone as follows: < 10 mm – no antimicrobial activity; 10 - 15 mm – weak antimicrobial activity; 16 - 20 mm – moderate antimicrobial activity; 20 mm > – high antimicrobial activity. The mean zone of inhibition was determined for formulations F1 (18.6 mm), F2 (17.7 mm), F4 (17.3 mm), F5 (18 mm) and F8 (18.4 mm), respectively.

Ocular irritation test[13]

Ocular irritation study was performed using healthy albino rabbits after getting prior permission from the institutional animal ethics committee. The eyes of each rabbits were examined at particular time interval after instillation of the selected formulations (F1, F2, F4,

F5, F8). The results showed that 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. It was observed that the selected formulations were non-irritant to rabbit eye.

***In- vitro* drug release study:** The percentage cumulative drug release of the prepared formulations showed sustained drug release up to 8 hours duration. Formulation F4 showed more sustained release compared to other formulations. The comparative percent cumulative drug release (%CDR) of formulations (F1, F2, F4, F5, F8) is given in figure 2.

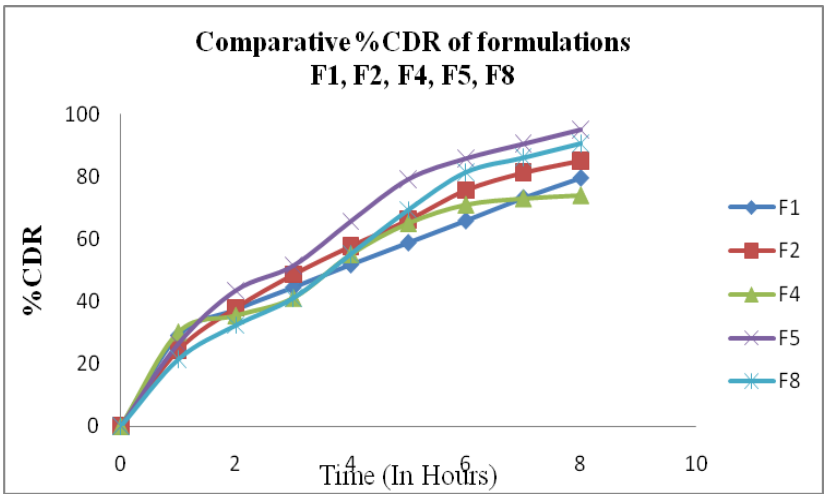

Fig. 2: A Comparative percent cumulative drug release (%CDR) of formulations

Stability studies: The samples were analyzed periodically for the 7th, 15th and 30th day and it was found that there were no changes in visual appearance, drug content, clarity, pH, and gelling capacity. All the formulations (F1, F2, F4, F5 and F8) had pH ranging between 5.0 to 5.1 and were clear. Formulations F4 and F5 had maximum in-situ gelling capacity.

CONCLUSION

The *in situ* gel formulation of Gentamicin sulphate is a viable alternative to conventional eye drops by virtue of its prolonged precorneal residence time and ability to sustain drug release. Another benefit is the ease of administration afforded and decreased frequency of administration resulting in better patient compliance.

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