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RESEARCH ARTICLE

FORMULATION AND EVALUATION OF SUSTAINED RELEASE *IN SITU* OPHTHALMIC GEL OF NEOMYCIN SULPHATE

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The aim of the present work was to formulate and evaluate *in situ* gelling system of Neomycin sulphate. Neomycin sulphate is an antibacterial agent which exhibits rapid precorneal elimination and poor ocular bioavailability, when given in the form of conventional ophthalmic drops. To overcome this, an attempt has been made to formulate temperature-triggered *in situ* gelling system of Neomycin sulphate to provide sustained release of drug based on polymeric carriers that undergo sol-to-gel transition upon change in temperature. The Neomycin sulphate in situ gelling system was formulated by using Poloxamer 407 in combination with hydroxyl propyl methyl cellulose (HPMC) which acted as viscosity enhancing agent. The formulations were evaluated for clarity, pH measurement, gelling capacity, drug content estimation, rheological study, *in vitro* diffusion study and antibacterial activity. The developed formulation was stable and provided sustained release up to a time period of 8 h and it is a viable alternative to conventional eye drops.

Key words: Neomycin sulphate, *In situ* gel, Sustained release, Neomycin sulphate, Poloxamer 407.

INTRODUCTION

Ocular drug delivery is an extremely important topic, especially with the recent development of new drugs for the treatment of different eye diseases. An ideal drug therapy achieves effective concentration of drug at the target for a specified period of time in order to minimize general and local side effects (Lee and Robinson, 1986; Sasaki *et al* 1996). Eye is most interesting organ due to its drug disposition characteristics. Generally, topical application of drugs is the method of choice under most circumstances because of its convenience and safety for ophthalmic chemotherapy.

Since past few decades, there has been plenty of research reports exhibiting potential of controlled and sustained drug delivery systems (Chitra *et al* 2012; Basarkar *et al* 2013; Nagpal *et al* 2014; Mishra and Jain, 2014). Moreover, 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 and the second one involves maximizing corneal drug absorption and minimizing pre-corneal drug loss. Ideal ophthalmic drug delivery must be able to sustain the drug release and to remain in the vicinity of front of the eye for prolong period of time. Consequently, it is imperative to optimize ophthalmic drug delivery; one of the ways to do so is by addition of polymers of various grades, development of *in situ* gel or colloidal suspension or using erodible or non-erodible insert to prolong the pre-corneal drug retention (Robinson, 1993).

Neomycin sulphate is an antibacterial agent widely used in various eye infections *i.e.* conjunctivitis, endophthalmitis. It is highly soluble but poorly absorbed from the normal gastrointestinal tract having half life of 2-3 h. Approximately, 3% of Neomycin is absorbed through intact intestinal mucosa. In situ gel solution increases the residence time and also sustain the release mechanism of the drug. So, to enhance its bioavailability and local as well as systematic effect for longer duration of time, ophthalmic *in situ* gel for sustained delivery of Neomycin sulphate was attempted.

MATERIALS AND METHODS

Neomycin sulphate was obtained from West-coast Pvt. Limited, Ahmedabad, India. Poloxamer 407, HPMC-K4M, HPMC-E50LV, Sodium chloride and Benzalkonium chloride was obtained from ACS chemicals, Ahmedabad, India. All the polymers received were of pharmaceutical grade and were used as received. Other materials and solvents used were of analytical grade.

Preparation of *in situ* gel

In-situ gel formulations

Required quantity of sodium chloride was dissolved in 75 ml of distilled water. To this, HPMC E50LV or HPMCK4M was added to the above solution and stirred slowly with magnetic stirrer. Care was taken to avoid lumps of HPMC during stirring. Poloxamer 407 was sprinkled over this solution and allowed to hydrate overnight (4°C) resulting in clear and transparent solution without swelling or lumps. A preservative, benzalkonium chloride was then added to it. The solution was again stirred with magnetic stirrer after 24 h.

From this solution, 40 ml was withdrawn and used for further preparation. Neomycin sulphate was added in distilled water now; the drug solution was added to the poloxamer 407/HPMC solution under constant stirring until a uniform solution was obtained. Distilled water was then added to make up the volume to 40 ml. The composition of *in situ* gel formulations are depicted in **Table 1**.

Table 1. Formulation of *in situ* gels of Neomycin sulphate

Sr. No.	Ingredients (% *w/v*)	F1	F2	F3	F4	F5	F6
1	Neomycin sulphate	0.5	0.5	0.5	0.5	0.5	0.5
2	Poloxamer 407	15	16	18	19	20	20
3	HPMC-E50LV	0.5	0.5	0.5	1.0	1.0	1.5
4	Sodium Chloride	0.9	0.9	0.9	0.9	0.9	0.9
5	Benzalkonium Chloride (ml)	0.01	0.01	0.01	0.01	0.01	0.01
6	Water (up to ml)	40	40	40	40	40	40

Evaluation of *in situ* gel

Visual appearance and clarity

Visual appearance and clarity was tested under fluorescent light against a white and black back ground for presence of any particulate matter (Mohanambal *et al* 2011).

pH

pH of the in-situ gels after addition of all ingredients were measured using digital pH meter (Mohanambal *et al* 2011).

Gelling capacity

All prepared formulations were evaluated for gelling capacity in order to identify the compositions suitable for use as in situ gelling systems. The gelling capacity was determined by placing a drop of the formulation in a vial containing 2 ml of artificial tear fluid freshly prepared (pH 7.4) and equilibrated at 37 °C and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve. The composition of artificial tear fluid used was sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride dihydrate 0.008 g, purified water *q. s.* 100.0 g (Qi *et al* 2007).

Rheological studies

The formulations were poured into the sample adaptor of the Brookfield DV-111+rheomoter and angular velocity was increased gradually from 1 to 60 rpm using spindle no. 4. The hierarchy of angular velocity was reversed and the average dial reading was considered to calculate the viscosity (Mohanambal *et al* 2011).

Drug content

Distribution of active ingredient is important to achieve dose uniformity. Drug content was determined by suitably diluting formulation with ATF and analyzed by UV spectroscopy at 277 nm

for Neomycin sulphate (Miyazaki *et al* 2001).

In vitro release study

Drug released study from prepared formulation was studies using Franz-diffusion cell. Cellophane membrane and artificial tear fluid (ATF) pH 7.4 was used as a diffusion membrane and medium respectively. The cellophane membrane (previously soaked overnight in the receptor medium) was tied at one end of the glass diffusion cell. Accurately weighed 1ml of gel was spread uniformly on a cellophane membrane, which was in contact with receptor medium. The receptor medium was stirred continuously at 20rpm to simulate blinking action of eyelids. The whole assembly was adjusted on magnetic stirrer and maintained at 37±1°C. At specify intervals (0.5 h, 1 h, 2 h, 8 h) 2 ml of sample was withdrawn from receptor compartment, replace with 1 ml of fresh ATF and analyzed by UV spectroscopy (Miyazaki *et al* 2001).

Antibacterial activity

Antimicrobial efficiency studies were carried out to ascertain the biological activity of sol-to-gel systems against microorganisms. This was determined in the agar diffusion medium employing cup plate technique. Sterile solution of marketed Neomycin sulphate eye drops was used as a standard. The standard solution and the developed formulations (test solution) were taken into separate cups bored into sterile SCDM Agar previously seeded with organisms *Staphylococcus aureus* and *Escherichia coli*. After allowing diffusion of solutions for two hours, the plates were incubated for 24 h at 37°C. The zone of inhibition (ZOI) was compared with that of the standard (Indian Pharmacopoeia, 2010).

Kinetic modeling

In order to determine drug release mechanism that provide the best description to the pattern of drug release, the *in vitro* drug release data were fitted to zero order, first order, Hixon Crowell equation, Higuchi matrix model and korsmeyer peppas model. Regression coefficient was calculated for each model (Gupta and Vyas, 2010).

Accelerated stability study

Accelerated stability study was carried out for the period of 30 days for the formulations. Selected sterilized formulations store at 40±2°C at 75±5% RH, for a period of 1 month. The formulation was evaluated at periodic intervals for drug content, clarity, pH, sol gel transition, rheology and *in vitro* drug release (Mandal *et al* 2012).

RESULTS AND DISCUSSION

In the present investigation, efforts were made to prepare the sustained release Neomycin sulphate in situ gel forming ophthalmic solution using polymers such as HPMC K4M, HPMC E50LV, Poloxamer 407 as novel ophthalmic gel-forming mucoadhesive polymer, which gets converted to stiff gel when its temperature is raised, was used as excipients in temperature-triggered *in situ* gelling system. The prepared in situ gel formulations were evaluated for clarity, pH measurement, gelling capacity, drug content estimation, rheological study, *in vitro* diffusion study. The pH of *in situ* gel solution was found to be around 6.5. The formulation should have an optimum viscosity that will allow for easy instillation into the eye, which would undergo a rapid sol-to-gel transition (triggered by temperature change) as shown in **Table 2**.

Table 2. Evaluation parameters of formulations

Formulations	Visual appearance	Clarity	pH±S.D.	Drug content ±S.D. (%)	Gelling capacity
F1	Transparent	Clear	6.5±0.21	93.62±0.65	+
F2	Transparent	Clear	6.5±0.19	90.98±0.87	++
F3	Transparent	Clear	6.6±0.16	94.06±0.13	++
F4	Transparent	Clear	6.7±0.19	91.86±0.59	++
F5	Transparent	Clear	6.4±0.27	92.30±0.32	+++
F6	Transparent	Clear	6.6±0.24	93.84±0.48	+++

* +: Gel dissolves fastly, ++: Gelation immediate and remains for a 1-2 h,
+++: Gelation immediate and remains for an extended period

Rheological evaluation of all the formulations exhibited Newtonian flow before gelling and exhibited pseudoplastic flow after gelling in the eye. There was increase in the viscosity after gelling. Additionally, the gel formed *in situ* should maintain its integrity without dissolving or eroding for a prolonged period. Results are as shown in **Table 3**.

Table 3. Viscosity (cps) of formulations at different speed

RPM	Viscosity (in cps) (Before gelling) (Spindle No.4)						Viscosity (in cps) (After gelling) (Spindle No. 4)					
	F1	F2	F3	F4	F5	F6	F1	F2	F3	F4	F5	F6
30	151±1.3	162±8.5	199±11.2	189±1.9	210±2.7	309±9.1	200±13.8	250±4.7	300±8.1	300±1.8	400±7.4	500±5.8
60	89±9.4	121±12.8	161±0.2	201±0.8	241±7.5	321±1.7	100±14.5	150±9.6	200±6.9	250±9.6	350±7.2	400±2.1

From the *in vitro* results, it was observed that percentage release of the drug from the developed formulations F1- F6 as shown in the **Figure 1**. Formulation F5 showed more sustained release compared to other formulations. This may be due to the optimum concentration of Poloxamer 407 (20%) along with HPMC E50LV (1.0%) in the formulation F5. By observing the drug release profile, it was concluded that release was not stagnant even end of eight hours.

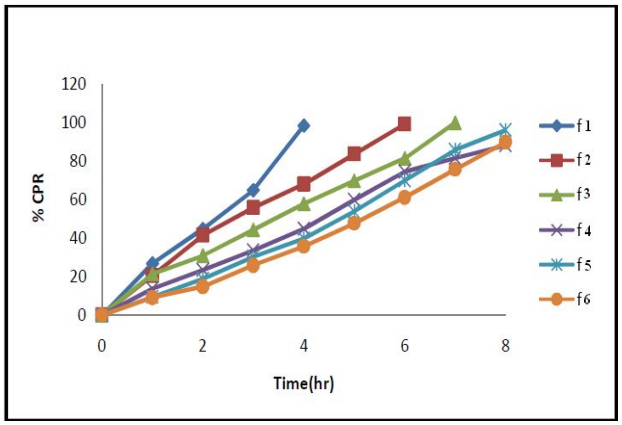

Fig. 1. Drug release study of batch F1 to F6

The *in vitro* release profile of F5 was compared with the marketed formulation of Neomycin sulphate. From the release studies, it was found that the drug release was about 56.78% and 9.35% for marketed product and F5 respectively after 1 h and at the end of two hours the drug release was 83.46% and 29.98% for marketed product and F5, respectively. The comparative release is shown in **Figure 2**. Results indicated that, the drug release was significantly prolonged by using the *in situ* gelling system due to the addition of the polymers Poloxamer 407 and HPMC E50LV.

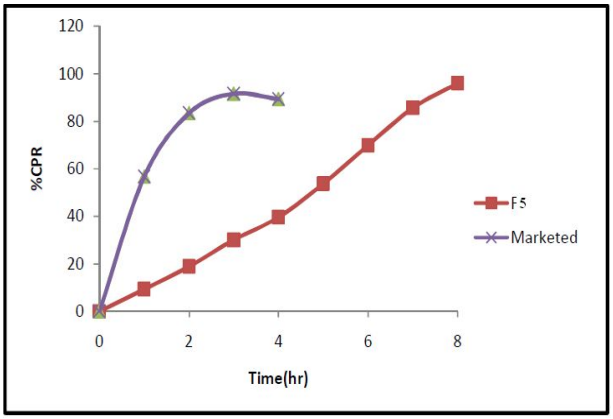

Fig. 2. Drug release study of batch F5 and marketed formulation

Formulation F5 showed highest zone of inhibition values against *S. aureus* (15 mm) and *E. coli* (17 mm), respectively, compared to other developed formulations (**Table 4**). Hence, F5 formulation was taken for further study.

Table 4. Zone of inhibition values of formulations

Micro-organism	Conc. (µg/ml)	Zone of Inhibition (mm)						
		Standard (Pure drug)	F1	F2	F3	F4	F5	F6
S. aureus	2 µg/ml	16.99±0.2	13.25±0.1	14.23±0.1	13.65±0.3	14.32±0.12	15.67±0.01	12.01±0.2
E. coli	2 µg/ml	18.19±0.31	15.32±0.4	16.21±0.23	15.54±0.4	16.54±0.34	17.89±0.89	14.12±0.23

The release kinetics of all the formulations (F1-F6) are shown in **Table 5**. The release kinetics of the optimized formulation was well fitted to Korsemeyer-Peppas model based on the concepts of the highest regression coefficient (R^2) value. The 'n' value for the optimized formulation was found to be 0.579. The 'n' value obtained from Peppas equation was greater than 0.5, which indicated that the formulation showed drug release by the non-fickian diffusion mechanism. Accelerated stability studies were carried out at 40±2°C at 75±5% RH for 1 month using stability chamber. Samples were analyzed periodically every week and found that there were no changes in visual appearance, clarity, pH, and gelation. Assay values after 1 month of storage were found almost same. Release profiles were similar to that of zero days.

Table 5. Kinetic modeling of batches F1 to F6

Formulation	R^2 value				
	Zero order	First order	Higuchi Model	Hixson Crowell Model	Korse Meyer Peppas Model
F1	0.9878	0.9979	0.9690	0.9979	0.9890
F2	0.9973	0.9564	0.9957	0.9973	0.9968
F3	0.9974	0.9855	0.9821	0.9974	0.9919
F4	0.9950	0.9660	0.9873	0.9950	0.9961
F5	0.9960	0.9708	0.9764	0.9960	0.9992
F6	0.9941	0.9795	0.9699	0.9941	0.9908

CONCLUSION

Neomycin sulphate, a broad spectrum antibacterial agent used in the treatment of ocular infections, was successfully formulated as *in situ* gel-forming eye drops using Poloxamer 407 and HPMC E50LV as a gelling agent in combination with HPMC as a viscosity enhancing agent. Thus, the developed formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer pre-corneal residence time and ability to sustain drug release.

Also important is the ease of administration afforded and the decreased frequency of administration resulting in the better patient acceptance.

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