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Novel hydrogel-based ocular drug delivery system for the treatment of conjunctivitis

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Abstract
Purpose Conventional dosage form like eye drops showed poor therapeutic response and also require frequent dosing. Therefore, developing the dosage form to deliver the drug to the target site without much loss of drug or without causing any systemic side effects is the challenging job for the researchers in pharmaceutical industries.
Objective The main aim of the present work was to formulate and evaluate hydrogel-based drug delivery containing combination of neomycin sulphate and betamethasone sodium phosphate in order to provide prolonged release and also better bioavailability of drugs for the treatment of eye infections.
Methods In this study, poloxamer 407 and chitosan at different concentrations were used as the gelling agents. The prepared formulations were evaluated for clarity, pH, drug content, gelling capacity, gelling temperature and in vitro drug release study.
Results From the preliminary studies, F5 formulation was selected as an optimized formulation. The optimized formulation was further evaluated for ex vivo permeation study, sterility test, HET-CAM and ocular irritation testing using rabbits. Ocular irritation by HET-CAM assay showed that the formulated gel does not cause any irritation to the blood vessels. Draize irritation test was performed using rabbits and results showed that formulation was non-irritant to the eye.
Conclusion The formulated hydrogel formulation can be used as an alternative to conventional ophthalmic eye drop formulation of drugs neomycin and betamethasone for the purpose of providing prolonged therapy for the treatment of conjunctivitis.

Keywords Hydrogel · Neomycin · Betamethasone · Poloxamer 407 · Chitosan

Introduction

In the past few years, there has been a tremendous increase in the research of ocular drug delivery systems. Loss of drug and poor bioavailability of drugs are the major drawbacks of ocular drug delivery. Conventional dosage form like eye drops showed these types of disadvantages and sometimes fail in providing therapeutic response and also require frequent dosing. Therefore, developing the dosage form to deliver the drug to the target site without much loss to the drug or without causing any systemic side effects is the challenging job for the researchers in pharmaceutical industries [1].

Sustained drug delivery systems to eye have been received a promising attention in past few years.

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Therefore, nowadays, the ophthalmic eye drops have been replaced by polymeric solution which has properties like it is liquid before instillation and changes into gel when exposed to body temperature or by changes in pH and ionic configuration [2]. Several hydrogels have been developed nowadays to enhance the contact time of the formulation. There are various types of hydrogels like temperature-triggered, pH-triggered and ion-activated hydrogels. Researchers over the years have been defined hydrogel in different ways. "Hydrogel is defined as water swollen and cross-linked polymeric network produced by the simple reaction of one or more monomer [3].

The in situ hydrogels are capable of maintaining the constant drug level in plasma by releasing the drugs in a sustained manner [3]. Various combinations of polymers are used in hydrogel formulation to enhance the drug delivery to eyes. Researchers have been showed that combination of natural and synthetic polymers provides better stability. In situ forming hydro gels have an ability to undergo gelation after its administration in the ocular *cul de sac*. It is one of the newer approaches which combine the benefits of both solutions and gels like accuracy of dose, easy administration of dosage form and extended residence time, thereby it produces sustained release of drugs which in turn enhances the bioavailability and improves patient compliance [4].

Conjunctivitis is one of the most common eye disorder seen by medical and pediatric practitioners. Without treatment, the infection may improve spontaneously or worsen to cause corneal ulceration, scarring, and sometimes it leads to blindness. Neomycin is a wide spectrum amino glycoside antibiotic produced by the growth of *Streptomyces fradiae* of the family *Streptomycetaceae*. Betamethasone is a corticosteroid. Combination of these two drugs is used in effective treatment of conjunctivitis. In this combination of drugs, steroids reduce inflammation while an antibiotic treats/prevents infections. The aim of the research work was to investigate the potential of hydrogel containing combinations of neomycin and betamethasone as a novel drug delivery system for the treatment of conjunctivitis so as to increase the time of contact of the drug and improves patient compliance [5]. In this study, combinations of two polymers were used like poloxamer and chitosan in different concentrations. Poloxamer is a tri-block soluble polymer. It has good thermo-gelling properties. At 25 °C, it forms viscous solution and at 37 °C it forms colorless transparent gel [6]. Chitosan is a natural cationic copolymer which is also useful in hydrogel preparation. It is obtained by partial deacetylation of naturally occurring chitin. Partial deacetylation increases number of amino groups, aqueous solubility and also its bio-degradability and biocompatibility [7].

Materials and methods

Materials

Neomycin sulphate was procured as a gift sample from Nanz Med Sciences Pharma Pvt Ltd. Sirmour, India. Betamethasone sodium phosphate obtained as a gift sample from Micro Labs, Bangalore, India. Poloxamer 407 was purchased from Yarrow Chem Product, Mumbai, India. Chitosan was obtained from Nice chemicals Pvt Ltd., Kochi, India. All other reagents used were of analytical grade.

Formulation of hydrogel

Hydrogel of neomycin sulphate (NS) and betamethasone sodium phosphate (BSP) was prepared by cold method. The different concentrations (0.1–0.3% w/v) of chitosan solutions (as shown in Table 1) were dispersed in acetic acid solution with continuous stirring until completely dissolved to obtain clear solution. Then, the poloxamer 407 (20–25% w/w) solution was added to the previously prepared chitosan solution. Then, the dispersion was kept in a refrigerator at 4 °C for overnight until the entire poloxamer dissolved and finally formed clear solution. Then, to this solution, 0.3% of neomycin sulphate and 0.1%

Table 1 Composition of hydrogel formulation

Formulation code	Pluronic F127 (w/w)	Chitosan (w/v)
F1	20	0.1
F2	20	0.2
F3	20	0.3
F4	25	0.1
F5	25	0.2
F6	25	0.3

betamethasone sodium phosphate were added and it was mixed thoroughly by continuous stirring at 200 RPM (Revolutions per Minute). Benzalkonium chloride was added as a preservative to the formulation (0.02% w/v). All the formulations were adjusted to the neutral pH range by adding 0.5 M sodium hydroxide solution (0.6–1 mL). Finally, the formulations were sterilized by autoclave at 121 °C for about 15 min using saturated steam under 15 psi of pressure. After sterilization, formulations were kept in a refrigerator prior to evaluations [8].

Compatibility studies by Fourier transform infra red spectroscopy (FTIR)

The FTIR spectroscopy was done to check the compatibility between drug and the gelling agents. The FTIR spectra of neomycin sulphate, betamethasone sodium phosphate, poloxamer 407, chitosan and the optimized formulation were carried out by this technique. The IR spectrum of formulation was compared with pure drugs to check the appearance and disappearance of characteristic peaks in IR spectrum [9].

Characterization and evaluation of hydrogel formulations

Clarity test/visual appearance

The clarity of the formulation was observed by visual inspection against white and black background under strong light [10].

Determination of pH

The prepared ophthalmic formulations were tested for pH using digital pH meter. This was done by dipping tip of the pH meter in standard solution followed by rinsing with distilled water and then checking pH by placing tip of the pH meter in formulations contained in a beaker [10].

Gelling capacity

The gelling capacity of the formulated hydrogels was evaluated by placing a drop of the hydrogel solution in a beaker containing artificial tear fluid of pH 7.4 (it was prepared using NaCl 0.67 g, $NaHCO_3$ 0.20 g, $CaCl_2$ $2H_2O$ 0.008 g and distilled, deionized water to 100 ml) and it was observed for gelling time and categorized accordingly [11].

− No gelation
+ Gelation occurred in few min and remained for few hours
++ Gelation immediate remained for few hours
+++ Gelation immediate and remained for extended period.

Gelling temperature

Transfer 10 ml of the prepared ophthalmic hydrogel to 50 ml of beaker and it was kept under magnetic stirring in a thermostatically controlled heater with a magnetic bead inside. The temperature was gradually increased with an increment of 1 °C and the temperature of the formulation was checked using thermometer. The speed of rotation of the bead gradually decreases, as the viscosity increases. The gelation temperature was taken as the temperature at which the magnetic bead completely stopped rotating or the gel was said to have formed when there was no flow of the formulation when the container was overturned [12].

Rheological studies

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. The viscosity was measured using Brookfield viscometer. The viscosity of formulation was analyzed at two different temperatures like room temperature (25 °C) and at body temperature (37 °C) which was maintained by circulating bath connected to the viscometer. The viscosity was measured at different angular velocities (5, 10, 15 and 20) using spindle number 62 and rheogram was constructed by plotting rate of shear versus shear stress [13].

In vitro drug release profile

The in vitro drug release studies of hydrogel formulations were carried out by donor–receptor compartment model. The donor compartment consists of two open ends where one end is covered with the dialysis membrane (MWCO = 1000 D), which was presoaked overnight in the freshly prepared simulated

tear fluid of pH 7.4. Accurately weighed 1 g of in situ *gel* was placed on the dialysis membrane attached to the donor compartment [14]. A beaker containing 50 ml of simulated tear fluid was considered as receptor compartment which was maintained at 37 ± 0.5 °C under stirring. The donor compartment was suspended in simulated tear fluid in the receptor compartment so that membrane touches the receptor medium. For a scheduled interval of 8 h, aliquot of 3 ml was withdrawn from the receptor compartment. To maintain the perfect sink conditions, the receptor compartment was replenished with equal volume of dissolution medium. Then, the absorbance of the samples was taken for neomycin at 217 nm and for betamethasone at 240 nm after suitable dilution [15, 16].

Ex vivo permeation study

The goat cornea was used to carry out the ex vivo permeation study. The goat's whole eye ball was collected from the local slaughter house. Then, eye ball was kept in normal saline maintained at 4 °C. The cornea along with 5–6 mm of surrounding scleral tissue was carefully removed and washed with cold saline [17]. The cornea was kept in cold simulated tear fluid of pH 7.4. The experimental setup was similar to the in vitro study. This evaluation test was carried out by placing the formulation on the cornea which is tied to the one open end of the donor compartment [18]. This assembly was kept in contact with the receptor compartment. The receptor compartment was filled with simulated tear fluid of pH 7.4 as dissolution medium maintained at 37 ± 0.5 °C and it was kept under stirring at 600 RPM (Revolutions Per Minute). For a scheduled interval of 12 h, 3 ml of the sample was withdrawn from the receptor compartment and the same volume was replaced, then the absorbance of the samples was measured at respective wavelengths after suitable dilutions [19, 20].

Isotonicity studies

One of the ideal requirements of ophthalmic preparation is that it should be isotonic with the tear fluids. Few drops of freshly drawn blood were added to two slides and then few drops of optimized formulation were added to one of the slides and mixed. The other slide was kept as control [21]. Both the slides were observed under microscope and compared for any changes in the structure of RBCs.

Sterility test

Growth promotion test

This test was to assess whether medium supports the growth of microorganisms or not. Inoculate *S. aureus* in a test tube containing 20 ml of fluid thioglycolate media and inoculate *C. albicans* into test tube containing 20 ml of soyabean casein digest media. Incubate the test tubes at 20–25 °C (fungus) and 30–35 °C (bacteria) and note the observations.

Sterility test for the formulation

Test for aerobic bacteria

About 2 ml of the optimized ophthalmic formulation was aseptically transferred into the sterile test tube containing 20 ml of fluid thioglycolate medium. The medium was incubated for about 14 days at 30–35 °C after proper mixing [22].

Test for fungi

Sterile soyabean casein digest medium of about 20 ml was aseptically transferred to sterile test tube. Then, 2 ml of the ophthalmic formulation was aseptically transferred to test tube. The medium was incubated for about 14 days at 20–25 °C after proper mixing [22].

Both the medium were observed for every day for the presence or absence of turbidity and compared with negative and positive control.

Ocular irritation by Hen's egg test chorioallantoic membrane (HET-CAM)

Selection of eggs

The eggs were collected less than 1 week after laying and incubated for about 7 days; on 8th day, their blunt ends are tested by the candling lamp. Only the eggs with emergent vascular system were selected for the test [23].

Preparation of the eggs for test

Candling procedure helps in identifying the air space and it was marked on the eggs. Then, the shells of the egg are opened at that marked portion on the blunt ends [23]. The underlying membrane was moistened with 0.9% of NaCl and the moistening solution was slowly poured out from the opened egg and then the membrane was carefully removed in such a way that underlying blood vessels are not damaged [24].

Exposed chorioallantoic membrane (CAM) was treated with 0.3 ml of the optimized test formulation. The chorioallantoic membrane was also treated with 0.3 ml of 1 N NaOH and considered as positive control and 0.3 ml of 0.9% w/v NaCl as negative control. The effects were observed near the surroundings of the applied sample within 5 min [25]. After 5 min, change in CAM was observed and scored according to the scoring chart for HET-CAM test and the time point for the observation of parameters like hemorrhage, coagulation and lysis was noted [26]. An IS (irritation score) was calculated and the optimized test formulation was classified with this score. The irritation score was calculated using the following formula [27]:

$$\mathrm{IS} = \frac{(301 - \mathrm{Hemorrrrhage})}{300} \times 5 + \frac{(301 - \mathrm{lysis})}{300} \times 7 + \frac{(301 - \mathrm{coagulation})}{300} \times 9$$

where all the values of hemorrhage, lysis and coagulation are noted in seconds. After 5 min of treatment, the main reactions are scored (lysis, hemorrhage or coagulation) according to the following scheme (0 = no reaction, 1 = slight reaction, 2 = moderate reaction and 3 = severe reaction) and the mean irritation score was determined [28] (Fig. 1).

Stability studies

The stability study of hydrogel formulation was carried out for 3 months. This study was carried out at 25 °C/60% RH and 40 °C/75% RH. After the storage, the samples were tested for their physical appearance, pH, drug content and gelation temperature [28].

Fig. 1 Exposure of CAM with optimized formulation

Results and discussion

Formulation and evaluation of hydrogel containing neomycin and betamethasone

There are various techniques for the delivery of drugs to the eyes. Liang et al. developed noncross-linked collagen discs and cross-linked collagen shields for delivery of gentamicin [29]. The ocular penetration of topical drugs can be enhanced by use of soft contact lenses [30]. There are various techniques to prolong the release of drugs like modification of collagen molecules [31] and bionite hydrophilic bandage lenses used as cosmetic devices [32, 33]. Certain hydrophilic polymers were used in the manufacture of contact lenses. Gasset et al. designed hydrogel-based contact lenses for the treatment of low-grade infections or dry eyes [34]. In our study, we prepared hydrogels containing combination of neomycin and betamethasone in different concentration of polymers and the developed formulations were evaluated for various parameters. The results of clarity, pH, gelling capacity and gelling temperature are given in Table 2. All the formulations were found to be transparent and clear, the pH of the formulation was found to be in the neutral range which thereby indicates the absence of any irritation to the eyes. The gelation temperature of the formulations was found to be near to the body temperature.

Table 2 Data for pH, gelation temperature and gelling capacity of hydrogels

Formulation code	Clarity/visual appearance	pH	Gelling temperature (°C)	Gelling capacity
F1	Transparent and clear	6.9	39 ± 0.2	+
F2	Transparent and clear	6.9	37 ± 0.1	++
F3	Transparent and clear	6.7	38 ± 0.3	++
F4	Transparent and clear	6.4	36 ± 0.4	++
F5	Transparent and clear	6.9	37 ± 0.2	+++
F6	Transparent but turbid	6.5	36 ± 0.5	+++

Compatibility studies

The characteristic peaks of drugs, chitosan, poloxamer and optimized formulation are shown in Fig. 2a–e, respectively. The IR spectra of pure drug neomycin sulphate showed characteristic peak at 1721, 2885, and 1655 cm^{-1} due to C–F, CH- aliphatic and C=C, respectively. Betamethasone sodium phosphate showed IR spectra at 2946 and 1004 cm^{-1} for C–H and C–O–C, respectively. These peaks were retained in the final formulation. The spectrum indicated that the drugs and the gelling agents were compatible with each other.

Drug content estimation

The percentage drug content of formulations was found to be in the range of 91–95% for betamethasone and 90–98% for neomycin. This proved that method adopted for the preparation of hydrogel was found to be suitable and there was not much loss of drug during the process. This also indicated that maximum entrapment took place in all the formulations. The percentage drug content is given in Table 3.

Viscosity measurement

Viscosity measurement of sol and gel form of the formulations was shown in Figs. 3 and 4, respectively. The formulations showed less viscosity in solution form than gel form and as the concentration of poloxamer 407 increased, the viscosity also increased both in solution and gel form. The addition of chitosan also enhanced the viscosity of the formulation.

In vitro drug release study

The in vitro release from hydrogel depends on the concentration of polymers used. The drug release profiles of different formulation are given in Figs. 5 and 6. From the release study, it was revealed that concentration of polymer affects the release of drug from the hydrogel formulations. The prepared formulations showed sustained release action due its thermo conversion to gel form. Such system can overcome the frequent dosing of conventional dosage forms [28]. According to the results, as the concentration of the polymer increases, the drug release decreases. The result also showed that the in vitro drug release of F5 formulation was found to be 95.46% for neomycin and 94.90% for betamethasone.

The in vitro drug release data were fitted to different mathematical models like zero order, first-order, Higuchi and Korsmeyer-peppas models, to predict the kinetics and release mechanism of drugs. The regression value (R^2) of various release models was calculated. The results indicated that in vitro drug release from all the formulations followed Higuchi model. The F5 formulation was selected as optimized formulation based on the parameters like pH, gelling capacity, gelling temperature, viscosity, in vitro drug release profile and same was used for further studies.

Ex vivo permeation study

The ex vivo permeation study showed that drug release was slower compared to that of in vitro release because it was difficult for the drugs to pass through cornea due to its lipophilic nature. The results of ex vivo study are shown in Fig. 7.

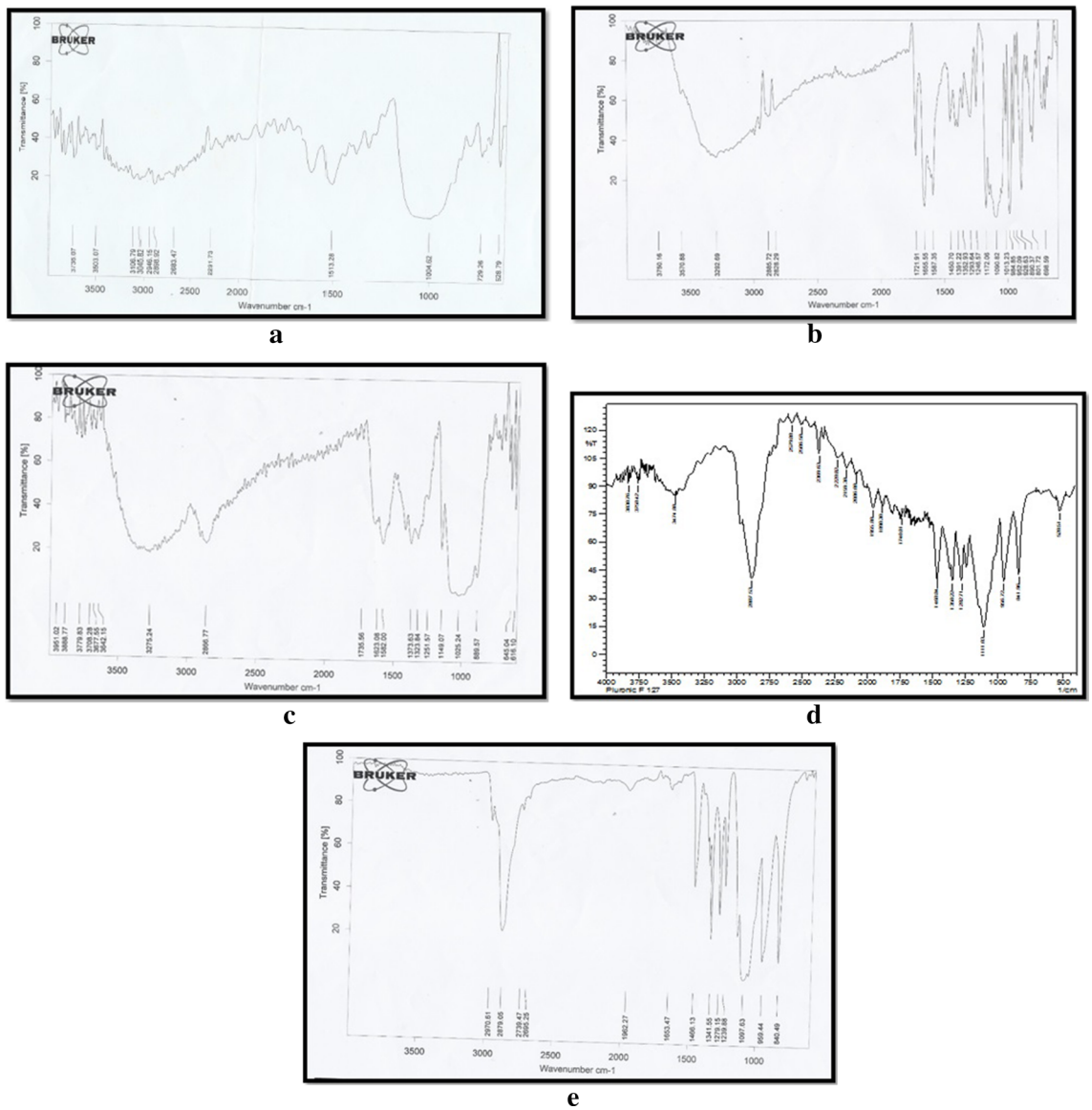

Fig. 2 **a** FTIR spectrum of Neomycin sulphate. **b** FTIR spectrum of Betamethasone sodium phosphate. **c** FTIR spectrum of Chitosan. **d** FTIR spectrum of Pluronic F127. **e** FTIR spectrum of optimized formulation F5

Table 3 Drug content of prepared hydrogels

Formulation code	Drug content (%)	
	Neomycin sulphate	Betamethasone sodium phosphate
F1	93.20 ± 0.35	90.30 ± 0.29
F2	95.61 ± 0.26	95.47 ± 0.32
F3	94.40 ± 0.23	96.86 ± 0.26
F4	95.36 ± 0.22	96.06 ± 0.35
F5	98.70 ± 0.35	98.43 ± 0.37
F6	95.70 ± 0.34	96.69 ± 0.40

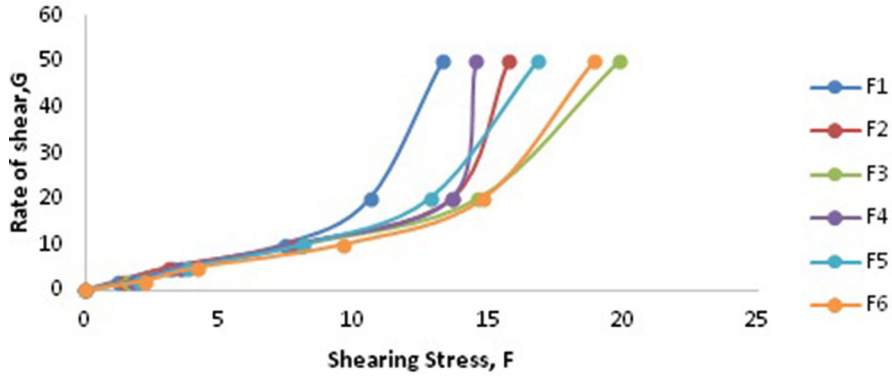

Fig. 3 Viscosity of formulations (sol form)

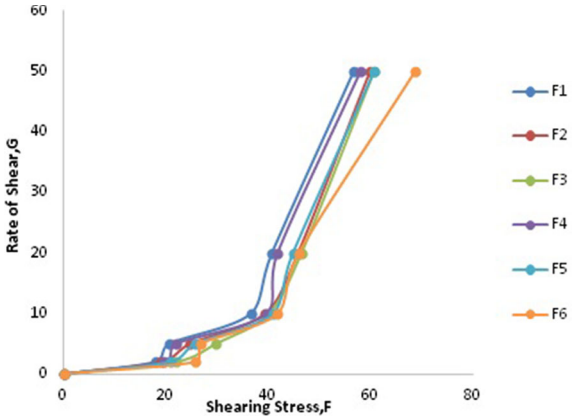

Fig. 4 Viscosity of formulations (gel form)

Isotonicity studies

The results isotonicity testing showed that the optimized formulation did not show any significant change in the structure of RBC-like shrinking or bulging. This study revealed that the formulation was isotonic in nature.

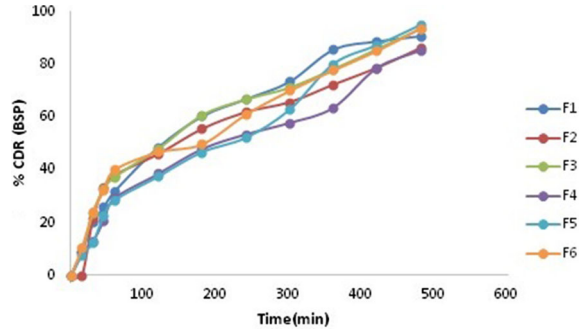

Fig. 6 In vitro drug release profile of betamethasone sodium phosphate

Sterility test

In case of sterility test for medium, the medium was found to be sterile due to the absence of growth (shown in Table 4). From the growth promotion test (Table 5), it was concluded that the medium supports the growth of test organisms. The sterility test of formulation showed that there was no growth in the

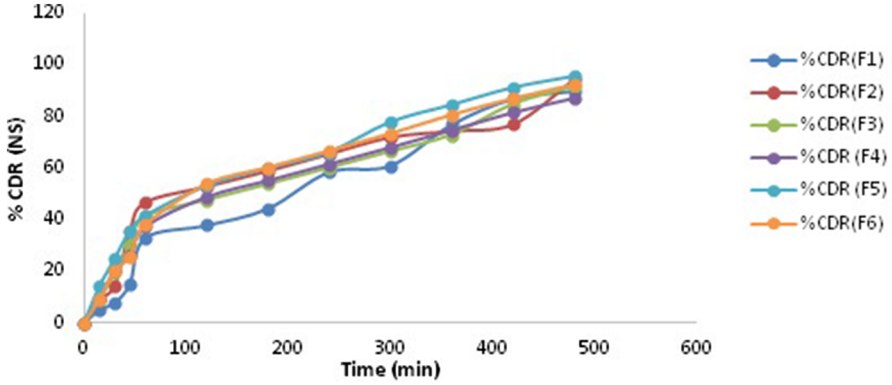

Fig. 5 In vitro drug release profile of neomycin sulphate

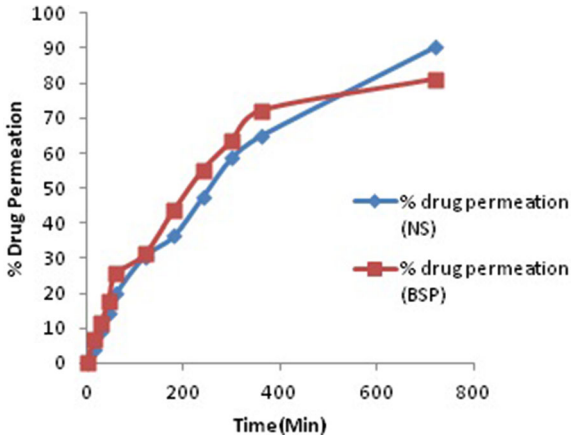

Fig. 7 Ex vivo permeation study of F5 formulation

medium which reveals that formulation was found to be sterile (shown in Fig. 8) (Table 6).

Ocular irritation test by Hen's chorioallantoic membrane model

The experimental method for testing eye irritation potential of optimized hydrogel formulation by HET-CAM (Hen's chorioallantoic membrane) and the results are shown in Table 7. The effects of formulation as well as controls on the chorioallantoic membrane were noted before and after the treatment [26].

By observing the changes in the chorioallantoic membrane, there was a great difference between the test formulation and positive control. The positive control (0.1 N NaOH) induced major damage to the CAM as shown in Fig. 9. The 0.1 N NaOH causes hemorrhage followed by the lysis of blood vessels, whereas not much severe changes occurred in the chorioallantoic membrane after the application of hydrogel formulation and 0.9% NaCl. The severity of ocular irritation of the hydrogel formulation was compared with that of positive and negative controls [27]. The images showed that there was no considerable change in the blood vessel morphology of isolated CAM and formulation did not cause any damage or irritation to the eye upon instillation.

Stability studies

The prepared formulations were subjected to stability studies as described under methodology and were checked for any changes in the visual appearance. All the formulations showed good stability at 25 °C/60% RH (room temperature) and 40 °C/75% RH (accelerated stability conditions).

Table 4 Sterility test for medium

Medium	Growth						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Fluid thioglycolate medium	–	–	–	–	–	–	–
Soyabean casein digest medium	–	–	–	–	–	–	–

'+' indicates presence of growth. '–' indicates absence of growth

Table 5 Growth promotion test

Medium	Growth							
	Microorganism	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Fluid thioglycolate medium	*S. aureus*	+	+	+	+	+	+	+
Soyabean casein digest medium	*C. albicans*	+	+	+	+	+	+	+

'+' indicates presence of growth. '–' indicates absence of growth

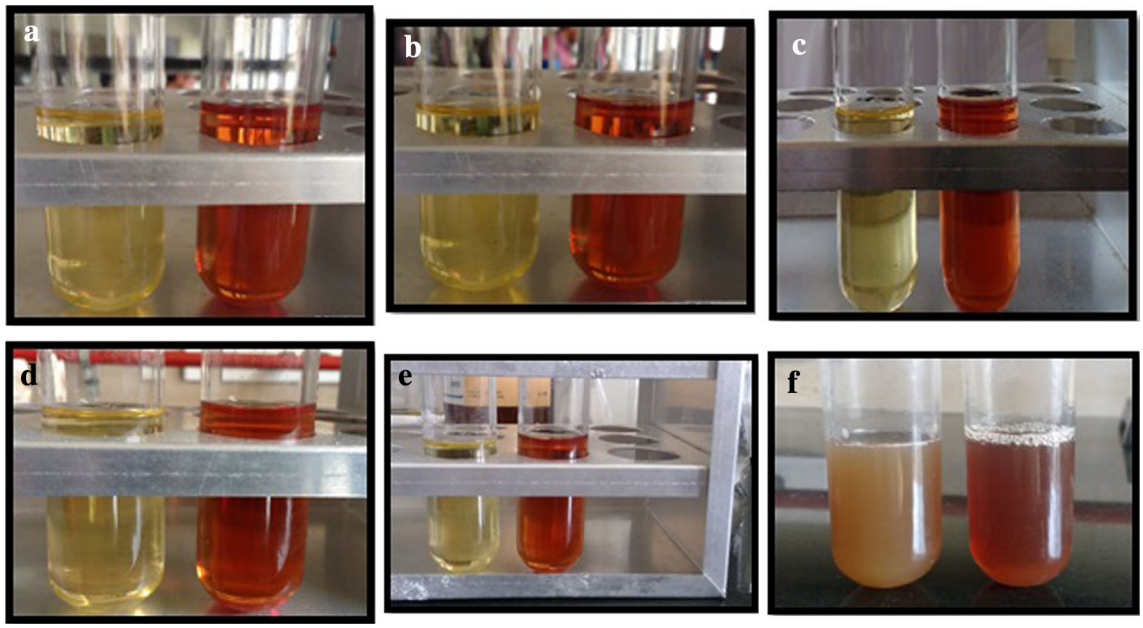

Fig. 8 Sterility test: **a** media containing formulation(F5) before incubation, **b** media with F5 formulation after 7th day of incubation, **c** negative control before incubation, **d** negative control after 7th day of incubation, **e** positive control with *S. aureus* before incubation, **f** positive control with *S. aureus* after 7th day of incubation

Table 6 Sterility test for F5 formulation

Medium	Growth						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Fluid thioglycolate medium	–	–	–	–	–	–	–
Soyabean casein digest medium	–	–	–	–	–	–	–

'+' indicates presence of growth. '–' Indicates absence of growth

Table 7 Irritation score, severity, and effect classification in the in vitro HET-assay

Compound	Irritation score (mean)	Irritation severity (mean)	Classification of the effect
0.9% NaCl (negative control)	0.06	0	No reaction
0.1 N NaOH (positive control)	13.76	3	Severe reaction
Hydrogel formulation (F5)	0.06	0	No reaction

Conclusion

The hydrogel formulation containing neomycin and betamethasone was successfully formulated using poloxamer 407 and chitosan as gelling agents. The hydrogel provides sustained release of drug, thereby providing better bioavailability. The problem associated with the conventional eye drop like frequency of dosing and loss of drug by drainage can be overcome by use of hydrogel approach. Therefore, this formulated hydrogel formulation can be used as an alternative to conventional ophthalmic eye drop formulation of drugs neomycin and betamethasone

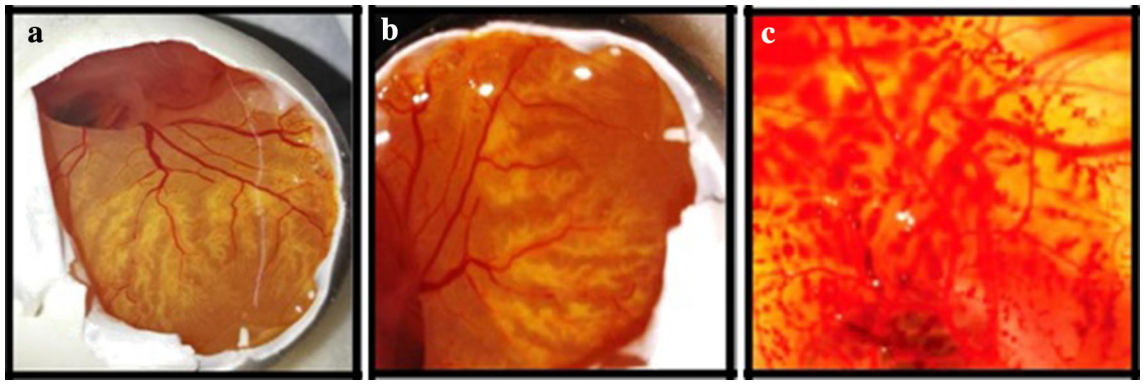

Fig. 9 Ocular irritation: Chorioallantoic membrane assay **a** CAM treated with optimized formulation, **b** CAM treated with 0.9% NaCl, **c** CAM treated with 0.1 N NaOH

for the purpose of providing prolonged therapy for the treatment of conjunctivitis.

Compliance with ethical standards

Conflict of interest The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institutional and/or national research committee or comparable ethical standards.

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