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Preparation and in Vitro Characterization of in Situ Ophthalmic Gel of Norfloxacin

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Abstract

Topical anti-infectives are commonly used to treat bacterial conjunctivitis and infection of cornea caused by susceptible strains of bacteria such as *S. aureus, S.epidermidis, S. pneumoniae, Enterobactercloacae*. The seantiinfectives are delivered in to the eyes by the formulations like eye drops, eye ointment and other conventional formulation but since they are associated with the problems like high flushing rate, irritation and lack of retention of the formulation in to the eye A new formulation In situ gels have come which is devoid of above mention lacking and suitable for the application of anti-infective agents in ophthalmic route .In this research work *in situ* Gel of Norfloxacin was developed by using different grade of Hydroxy propyl methyl cellulose and Carbopol 934 .Different *in situ* gel formulations were prepared and evaluated for Visual Appearance and Clarity, pH, *In vitro* gelation study, Rheological studies, Drug content analysis, and *in vitro* drug release study. In this research work total Eight formulations were developed and after optimization of batches F8 formulation revealed, good gelling strength, clarity, acceptable pH and better *in vitro* drug release .This was considered as the promising *in situ* gel formulation of Norfloxacin.

Keywords: In Situ, Norfloxacin, Gel, Retention, Formulation, Gel, Ophthalmic.

Introduction

Eye is unique most vital organ of human body. There are number of eye ailments which severely affect this organ and even can loss the eye sight. Therefore for the treatment of number of eye ailment many ophthalmic drug delivery systems are available. These are classified as conventional and newer drug delivery systems. Eye drops were considered as conventional ophthalmic delivery systems often result in poor bioavailability and therapeutic response, because high tear fluid turnover and dynamics cause rapid precorneal elimination of the drug.[1-2]

Various ophthalmic Drug delivery system has been used to get the therapeutic benefit of the entrapped drug such as inserts, ointments, suspensions, and aqueous gels lengthen the

residence time of instilled dose but have some drawbacks such as blurred vision from ointments, flushing and low patient compliance from inserts.

This problem can be easily overcome by using in situ gel drug delivery system prepared from different polymers that exhibit property of Sol to Gel transition when they get triggered with certain pH and Temperature condition.[3]

In situ drug delivery systems are liquid initially but when insertion, experience phase conversion in the cul de sac of eye to form a visco-elastic gel and these formed tender gels. In the past few years, a significant number of pH induced, temperature induced, and ion induced in situ gel forming systems have been reported for formulating better ocular delivery system.[4]

In-situ gel mechanisms including ion-activated systems, temperature and pH induced. Temperature triggered in-situ gel system contains temperature sensitive polymers which normally exist as a liquid form below its low critical solution temperature and undergoes gelation when temperature reaches or is above the low critical solution temperature. Osmotic ally triggered in-situ gel systems or Ion-activated systems contains polymer which undergoes a sol-gel transition due to changes of ionic concentration, which could be triggered by mono or divalentcations in tear fluid especially Na^{+}, $Mg^{2+}$and Ca^{2+}.The pH induced in-situ gel contains such sort of polymers which possess acidic or alkaline functional groups within the chain molecule and exhibits a sol-gel phase transition on change from a low pH to high pH environment.[5]

Topical anti-infective are commonly used to treat bacterial conjunctivitis and infection of cornea caused by susceptible strains of bacteria such as *S. aureus, S. epidermidis, S. pneumoniae*, *Enterobacter cloacae*, *H. influenzae*, *P. mirabilis* and *P. aeruginosa*.[6] In the present study an endeavor was prepared to develop an in situ ocular gel of Norfloxacin to increase ocular contact time, enhance the corneal permeability and site specificity for the better treatment.

Materials and Methods

Norfloxacin were obtained as a gift sample from Ranbaxy Dewas Labs Ltd, Carbopol(934), HPMC (K4M&K100M), Benzalkonium Chloride, Calcium Chloride Dihydrate, Sodium Bicarbonate and Sodium Hydroxide were purchased from Chemical drug house Delhi. All the other solvent were of Analytical grade.

Preparation of *In Situ* Ophthalmic Gel by Ph Triggered Based Method

Hydrxypropyl methyl cellulose (viscosity enhancing agent) was dissolve in18ml citrophosphate buffer solution and carbopol (gelling agent) was further added in solution and allowed to hydrate for overnight. Then the solution was stirred at magnetic strirrer for two hrs than drug solution was added after that purified water was added to make up the volume to

25ml.then benzalkonium chloride was added as a preservative in that solution Than final formulation kept in autoclave at 121^0C for 15minutes as shown in table: 1

Table 1.Formulation of *in situ* gels of Norfloxacin

S.N.	Ingredients	Concentration(w/v) in %							
		F1	F2	F3	F4	F5	F6	F7	F8
1.	Norfloxacin	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
2.	Carbopol 934	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
3.	HPMC(K4M)	0.6	0.7	0.8	0.9	-	-	-	-
4.	HPMC(K100M)	-	-	-		0.6	0.7	0.8	0.9
5.	Benzalkonium Chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
6.	Deionized Water	25	25	25	25	25	25	25	25

Evaluation Of *In Situ* Gels Of Norfloxacin

Visual Appearance And Clarity

Visual appearance and clarity was checked under a white and black back ground for presence of any particulate matter (Table 2).

PH

The pH of the prepared *in situ* gelling system was measured using Digital pH meter (Table 2). By dipping probe of pH meter into gel. It was carried out by taking 25ml of gel in 100ml of beaker and results were recorded and similar procedure was used for all the formulation.

Table 2.Preliminary evaluation of visual appearance, clarity, pH and drug content

Formulation	pH	Drug Content (%)	Appearance
F1	6.12	89.01	Translucent
F2	6.24	91.66	Translucent
F3	6.26	92.08	Translucent
F4	6.25	90.09	Translucent
F5	6.31	88.43	Translucent
F6	6.38	92.97	Translucent
F7	6.40	91.13	Translucent
F8	6.17	91.01	Translucent

In Vitro Gelation

Gelling capacity of formulations was evaluated in order to identify the formulations suitable for the formulation of *in situ* gelling systems. Gelling capacity was determined by placing 100µl of the gel in vial containing 2 ml of simulated tear fluid and visually assessing the gel formation and noting the time of gelation and the time taken for the gel formed to dissolve. (Table: 3).

Table 3 Evaluation of gelling capacity

Formulations	Gelling Capacity
F1	+
F2	++
F3	++
F4	+++
F5	++
F6	++
F7	+++
F8	+++

Note: + gelation immediate and remain for few minute,
++ gelation immediate and remains for few hours,
+++ shows gelation immediate and remains for extended period.

Rheological Studies

Viscosity of the *in situ* gel is an important factor in determining residence time of drug in the eye. The prepared solutions were allowed for gelation in the simulated tear fluid and then the viscosity was determined by using Brooke field viscometer LVDV II model in spindle no S-E, The rotation speed was set at 10,20,30,40 and 50 rpm than to study effect of shear on viscosity. The hierarchy of shear rate was found to be reversed and average of two readings was used to calculate viscosity in cp. (Table: 4).

Table 4.Rheological studies of *in situ* gels before gelation

Shear rate	Viscosity(cps)							
	A1				A2			
	F1	F2	F3	F4	F5	F6	F7	F8
10	350	400	497	579	400	450	500	570
20	230	298	405	487	320	390	440	510
30	150	210	318	394	250	310	370	440
40	100	167	236	302	180	240	300	370
50	75	118	178	213	100	170	230	310

Table 5.Rheological studies of *in situ* gels after gelation

Shear rate	Viscosity(Cps)							
	A1				A2			
	F1	F2	F3	F4	F5	F6	F7	F8
10	650	710	820	920	695	850	950	1050
20	564	650	720	865	570	710	810	950
30	400	540	650	750	450	560	690	780
40	300	430	540	650	360	480	560	670
50	210	320	420	510	250	350	450	540

Drug Content Analysis

One ml of the formulation was transferred into 100 ml volumetric flask with 1 ml graduated pipette, 50 ml of simulated tear fluid with pH 7.4 was added gel was completely mixed with the help of magnetic stirrer followed by vigorous shaking until the formed gel gets completely dispersed to give clear solution. Final volume was adjusted to 100 ml with STF, aliquot of 1ml was taken and further diluted to 10 ml with STF, obtained solution was filtered through filter membrane and the drug concentration was determined by UV Visible spectrophotometer at 272 nm (Table:6).

Table 6.*In-vitro*- drug release studies of best formulation F8

S.N.	Time(hrs)	% cumulative drug release
1	1	18.75
2	2	31.63
3	3	44.06
4	4	55.33
5	5	66.82
6	6	75.04
7	7	79.24
8	8	81.40

In Vitro Release Studies

The In vitro drug release studies were carried out using bichambered donor receiver compartment model (Franz diffusion cell) using cellophane membrane soaked overnight on brim of the receptor compartment having simulated tear fluid, pH 7.4and it was stirred at 20rpm kept $37^0C \pm 0.5^0C$. Than 1ml formulation were spread on the cellophane membrane and membrane was placed on such a way that cellophane membrane surface were constantly in the touch of Simulated Tear Fluid present in receptor compartment. The drug samples were withdrawn at the interval of one hour for the period of 8 hrs from diffusion medium and analyzed by a UV spectrophotometer at 272 nm using simulated tear fluid as blank.

Pharma Cokinetic Release Studies

All the optimized formulations were subjected to study the release kinetics and the best fit kinetic model was determined for the optimized formulations. (Table 7)

Tables 7.Regression co-efficient (r^2) values of Formulation F8 of different kinetic models

Formulation	Zero order kinetic model(r^2)	Firstorder kinetic model(r^2)	Higuchi kinetic model (r^2)	Korsmeyer-peppas kinetic model (r2)
F8	0.956	0.616	0.961	0.918

Evaluation of Prepared *In Situ* Gelling System

Evaluation of Visual Appearance, Clarity Ph and Drug Content

All the prepared *in situ* gelling systems were evaluated for preliminary steps such as visual appearance, clarity, pH and Drug Content. These formulations were translucent and clear. The pH of the formulations was found to be in the range of 6.1 to 6.4and drug content were in 89 to 93%.

In Vitro Gelation

Prepared *in situ* gelling systems were evaluated for the *in vitro* gelation capacity. All the formulations gave satisfactory results (Table: 3).

Rheological Studies

For the development of optimum *in situ* gelling system, two major prerequisites viscosity and gelling capacity were studied since the ocular shear rate is very high ranging from 0.03 S^{-1} during inter-blinking periods to 4250-28500 S^{-1} during blinking, viscoelastic fluid with a viscosity that is high under low shear rate condition and low under high shear rate condition, which is called Pseudoplastic fluid, is often preferred, so dynamic viscosity of formulations were measured by the change of shear rate before and after gelation (Tables 4-5)

In Vitro Release Studies

The *in vitro* release of Norfloxac in from the prepared formulations was studied through cellophane membrane using modified diffusion cell. The release studies of prepared *in situ* gelling systems were carried out up to 8 hours.

Differential Scanning Calorimetry

It was conducted by JADE DSC V1.12 Nov 12 DSC Model, Loading temperature was -30^0C and the temperature was raised at the rate of 10^0C/ minute up to temperature-300^0C .It shows DSC of the rmogram of blend in which asharp endotherm was observed at 220^0C which reveals melting point of drug as per the literature available similarly one more exothermwas observed at 174^0C which may be melting point of the HPMC K100M as per the literature available. At 49.98^0C. One endotherm was observed in DSC thermogram of blend this could be because of moisture loss, removal of water from the drug and polymeric blend. It seems no such transformation or the adjustment of endothermic peak of drug was observed in the range of blend which shows no interaction between the drug and polymer indirectly increases the bioavailability of the formulation. (As shown in Figure no 1

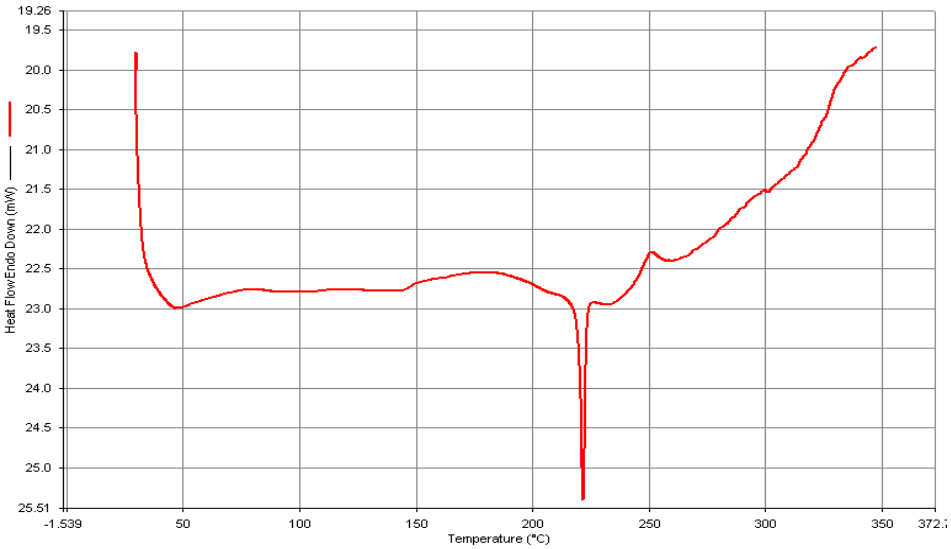

Figure 1.DSC of Physical mixture of drug and Polymer

Discussion

Optimized *in situ* gels were subjected for preliminary evaluation such as visual appearance, clarity and pH. All formulations were found translucent and clear, pH of the formulations was within the range of 6.1 to 6.4.and drug content were in range of 89-93% in all optimized *in situ* gelling systems.

Using a simple spectrophotometric method for calibration curve of drug Norfloxacin was prepared in STF solution at 272 nm. The concentration used for calibration curve in the range of 10-50µg/ml.

In order to evaluate the rheological especially behavior, viscosity of the formulations before and after addition of Simulated Tear Fluid was evaluated using Brook Field viscometer (LVDV MODEL). It showed that viscosity of all formulations decreases as the shear rate increased, which indicates the character of pseudoplastic fluid. This revealed that F8 showed better sustaining effect due to higher concentration of Carbopol and HPMC.

The *in vitro* release studies were carried out for all formulations using cellophane membrane and simulated tear fluid as the medium. Release kinetic studies of prepared *in situ* gels showed that the *in situ* gels followed Higuchi drug release kinetic for the release of drug. Release study revealed that F8 showed better sustaining effect amongst all formulations. This may be due to the higher concentration of Carbopol (934) and HPMC (K100M) in the formulation batch.

Above mentioned results can conclude that *in situ* ophthalmic gels of Norfloxcin using Carbopol (934) and HPMC (K100M) would be the promising formulation for treatment of Conjuctivitis because of its higher retention time and its feasibility of application.

Conclusion

In the present work we have prepared *in situ*opthalmic gel of norfloxacin, which is broad spectrum anti bacterial agent used in the treatment of ocular infections, by using Carbopol (934) as a gelling agent and HPMC(K100M) as a viscosity enhancing agent.

Out of 8 different batches prepared only one batch was showing optimum results. Optimized formulation F8 (0.5%w/ v, Carbopol (934) and HPMC(K100M).0.9%w/v) were liquid in consistancy before instillation in to eye and underwent rapid gellation upon instillation in to eye, the formulations were found to be clear, translucent having good *in situ* gelling capacity .

The *in vitro* release kinetic revealed that the formulation followed Higuchi diffusion release mechanism by pertaining kinetic study of release data.

Hence from the above results this can beconcluded that the *in situ* ophthalmic gels of norfloxacinis the better formulation for treatment of conjunctivitis. It is the best mode of retaining the drug in to the site of action and got better bioavailability of drug in formulation.

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