FORMULATION AND THE EVALUATION OF A NOVEL IN SITU GEL OF ANTIBACTERIAL DRUG FOR SUSTAINED OCULAR DRUG DELIVERY

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ABSTRACT: -

Extensive research has been carried in designing of polymeric drug delivery systems. The development of in situ gel systems has received considerable attention over the past few years.9 This interest has been sparked by the advantages shown by in situ forming polymeric delivery systems such as ease of administration and reduced frequency of Administration, improved patient compliance and comfort.10 Smart polymeric systems represent promising means of delivering the drugs; these polymers undergo sol-gel transition, once administered.11 In situ gel formation occurs due to one or combination of different stimuli like pH change, temperature modulation and solvent exchange. From the early 1970's natural and synthetic polymers began to be investigated for controlled release formulations. Various natural and synthetic polymers are used for formulation development of in situ forming drug delivery systems.

Keywords: *Barriers for Ocular delivery: Lacrimal fluid-eye barriers, Blood-ocular barriers, In Situ Hydrogels, Introduction Of Drug: Norflox*acin, *Introduction To Polymer: Sodium Alginat*.

INTRODUCTION OF DISEASE

Conjunctivitis:

Conjunctivitis (commonly called "pink eye" or "Madras eye"[1]) is an acute inflammation of the conjunctiva (the outermost layer of the eye and the inner surface of the eyelids), most commonly due to an allergic reaction or an infection (usually viral, but sometimes bacterial [2]).

Classification:

Classification can be either by cause or by extent of the inflamed area.

1. By cause
- Allergic conjunctivitis
- Bacterial conjunctivitis
- Viral conjunctivitis
- Chemical conjunctivitis
- Neonatal conjunctivitis is often defined separately due to different organisms

2. By extent of involvement

Blepharoconjunctivitis (inflammation of the eyelids).

Keratoconjunctivitis (corneal inflammation).
Episcleritis is an inflammatory condition that produces a similar appearance to conjunctivitis, but without discharge or tearing.
Signs and symptoms:
Redness (hyperemia), irritation (chemosis) and watering (epiphora) of the eyes are symptoms common to all forms of conjunctivitis.

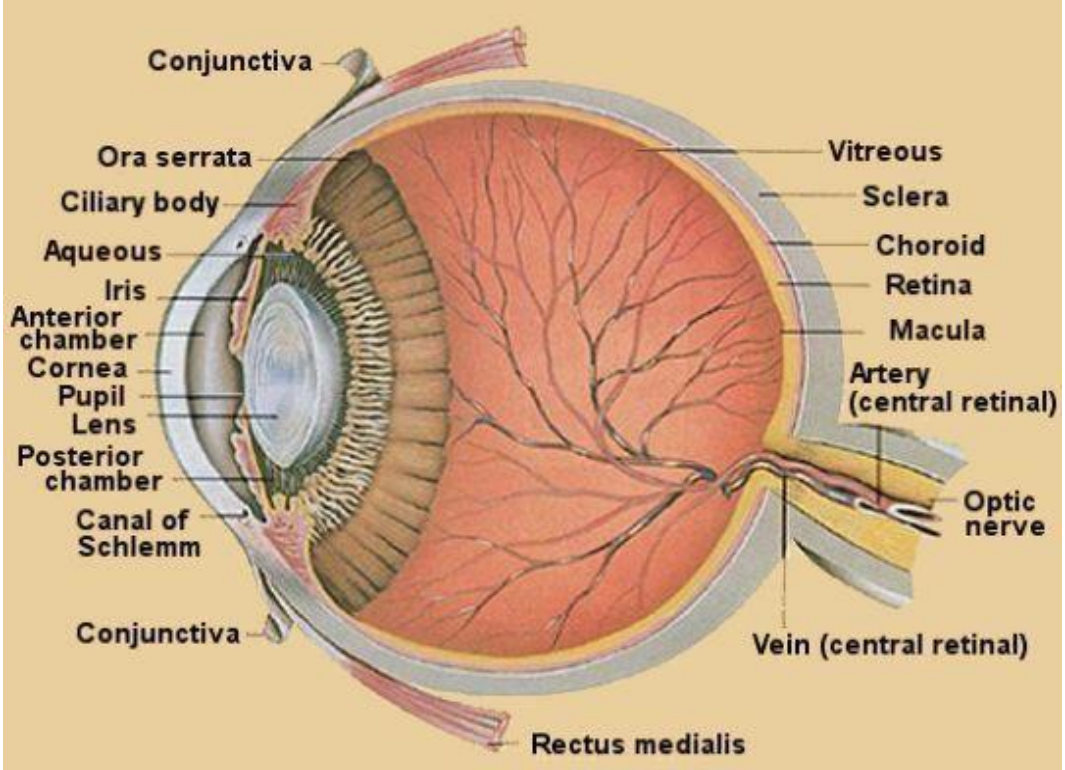

Figure 1.2: Diagram of the eye

OCULAR DRUG DELIVERY

Routes of Ocular Drug Delivery

There are several possible routes of drug delivery into the ocular tissues. The selection of the route of administration depends primarily on the target tissue.[19]

1. Topical route
2. Subconjunctival administration
3. Intravitreal administration

Barriers for Ocular delivery:
Drug loss from the ocular surface after instillation, the flow of lacrimal fluid removes instilled compounds from the surface of the eye. Even though the lacrimal turnover rate is only about 1 µl/min the excess volume of the instilled fluid is flown to the nasolacrimal duct rapidly in a couple of minutes. Another source of non-productive drug removal is its systemic absorption instead of ocular absorption.
Lacrimal fluid-eye barriers
Blood-ocular barriers

Mechanism of ocular drug absorption:

Drugs administered by instillation must penetrate the eye and do so primarily through the cornea followed by the non-corneal routes. These non-corneal routes involve drug diffusion across the conjunctiva and sclera and appear to be particularly important for drugs that are poorly absorbed across the cornea.

Corneal permeation

The permeation of drugs across the corneal membrane occurs from the precorneal space. Thus, the mixing and the kinetic behavior of drug disposition in tears have a direct bearing on efficiency of drug absorption into the inner eye. The productive absorption of most ophthalmic drugs results from diffusional process across corneal membrane.

Non-corneal permeation

Primary mechanism of drug permeation is the sclera is likely to be diffusion across the intercellular aqueous media in the case of structurally similar corneal stroma. Therefore the possibility of partitioning mechanism cannot be eliminated. Although like cornea, the conjunctiva is composed of an epithelial layer covering an underlying stroma, the conjunctival epithelium offers substantially less resistance than does the corneal epithelium.

In Situ Hydrogels

Hydrogels are polymeric networks that absorb large quantities of water while remaining insoluble in aqueous solutions due to chemical or physical crosslinking of individual polymer chains.

***In Situ* gel system[36]**

The use of preformed hydrogels still has drawbacks that can limit their interest for ophthalmic drug delivery or as tear substitutes. They do not allow accurate and reproducible administration of quantities of drugs and, after administration; they often produce blurred vision, crusting of eyelids, and lachrymation.

Based on different stimuli, in situ forming hydrogels can be classified as follow:

1. Ion-sensitive hydro gels
2. pH-sensitive hydro gels
3. Temperature-sensitive hydrogels

Advantages of *in-situ* forming gel:

Generally more comfortable than insoluble or soluble insertion.

- ✓ Less blurred vision as compared to ointment.
- ✓ Increased bioavailability due to –Increased precorneal residence time, Decreased nasolacrimal drainage of the drug
- ✓ Chances of undesirable side effects arising due to systemic absorption of the drug through naso-lacrimal duct is reduced
- ✓ Drug effect is prolonged hence frequent instillation of drug is not required

INTRODUCTION OF DRUG: DRUG PROFILE:

Generic name: Norfloxacin
Chemical Structure:

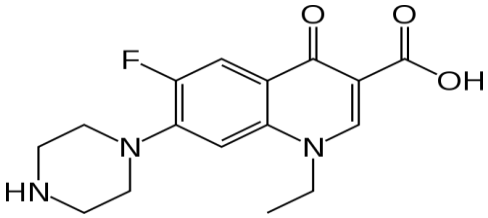

Figure 2 : Chemical Structure of Norfloxacin

Descriptions: Norfloxacin is a white to light yellow crystalline, odorless powder with a bitter taste.
Solubility: 43, 44 It is freely soluble in acetic acid; slightly soluble in chloroform, acetone and in ethanol (95%); very slightly soluble in methanol, water and ethyl acetone; insoluble in ether.
Storage 43, 44: Store protected from light and moisture.
Availability [50] : Norfloxacin is available as:

1. Tablets
2. Eye drops

MARKETED PRODUCTS [53-56] :

Table 1. : Marketed Products of Norfloxacin		
Sr.No.	Product name	Manufacturer name
1	Norflox	Cipla
2	Alflox	Alken
3	Bacigyl	Aristo
4	Biofloxin	Biochem
5	Norbactin	Ranbaxy

Hydroxy Propyl Methyl Cellulose (HPMC) [64-68]

Introduction:

Hydroxypropylmethylcellulose is cellulose having some of the hydroxyl groups in the form of the methyl ether and some in the form of the 2-hydroxypropyl ether. The various grades commercially available are distinguished by a number indicative of the apparent viscosity in millipascal seconds of a 2% w/v solution measured at 20 ^{0} C.
Description: Odorless, tasteless, white to creamy white fibrous and granule powder.
Chemical name: Cellulose, 2- hydroxypropyl methyl ether.

Structural formula:

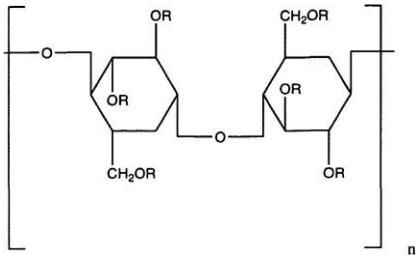

Figure 3: Chemical structure of HPMC

Solubility: Soluble in cold water, forming a viscous colloidal solution. Practically insoluble in chloroform, ethanol, and ether. But soluble in mixture of ethanol and dichloromethane, and mixture of water and alcohol.

Storage: It is stable, although it is hygroscopic after drying. But it should be Store in well closed container in cool and dry place.

MATERIALS:

LIST OF MATERIALS USED IN PRESENT INVESTIGATION:

Table 2: List of materials used in present investigation

Sr. No.	Name of Materials	Suppliers
1	Norfloxacin	ICPA, Ankleshwer, India
2	Carbopol-934	S.D. Fine Chem. Ltd, Mumbai, India
3	Pluronic F- 127	Yarrow Chemicals, India
4	HPMC K4M	Yarrow Chemicals, India
5	Sodium Alginate	Yarrow Chemicals, India
6	Calcium chloride dihydrate	Yarrow Chemicals, India
7	Sodium chloride	Yarrow Chemicals, India
8	Sodium bicarbonate	Yarrow Chemicals, India
9	Glacial acetic acid	Yarrow Chemicals, India
10	Citric acid monohydrate	Yarrow Chemicals, India
11	Disodium hydrogen Phosphate	Yarrow Chemicals, India
12	Edetate disodium	Yarrow Chemicals, India
13	Benzalkonium Chloride	Yarrow Chemicals, India
14	Tween 20	S.D. Fine Chem. Ltd, Mumbai, India

LIST OF INSTRUMENTS USED IN PRESENT INVESTIGATION:

Table 3 : List of instruments used in present investigation

Sr. No.	Name of Instruments	Manufacturer / Supplier
1	Electronic balance	Shimadzu Weighing Balance AUX-220
2	Dissolution test apparatus	Modified Dissolution apparatus-XXIII
3	Magnetic stirrer	Remi Instruments Private Limited, Mumbai
4	Digital ph meter	Electrolab, India
5	Brookfield Viscometer	Brookfield Viscometer DV-III ULTRA, Brookfield Engineering Laboratories Inc., USA
6	UV-Vis Spectrophotometer	Shimadzu UV-Spectrometer,Japan
7	Autoclave	Equitron, Medica instrument
8	Incubator	Hicon lab, Mumbai
9	Stability chamber	Thermolab

METHOD:

SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF NORFLOXACIN 96

The calibration curve for estimation of norfloxacin for determination drug content and cumulative percent release (CPR) were prepared in 1% v/v acetic acid and artificial tear solution respectively.

Preparation of stock solution 10 mg of norfloxacin was weighed accurately and transferred to 100 ml volumetric flask. Dissolve the drug in 1% v/v acetic acid and artificial tear solution and the volume was made up to 100 ml with respective solution to get the final concentration of 100 µg/ml.

Preparation of calibration curve. The above stock solutions were scanned for the maximum absorbance using Shimadzu 1700 UV-Visible spectrophotometer. The λmax for norfloxacin was found to be 273 nm in 1% v/v acetic acid and artificial tear solution.

Independent Variables

- Concentration of Gelling agent (X1)
- Concentration of Viscofying agent HPMC K4M (X2)

Dependent Variables

- Viscosity (Y1)
- Gelling capacity (Y2)
- Drug release (Y3)

Validity of equations

In order to assess the reliability of the equations that describe the influence of the factors on the mechanical properties of *in situ* gel, two additional checkpoint experiments (batch C1 and batch C2) were conducted. The % relative error between predicted values and experimental values of each response was calculated using the following equation:

$$\text{Percentage Relative Error} = \frac{\text{(Predicted value – Experimental value)}}{\text{Predicted value}} \times 100$$

EVALUATION:

Determination of visual appearance, clarity, ph and Drug Content:

The appearance and clarity were determined visually. The ph of the formulations was measured by using ph meter. The drug content was determined by diluting 1 ml of the formulation to 50 ml freshly prepared simulated tear fluid (ph 7.4). The formed gel was completely crushed with the help of a glass rod, followed by vigorous shaking until the formed gel got completely dispersed to give a clear solution. The volume was adjusted to 100 ml with simulated tear fluid. The solution was filtered through a 0.45-mm filter membrane and Norfloxacin concentration was then determined at 272 nm by using UV- Vis spectrophotometer. The results were the means of three runs.

In vitro Gelation Studies:

The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of artificial tear fluid freshly prepared and equilibrated at 37oc and visually assessing the gel formation, noting the time of gelation and the time taken for the gel formed to dissolve. The results were the means of three runs.

In vitro Drug Release Studies:

The in vitro release studies were carried out on formulation codes F1 to F9 using a modified USP dissolution testing apparatus. The dissolution medium maintained at temperature of 37±1°C. The shafts were allowed to rotate at a constant speed (50 rpm). At predetermined

time intervals for 8 hrs, aliquots were withdrawn and replaced by an equal volume of the receptor medium. The drug content in the withdrawn samples was determined at 272 nm using UV-visible double beam spectrophotometer. The results were the means of three runs. The results of in vitro data were analyzed by statistical software PCP Disso, version 2.04, to obtain the best fit kinetic model for in vitro drug release. The formulations were optimized on the basis of viscosity and in vitro release studies. The optimized formulation was subjected to antimicrobial efficacy, sterility testing and ocular irritancy studies.

Kinetic Modeling and Mechanism of Drug Release:

Data obtained from in vitro drug release study were fitted to following kinetic models.

Zero order release kinetics

To study the zero order release kinetics the release data was fitted into the following equation:
$Dq / dt = K0$
Where, Q is the amount of drug release, K0 is the zero order release rate constant, t is drug release time.

First order release kinetics

To study the first order release kinetics the release rate data are fitted into the following equation:
$Dq / dt = K1 Q$
Where, Q is the fraction of drug release, K1 is first order release rate constant, t is the release time. The graph is plotted log % CDR remaining v/s time.

Where, $Mt/M\infty$ is the fraction of drug release, KKP is the release rate constant; t is the release time, n is the diffusion component related to mechanism of drug release. The graph is plotted log %CDR v/s log t.

RESULT AND DISCUSSION:

Determination of melting point: Melting point of Norfloxacin was found to be in the range of 220- 225ºc as reported in literature, thus indicating purity of the drug sample. Any impurity, if present, will cause variation in the melting point of a given drug substance.

Solubility: Norfloxacin was found soluble in acetic acid.

Identification of drug by IR Spectroscopy

The IR spectrum of the pure Norfloxacin sample recorded by FTIR spectrometer is shown in ,functional group frequencies of Norfloxacin were in the reported range which indicates that the obtained sample was of Norfloxacin and was pure.

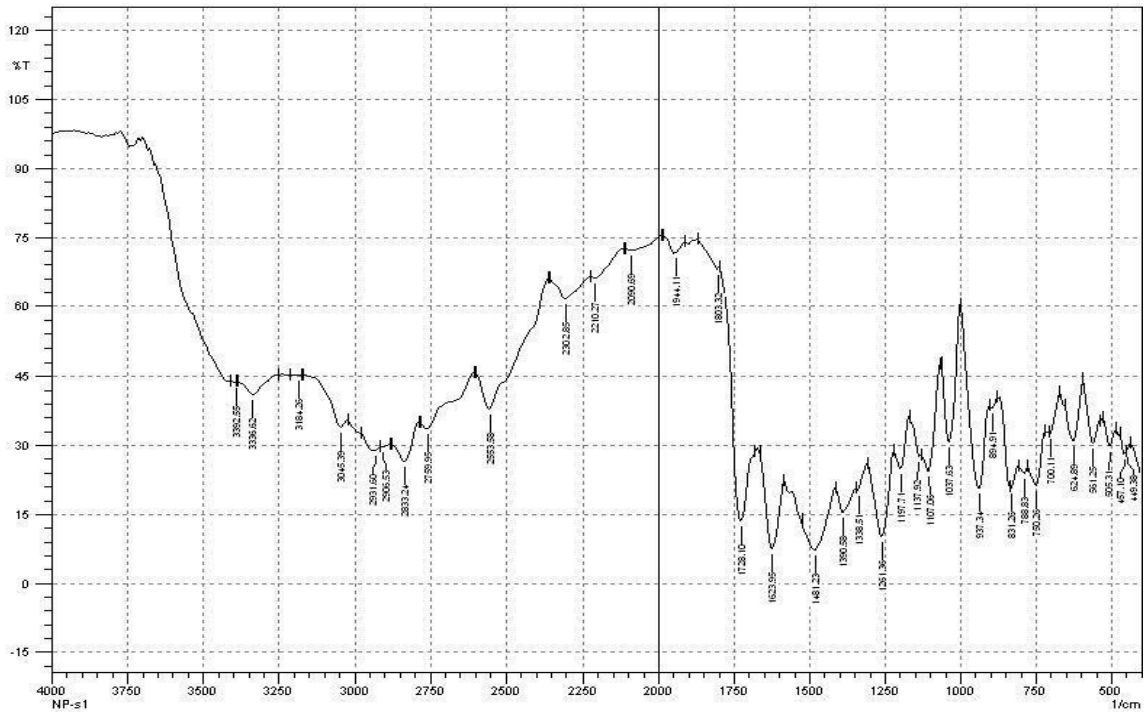

Figure 4 : FT IR spectra of Norfloxacin

Calibration curve in Artificial Tear Solution (For *In Vitro* Release)

Table 4. shows the absorbance of Norfloxacin standard solutions containing 1-5 µg/ml of drug in Artificial Tear Solution. **Figure 5.** shows a representative standard calibration curve with slope, regression coefficient of 0.119, 0.998 respectively. The curve was found to be linear in the range of 1-5 µg/ml at λmax 273 nm.

Table 4 : Calibration curve of Norfloxacin in Artificial Tear Solution

Sr.No.	Concentration (µg/ml)	Absorbance			Average Absorbance ±std.dev.
		I	II	III	
1	1	0.1594	0.1567	0.1572	0.1577 ± 0.001436
2	2	0.2598	0.2571	0.2599	0.2589 ± 0.074691
3	3	0.3961	0.3929	0.3968	0.3952 ± 0.002079
4	4	0.5151	0.5168	0.5118	0.5145 ± 0.002542
5	5	0.6298	0.6294	0.6276	0.6289± 0.001172

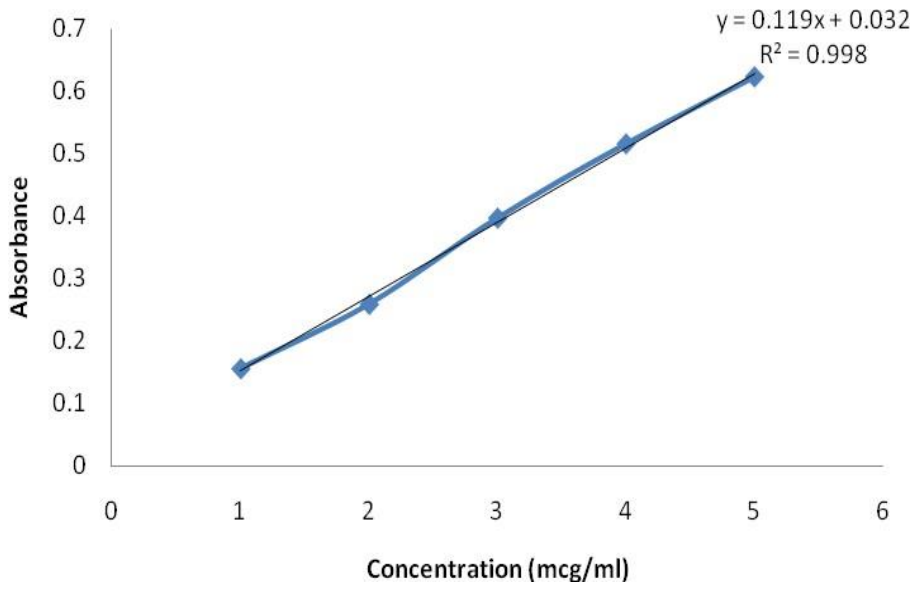

Figure 5 : Calibration curve of Norfloxacin in Artificial Tear Solution

Preparation of check point batch from overlay plot:

Check point batch C1 and C2 were selected from the overlay plot of responses.

The amount of Sodium alginate and HPMC K4M and according to their amounts the predicted responses were given in the Overlay plot flag or in the solution of overlay data. From that any two batches C1 and C2 were selected for the verification of the model.

Following table is showing the formula for C1 and C2 batches :

Table 5.: Formulation of Checkpoint Batches for sodium alginate and HPMC K4M based *in situ* gelling system of Norfloxacin

Ingredients	Batch C1	Batch C2
	Quantity (% w/v) (mg)	
Norfloxacin	0.3	0.3
Sodium alginate	0.70	0.91
HPMC K4 M	0.50	0.42
Edetate disodium	0.01	0.01
Benzalkonium chloride	0.01	0.01
Citric Acid I.P.	0.407	0.407
Disodium hydrogen phosphate I.P	1.125	1.125
Purified Water I.P.	100ml	100ml

Verification of model by comparing predicted response to actual response.

Table 6 : Predicted Response And Actual Response of Checkpoint Batch for sodium alginate and HPMC K4M based *in situ* gelling system of Norfloxacin

Evaluation parameters	Batch C1			Batch C2		
	Predicted value	Actual value	% error	Predicted value	Actual value	% error
Viscosity	517 cps	501 cps	3.09	589 cps	570cps	3.22
Gelling Capacity	1.87	1.80	3.74	2.10	2.0	4.76
Drug release	88.42 %	85.0 %	3.86	89.68 %	86 %	4.10

Actual response of C1 and C2 batch was measured and compared with the predicted response of check point batch. Error was found to be less than 5 of all the responses. Hence, this model was valid and optimized batch can be selected from the overlay plot of this model.

CONCLUSION:

Infrared spectroscopy studies of Norfloxacin, Sodium alginate, Carbopol 934, Pluronic F 127 and HPMC K4M alone and their physical mixture revealed that, Norfloxacin is compatible with all the polymers used. Ophthalmic in situ gelling system of Norfloxacin was successfully formulated using three different gelling agents viz. Sodium alginate, Carbopol 934 and Pluronic F 127 as ion-sensitive, pH-sensitive and temperature sensitive respectively along with HPMC K4M as viscosity enhancing agent. 32 full factorial design was applied to all the three method of in situ gel to select optimized formulations.

Present work was a satisfactory preliminary study in developing in situ gelling system of Norfloxacin. The in vitro – in vivo correlation need to be established to guarantee the bioavailability of prepared formulations.

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