

Formulation and Evaluation of A Novel in Situ Gel of Moxifloxacin Drug

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Abstract

The present work describes the formulation and evaluation activated of an ophthalmic delivery system of an anti-bacterial agent- Moxifloxacin. Sodium alginate was used as the gelling agent for Ion activated in situ gel, Polyacrylic acid (Carbopol- 934) was used as the gelling agent for pH-triggered in situ gel and Poloxamer was used as the gelling agent for temperature activated in situ gel in combination with HPMC K4 M which acted as a viscosity enhancing agent. The evaluated for determination of visual appearance, clarity, pH and Drug Content, In vitro gelation studies, Rheological studies, In vitro drug release study, antimicrobial efficacy Studies, Ocular irritation studies, Sterility testing, Stability studies. Cumulative percent release of 86.89 %, 63.23 % and 76.37 %, was observed for optimized formulations

Keywords: In situ-forming system, ophthalmic hydrogel, Moxifloxacin, Sodium alginate, Carbopol 934, Poloxamer

Introduction

- ✓ Mechanism of Drug Absorption in Ocular Delivery
- ✓ 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.
- ✓ Ophthalmic drug preparations
- ✓ Ophthalmic drugs are formulated to bring the active drugs in contact with the eye surface to allow for absorption. Extension of corneal contact time may result in increased drug penetration & higher intraocular medication administration. In addition to the active drug, eye-related preparations should contain other ingredients to control various characteristics of the formulation, such as the buffering and pH, osmolality & tonicity, viscosity & antimicrobial preservatives. Although these ingredients are listed inactive, they can affect permeability of drug across the ocular surface barriers & alter the therapeutic effectiveness of the drug.
- ✓ In Situ gel system

- ✓ The use of preformed gel matrixs still has drawbacks that can limit their interest for eye-related medication administration 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. A new approach is to try to combine advantages of both solutions and gels, such as accuracy and facility of administration of the former and prolonged residence time of the later. Thus, in situ gel matrixs can be instilled as eye drops and undergo an immediate gel formation when in contact with eye. The liquid to semisolid phase change can be triggered by increased temperature, increased pH and ionic strength of the tear film. Based on different stimuli, in situ forming gel matrixs can be classified as follow:
 - ✓ Ion-sensitive hydro gels
 - ✓ pH-sensitive hydro gels
 - ✓ Temperature-sensitive gel matrixs
 - ✓ Ionically induced gel formation
 - ✓ Gellan gum is an anionic exocellular polysaccharide by the bacterium pseudomonas elodea, having the characteristic property of cation-induced gel formation. The acetylated form is commercially available as gelrite (Kelco

division of Merck and Co, USA). The sol-gel transition process is induced by the presence of monovalent or divalent ions such as Na⁺ and Ca⁺. Some other parameters influence the phase transition. e.g.: The concentration of polysaccharide, the temp of the preparation, and the nature and the concentration of cations. It was determined that divalent ions such as magnesium or calcium were superior to monovalent cations in promoting the gel formation of the polysaccharide.

- ✓ pH induced gel formation
- ✓ Pseudolatexes can be defined as artificial latexes prepared by the dispersion of a preexisting polymer in aqueous medium on-site gelling formulationling pseudo latexes for eye-related use can be described as aqueous colloidal dispersions of polymer, which become viscous gels after instillation in the conjunctival cul-de-sac due to modification of the pH. Pseudo latexes are obtained by dispersion of an organic solution of a preformed polymer in an aqueous medium, leading to an o/w emulsion. Two principal methods are commonly used to prepare eye-related pseudo latexes, the solvent evaporation process and the salting out process. Both methods allow the production of a lyophilized and easily re dispersible power. Thus, pseudo latexes have the advantage of the latex as well as the stability of active compounds such as pilocarpine, which is sensitive to aqueous media. In addition, such systems represent an interesting technological alternative that avoids the use of organic solvents, which can cause problems such as toxicity.
- ✓ Thermo reversible hydro gels
- ✓ These hydro gels are liquid at room temperature (20-250 C) and undergo gel formation when in contact with body fluids (35-370 C), due to an increase in temperature. Different thermal settings gels have been described in this Review.

PREFORMULATION STUDY

PREFORMULATION STUDY

Determination of melting point

Melting point of Moxifloxacin was determined by capillary method. Moxifloxacin was filled in

For example acrylic acid copolymers and N-isopropylacrlamide derivatives eye-related administration such as tolerance have limited the choice of such polymers. Poloxamers, commercially available as pluronic (BASF–Wyandotte, USA), are the most commonly used thermal setting polymers in ophthalmology. They are formed by a central hydrophobic part (poly oxy propylene) surrounded by hydrophilic part (ethylene oxide). Depending on the ratio and distribution along the chain of the hydrophobic and hydrophilic sub units, several molecules weights are available, leading to different gel formation properties. Pluronic F-127, which gives colorless and transparent gels, is the most commonly prepared by solubilization of the polymer in cold water (5-100 C) followed by gel formation up on warming to ambient temperature.

- ✓ Benefits of On-Site Gelling Systems
- ✓ 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
- ✓ Less blurred vision as compared to ointment.
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- ✓ Drug effect is prolonged hence frequent instillation of drug is not required capillary and tied with a thermometer.

Solubility

Solubility is an important consideration in eye-related preparations as clarity of the solution is an essential requirement. The solubility of Moxifloxacin was tested in various solvents such

as Distilled water, Ethyl alcohol, Acetic acid, Chloroform and Acetone.

IR Spectroscopy and Compatibility studies

The FT-IR spectrum of the obtained sample of the drug was compared with the standard FT-IR spectra of the pure drug, using potassium bromide (KBr) discs

Spectrophotometric method for estimation of Moxifloxacin

The calibration curve for estimation of Moxifloxacin 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 Moxifloxacin 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 λ_{max} for Moxifloxacin was found to be 273 nm in 1% v/v acetic acid and artificial tear solution.

Calibration curve in 1% v/v acetic acid

The above stock solution (100 µg/ml) prepared in 1% v/v acetic acid was further diluted to get concentration in the range of 1-5 µg/ml for 1% v/v acetic acid. From the stock solution aliquots of 1-5 ml were withdrawn and further diluted to 100 ml with 1% v/v acetic acid to obtain a concentration range of 1-5 µg/ml. Absorbance of solution was measured at 273 nm using Shimadzu 1700 UV-Visible spectrophotometer by putting reference standard of medium. The experiment was performed in triplicate and based on average absorbance; the equation for the best line was generated.

Calibration curve in artificial tear solution

The above stock solution (100 µg/ml) prepared in artificial tear solution was further diluted to get concentration in the range of 1-5 µg/ml for artificial tear solution. Absorbance of solution

was measured at 273 nm using Shimadzu 1700 UV-Visible spectrophotometer by putting reference standard of medium. The experiment was performed in triplicate and based on average absorbance; the equation for the best line was generated.

Preparation of on-site gelling formulationling system

Preliminary Trials for sodium alginate based Ion activated on-site gelling formulation system

For Sodium alginate: In batches P1 to P8 (table 5.4), the concentration of sodium alginate were 0.2 to 1.5% and all the preparations were checked for their pH, viscosity, drug content and gelling capacity.

Formulation of factorial batches of Ion activated Moxifloxacin in-situ eye-related gel

The table 5.6 shows the composition of all the preparations. Sodium alginate and HPMC K4 M were dissolved in a beaker containing purified water, and this solution was heated about 85°C for 15 min, then beaker was cooled with stirring. Moxifloxacin (0.3% W/V) was dissolved in 0.1 N NaOH to get clear solution. After cooling benzalkonium chloride and drug solution were added to the polymer solution and volume was made up to 100 ml with distilled water and this solution was filtered through 0.2 mm filter paper. The preparations were sterilized by terminal autoclaving at 121°C for 20 min at 15 psi. All glassware used during the preparation of the in situ forming gels was sterilized by autoclaving and the entire procedure was carried out in a laminar flow hood. STF was prepared using NaCl 0.67 g, NaHCO₃ 0.20 g, CaCl₂ · 2H₂O 0.008 g and water up to 100.0 g.

Evaluation of formulation

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

The appearance and clarity were determined visually. The pH of the preparations 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 Moxifloxacin concentration was then determined at 272 nm by using UV- Vis spectrophotometer. The results were the means of three runs.

Rheological studies:

The rheological properties of solutions and gels were measured using a Brookfield synchroelectric viscometer.

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.

Sterility Testing

The sterility test was performed according to Indian Pharmacopoeia. Direct inoculation method served as. 2 ml of liquid from test container was removed with a sterile pipette or with a sterile syringe or a needle. The test liquid was aseptically transferred to fluid thioglycolate medium (20 ml) and soyabean-casein digest medium (20 ml) separately. The liquid was mixed with the media. The inoculated media were incubated for not less than 14 days at 30°C to 35°C in the case of fluid thioglycolate medium and 20°C to 25°C in the case of soya bean casein digest media. Both positive and negative controls were maintained the study.

RESULT AND DISCUSSION

Determination of melting point:

Melting point of Moxifloxacin was found to be in the range of 220- 225°C 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: Moxifloxacin was found soluble in acetic acid.

Identification of drug by IR Spectroscopy

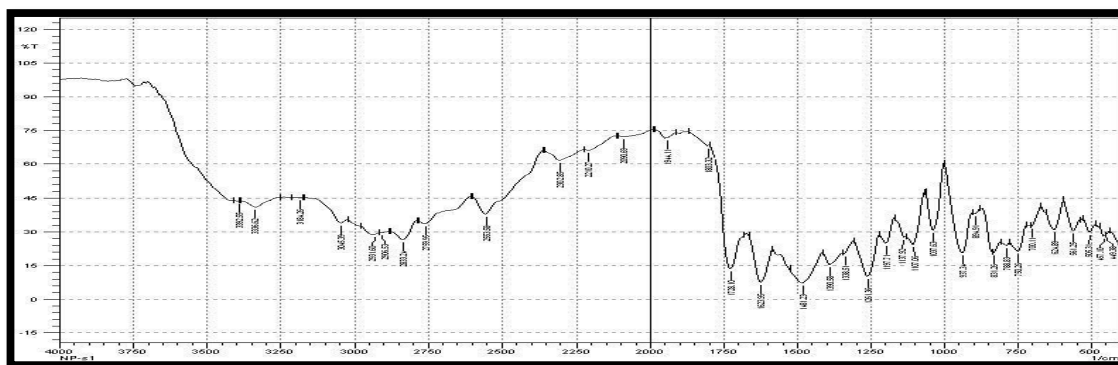


Figure : FT IR spectra of Moxifloxacin

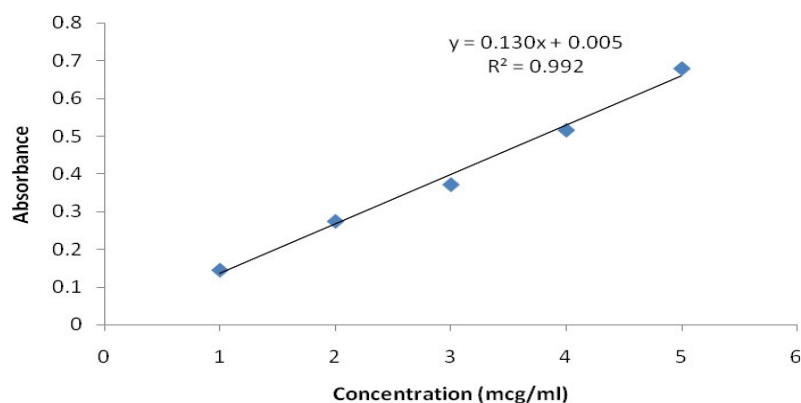
Selection of Vehicle

Buffers play a pivotal role in formulating ophthalmic drops. They contribute significantly to chemical stability and clinical response and also influence the comfort and safety of the product; hence the importance of selecting a suitable buffer ensures product stability and desired drug solubility. The studies in various buffer solutions indicated

the drug was soluble in acetate buffers of pH 6 & 6.5 at the dosage level desired (0.3% w/v). The solutions were stable to elevated temperatures and autoclaving. However, their instability to light as evidenced by discoloration of the exposed solutions necessitated their packing in amber via

**Spectrophotometric method for estimation of Moxifloxacin:
Calibration curve in 1% V/V acetic acid (For Drug Content)**

The curve was found to be linear in the range 1-5 µg/ml at λ_{max} 273 nm.



Formulation of Optimized Batch

Table: Formulation of Optimized Batch	
Ingredient	Quantity (% w/v)
Moxifloxacin	0.3
Sodium alg	0.94
HPMC K4M	0.51
Edetate disodi	0.01
Benzalkonium chlorid	0.01
Citric Acid I.P.	0.407
Disodium hydrogen phosphate I.P	1.125
Purified Water I.P.	100ml

Table : Evaluation of selected optimized batch			
Values	Viscosity (cps)	Gelling Capacity	Drug release(%)
Predicted value	623.93	2.6	89.73
Actual value	618	2	86.89

CONCLUSION

Infrared spectroscopy studies of Moxifloxacin, Sodium alginate, Carbopol 934, Pluronic F 127 and HPMC K4M alone and their physical mixture revealed that, Moxifloxacin is compatible with all the polymers used. Ophthalmic on-site gelling formulationling system of Moxifloxacin 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 on-site gelling formulation to select optimized preparations.

The clarity of the prepared preparations was found satisfactory. The pH of all preparations was found to be satisfactory in the range of 6 - 7.4. The drug content of the prepared formulation was within the acceptable range, and ensures dose uniformity. All the optimized preparations showed instantaneous gel formation when contacted with simulated tear fluid (STF).

Cumulative percent release of 72.25 %, 62.10 % and 76.37 %, was observed for formulation F6, F18 and F23 respectively. Cumulative percent release of 86.89 %, 63.23

% and 76.37 %, was observed for optimized preparations NF1, NF2 and NF3 respectively. Optimized preparations showed sustained drug release for a period of 8 hour. It was observed that two optimized preparations NF1 and NF2 followed the zero order drug release, suggesting drug release in a controlled manner. Optimized formulation with pluronic F 127, NF3 showed higuchi release order suggesting drug release in a sustained manner. The formulation passed the antimicrobial efficacy studies. The results of the ocular irritation studies indicate that all the three preparations were non-irritant and excellent ocular tolerance was noticed. Results of sterility test confirmed that all the preparations were sterile. From the stability studies it was confirmed that on-site gelling formulationling preparations

of Moxifloxacin remained more stable at ambient temperature (25°C) and humidity. The maximum instability of on-site gelling formulationling preparations was observed at 40°C and 4°C (significant decrease in drug content and in vitro drug release).

preliminary study in developing on-site gelling formulationling system of Moxifloxacin. The in vitro – in vivo correlation need to be established to guarantee the bioavailability of prepared formulation

REFERENCES

Retrieved 2008-10-30.

J. M. Langley. Adenoviruses. *Pediatr Rev.* 2005;26:238-242.

Fisher, Bruce, Harvey, P. Richard, Champe, C. Pamela. *Lippincott's Illustrated Reviews: Microbiology.* Lippincott's Illustrated Reviews Series. Hagerstown, MD: Lippincott Williams & Wilkins. ISBN 0-7817-8215-5. 2007.

P Rose. Management strategies for acute infective conjunctivitis in primary care: a systematic review. *Expert Opin Pharmacotherapy.*

Visscher et al. Evidence- based treatment of acute infective conjunctivitis: Breaking the cycle of antibiotic prescribing. 55(11):1071 -- *Canadian Family Physician.* 7. A Sheikh, B Hurwitz. Antibiotics versus placebo for acute bacterial conjunctivitis. *Cochrane Database Syst Rev.* 2006;(2): CD001211. doi:10.1002/14651858.CD001211.pub2. PMID 16625540.

H. A. Everitt, P. S. Little, W. F. Smith. A randomised controlled trial of management strategies for acute infective conjunctivitis in general practice. *BMJ.* 2006;333:321. doi:10.1136/bmj.38891.551088.7C. PMID 16847013. PMC

1539078.

Peppas NA, Langer R. New challenges in

biomaterials. Science. 1994;263:1715– 20.

Zhidong L, Jaiwei L, Shufang N, Hui L, Pingtian D, Weisan P. Study of an alginate/HPMC based on-site gelling formulationling eye-related delivery system for gatifloxacin. Int J Pharm. 2006;315:12–7.

Peppas NA, Langer R. New challenges in biomaterials. Science. 1994;263:1715– 20.

Sasaki H, Yamamura K, Nishida K, Nakamurat J, Ichikawa M, —Delivery of drugs to the eye by topical application. Progress in Retinal and Eye Research . 1996, 15(2), 553-620.

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