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Fabrication, Characterization and Evaluation of *In Situ* Gel for the Treatment of Conjunctivitis

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Abstract

The aim of the work was to develop the *in situ* hydrogel composed of chitosan, sodium glycero-phosphate, benzalkonium chloride loaded with Ofloxacin to extend the drug delivery in the ocular socket using a suitable transporter. The technique used to formulate *in situ* gel was dispersion method. Thedeveloped delivery system decreases the side effects, improves the efficiency and patient compliance. The preparedformulation wascharacterizedby FT-IR, DSC and evaluated forgelation temperature, gelation capacity, viscosity, content uniformity, sterility and*in vitro* release. Gel viscosity increases as polymer concentration and temperature increase, concentration of drug had little or no effect on the viscosity of formulations. All the formulations were made certain to be sterile as there was no bacterial growth even after 14 days. The best formulation showed good gelling and rheology properties. The optimized *in situ* gel was F5 which improved ability to sustain the drug compared to other formulations by *in vitro* release studies. Therefore developed *in situ* gel formulation of Ofloxacin was effective in terms of treatment of conjunctivitis.

Keywords: Chitosan, Conjunctivitis, *Insitu* gels, Ofloxacin, Sodium Glycerophosphate;

1. INTRODUCTION

The sensitive and important organ of the body is eye and is considered as window hinge. Many eye diseases like conjunctivitis, uveitis, glaucoma etc.can cause vision loss. Due to activedefensive mechanisms like blinking of eye, lachrymation, and drainage bioavailability of ophthalmic drugs administered is poor [1].

The usage of eye drops is the best method to treat ocular diseases [2]. These are delivered into the lower cul-de-sac by topical instillation. The most frequently available ophthalmic formulations in market [3] are eye drops and eye ointments. Desired drug concentration and therapeutic effectcan be attained by repeated usage of eye drops.

In order to extend the residence time of instilled dose several ophthalmic vehicles such asviscous solutions, ointments, polymeric inserts, suspensions and gels have been developed andalso increasebioavailability of eye.An ideal ophthalmic dosage form can withstand the drug releaseand persist in contact with pre-cornea for aprolonged period.

Based on*in-situ* gelationconcept [4],residence time of the formulation can be increased and bioavailability of the drug can also be achieved. Due to the change in physiological conditions like pH, temperature, and ionic strength in the eye [5], these delivery systems along with polymers show sol to gel phase transition. Three types of delivery systems are predicted, i.e.,pH-triggered system-pluronic and tetronic and ion activated systemgellan gum and sodium alginate.

The main objective of the current work was involved in the development of an *in-situ* gel formulation using an ion-activated phase transition polymer to deliver the drug effectively into the eye for sustained drug release and enhanced ocular drug bioavailability.

Chitosan is a linearpolysaccharide which is composed of deacetylated chitin. It is biodegradable, biocompatible, pH-dependent cationic polymer, water soluble up to pH6.2. Itfreely binds to negatively charged surfaces andtransports polar drugs across epithelial surfaces. Chitosan is used to formulate ophthalmic dosage forms due to its low toxicity, good ocular tolerance, bio-adhesion and permeability-enhancing properties[6].

Sodium glycerophosphate is an organophosphate used as a phosphate source, in tonics and emulsifier.

2. MATERIALS AND METHODS

2.1 Materials

Ofloxacin (Qwest International, Bangalore); Chitosan (Loba Chemie, Mumbai); Sodium Glycerophosphate (Merck, India); Benzalkonium Chloride (Merck, India) were obtained from approved sources.

3.0 METHODS

3.1 Formulation of *in situ* gel loaded with ofloxacin by Dispersion method

A weighed amount of chitosan was solubilized in distilled water at ambient temperature.Different concentration of sodium glycerol-phosphate solutions were prepared by dissolving glycerol-phosphate in distilled water.Both these solutions were refrigerated for 2 hours to attain complete solvation.Drop by drop glycerol-phosphate solution was addedinto the chitosan solutionwith stirring.Take little quantity of Ofloxacin (0.5% w/v) in distilled water and dissolved. This dissolved solution was furtheraddedto polymeric solution with constant stirring for 10 minutes to gain homogenous mixture [7, 8]. To preserve the developed *in-situ* gel add Benzalkonium chloride. The different compositions of the developed in situ gels were given in table 1.

Table 1: Composition of 0.5% w/v Ofloxacin in situ hydrogel

Sl. No.	Materials	Formulations					
		F1	F2	F3	F4	F5	F6
1	Ofloxacin(%)	0.50	0.50	0.50	0.50	0.50	0.50
2	Chitosan (mg)	175	200	225	175	200	225
3	Sodium Glycerophosphate (mg)	2500	2500	2500	2750	2750	2750
4	Benzalkonium chloride (% w/v)	0.001	0.001	0.001	0.001	0.001	0.001

3.2 CHARACTERIZATION:

3.2.1 FTIR studies:

During the process of gel transition, the nature of drug-excipient interacting forces can be determined by FT-IR by employing potassium bromide pellet method [9].

3.2.2 DSC studies

DSC studies of pure drug ofloxacin and its mixture was done using Shimadzu thermal analyser (DSC-60). A few mg of sample were entirely sealed into aluminium cells. Under nitrogen atmosphere, it was heated at constant temperature of 10ºC /min [10].

3.3 EVALUATION OF PREPARED INSITU GELS:

3.3.1 Visual Appearance and Clarity

The formulated gels were examined for visual appearance and clarity by observing the formulated *in* situ gels against awhite and black background to check the presence of any particulate matter [11].

3.3.2 pH:

The most accurate common means of measuring pH is through a lab device called a probe pH meter. Measurement is made by submerging the probe in the liquid until a reading is registered by the meter.

3.3.3 Viscosity:

The formulations are evaluated for rheological properties by using Brookfield viscometer using spindle no. 6 at 30 rpm at two different temperature viz. 8±1^{o}C and 37 ±1^{o}C [12].

3.3.4 Drug Content:

1 ml of *in situ* gel was taken and solubilized in simulated lachrymal fluid in 100ml volumetric flask.After diluting appropriately,the solution was examined in UV-spectrophotometer (UV-1800, Shimadzu) by measuring the absorbance at 215 nm.

3.3.5 Gelation Temperature:

Evaluation of gelation temperature was done by taking the transparent vialcontaining 10 ml of sample solution andmagnetic bar was placedin a thermostatically controlled water bath. A thermometer of 0.1˚C was immersed in the sample solution. At the rate of 1˚C/min the solution was heated with continuous agitation (100 rpm). The temperature at which the magnetic bar stops due to gelation is noted as gelation temperature[13].

3.3.6 *In vitro* dissolution Studies

The *in vitro release* study of the formulated*in-situ* gel was done by using modified USP dissolution apparatus-1. A whatman filter paper No 41 was taken in the basket and wetted by dipping in simulated lacrimal fluid for a time period of atleast one minute to ensure the contact of release medium with the formulation. 100µl of the formulation was applied to the whatman filter paper and 50 ml of simulated lacrimal fluid was filled in a beaker and basket was rotated over its surface.3ml of samples were withdrawn at regular time intervaland replaced with an equal amount of fresh simulated lacrimal fluid. The samples were analyzed using UV-visible spectrophotometer (Shimadzu 1700) at 286 nm [14].

3.3.7 Sterility Test:

Sterility test was performed to examine the growth of bacteria or fungus. The media used to detect aerobic and anaerobic bacteria is fluid thioglycollate media and soyabean casein digest media to detect fungal organisms. The prepared*in situ* gel was incubatedin an incubator at 37±1^{o}C for a period of 14 days using fluid thioglycollate media and soyabean casein digest media.

4. Resultsand Discussions:

4.1Compatibility studies by FT-IR studies:

The FT-IR spectra of pure drug Ofloxacin were compared with the IR spectra of drug and excipients combination. The comparison between two spectra was studied and it was observed that the interaction between pure drug and drug-excipients mixture was absent which is depicted in figure 1.

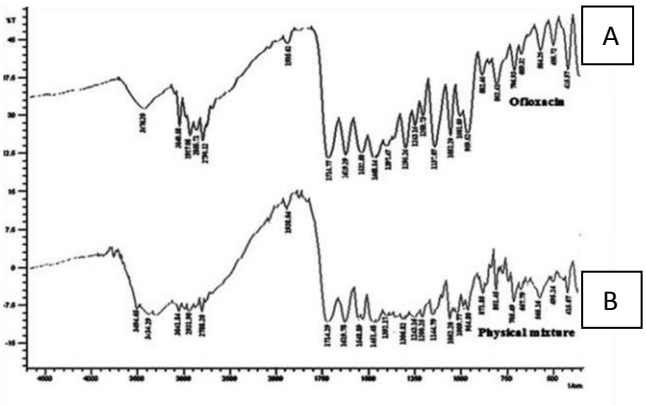

Figure 1: FTIR spectrum of pure ofloxacin (A) &Physical mixture of ofloxacin and excipients(B)

4.2 DSC Studies:

Figure 2 represents the DSC thermogram of Ofloxacin and excipients which yields sharp endothermic peak at 277^{o}C and 283^{o}C respectively. Hence, there was no incompatibility between the drug and physical mixture.

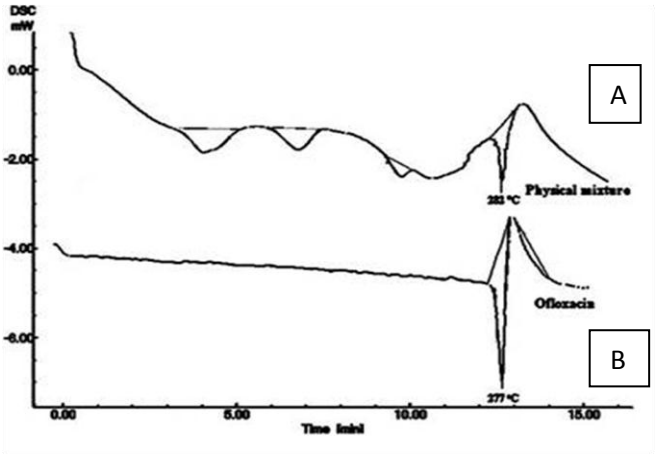

Figure 2: DSC Thermograms of physicial mixture of ofloxacin and excipients (A) & Pure ofloxacin(B)

4.3 Visual Appearance and Clarity:

The visual appearance of formulated gel (F1-F6) was found to be clear and satisfactory when observed against a white and black background and results were depicted in Table 2.

4.4 pH:

The optimal pH range for *in-situ* ophthalmic gel is 4.7 to 4.9 and the formulated gel loaded with Ofloxacin had shown within the range of 4.6 to 4.9 as depicted in Table 2. From the results obtained it can be inferred that pH of the formulated in-situ gels were within the range.

4.5 Effect on Viscosity

The viscosities of the formulated gels (F1 - F6) were found in the range of 388.67±24 to 877.6±21 CPS at 15°C and 6818.6±38 to 10132±48 at 37°C which is depicted in figure 3. From the results it can be inferred that as the concentration of chitosan increases there was an increase in viscosity. The optimized formulations are F3 which showed the maximum viscosity value of 877.67± 21.2 cps at 15°C and F2 showed the maximum viscosity value at 10132.00±48.1 cps at 37°C.

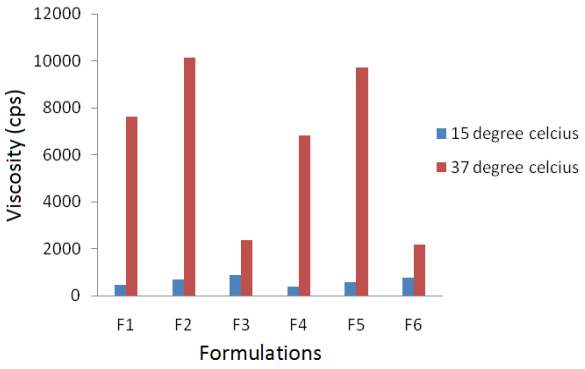

Figure 3: Viscosities of Formulations

4.6 Drug Content:

The drug content for the formulations from F1 to F6 are found to be in the range of 97.82-99.20% indicating the uniform distribution of the drug.The evaluation results are mentioned in figure 4.

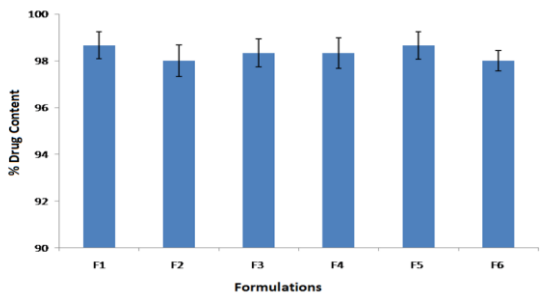

Figure 4: Bar graph showing % drug content of formulations

4.7 Gelation Temperature:

As depicted in Table 2, *in situ* gel formulations F1, F2, F4 and F5 showed gelation temperature between 31°C to 37°C thus they readily become gels, making them ideal to function as injectable drug depot whereas gelation temperature of formulations F3 and F6 was more than the body temperature (37°C). Hence these formulations cannot be used as injectable drug depot.

4.8 *In vitro* drug release

The formulated gels F2, F4, F5 prolonged the release of drug for 10-12 hr whereas F1, F3, F6 release drug quickly within 6-8 hr. Comparatively, the release rates of F3 and F6 were faster than their former formulations.

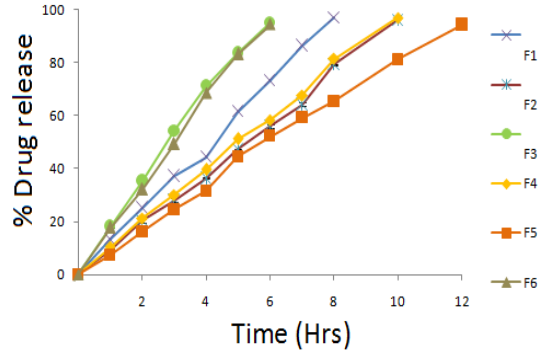

Figure 5: *In vitro* drug release from the hydrogel

4.9 Sterility Test:

Absence of turbidity indicates the prepared formulations passed the test for sterility.Hence revealed that no growth ofmicro-organismsin the formulation.

Table 2: Results of evaluated physiochemical parameters.

	F 1	F2	F3	F4	F5	F6
Clarity	Clear	Clear	Clear	Clear	Clear	Clear
Initial pH	4.9 ±0.05	4.6 ±0.100	4.7 ±0.058	4.9 ±0.100	4.72 ±0.115	4.8 ±0.058
Gelation Temperature (°C)	33.00 ±1.00	36.33 ±0.57	39.66 ±0.75	31.33 ±0.46	34.00 ±0.68	38.3 3±0.39
Gelation Time (sec)	26.2 ±1.23	31.5 ±0.98	37.1 ±1.06	23.6 ±1.26	28.4 ±1.14	35.3 ±1.34

5.0 Conclusion

Ofloxacin is effectively formulated as *in situ* gels forming ophthalmic solution using Chitosan as a gelling agent by dispersion technique which showed good gelation, rheological properties and exhibited better ability to retain drug. Based on results it was observed that the prepared Ofloxacin *in situ* gel can overcome limitations of the conventional ocular dosage form. This technique helped to increase patient compliance. They offer many advantages like easy installation,improves ocular bioavailability, prolongs the duration of contact with corneal tissue, lessensfrequency of administration.

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