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PREPARATION AND EVALUATION OF IN SITU GEL OF CIPROFLOXACIN HCL FOR BACTERIAL CONJUNCTIVITIS

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ABSTRACT

In situ gelling system, are the systems, when instilled into the eye it under goes phase transition from sol to gel. Gels are having more residence time and contact than conventional ocular formulation which make in situ formulation as a best formulation for ocular drug delivery. In this work a effort was made to formulate in situ gel of Ciprofloxacin Hcl by using Carbopol and Hydroxymethylcellulose K4M in different concentration. Prepared Formulations were further evaluated for Visual appearance and Clarity, pH ,Gel forming capacity , Rheological study ,Drug content analysis ,in vitro drug release and differential scanning calorimeter .Among eight formulations from formulation code F1 to F8 ,Formulations F7 and F8 revealed acceptable results of all the evaluation parameters . The drug release study and drug content of the formulation revealed that formulation batch F8 showed good release than formulation batch F7 and Formulation F8 was finally considered as optimized and promising in situ gel formulation of ciprofloxacin HCL.

KEYWORDS: Rheological, Evaluation, In situ gel, Ciprofloxacin Hcl, Optimized.

INTRODUCTION

In situ system is an alternative and novel approach to overcome the problems of conventional dosage form. *In situ* gelling system, which is liquid but when instilled into the eye it under goes phase transition from sol to gel. This consists of polymer that exhibit sol-to-gel phase transitions and the triggering factors that favours formation of gel are temperature modulation, pH change, presence of ions and ultraviolet irradiation from which the drug gets released in a sustained and controlled manner.[1-2] These systems consist of polymers that exhibit sol to gel phase transitions due to change in specific physicochemical parameters (pH,

temperature, ion concentration) in the cul de sac.[3] In situ gelling systems possess following advantages.

1. They overcome poor bioavailability problem of conventional ophthalmic solution by instilled gel into eyes as drops.
2. It increases the contact time of drug at the site of absorption.
3. Reduced frequency of administration.
4. Reduced systemic absorption of drug drained through the nasolacrimal duct.[4-5]

Conjunctivitis is swelling (inflammation) or infection of the membrane lining the eyelids (conjunctiva). It is characterized by cellular infiltration and exudation. *Staphylococcus aureus* is the most common cause of Bacterial conjunctivitis and Blepharo-conjunctivitis. Many other organisms like *Haemophilus influenza*, *Streptococcus pneumoniae* also cause conjunctivitis. Conjunctivitis can be classified as (1) Infective – Acute, Sub acute & Chronic (2) Allergic conjunctivitis. Uncomplicated bacterial conjunctivitis may be self limiting but empirical treatment with topical antibacterial is often given.[6-11]

The aim of present work is to prepare and evaluate *in situ* opthalmic gel of an anti infective drug for sustained ocular delivery which is used for the treatment of various infective diseases of the eye, to get better patient compliance by increasing residence time and bioavailability.

MATERIAL AND METHOD

Ciprofloxacin HCL obtained from Mariya Pharmaceuticals, Indore, India. Polyvinylpyrrolidone, polyvinyl alcohol, Polyethylene glycol 400, beeswax, dextrose, calcium carbonate, peppermint, ascorbic acid (CDH (P) Ltd, New Delhi and other reagent were of analytical grade.

Preparation of *in situ* ophthalmic gel by pH Triggered method[12]

Citrophosphate buffer was prepared and HPMC K4M was dissolved in 18ml of the buffer solution. Now, this solution was stirred at magnetic stirrer for 2 hrs at 100rpm. Then carbopol 934 was added to the solution and this solution was allowed to hydrate for overnight. The solution was again stirred at magnetic stirrer for 2hrs at 100 rpm. Ciprofloxacin HCl was dissolved in distilled water and benzalkonium chloride was added to it. The drug solution was

added to the Carbopol-HPMC solution under constant stirring until the uniform solution was formed. Citrophosphate buffer was added to the solution to make up the volume to 25ml.

The optimization of the formulation was done using concentration of Carbopol 934 0.5% w/v because quantity less than 0.5%w/v was not able to form gel and the quantity more than 0.5% was showing more acidity. As the concentration of the carbopol 934 increase it will show more acidity.

EVALUATION OF *IN SITU* GEL

1. Visual appearance and Clarity

Visual appearance and clarity was checked under a white and black background for presence of any particulate matter as shown in Table no 2.

2. pH

The pH of the prepared *in situ* gelling system was measured using glass electrode pH meter. The pH was measured by dipping the glass electrode into the 25 ml of formulation was kept in a 100ml of beaker and the results were recorded. The pH of all the formulations was measured by this method. The averages of the three readings were used to calculate pH as shown in Table no 3.

3.Gel forming capacity

Gel forming capacity of formulation was evaluated in order to identify the best suitable formulation for the preparation of *in situ* gelling systems.

Gel forming capacity was determined by placing a drop or two of the formulation in a 25 ml beaker containing 2ml of simulated tear fluid which was freshly prepared and visually assessing the gel formation and the results were recorded as shown in Table no 4.

4.Rheological studies

Viscosity of the *in situ* gel is an important factor in determining the residence time of drug in the eye. The prepared solutions were allowed for gelation in the STF and then the viscosity was determined by using Brooke field Viscometer DV II + Pro model in spindle T95. The viscosity was measured using 25 gm of gel in 100ml beaker. The T 95 bar spindle was lowered perpendicular in the center taking care that spindle does not touch the bottom of the beaker. T 95 spindle was used for determining the viscosity of the gels. Factors like temperature, pressure and sample size was maintained during the process. The helipath T-bar

spindle was moved up and down giving viscosities at number of points. The rotation speed was 50 and 100 rpm to study effect of shear on viscosity. The averages of the three readings were used to calculate viscosity in cps. The viscosities of the formulations before and after gel formation are mentioned below in table no.5 and 6.

5.Drug content analysis

1ml of the formulation was transferred into 100ml of volumetric flask with the help of 1ml pipette, 50ml of STF (pH 7.4) was added to gel and the gel was completely mixed with the help of vigorous shaking until the formed gel gets completely dispersed to give clear solution. Final volume was adjusted to 100ml STF, aliquots of 1ml was taken and further diluted to 10ml with STF and absorbance was determined by UV Visible spectrophotometer at 271nm.

Preparation of simulated tear fluid: Dissolve 0.670g of sodium chloride, 0.2g of sodium bicarbonate and 0.008g calcium chloride di hydrate in 100ml of distilled water and adjust the pH to 7.4 using 0.5M sodium hydroxide (in distilled water) or 0.5M hydrochloric acid.

***6.In vitro* release studies**

The *in vitro* release of Ciprofloxacin HCL from the prepared formulations was carried out in a Modified Diffusion Cell. The semi permeable membrane (cellulose nitrate membrane pore size 0.2µm and 47 mm in diameter) was soaked in STF for 6-8 hours. It was clamped carefully to one end of the hollow glass tube of 17 mm (area 2.011 cm^2) (dialysis cell). This acted as donor compartment. 50 ml of STF 7.4 pH was taken in a beaker of 250 ml which was used as a receptor compartment. A weighed amount of *in situ* gel containing drug equivalent to 6 mg of Ciprofloxacin HCl, was spread uniformly on the membrane. The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at 37 ± 2 ºC. The solutions of the receptor side were stirred by externally driven magnetic bars using magnetic stirrer at 50 rpm. 1 ml sample was withdrawn at every 1 hr interval and replaced by fresh STF 7.4 pH to maintain the simulated condition. These samples were diluted upto 5 ml with STF 7.4 pH .The drug concentrations in the aliquots were determined at 271 nm using UV spectrophotometer. The amount of Ciprofloxacin HCL released at each time was calculated from the equation: $y= 0.017x+0.13$

7.Differential scanning calorimetry

It was performed on Instrument version: Jade DSC Pyris 6 DSC typen DSC instrument. The Load temperature was 30^0 and it was raised up to High temperature: 355^0C.The heat flow rate was 10^0C/min.The thermogram of the formulation shown in fig no 1.

RESULT AND DISCUSSION

Visual appearance and clarity

All the prepared *in situ* gelling systems were evaluated for preliminary steps such as visual appearance and clarity. These formulations were transparent and clear. The results obtained are shown in table no.2.

pH

The pH of the formulations was found to be in the range of 5.01 to 5.21. The results are shown in table no.3.

Rheological Studies

The viscosity of the formulations before and after addition of STF was evaluated using Brook Field Viscometer (DV II + Pro Model). The results obtained, are shown in table no.5 and 6. The results showed that viscosity of all formulations decreases as the shear rate increased, which indicates the character of pseudoplastic fluid. The results revealed that formulation F8 showed better sustaining effect due to higher concentration of polymers used.

In vitro Gel Forming Capacity

Prepared *in situ* gelling systems were evaluated for the *in vitro* gel forming capacity. The results of all the formulations are shown in table no.7.

From the results obtained, it revealed that as the concentration of HPMC K4M increases the gelling capacity of the formulation increases as it was used as the viscosity enhancing agent.

Drug content analysis

The drug content of the best formulations selected was found to be 70% and 74.6% of formulation F7 and F8 respectively.

In vitro release studies

The two optimized formulation F7 and F8 were subjected to *in vitro* drug release. The release study of the prepared formulation was carried out up to 7 hrs. The results showed, that F7 and F8 showed 48.83% and 53.16% respectively.

Differential Scanning Calorimetry

DSC thermogram of a mixture shows a endotherm at 320^0C which revels the melting point of the drug as the literature available similarly one more exotherm was observed at 179^0C which

may be the melting point of HPMC K4M as per the literature available and another endotherm was observed at around 280^{0}C which is the melting point of Carbopol 934 as per the literature available. At around 80^{0}C one endotherm was observed in DSC thermogram could be because of moisture loss, removal of water from the drug and the polymeric mixture.

Table no 1. Different formulation of *in situ* gel of Ciprofloxacin HCl

Formulation code	Drug	COMPOSITION % w/v			
		Carbopol 934	HPMC K4M	Benzalkonium chloride	Buffer solution
F1	150mg	0.5	0.4	0.01	Upto 25 ml
F2	150mg	0.5	0.5	0.01	Upto 25 ml
F3	150mg	0.5	0.6	0.01	Upto 25 ml
F4	150mg	0.5	0.7	0.01	Upto 25 ml
F5	150mg	0.5	0.8	0.01	Upto 25 ml
F6	150mg	0.5	0.9	0.01	Upto 25 ml
F7	150mg	0.5	1.0	0.01	Upto 25 ml
F8	150mg	0.5	1.4	0.01	Upto 25 ml

Table no.2: Preliminary evaluation by visual appearance

Formulation code	Appearance
F1	Transparent
F2	Transparent
F3	Transparent
F4	Transparent
F5	Transparent
F6	Transparent
F7	Transparent
F8	Transparent

Table no. 3: pH of formulations

Formulations	pH
F1	5.01
F2	5.05
F3	5.11
F4	5.13
F5	5.14
F6	5.15
F7	5.19
F8	5.21

Table no.4: Gel forming capacity of different formulations

Formulation	Gelling capacity
F1	-
F2	+
F3	+
F4	+
F5	++
F6	++
F7	+++
F8	+++

Table no.5: Rheological studies of *in situ* gels before gelation.

Shear rate (rpm)	Viscosity (cps)							
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
50	75	118	158	178	195	211	230	310
100	31	39	48	56	65	78	82	98

Table no.6: Rheological studies of *in situ* gels after gelation.

Shear rate (rpm)	Viscosity (cps)							
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
50	213	320	430	497	687	883	1080	1160
100	119	210	246	302	354	443	660	920

Table no. 7: % Cumulative Drug Release

Time (hrs)	F7	F8
1	21.3	24.16
2	27.33	29.33
3	30	33.5
4	34.16	38.83
5	38.5	43.5
6	43.66	48.83
7	48.83	53.16

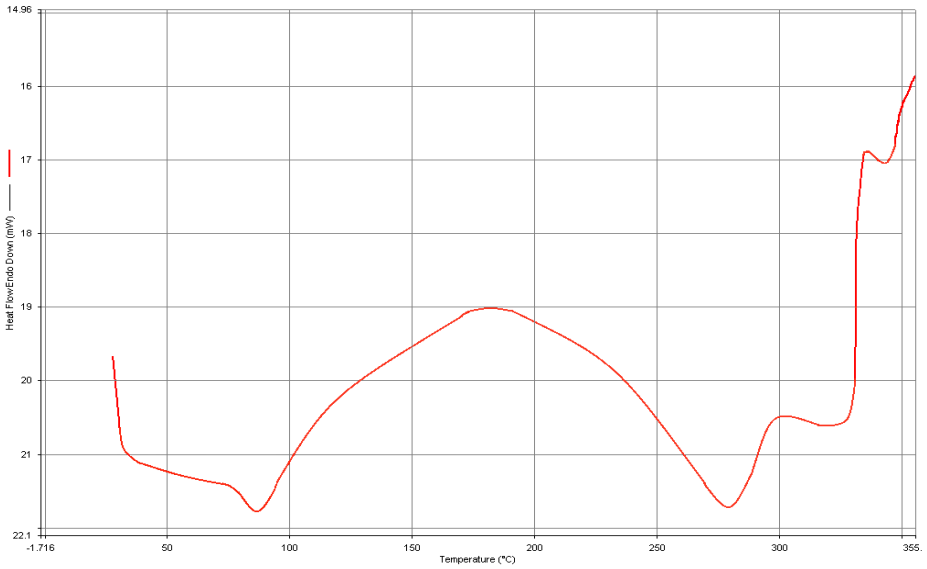

Figure no. 1 DSC of the Formulation

CONCLUSION

In the present work, pH triggered *in situ* gel of Ciprofloxacin HCl was prepared , which is a broad spectrum antibacterial agent used in the treatment of ocular infection, by using Carbopol 934 as a gelling agent and HPMC K4M as a viscosity enhancing agent. Out of 8 different batches prepared only two formulations F7 and F8 showed the good gelling capacity.

The formulations were found to be transparent and clear having good *in situ* gelling capacity. The pH and the drug content was found to be was within the acceptable rage.

Both the formulations were subjected to *in vitro* dissolution study. The drug release study and drug content of the formulation revealed that F8 showed good release than F7.

Hence from the above results, it was concluded that *in situ* gelling system of Ciprofloxacin HCl is the best mode of retaining the drug in the site of action and got better bioavailability of the drug in the formulation.

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