DESIGN AND EVALUATION OF IN SITU GELS FOR OPHTHALMIC DRUG DELIVERY OF FLUROQUINOLONE

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ABSTRACT

The aim of the present work was design and evaluation of in situ gelling system of Gatifloxacin Sesquihydrate. Gatifloxacin Sesquihydrate is an antibacterial agent which exhibits rapid precorneal elimination and poor ocular bioavailability, when given in the form of conventional ophthalmic solutions. To overcome this, an attempt has been made to formulate pH-triggered in situ gelling system of Gatifloxacin Sesquihydrate to provide sustained release of drug based on polymeric carriers that undergo sol-to-gel transition upon change in pH.The Gatifloxacin Sesquihydrate in situ gelling system formulated by using poly acrylic acid (Carbopol 940P) in combination with Hypermellose which acted as viscosity enhancing agent. The developed formulation was stable, non-irritant and provided sustained release over 8-hour period and it is a viable alternative to conventional eye drops.

KEYWORDS: In situ gel,Hypermellose,Carbopol 940P, pH,gelation,Gatifloxacin Sesquihydrate

INTRODUCTION

Eye drops that are conventional ophthalmic delivery systems often result in poor bioavailability and therapeutic response because high tear fluid turnover and dynamics cause rapid pre corneal elimination of the drug . A high frequency of eye drop instillation is associated with patient non-compliance. Inclusion of excess drug in the formulation in an attempt to overcome bioavailability problem is potentially dangerous if the drug solution drained from the eye is systemically absorbed from the nasolacrimal duct.[1,2]. Various ophthalmic vehicles such as inserts, ointments, Suspensions, and aqueous gels, have been developed in order to lengthen the residence time of instilled dose and enhance the ophthalmic bioavailability.[3,4]. These ocular drug delivery systems, however, have not been used extensively because of some drawbacks such as blurred vision from ointments or low patient compliance from inserts.[5,6]

Several in situ gel forming system have been developed to prolong the precorneal residence time of a drug and improve ocular bioavailability. These systems consist of polymers that exhibit sol- to-gel phase tansititions due to change in specific physico chemical parameter (pH, temperature), in their environment, the cul-de-sac in this case.[4,7]. Depending on the method employed to cause sol-to-gel phase transition on the eye surface, the following three types of systems are recognized. PH triggered system, temperature dependant system[23] and ion activated system[8]. Using these three methods above in-situ gelling ophthalmic delivery system is developed. How ever most of the systems require the use of high concentration of polymers. For instance, it needs 25% (w/v) pluronics and 30% (w/v) CAP, respectively, to from stiff gel upon instillation in the eye. As the concentration of carbopol increases in the vehicle, its acidic nature may cause stimulation to the eye tissue[9, 10, 11]. The present study aim was to develop the in-situ gelling ophthalmic delivery system of Gatifloxacin Sesquihydrate a fluoro quinoline derivative used in external infections of eye such as acute and sub acute conjunctivitis, bacterial keratitis and keratoconjunctivitis .

MATERIALS AND METHODS

Materials:

Gatifloxacin Sesquihydrate was obtained from Chethana pharmaceuticals Pvt Ltd, Carbopol 940P and Hydroxypropylmethyalcellulose were obtained from Micro labs Pvt Ltd, Benzalkonium chloride,Sodium chloride, Disodium hydrogen phosphate, calcium chloride and sodium hydroxide pellets were purchased from Nice chemicals Pvt Ltd, Cochin, India.

Estimation of Gatifloxacin Sesquihydrate using spectrophotometermethod:

Gatifloxacin sesquihydrate 100 mg pure drug was dissolved in simulated tear fluid and was diluted to give a concentration of 10µg/ml and was scanned between 200 nm and 400 nm for the determination of λmax. The wavelength of 289 nm was selected as λmax for Gatifloxacin sesquihydrate. The same was used for further analysis of drug.[14]

Preparation of formulation

P^H-triggered system:

In situ gel preparation was prepared in sterile buffer solution. The polymers were dissolved in the freshly prepared buffer solution with deionized water and allowed for proper hydration. The drug was dissolved in solution separately and the P^H was adjusted to required level and preservative solution was added to the drug solution. Then which was added to the polymer solution slowly with constant stirring until uniform clear solution obtains and made up to required volume with distilled, deionized water.[15,16]

Preparation of polymer phase

The buffer salts were dissolved in distilled water and hypromellose was added and allowed to hydrate. Carbopol 940P was sprinkled over this solution and allowed to hydrate overnight. The solution was stirred with an over head stirrer.

Preparation of drug solution

Gatifloxacin sesquihydrate was dissolved in sodium hydroxide and P^H was adjusted. Benzalkonium chloride was then added and the solution was filtered through 0.2 µm cellulose acetate membrane filter.

Preparation of in situ gel system

The drug solution was added to the Carbopol 940P - Hydroxypropyl methyl cellulose solution under constant stirring until a uniform solution was obtained. Purified water then added to make up the volume to required volume. The developed formulation were filled in 5 ml capacity amber glass vials, closed with gray butyl rubber closures and sealed with aluminum caps. The formulations were subjected to terminal sterilization by autoclaving at 121^0C and 15 p.s.i for 20 min[12,13]

Rheological studies

The viscosity of the formulation was carried out on a cone and plate geometry viscometer (Brookfield RVCP DV-II). The viscosity and shear stress of the sample solution were measured at various shear rates of 0.5 – 50 rpm at 25^0C. The temperature was maintained within $\pm 0.1^0$C by a recirculating bath (Wisdom) connected to the sample cup of viscometer. The samples were equilibrated on the plate for 5 min. to reach the running temperature prior to each measurement. A typical run comprised of changing the shear rates from 0-200s^{-1} at the same controlled ramp speed.[17,18]

***In-Vitro* release studies of Ciprofloxacin Hydrochloride *In-Situ* gels:**

The in vitro release from Gatifloxacin sesquihydrate formulations was carried out by the apparatus suggested by Sasaki et al. the apparatus consist of cylindrical glass container with surface area of 1.766 cm^2, which was opened at both ends. Cellophane membrane, molecular weight cut-off: 6000 – 8000, previously soaked in simulated tear fluid, was placed over one end of the cylindrical container and clamped into position. The cellophane membrane acts as a barrier between the in situ gel system and the simulated tear fluid (sink phase). The dissolution medium was freshly prepared artificial tear fluid (P^H 7.4). An aliquot of 1 ml of the examined

formulation was placed over the cellophane membrane and the end of cylindrical container was immersed in 100 ml of simulated tear fluid kept at 37^0C and stirred with magnetic stirrer at 50 rpm. The whole assembly was fixed in such a way that the lower end of the tube containing the formulation just touched the surface of simulated tear fluid. A quantity of 2ml sample was withdrawn from receptor fluid at the regular interval and same time samples were replaced by fresh simulated tear fluid. The concentration of Gatifloxacin sesquihydrate was analyzed spectrophotometrically at 289 nm. Each experiment was performed in triplicate.[19,20]

Antimicrobial studies

Antimicrobial microbial efficacy was determined by Kirby-Bauer disk diffusion method. In testing, a disc was placed on the Lowenstein slope, near the top of the medium, as soon as convenient after seeding the culture suspension. Sterile solution of Gatifloxacin sesquihydrate in citrophosphate buffer, P^H 6.0 as standard solution and the developed formulation diluted suitably with citrophosphate buffer, P^H 6.0 as test solution impregnated 6 mm diameter disc was put in the culture suspension and incubated. Zones were obtained by measurement of the radius from the centre of the disc to the edge of the inhibition of growth. Measurements were made from both sides of the slope and their average. With discs it was possible to record sensitivities in 10 to 14 days, and no encroachment on the zone of inhibition occurred within 21 days. The entire operation without incubation was carried out in a laminar flow unit.[21,22]

Accelerated stability studies

Stability studies were carried out on the optimized in situ gel formulation. Optimized in situ gel formulation were stored at the below mentioned storage condition for three month and evaluated for the parameters viz. PH, appearance, gelation studies, drug content and in vitro drug release studies. The prepared formulations were filled in tightly closed vials and stored at 4±1°C, 27±1°C, 45±1°C. The drug content of formulation was detected at each month interval.[15,16]

RESULTS AND DISCUSSION

The developed formulations were evaluated for visual appearance, pH and drug content by UV spectrophotometer at 289 nm, clarity by visual observation against a black and white back ground, pH (Digital pH meter), gelation study, In-vitro release studies, antimicrobial studies and accelerated stability studies. The obtained results of preliminary evaluation studies are represented in table 2.

The two main requisite of an in situ gelling system are viscosity and gelling capacity. Except for the formulation F III and F IV all the formulation gelled instantaneously but the F VI only shows the intermediate gelation which remains for few hours and F II is highly viscous solution having less pourability. Table 6.10 shows the gelling capacity Table 6.9 shows the viscosity of formulation F I – F VI. A concentration of 1.5 % Hydroxypropyl methyl cellulose E50 LV and 0.5% Carbopol (F VI) was selected as it had satisfactory attributes of viscosity value to improve the contact time in the eye. The formulations were shear thinning and an increase in shear stress was observed with increase in angular velocity. The administration of ophthalmic preparation should influence as little as possible the pseudoplastic character of the precorneal tear film. An increase in P^H to 7.4 caused the solutions to transform into gels.

The P^H of all formulations was acceptable range and hence would not cause any irritation upon administration. The drug content determination in the Table 6.11 showed that even if the polymer composition was changed the process was highly efficient to give the formulation with maximum drug loading.

In vitro release studies indicated that the amongst all the formulation F VI sustained drug release up to 9hr. due to the concentration of the polymer combination and gel formation. The higher regression values for each formulation suggested that all the formulation F I –F VI behaved as Korsmeyer - Peppas release model and interpretation of release exponent values enlightens in understanding the release mechanism from the dosage form. In the formulations except F III and F IV exhibited anomalous (non-fickian transport) diffusion mechanism. The drug release was diffusion controlled as the plot of Higuchi model was found to be linear ($r^2 > 0.9830$). These formulations also showed as highest r values of zero order kinetic ($r^2 > 0.9754$)

indicating Gatifloxacin sesquihydrate release from these in situ gel were by both diffusion and gel solvation.

The comparative in vitro drug release profile of formulation Table 6.18 and Figure.6.44 shows the formulation F II and F VI only take more than 8 hr to release the 90% of drug. But from the previous data indicates that F VI only had the satisfactory rheological properties and gelling capacity when compared to Formulation F II. This indicates the system is suitable for the in situ gel forming delivery.

The antimicrobial efficacy tests are shown in Table.6.20 The study indicates that F VI retained its antimicrobial efficacy when formulated as an in situ gelling system. The ocular irritation studies indicate that F VI was non irritant. Excellent ocular tolerance was noted. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae were visible.

Stability studies were carried out on Formulation F VI shown that formulation was physically and chemically stable with no significant change in any of the parameters studied after 3 month of study. It was found that the in situ gelling system of Gatifloxacin sesquihydrate was stable at selected storage condition with most suitable storage condition at the refrigeration temperature.

CONCLUSION

Gatifloxacin sesquihydrate, a broad spectrum antibacterial agent used in the treatment of ocular infection, was successfully formulated as P^H activated in situ gel forming ophthalmic solutions (0.3 % w/v) using Carbopol 940P as gelling agent in combination with Hydroxypropyl methyl cellulose as a viscosity enhancing agent. The formulation was liquid at the formulated P^H and underwent rapid gelation in the cul-de-sac upon instillation as drops into the eye. The gel formed in vitro produced sustained drug release over an 8-h period and the formulations were therapeutically efficacious. Stability data recorded over a 3-month period under accelerated temperature conditions indicated the formulation to be stable. The developed new formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its sustained drug release and longer pre-corneal residence time. Also important is the ease of administration resulting in better patient acceptance and this study encourages further clinical trials and long term stability study on this formulation.

SL. No	Materials	Quantity gm % w/v					
		Formulation Number					
		I	II	III	IV	V	VI
1	Gatifloxacin sesquihydrate	0.3	0.3	0.3	0.3	0.3	0.3
2	Carbopol 940P	0.3	0.5	0.3	0.5	0.3	0.5
3	HPMC K4 M	1.5	1.5	-	-	-	-
4	HPMC E15 LV	-	-	1.5	1.5	-	-
5	HPMC E50 LV	-	-	-	-	1.5	1.5
6	Hydrochloric acid	0.16	0.16	0.16	0.16	0.16	0.16
7	Benzalkonium chloride	0.02	0.02	0.02	0.02	0.02	0.02
8	Tween 20	1.0	1.0	1.0	1.0	1.0	1.0
9	Citric acid	0.407	0.407	0.407	0.407	0.407	0.407
10	Disodium hydrogen phosphate	1.125	1.125	1.125	1.125	1.125	1.125

Table No: 1 Data for ingredients of the developed formulations

SL. No.	Revolution per min.	Viscosity of Formulation (cp)					
		Formulations					
		I	II	III	IV	V	VI
1	0.5	7600	9600	160	800	1600	2400
2	1	6000	8000	120	600	1400	1800
3	2	4900	7200	90	500	1200	1500
4	4	4050	6350	80	400	1100	1250
5	5	3640	5760	72	280	1000	1120
6	10	3320	4860	60	220	860	1000
7	20	2980	4120	50	180	720	900
8	50	2652	3772	40	152	660	812

Table No:2 Rheological Profile of Formulation I – VI

SL. No.	Time hour	CUMULATIVE PERCENTAGE DRUG RELEASE					
		FI	FII	FIII	FIV	FV	FVI
1	0.5	9.8878	5.7045	19.77562	8.74691	15.5923	6.46511
2	1	18.2544	10.2681	48.31002	19.40057	31.9546	11.0326
3	1.5	30.8043	15.5923	66.21312	30.44092	47.1858	16.7437
4	2	39.1710	20.1559	91.73295	41.10759	62.0458	23.2189
5	3	63.5102	26.2407	-	66.6136	72.7314	34.6418
6	4	77.9616	31.9452	-	90.6112	86.8461	45.311
7	5	93.5539	39.171	-	-	99.4480	55.6062
8	6	-	50.1997	-	-	-	67.4288
9	7	-	59.3269	-	-	-	78.8783
10	8	-	66.5526	-	-	-	86.5315
11	9	-	73.3980	-	-	-	94.9499
12	10	-	81.3843	-	-	-	-
13	11	-	90.1312	-	-	-	-
14	12	-	98.1175	-	-	-	-

Table No: 3 Data for In vitro drug release profile of Formulation I – VI

SL. No.	Organism used	Zone of inhibition (mm)		Mean Percentage efficacy
		Standard	*Test	
1	Moraxella catarrhalis	32	27	84.38
2	Haemophillus Influenza	33	25	75.75
3	Klebsiella Pneumonia	35	28	80
4	Mycobacterium Marinum	35	27	77.14
5	Staphylococcus Aureus	34	29	85.29
6	Streptococcus Pneumoniae	35	29	82.86

Table No:4 Data for antimicrobial efficacy of formulation I - VI

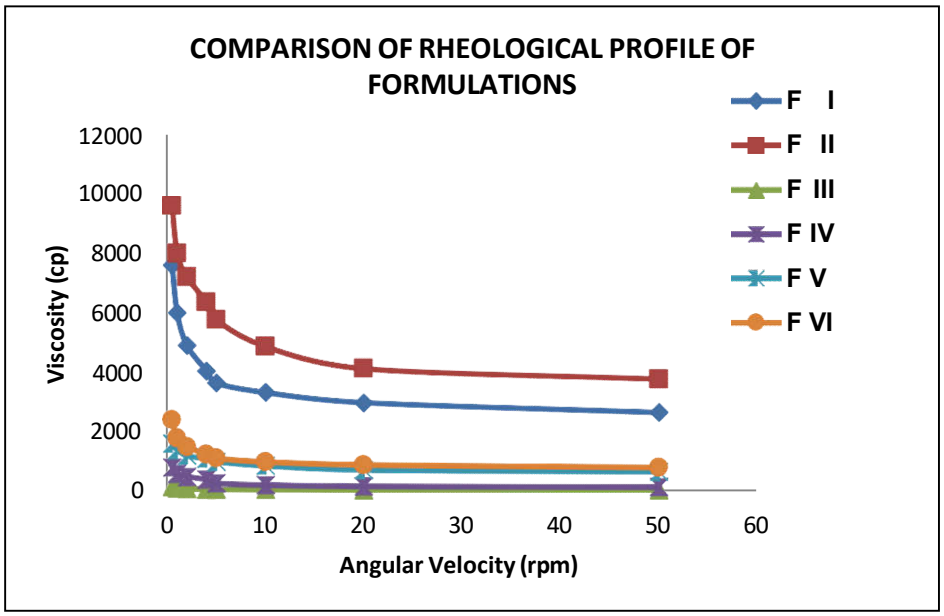

Figure No: 1 Rheological Profile of Formulation I - VI

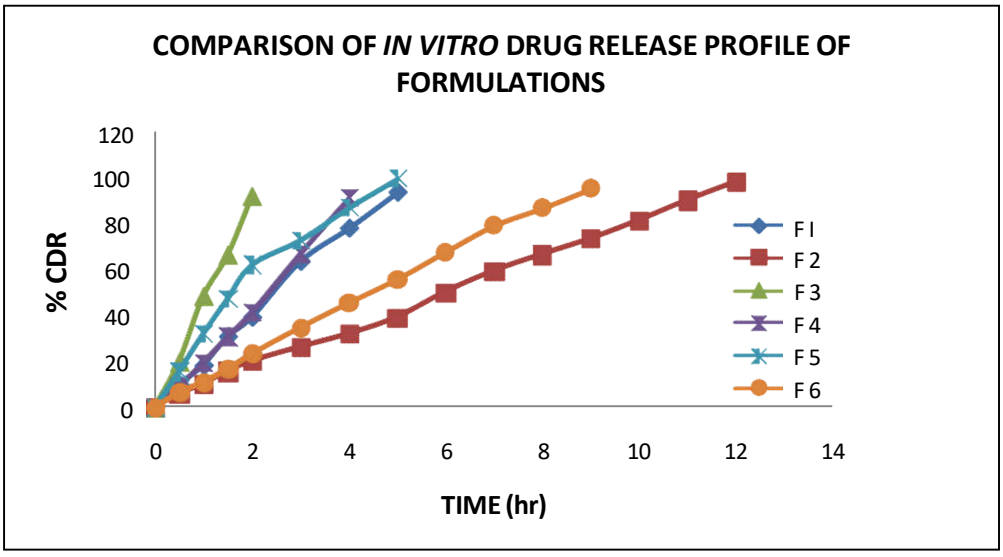

Figure No: 2 In vitro drug release profile for Formulation I – VI

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