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A novel *in situ* gel for sustained ophthalmic delivery of Ciprofloxacin hydrochloride and Dexamethasone- design and characterization

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ABSTRACT

A major problem in ocular therapeutics is the attainment of optimal drug concentration at the site of action, which is compromised mainly due to precorneal loss resulting in only a small fraction of the drug being ocularly absorbed. The effective dose administered may be altered by increasing the retention time of medication into the eye by using in situ gel forming systems. The aim of the present investigation is to prepare and evaluate novel in situ gallen gum based ophthalmic drug delivery system of ciprofloxacin hcl and dexamethasone. All the formulations were sterilized in an autoclave at 121°C for 15mins. The formulations were evaluated for clarity, pH measurement, gelling capacity, drug content estimation, rheological study, in vitro diffusion study and ICH stability studies. The developed formulations exhibited sustained release of drug over a period of 7 hours thus increasing residence time of the drug and optimized formulations also found satisfactorily stable, thus these in situ gelling systems may be a valuable alternative to the conventional systems.

Keywords: Ophthalmic *in situ gel*, Ciprofloxacin hydrochloride, Dexamethasone, Gallen gum

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INTRODUCTION

Ocular drug delivery has remained as one of the most challenging task for pharmaceutical scientists. The unique structure of the eye restricts the entry of drug molecules at the required site of action. Drug delivery to the eye can be broadly classified into anterior and posterior segments. Conventional systems like eye drops, suspensions and ointments cannot be considered optimal in the treatment of vision threatening ocular diseases however, more than 90% of the marketed ophthalmic formulations are in the form of eye drops. These formulations mainly target the anterior segment eye diseases. Most of the topically applied drugs are washed off from the eye by various mechanisms (lachrymal drainage, tear dilution and tear turnover) resulting in low ocular bioavailability of drugs. Moreover, human cornea comprising of epithelium, substantia propria and endothelium also restricts the ocular entry of drug molecules. As a result of these factors less than 5% of administered drug enters the eye. [1,2]

The conventional drug delivery systems like solutions, suspensions and ointments are no longer sufficient to fulfill the present day requirements of providing a constant rate delivery and prolonged time. One of the main reason for that is poor residence time of drug at the site of action, which results into poor bioavaibility. [3]

For this project work we had selected Ciprofloxacin hydrochloride and Dexamethasone combination because of Ciprofloxacin is potent antibiotic and it regularly preferred for many eye infection such as, Conjunctivitis and Dexamethasone is glucocorticoid which preferred for intraocular inflammation and for other allergic reaction. So combination of these to drug proved effective against many intraocular infection and inflammatory diseases. But marketed product of this combination mostly present in solution form (eye drops). Which have certain drawback like low residence time, poor bioavaibility, lower drug concentration at the the site of action due to lachrymal drainage.

The present work is intended to formulate & evaluate the *in situ* gel of Ciprofloxacin hydrochloride and Dexamethasone. Gelling agents Gallen gum (ion sensitive) was used to formulate *in situ* gel. So formulating *in situ* gel we can achieve increase residence time of drug at the site of action, increase bioavaibility of drug and patient compliance.

MATERIALS AND METHODS

Material

Ciprofloxacin hcl (1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid) and Dexamethasone [8S, 9R, 10S, 11S, 13S, 14S, 16R, 17R - 9 - fluoro – 11 – 17 - dihydroxy – 17 - (2-hydroxyacetyl) - 10,13,16- trimethyl 6,7,8,9,10,11,12,13,14,15, 16, 17- dodecahydro - 3H - cyclopentane phenanthrene-3-one] obtain as gift sample from Redchem lab Mumbai. Gellan gum obtain as gift sample Applied bioscience Mumbai. All other chemical used either AR/LR Grade.

Method for *in situ* hydrogel preparation: [4,5]

Gallen gum ophthalmic *in situ* gel: For the preparation of gallen gum containing *in situ* gel, a gelling agent, gallen gum was firstly sprinkled over 60 ml distilled water with constant heat up to 60^0C to 80^0C and constant stirring. Ciprofloxacin hcl was dissolved to polymer solution under constant stirring until a uniform solution was obtained. Dexamethasone and BCD solid dispersion was prepared in 1:6 ratio respectively. After getting smooth and fine powder of mixture, the prepared powder slowly dissolved in 40 ml separate distilled water with constant heating and stirring. When mixture get completely dissolved this solution mixed with polymer solution. The manitol and benzalkonium chloride were added as tonicity adjuster and preservative respectively. The final formulations, in their final pack were subjected to terminal sterilization by autoclaving at 121ºC and 15 psi for 20 min.

Table 1: Composition of gallen gum formulation

Formulation code	Ciprofloxacin (%w/v)	Dexamethasone (%w/v)	Gallen gum (%w/v)
G1	0.3	0.1	0.1
G2	0.3	0.1	0.2
G3	0.3	0.1	0.3
G4	0.3	0.1	04
G5	0.3	0.1	0.5
G6	0.3	0.1	0.6

Benzalkonium chloride: 0.01%
Manitol : 5% w/v

Evaluation parameters:

✓ **Appearance:** [6] Clarity is one of the most important characteristic features of ophthalmic preparations. All developed formulations were evaluated for clarity by visual observation against a black and white background.

✓ **pH:** [7] pH is one of the most important parameter involved in the ophthalmic formulation. The two areas of critical importance are the effect of pH on solubility and stability. The pH of ophthalmic formulation should be such that the formulation will be stable at that pH and at the same time there would be no irritation to the patient upon administration of the formulation. Ophthalmic formulations should have pH range in between 5 to 7.4. The developed formulations were evaluated for pH by using digital pH meter.

✓

Simultaneous estimation of Ciprofloxacin hydrochloride and Dexamethasone: [8, 9, 10]

A simple, precise, accurate and reproducible method was developed for simultaneous estimation of Ciprofloxacin hcl and Dexamethasone. The method involved the measurement of absorptivity data of Ciprofloxacin hcl and Dexamethasone at 271 nm and 242 nm within beer's range respectively. The method was validated for recovery, repeatability and ruggedness.

Table 2: The absorptivity value of both drugs at 271nm and 242 nm

Sr. No.	Drugs	At 271 nm	At 242 nm
1	Ciprofloxacin	91.82 (X_1)	36.44 (X_2)
2	Dexamethasone	19.10 (Y_1)	44.28 (Y_2)

The quantitative estimation of Ciprofloxacin and Dexamethasone was carried out using following equations:

$$Cx = A_1.Y_2 – A_2.Y_1 / X_1.Y_2 – X_2.Y_1$$
$$Cy = A_2.X_1 – A_1.X_2 / X_1.Y_2 – X_2.Y_1$$

✓ **Drug Content:** [11, 12] Uniform distribution of active ingredient is important to achieve dose uniformity. The drug content was determined by diluting 1 ml of the formulation to 100 ml with ATF solution pH 7.4. Aliquot of 1 ml was withdrawn and further diluted to 10 ml with ATF. Ciprofloxacin hcl and Dexamethasone concentration were then determined by simultaneous method at 271 nm and 242 nm by using UV-Vis spectrophotometer.

✓ **Gelation Studies:** [13] All prepared formulations were evaluated for gelling capacity and viscosity in order to identify the compositions suitable for use as *in situ* gelling systems. 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 37^0C and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve. The composition of artificial tear fluid used was sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride 2H2O 0.008 g, purified water Q.S. 100.0 g.

✓ **Rheological Studies:** [14] The formulations were poured into the sample adaptor of the Brookfield DV-111+ rheomoter and angular velocity was increased gradually from 1 to 50 rpm using spindle no. 4. The hierarchy of angular velocity was reversed and the average dial reading was considered to calculate the viscosity. The temperature was maintained within 37 ± 0.1°C.

✓ ***In vitro* release studies:** [15,16] The *in vitro* release of Ciprofloxacin hcl and Dexamethasone from the formulations was studied through cellophane membrane using a fabricated dissolution testing apparatus. The dissolution medium used was artificial tear fluid freshly prepared (pH 7.4). Cellophane membrane, previously soaked overnight in the dissolution medium, was tied to one end of a specifically designed glass cylinder (open at both ends and of 5 cm diameter). A 1-ml volume of the formulation was accurately pipetted into this assembly. The cylinder was suspended in 50 ml of dissolution medium maintained at 37^0C so that the membrane just touched the receptor medium surface. The dissolution medium was stirred at 50 rpm using magnetic stirrer. Aliquots, each of 1-ml volume, were withdrawn at regular intervals and replaced by an equal volume of the receptor medium. The aliquots were suitablely diluted with the receptor medium and analyzed by UV-Vis spectrophotometer at 271 nm and 242 nm with the use of simultaneous estimation method.

✓ **Stability studies:** [17] The accelerated stability studies were carried out according to the ICH guidelines. Optimized formulations G6 and G5 were seal in amber colored bottles which cap covered by aluminum foil and these packed formulation was stored in ICH certified stability chambers (Thermo labs, Mumbai) maintained at 40^0C ± 2^0C and 75% RH ± 5 % for 3 month. The formulations were evaluated before and after periodic interval for change in appearance, the drug content, and *In vitro* drug release.

RESULTS AND DISCUSSION

Appearance: Clarity of all the formulations was found to be satisfactory. Terminal sterilization by autoclaving had left no effect on the clarity and other physicochemical properties of the formulations, no changes were observed after autoclaving.

pH: The pH of the formulations was found to be satisfactory and lies in the range of 6-7.4. The formulations were liquid at room temperature and at the pH formulated. Terminal sterilization by autoclaving had no effect on the pH.

Drug Content: The percent drug content for formulations was found to be in acceptable range for all the formulations. Solubility of drug in vehicle may affect drug content.

The drug content determined for all formulations and results are shown in Table2. The percent drug content of gallen gum formulations was found to be in between 96.91% to 97.91 % for ciprofloxacin hcl and for dexamethasone it lies between 91.44% to 94.01 %.

Table 3: Gallen gum formulations % drug content of Ciprofloxacin hcl and Dexamethasone by simultaneous estimation method.

Drugs	Formulation code	% drug content (n=3 mean± SD)
Ciprofloxacin	G1	97.28± 0.590
	G2	96.91± 0.252
	G3	97.12± 0.163
	G4	97.89± 1.334
	G5	97.66± 1.030
	G6	97.91± 0.143
Dexamethasone	G1	92.54± 1.129
	G2	92.71± 0.624
	G3	91.44± 3.107
	G4	92.55± 0.854
	G5	92.36± 3.414
	G6	94.01± 0.948

***In vitro* Gelation Studies:** One the main prerequisites of an *in situ* gelling system is gelling capacity. The formulation should have an optimum gelling capacity to facilitate sustained release of drug to the ocular tissue, the gel formed *in situ* should preserve its integrity without dissolving or eroding for a prolonged period of time.

Table 4: Gelling capacity of gallen gum formulation

Sr. no.	Formulation code	Concentration (% w/v)	Gelling capacity
1	G1	0.1	+
2	G2	0.2	++
3	G3	0.3	++
4	G4	0.4	++
5	G5	0.5	+++
6	G6	0.6	+++

Gelling capacity of gallen gum formulations were showed in table 3. Sign depicted as + (gels after few minutes and dissolves rapidly), ++ (gelation immediate, remains for few hours) and +++ (gelation immediate, remains for extend period). In gallen gum formulation G5, G6 were found with good gelling capacity However, the nature of the gel formed depended on the concentration of polymers used. The formation of instantaneous gels can be attributed to the buffering capacity of the simulated tear fluid.

Rheological studies:

Table 5: Viscosity (in cps) profile of gallen gum formulations

Angular velocity (rpm)	G1 (cps)	G2 (cps)	G3 (cps)	G4 (cps)	G5 (cps)	G6 (cps)
1	185	267	310	365	410	487
2	164	242	289	342	387	468
5	135	219	263	304	356	428
10	103	197	239	279	289	356
20	79	172	201	228	237	258
30	54	154	171	188	199	210
40	37	138	152	176	184	198
50	32	126	140	167	172	182

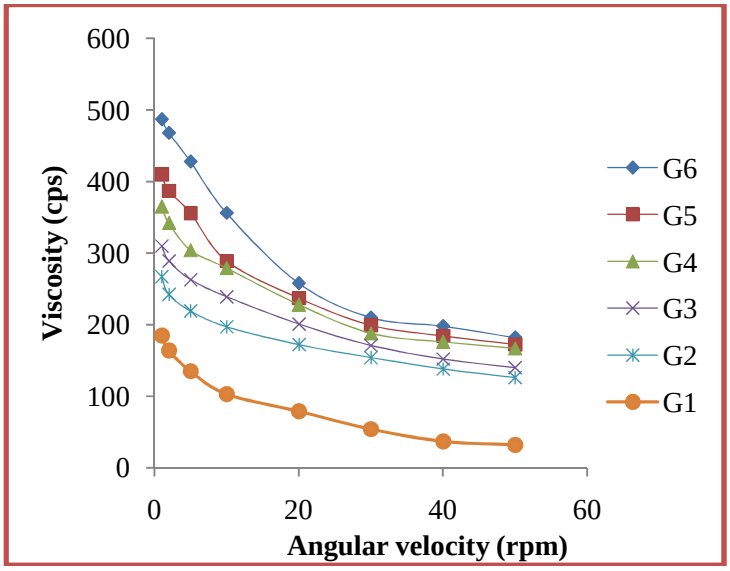

Figure 1: Gallen gum formulations rheograph. (Angular velocity (rpm) Vs Viscosity)

The results obtained from the rheological study of prepared in situ gelling system revealed that the viscosity decreases as the angular velocity increases. Furthermore results showed optimum viscosity and formulations were pourable at normal physiological conditions.

***In Vitro* Release Studies:**

The release profile of a drug predicts how a delivery system might function and gives valuable insight into its *in vivo* behaviour. All the *in situ gelling* formulations were subjected to *in vitro* release studies. These *in vitro* release studies were carried out using simulated tear fluid (STF) of pH 7.4 as the dissolution medium. The drug release data obtained for formulations shows the cumulative percent drug released as a function of time for Ciprofloxacin hcl in gallen gum formulation and It was found that cumulative percent drug release was 75.65%, 74.08%, 72.88%, 73.16%, 72.79% and 75.89% for G1-G6 formulation respectively. Dexamethasone cumulative percent drug released as a function of time was found to be, 73.30%, 72.63%, 71.84%, 72.34%, 71.04% and 73.43% in gallen gum formulations from G1-G6 respectively.

Table 6: Cumulative % release of Ciprofloxacin and Dexamethasone Vs Time in gallen gum formulations from G1 to G3.

Time (min)	Cumulative % drug release of Ciprofloxacin and Dexamethasone in formulation G1 to G3 (n=3) Mean±SD					
	G1		G2		G3	
	Cipro	Dexa	Cipro	Dexa	Cipro	Dexa
15	37.89± 0.32	35.64± 0.51	35.41± 0.57	33.60± 0.93	33.54± 0.46	31.22± 0.62
30	47.90± 0.71	45.70± 0.43	41.64± 0.44	39.09± 0.74	36.97± 0.28	35.80± 0.77
45	57.95± 1.04	54.61± 0.94	47.86± 0.20	45.23± 0.52	45.67± 0.10	43.65± 0.78
60	64.24± 0.73	62.73± 0.58	52.28± 0.41	51.12± 0.51	50.93± 0.18	49.48± 0.83
120	75.65± 1.14	73.30± 0.90	63.32± 0.88	62.11± 0.95	57.77± 0.14	55.48± 0.4364
180	-	-	74.08± 0.94	72.63± 0.83	64.20± 0.18	63.16± 0.58
240	-	-	-	-	72.88± 0.37	71.84± 0.99

Gallen gum formulations the initial burst release of the drug can be explained by the fact that, the *in situ* gelling system is formulated in water and hence the polymer was completely hydrated. When they come in contact with STF, gelation occurs and a prehydrated matrix is formed in which hydration and water penetration no longer limit drug release, leading to an apparent diffusion-controlled release. The *in vitro* drug release conditions may be very different from those likely to be encountered in the eye. However, the results clearly show that the formulation G6 have the ability to retain drug for prolonged period of time up to 7 and 5 hrs respectively. In the cul-de-sac, the gels will probably undergo faster dissolution due to the shearing action of the eyelid and eyeball movement. It is also observed that the dissolution in the cul-de-sac will proceed more slowly than that seen in the *in vitro* experiments, as

the normal resident volume of the lachrymal fluid in the human eye is 7.5-10 µl. The gels on visual inspection at periodic intervals during the *in vitro* drug release experiments showed a gradual swelling that resulted in an increase in volume of most gels. No discernible relationship between the extent of swelling and gel composition could be established. Also, no apparent changes or disruptions in the integrity of the gels were noticed during the course of experiment. This shows *in vitro* release of drug from the in situ formulation follows diffusion mechanism.

Table 7: Cumulative % release of Ciprofloxacin and Dexamethasone Vs Time in gallen gum formulations from G4 to G6

Time (min)	Cumulative % drug release of Ciprofloxacin and Dexamethasone in formulation G4 to G6 (n=3) Mean±SD					
	G4		G5		G6	
	Cipro	Dexa	Cipro	Dexa	Cipro	Dexa
15	31.07± 0.18	30.26± 0.41	30.27± 0.03	28.90± 0.91	28.6± 0.26	26.7± 0.51
30	37.05± 0.18	36.36± 0.51	35.35± 0.07	34.04± 0.85	34.19±0.31	31.4± 0.75
45	41.01± 0.24	39.83± 0.65	39.96± 0.74	37.68± 1.15	38.18±0.50	35.19±0.39
60	46.18± 0.23	43.89± 0.64	45.52± 0.75	43.40± 0.83	42.03± 0.62	39.8± 0.45
120	52.74± 0.35	50.385± 0.24	51.38± 0.48	48.35± 1.70	48.67± 0.84	46.587± 0.67
180	57.26 ± 0.71	56.00± 1.35	58.16± 0.35	56.07± 0.94	54.1± 0.82	52.33± 0.89
240	65.49± 0.13	63.61± 0.95	64.67± 0.24	61.79± 1.37	60.09± 1.24	56.8± 0.72
300	73.16± 0.29	72.34±0.36	68.17± 0.86	67.12±0.61	65.43± 0.82	63.6±0.62
360	-	-	72.79± 0.98	71.04±0.78	69.35± 0.72	68.3±0.50
420	-	-	-	-	75.8± 0.78	73.4±0.62

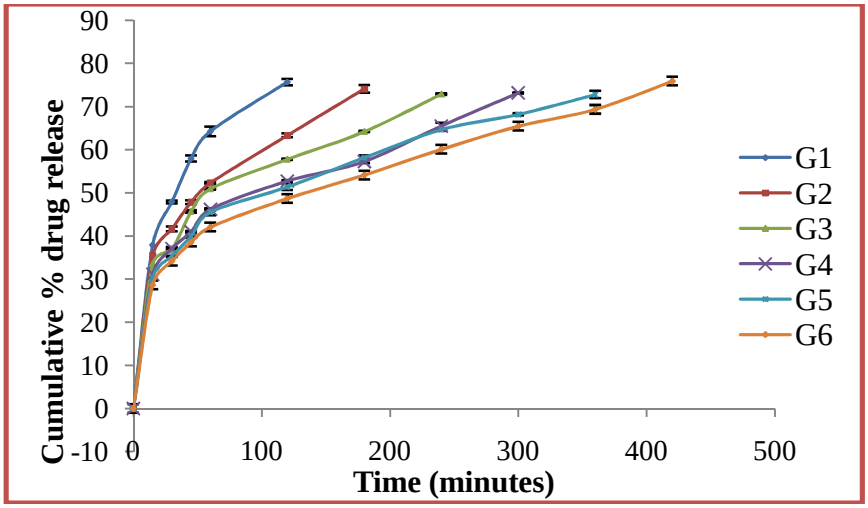

Figure 2: *In vitro* drug release Ciprofloxacin for G1 to G6

Stability studies : From stability study it reveals that both formulation G6 and G5 showed no change in physical appearance, but both formulation showed very less decrease in drug content and in vitro release. This may because of slight degradation of drugs at elevated temperature, but this changes will not occur if formulations store at room temperature. From study it reveals that tested formulation were satisfactorily stable.

CONCLUSION

Ophthalmic *in situ* gel of Ciprofloxacin hcl and Dexamethasone by using galllen gum as gelling agent prepared successfully and showed appreciable *in situ* gel forming capacity. The formulations were found to be clear and uniform. The *in vitro* study ensures that drug release over period of 7 hours. The polymers used are inexpensive and easily available. The formulation also promises to reduce the frequency of drug administration, thus improving patient compliance. As the concept involved is novel and the methodology used for the preparation is simple as that of conventional ophthalmic liquid dosage form, it is industrially oriented and economical.

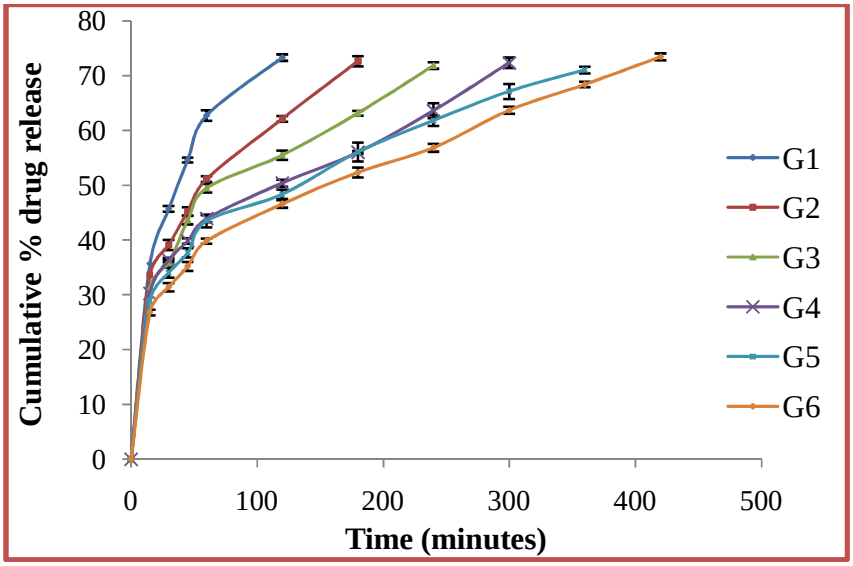

Figure 3*: In vitro* drug release for Dexamethasone for G1 to G6

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