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Creation, Profiling, and Assessment of an Innovative in-situ Gel Incorporating Doxycycline for Enhanced Ocular Drug Release

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ABSTRACT: Aim of the present study was to design and develop in-situ gel-forming containing Doxycycline Hyclate and evaluate against dry eye disease related bacterial infections. The formulation was systemically optimized using simplex lattice design model. The optimized formulation was evaluated for clarity, pH measurement, gelling capacity, Gel strength, drug content estimation, rheological study, viscosity, *in-vitro* diffusion study, antimicrobial activity, and osmolality study. The Gellan gum is a well-known natural source of gel-forming mucoadhesive polymer, which gets converted to gel in the presence of divalent-cations (calcium ion) present in the lachrymal fluid, was used as the gelling agent. The optimized *In-situ* formulations were evaluated for osmolality 260-310 mOs mol/kg, drug content 95-100% and pH 6.5-7.4, gelling time 22 seconds, gelling strength. The antimicrobial efficacy results showed that not less than 1.0 log reduction from the initial calculated count at 7 days, not less than 3.0 log reduction from the initial calculated count at 14 days, and no increase from the 14 days count at 28 days. The obtained results indicated that the elaborated in-situ gel system containing Doxycycline Hyclate has been successfully developed. Results showed that a suitable combination of polymers Gellan gum, Povidone K30 & Hypromellose were provide better Mucoadhesion and to increase the residence time. This may be helpful in term of patient compliance for the dry eye disease related bacterial infections. This in situ gelling system containing natural source of gums may be a valuable alternative to the conventional systems.

Keywords: Dry eye disease, In-situ gel, Doxycycline Hyclate, drug delivery, polymer, Gellan gum.

INTRODUCTION

The primary goal of ocular drug delivery for the researchers is to achieve an acceptable therapeutic drug concentration at the target area, whether it is the anterior or posterior eye segment. The anatomy, physiology, and biochemistry of human eye (barrier role of corneal epithelium, tear turnover, reflex blinking), as well as the intrinsic characteristics of the most frequently used ophthalmic dosage forms. e.g., eye drops-low viscosity, relatively large volume of applied drop lead to short precorneal contact time, rapid elimination and determined the poor bioavailability in ocular therapeutics (Ahmed *et al.,* 2023). The poor bioavailability of medication from conventional delivery system is resulted from a great extent of precorneal drug loss by nasolacrimal drainage. The rapid clearance of the topically applied drug into the eye often results in a short duration of pharmacological activity and, therefore, the need for a frequent dosing regimen. Moreover, 50%-100% of an instilled dose could undergo systemic absorption through drainage via the nasolacrimal duct. This could lead to a possible increased risk of unwanted systemic toxic effects (Kolawole *et al.,* 2023). The administered effective dose may be altered by increasing the retention time of medication into the eye by using *in-situ* gel forming system (Kilbinger and Mandal 2022; Mandal *et al.,* 2012). In order to overcome these limitations, the present study was to formulate the *in-situ* gel formulation using novel gum system. *In-situ* forming hydrogels are liquid upon instillation and undergo phase transition in the ocular clu-de-sac to form viscoelastic gel and this provides a response to environment changes (Kumar *et al.,* 2013). In which *In-situ* phase transition occurs on the surface of the cornea. At the time of instillation dosage form is in the solution phase and soon later upon coming in contact with calcium ion with surrounding pH of 7.4 it turns into transparent gel depot. Thus, this type of formulation has benefited of both solutions as well gels. They may improve retention time of the formulation as well the encapsulated drug, accuracy, and easy administration. The sol-gel phase transition on the eye surface depending on the different methods employed which consist of the thermosensitive, ion activated and electric sensitive, magnetic field sensitive, ultrasonic sensitive and chemical material sensitive varieties (Makwana *et al.,* 2016). The most commonly methods are as follow: (a) pH triggered system (b) Temperature dependent system and (c) Ion activated system. The present work is based on pH triggered system. Gellan gum is an

anionic water soluble, extracellular polysaccharide obtained from microorganism bacteria *Pseudomonas elodea* (Makwana *et al.,* 2016). The deacetylated gellan gum, gelrite, is synthesized by basic hydrolysis of the gellan gum parent polymer. Upon instillation, gelrite forms a gel due to the presence of mono-(Na^+, K^+) and divalent (Ca^{2+}) cation of the tear fluids. Gellan gum-based formulation forms a clear gel upon contact with cations in the tear due to the crosslinking reaction of the negatively charges polysaccharide helices by mono-valent and divalent drainage process and extends drug residence time (Samadi *et al.,* 2008; De Jong *et al.,* 2022).

MATERIALS AND METHODS

Materials: Doxycycline Hyclate received as a gift sample from Jagannath Chemical and Pharmaceutical works Pvt. Ltd., Odissa, India. Gellan gum from CP kelco (California, United state), Povidone 30 from Dow, Hypromellose from Colorcon, Benzalkonium chloride from Novo Nordisk Pharmatech A/S (supplied by signet chemical), Mannitol from Merck, Tromethamine from Merck, and dialysis tubing cellulose. Other reagents and solvents used in the study were of an analytical grade.

Methods

Solubility studies: The solubility of Doxycycline Hyclate was studied in water and in buffer solutions of different pH. The following buffers were selected; acetate buffer (pH 4.5), Phosphate buffer (pH 7.4) and borate buffer (pH 9.8). For evaluating the solubility in a particular solvent an excessive amount of the drug was dissolved in 5mL solvent and solution was stirred by using magnetic stirrer for 12 hrs at room temperature (25˚C-27˚C). After 12 hrs the sample was removed from stirrer and allowed to settle down. The supernatant solution was separated and filtered, and appropriate dilution was made with the respective solvent. The absorbance of diluted solution was measured at 276nm, and the concentration of soluble drug was calculated.

Preparation of In Situ Gel Formulation: In Situ ion responsive gel formulations containing Gellan gum were prepared on weight by volume basis. Add Gellan gum (0.4 to 0.5% w/v) slowly dispersed in a de-ionized under continuous stirring. Stir for 40 minutes at 450 rpm. Add Povidone K30 (0.2 to 0.3%w/v) under stirring and stir till dissolved. Add HPMC (0.3 to 0.4%w/v) in above solution under stirring and stir until dissolve completely. Heat the polymer phase up to 90 - 110˚C for 15 minutes and filter through 0.2µ filter (Millipore filter) to make the formulation sterile. Dissolve the remaining Excipients in de-ionized water (Doxycycline hyclate 0.05%), Tromethamine as a buffering agent to provide pH 6.5, and Benzalkonium chloride (0.01%) as a preservative. Filter the Drug phase through 0.2µ filter (Millipore filter) to make the formulation sterile and add the filtrate into the above prepared Gellan gum solution under continuous stirring stir for 30 minutes. Make the volume with filtered de-ionized water up to 100% of the batch size. The composition of the *in-situ* gel formulation is shown in the below Table 1.

Table 1: Composition of the *in-situ* gel formulation.

Doxycycline Hyclate (In mg)	BKC (in mg)	Gellan gum (%)	Povidone K30 (%)	HPMC (%)	Tromethamine (In mg)	Mannitol (In mg)	Sodium thiosulfate (In mg)	De-ionized water (In mL)
0.5	0.05	0.4	0.2	0.5	1.75	35	5.0	1
0.5	0.05	0.4	0.4	0.3	1.75	35	5.0	1
0.5	0.05	0.5	0.2	0.4	1.75	35	5.0	1
0.5	0.05	0.43	0.23	0.43	1.75	35	5.0	1
0.5	0.05	0.6	0.2	0.3	1.75	40	5.0	1
0.5	0.05	0.43	0.33	0.33	1.75	45	5.0	1
0.5	0.05	0.47	0.27	0.37	1.75	40	5.0	1
0.5	0.05	0.4	0.3	0.4	1.75	40	5.0	1
0.5	0.05	0.5	0.3	0.3	1.75	40	5.0	1
0.5	0.05	0.53	0.23	0.33	1.75	40	5.0	1

Note: BKC- Benzalkonium chloride, HPMC- Hypromellose

Physicochemical Characterization of Prepared Doxycycline *In-Situ* Gel Formulations

Description (Visual) and pH: Prepared formulations were evaluated visually for their clarity and appearance before and after conversion into gel form. pH of the formulations was determined by using calibrated pH meter.

Gelling time: The gelling time of the formulations were tested by adding a drop of the formulation (40µL) in to 2 ml of freshly prepared simulated tear fluid (pH 7.4) taken in test tube and equilibrated at room temperature (Kavitha and Rajas 2011). The composition of simulated tear fluid consists of sodium chloride 0.67g, sodium bicarbonate 0.2 g, calcium chloride dihydrate 0.008g, and purified water to 100mL. Gelling time was visually assessing the gel formation and noted down the time needed for gelation/gel formation. The experiment was carried out in triplicate.

Gelling capacity: The gelling capacities of the formulations were tested by adding a drop of the formulation (40µL) in to 5 ml of freshly prepared simulated tear fluid (pH 7.4) taken in Petri dish and equilibrated at room temperature (Liu *et al.,* 2006). The composition of simulated tear fluid consists of sodium chloride 0.67g, sodium bicarbonate 0.2 g, calcium chloride dihydrate 0.008g, and purified water to 100mL. Gel capacity was determined by visually

assessing the gel formation and noted down the time taken for gelation formation and the time taken in gel dissolution. The experiment was carried out in triplicate.

Gel strength: Took 25mL of prepared formulation into 50 mL glass beaker using measuring cylinder, added 7mL of freshly prepared simulated tear fluid (pH 7.4) into it and mix well using glass road then it will form a gel like mass (Harish *et al.*, 2009). Kept the gel for 30 minutes at room temperature covered with aluminum foil. Gel strength of above prepared gel was determined through texture profile analysis using Texture analyzer. Placed the gel in standard beaker below the probe, immersed the analytical probe in to the sample. The Texture Analyzer was set to the "gelling strength test" mode or compression mode with a test speed 1.0 mm/s. An acquisition rate of 50 points per seconds and a triggered force of 5g were selected. An aluminum probe of 7.6 cm diameter was used. The study was carried out at room temperature. The force required to penetrate the gel was measured as gel strength in terms of g.

Viscosity measurement: Viscosity of prepared formulation was determined using Brookfield viscometer with spindle no 62 at 10 – 100 rpm at temperature 37±0.5˚C by using method as reported previously (Shaikh *et al.*, 2021). Spindle was lowered perpendicularly into gel placed into a beaker taking care that spindle does not touch the bottom or side wall of beaker. Readings were noted after stabilization of displayed value.

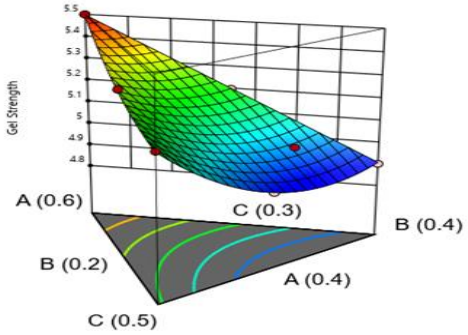
Osmolality: For ophthalmic preparation osmolality is the primary requirement to determination in order to avoid irritation and provide ocular tolerability. Osmolality of the formulations were tested using advanced instrument Osmometer (Model 3320). The experiment was carried out in triplicate.

Drug content: Determination of the content of Doxycycline Hyclate was carried out by external standard UV/Visible spectroscopy under the operation condition like wavelength as 276 nm and in this method purified water was used as solvent (Morsi *et al.*, 2017).

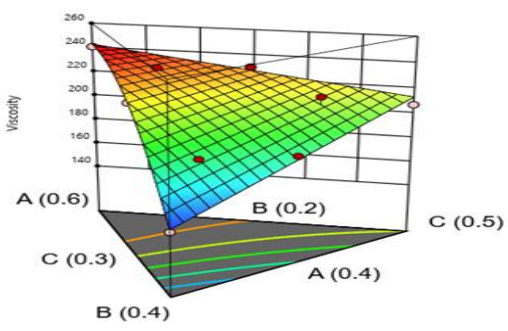
***In vitro* release of Doxycycline Hyclate from in situ gel:** *In-vitro* release study of *in-situ* gel solution was carried out by using Franz diffusion cell. The formulation is placed in donor compartment and freshly prepare simulate tear fluid in receptor compartment. Between receptor and donor compartment dialysis membrane was placed (0.22µm pore size). The whole assembly was placed on thermostatically controlled magnetic stirrer. The temperature of the medium maintained at 37˚C±0.5˚C. 1mL sample was withdrawn at predefined time interval of 5 minutes for 45 minutes, the sample volume of fresh medium was replaced. The withdrawn samples were dilute to 10mL in volumetric flask with respective solvent and analyzed by UV spectrophotometer at respective nm using reagent black. The drug content calculated using an equation generated from standard calibration curve. The percentage cumulative drug release (% CDR) calculated. The obtained data is further subjected to curve fitting for drug release data.

Antimicrobial efficacy testing: Possible antimicrobial effects of formulation were performed by method (Samadi *et al.*, 2008) proposed by USP using Fluid Casein Digest-Soy Lecithin – Polysorbate 20 media. The final *in-situ* gel formulation was subjected to the USP, preservative challenge test using *Escherichia coli*, *Pseudomonas aeuroginosa*, *staphylococcus aureus*, *Candida albicans* and *Aspergillus niger* as a test organism. To determine the microbial killing rate, the *in-situ* formulation was inoculated with challenging microorganism at a final concentration of 10^5 – 10^6 CFU/mL and the viable organisms were determined 30, 90 & 180 minutes after inoculation for bacteria and 24hrs for fungi. Aerobic viable cell count of 1:10 dilution of product in neutralizer was determined by plate count method and 0.5 log increase in colony forming units was accounted for variability.

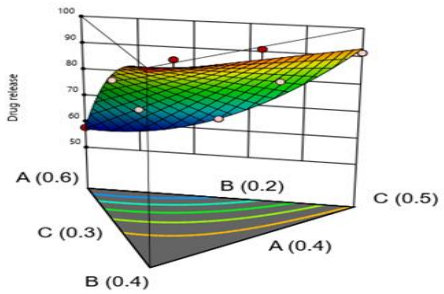
Results Optimization of proportion of polymers The QbD oriented approach was systematically implemented by using Simplex Lattice design, Design Expert 12.0 software. Simplex Mixture design was implemented for optimization of polymer concentration and statistical evaluation was done by using ANOVA Linear regression model. Numerical & Graphical optimization with the help of graph (Fig. 1) was carried to achieve the desire goals for response variables. The whole experimental domain was investigated for desirability function near or equals to 1 as an indicator for best fit model with desired composition.



A) Response: Gel strength 3D surface plot.



B) Response: Viscosity 3D surface plot.



C) Response: % Drug release 3D surface.

Fig. 1. 3D surface plot A, B, & C, showing the responses of polymer concentration on Gel strength, Viscosity and % drug release.

Characterization of optimized in situ gel formulation. *In-situ* gels were prepared in order to establish an optimal concentration necessary to develop a formulation with desired gelation properties. Selected *in-situ* gel bases were further subjected to rheological studies to establish the optimal in situ gel composition. Based on Design Experiment runs outcomes most desirable concentration of polymer i.e., runs R1, R2, R6 & R8 were selected for further trials with Doxycycline Hyclate.

Description (Visual) and pH: Selected in situ gel formulations were visually evaluated and found pale yellow colored, transparent and free flowing liquid at room temperature. The pH value was within the physiologically tolerable range between 6.5 to 7.5.

Gelling time: Gelling time of in situ gel formulations was evaluated and the results are presented in Table 2. From table it evident that, by increasing the gellan gum concentration the gelling time decreases due to greater number of ions interacting with gum and form the gel structure for shorter time. Out of four trial formulation R6 form gel in 22 seconds and the uniformity of gel was also smooth and remains for a minute which was comparable to market sample.

Table 2: Composition and gel properties of *In-situ* gel formulations.

Formulation	A: Gellan Gum (%)	B: Povidone K30 (%)	C: HPMC (%)	pH	Temperature	Gelation time (sec.) visually observe (avg. value)	Gel capacity
R1	0.4	0.2	0.5	7.2	RT	28	+++
R2	0.4	0.4	0.3	7.19	RT	38	++
R6	0.43	0.33	0.33	7.2	RT	22	+++
R8	0.4	0.3	0.4	7.21	RT	38	++

RT- Room temperature, (-) no gel formulation, (+) gel formulation takes time and disappear shortly, (++) Instant gel formation and thick, (+++) instant gel formation, thin uniform and remain for minutes.

Gelling capacity: Gelling capacity of formulations were visually evaluated results are mentioned in table-2, from the results it was observed that formulation R1 and R6 shown good gel capacity out of that R6 gelling capacity was comparatively better, uniform in nature and remain for a minutes and comparable to market sample. Whereas, R1 gels was disappeared very fast as compared to R6.

Gel strength: Gel strength is an important physicochemical parameter for in situ forming ophthalmic gel because it prevents the formulation from rapid drainage and hence increase the residence time. Gel strength of in situ formulations were evaluated and presented in Table 3, with increase of concentration of polymer concentration, gel strength increases. All the four trial results show satisfactory results and were comparable to in situ gel available in market.

Viscosity measurement: Viscosity is the most important factor evaluating the successfulness of in situ gelling system. For easy administration in the eye, the formulation must have optimum viscosity and for a prolong residence time, it should undergo rapid sol to gel transition upon instillation in the eye. Since, the ocular shear rate is very large, ranging from 0.03 s^{-1} during interlinking periods to 4250 – 28,500 s^{-1} during blinking, the formulations with pseudo plastic rheological characteristics are usually preferred for ocular delivery. The viscosity results of trials mentioned in Table 3 were found satisfactory and comparable to *in-situ* gel market sample.

Table 3: Gel strength of optimized in situ formulations.

Formulation	A: Gellan Gum (%)	B: Povidone K30 (%)	C: HPMC (%)	pH	Temperature	Gel Strength (g)	Viscosity (cP)	Drug release (%)
R1	0.4	0.2	0.5	7.2	37±0.5˚C	5.2	206	91.2
R2	0.4	0.4	0.3	7.19	37±0.5˚C	4.9	150	95.4
R6	0.43	0.33	0.33	7.2	37±0.5˚C	5	187	97.1
R8	0.4	0.3	0.4	7.21	37±0.5˚C	4.9	185	99.1

Osmolality: Osmolality is the most important physicochemical parameter and to maintain the structural integrity of eye, the osmolality of an ophthalmic formulation should be in the range of 270 to 340 mOsm/kg. In situ formulations were evaluated for osmolality and results were found within 260-310 mOsm/kg.

Drug content: The percent drug content for all formulations was found to be satisfactory in the range of 95–105% (data not shown).

***In vitro* release of Doxycycline Hyclate from in situ gel:** In vitro release studies comprise an essential element in the process of elaboration of novel delivery systems since drug release rate and the release patterns are important factor to be assessed as a prerequisite to achieve optimal delivery characteristics in vivo. The release of Doxycycline hyclate from selected in situ formulation R1, R2, R6 & R8 and obtained result presented in Fig. 2 Approx. 90% of drug release observed in 30 minutes.

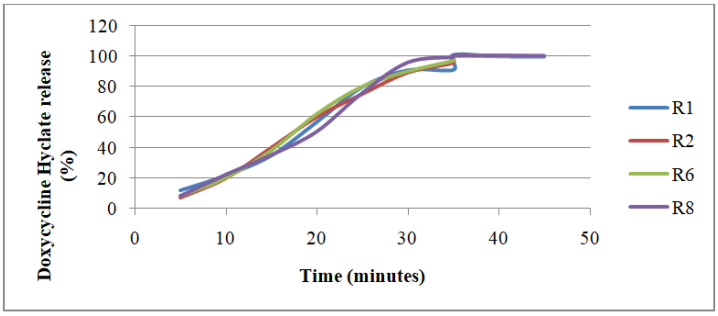


Fig. 2. *In-vitro* drug release plot of Doxycycline Hyclate.

Table 4: Antimicrobial study.

Organisms	Initial count CFU/mL	At 7th day CFU/mL	At 14th day CFU/mL	At 28th day CFU/mL
E. coli	12×10^6	04×10^5	03×10^4	08×10^2
S. aureus	05×10^6	07×10^5	08×10^4	04×10^2
P. aeruginosa	09×10^6	03×10^5	07×10^4	06×10^2
C. albicans	08×10^6	02×10^5	06×10^4	09×10^3
A. niger	04×10^6	06×10^5	05×10^2	Nil

DISCUSSION

In-situ gels were prepared in order to establish an optimal concentration necessary to develop a formulation with desired gelation properties. Selected *in-situ* gel bases were further subjected to rheological studies to establish the optimal in situ gel composition. The QbD oriented approach was systematically implemented by using Simplex Lattice design, Design Expert 12.0 software. Simplex Mixture design was implemented for optimization of polymer concentration and statistical evaluation was done by using ANOVA Linear regression model. The whole experimental domain was investigated for desirability function near or equal to 1 as an indicator for best fit model with desired composition.

CONCLUSIONS

In the present study, ion responsive *in-situ* gel forming solution containing doxycycline Hyclate was prepared and evaluated for antimicrobial activity. Gellan gum in a concentration of 6mg/mL (0.6% *w/w*) showed optimum gelation when mix with simulated tear fluid (STF). Benzalkonium chloride used as a preservative which showed good preservative efficacy at a concentration of 0.05mg/mL (0.005% w/w). In situ gel forming ophthalmic solution available in market containing Timolol was used to compare the other physicochemical parameters like gel strength, gelling time, viscosity, gelling capacity with prepared *in-situ* gel forming solution out of that formulation R6 found satisfactory and comparable.

FUTURE SCOPE

In-situ novel drug delivery system containing Doxycycline was developed which can be provide unmet need for ocular drug delivery.

Conflict of Interest. None.

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