ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 08, Issue, 02, pp.19028-19035, February, 2018

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

FORMULATION AND EVALUATION OF PH SENSITIVE *IN SITU* OCULAR GEL OF DOXYCYCLINE HYCLATE

Suresh Chand, *Diksha Sharma, Abhishek Sharma, Shweta Agarwal, Sharma, R.B. and Hem Raj Vashist

LR Institute of Pharmacy Jabli Kyar Oachhghat Solan HP-173223, India

ARTICLE INFO

Article History:
Received 14th November, 2017
Received in revised form
18th December, 2017
Accepted 23rd January, 2018
Published online 28th February, 2018

Key Words:
Ophthalmic drug,
Virulent diseases,
Therapeutic effect,
Ph sensitive, in situ,
ocular gel, Doxycycline hyclate.

ABSTRACT

Several new preparations have been developed for ophthalmic use, not only to prolong the Ophthalmic drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. The challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage. The primitive ophthalmic solutions, suspensions and ointment dosage forms are clearly no longer sufficient to combat some present virulent diseases. Due to tear drainage, most of the administered dose passes via the naso-lacrimal duct into the gastro intestinal tract (GIT), leading to side effects. Rapid elimination of the eye drops administered often results in a short duration of the therapeutic effect making a frequent dosing regimen necessary. To overcome such drawbacks of conventional ophthalmic drug delivery the present work is focused on Formulation and evaluation of Ph sensitive in situ ocular gel of deoxycycline hyclate.

Citation: Suresh Chand, Diksha Sharma, Abhishek Sharma, 2018. "Formulation and Evaluation of Ph Sensitive In Situ Ocular Gel of Doxycycline Hyclate", *International Journal of Development Research*, 8, (02), 19028-19035

INTRODUCTION

Ophthalmic drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. The challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage. The primitive ophthalmic solutions, suspensions and ointment dosage forms are clearly no longer sufficient to combat some present virulent diseases. Due to tear drainage, most of the administered dose passes via the naso-lacrimal duct into the gastro intestinal tract (GIT), leading to side effects. Rapid elimination of the eye drops administered often results in a short duration of the therapeutic effect making a frequent dosing regimen necessary. Ocular therapy would be significantly improved if the precorneal residence time of drugs could be increased. Several new preparations have been developed for ophthalmic use, not only to prolong the contact time of the vehicle on the ocular surface, but also to slow down drug elimination. However, these preparations have some disadvantages such as poor compliance, especially by elderly people and many patients sometimes lose the device without noticing it. From the point of view of patient acceptability, a liquid dosage form is preferable 42 (Aparna, *et al*., 2011). Although various formulation exists in market for ocular drug delivery but are not able to provide highest bioavailability related to administered dose. Whenever an ophthalmic drug is applied through a conventional dosage form to the anterior segment of the eye, only small amount (5%) actually penetrates the cornea and reaches the interior tissue of the eyes. Factors that affects drug bioavailability includes rapid solution drainage by gravity, induced lachrymation, blinking reflex, normal tear turnover, superficial absorption of drug into the conjunctiva and sclera, rapid removal by the peripheral blood flow and low corneal permeability (act as lipid barrier). The progress has been made in gel technology for the development of droppable gel. They are liquid upon instillation and undergo phase transition in the ocular cul-de-sac to form visco-elastic gel and this provides a response to environmental changes 43 (Mitali *et al*., 2012). Conventional liquid formulations demonstrate low bioavailability because of a constant lacrimal drainage in the eye. The normal drainage of an instilled drug dose commence immediately upon instillation and is especially

***Corresponding author:* Diksha Sharma**
LR Institute of Pharmacy Jabli Kyar Oachhghat Solan HP-173223, India.

completed within 5min. Typically ophthalmic bioavailabilities of only 1-10% are achieved due to short precorneal residence time of ophthalmic solutions. Consequently, there is a need for frequent instillation of concentrated solutions to achieve the desired therapeutic effect. Systemic absorption of the drug drained through the nasolacrimal duct may result in some undesirable side effects. To overcome these problems ophthalmic in situ gels have been investigated in an attempt to extend the ocular residence time of medications for topical application to the eye 44 (Swati and Suresh, 2010). In situ gel formation occurs due to one or combination of different stimuli like pH change, temperature modulation and solvent exchange. Smart polymeric systems represent promising means of delivering the drugs. These polymers undergo sol gel transition, once administered. From the early 1970's natural and synthetic polymers began to be investigated for controlled release formulation. The advantages of using biodegradable polymers in clinical applications are apparent. Various natural and synthetic polymers are used for formulation development of in situ common drug delivery systems 45 (Lalitkumar *et al*., 2011).

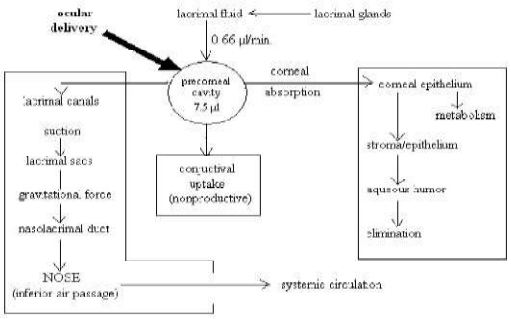

Figure 2. Routes of ocular absorption of drugs

The unique anatomy, physiology and biochemistry of eye offer many challenges to developing effective ophthalmic drug delivery systems. Topical delivery into cul-de-sac is, by far the most common route of ocular drug delivery (Bhoyar *et al*., 2011). Doxycycline is a broad spectrum tetracycline antibiotic that chelates metal ions and is frequently used as part of the treatment of ocular surface diseases. Its therapeutic value has been ascribed to an ability to inhibit matrix metalloproteinase (MMP) activity and both MMP and IL-1 synthesis (Smith and Cook, 2004). Doxycycline undergoes hepatic metabolism and its half-life is 18-22 hrs. It is an amphoteric compound with three pKa values. At 20˚C, pKa values of doxycycline are reported as 3.5(tricarbonyl system), 7.7 (ketophenolic system) and 9.5 (dimethyl ammonium group) (Jantratid *et al*., 2010). In the present study attempt is made to develop in situ ocular gel of doxycycline for better treatment of ocular surface diseases with reduced adverse effects, better patient compliance and also to increase ocular contact time, to enhance the corneal permeability and site specificity (Mitali *et al*., 2012).The doxycycline hyclate is a broad-spectrum antibiotic oxytetracycline synthetic derivative used in several countries. It has been used to treat infectious diseases and as an additive in animal nutrition to facilitate growth (Ana, 2012)

MATERIALS AND METHOD

Preformulation studies

Description: Drug was observed for its general appearance.

Table No.2. List of chemicals used in experimental work

Sr. No.	Instruments	Company
1	Electronic weighing balance	Sartorious
2	Magnetic stirrer	Globe Instruments
3	Mechanical stirrer	Perfit Instruments
4	Viscometer	Brookfilled
5	UV Spectrophotometer	Jasco V-630
6	Melting point apparatus	Perfit Instruments
7	Digital pH meter	Globe Instruments
8	Water bath	Perfit Instruments

Table 3 List of instruments utilized in experimental work

Sr. No.	Name of Chemicals	Supplier
1	Doxycyclin hyclate	Gift sample from Aurochem Pharmaceuticals (I) Pvt. Ltd.
2	Sodium alginate	Gift sample from Aurochem Pharmaceuticals (I) Pvt. Ltd
3	Hydroxypropylmethyl cellulose (HPMC)	SPB Laboratories
4	Carbopol 940	SPB Laboratories
5	Methanol	Avantor performace materials (I) ltd.
6	Sodium chloride	Avantor performace materials (I) ltd.
7	Sodium bicarbonate	Central drug house (P) ltd
8	Calcium chloride dehydrate	Central drug house (P) ltd
9	Sodium hydroxide	Avantor performace materials (I) ltd.

Melting point determination: Melting point of the drug was analyzed using digital melting point apparatus and observed value was compared with reported melting point (Nair *et al*., 2014).

Solubility analysis: The solubility of Doxycyclin hyclate was determined in different solvents like in distilled water, methanol, and simulated tear fluid having pH 7.4. The samples were added to each test tube containing 5 mL of different solvents with continuous shaking for 30 min to prepare saturated solution. All mixtures were allowed to equilibrate at room temperature (37 ^{o}C) for 24 h. The samples were filtered through what man filter and aliquots were suitably diluted and assayed spectroscopic ally at 270 nm. Each value for solubility was deermined in triplicate and average values were reported (Peng *et al*., 2008). The drug excipient compatibility study was determined by FTIR (Fourier Transformer Infrared Spectroscopy) using KBR pellets of 0.1 mm. The IR spectra of the pure drug (Doxycyclin hyclate) is compared with IR spectrum of combination of Doxycyclin hyclate and all the excipients to check the interaction (Kugular *et al*., 2010).

Differential scanning Calorimetry (DSC)

DSC used to examine the thermal behavior (melting point) of drug and compatibility between the drug and excipients also evaluated by it. Differential Scanning Calorimetry (DSC) measurements were carried out with a type of Perkin-Elmer DSC7, USA Instrument. The instrument was calibrated using Indium as standard. Samples (2 mg) were placed in sealed aluminium pans and heated from 25 ^{o}C to 250 ^{o}C at a rate of 10 ^{o}C/min. under nitrogen atmosphere (60 mL/min), with empty pan as reference (Sahoo *et al*., 2011; Rajkumar and Bhise, 2008; Mohima *et al*., 2015).

λmax scanning

A solution of drug was prepared in the Simulated tear fluid (pH 7.4) λmax was determined by scanning the above solution between 200-400 nm, using UV spectrophotometer (Nair *et al*., 2014).

Table No.4. Composition of In situ ocular gel

Sr. No.	Compositions	Concentration (% w/w)							
		F1	F2	F3	F4	F5	F6	F7	F8
1	Doxycycline Hyclate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
2	HPMC E50LV	0.2	0.3	0.4	0.5	0.2	0.3	0.4	0.5
3	Carbopol-940	0.1	0.1	0.1	0.1	-	-	-	-
4	Sodium alginate	-	-	-	-	0.5	0.5	0.5	0.5
5	Sodium chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
6	Sodium Hydroxide	Qs	Qs	qs	Qs	Qs	qs	qs	qs
7	Purified water	q.s. to 100 g	q.s. to 100 g	q.s. to 100 g	q.s. to 100 g	q.s. to 100 g	q.s. to 100 g	q.s. to 100 g	q.s. to 100 g

Establishment of calibration plot

Preparation of Stimulated tear fluid (pH 7.4)

Sodium chloride 0.67 g, sodium bicarbonate 0.20 g,calcium chloride dehydrate 0.008 g in distilled water q.s. to 100 ml [66] (Hong and Sung, 2000).

Preparation of stock solution

Stock solutions of drug samples were prepared in the simulated tear fluid (STF). 10 mg of Doxycycline Hyclate was accurately weighed and transferred to a 100 ml volumetric flask volume was make up the volume upto 100 ml with Simulated tear fluid pH 7.4 (i.e. 100 µg/mL solution was obtained). From this solution, aliquots of 5 mL, 10 mL, 15 mL, 20 mL and 25 mL were taken and diluted up to 100 mL in order to get the concentration 5,10,15, 20 & 25 µg/mL. These concentrations were used to determine absorbance at λ_{max} 270 nm against blank using UV-VIS spectrophotometer.

Method of preparation

The in situ gel formulation was prepared by changing the concentration and using different polymers. Different concentrations of polymers were used to prepare ophthalmic solutions as per the composition shown in Table 4. Accurately weighed HPMC was dispersed in 50ml of purified water, carbopol 940 was sprinkled over this solution and allowed to hydrate overnight. The solution was stirred with an overhead stirrer and Sodium hydroxide were dissolved in the solution. Doxycyclin hyclate was dissolved in small quantity of purified water. The drug solution was added to the polymer solution. Purified water was then added to make up the volume to 100ml and the prepared formulations were sterilized in an autoclave at 121°C for 20 min (Deshpande, 2013).

Formulation study

Determination of visual appearance, clarity, pH and drug content:

The appearance and clarity were determined visually. The pH of the formulations was adjusted by using pH meter. The drug content of in situ gel was determined by taking sample (2ml) of in-situ gel in a 100ml volumetric flask and diluted with simulated tear fluid of pH 7.4 to get the concentration of 10g/ml (approximately).Then the absorbance was measured at max (281nm) using UV-spectrophotometer to calculate the percentage of drug content (Eaga *et al*., 2009)

Gelling capacity

Determination of in-vitro gelling capacity was done by visual method. Colored solutions (1% Congo Red solution in water) of in-situ gel forming drug deli- very system were prepared. The in-vitro gelling capacity of prepared formulations was measured by placing 5ml of the gelation solution (pH 7.2 buffer) in glass test tube and maintained at 37±1°C temperature. Oneml of colored formulation solution was added with the help of pipette. As the solution comes in contact with gelation solution, it was immediately converted into stiff gel like structure. The gelling capacity of solution was evaluated on the basis of stiffness of formed gel and time period for which the formed gel remains. Color was added to give visualized appearance to formed gel (Kanoujia, 2012).

In Vitro drug release studies

In-vitro release studies were carried out using bichambered donor receiver compartment model (Franz diffusion cell) and this was placed on magnetic stirrer and temperature was adjusted to 37 ± 0.5 °C. Accurately measured 1 ml of the formulation spread uniformly on a dialysis membrane, which was in contact with receptor medium. The receptor medium was stirred continuously at 20 rpm to simulate blinking action of eyelids. Samples were withdrawn at periodic intervals and dilution was done with 10 ml of STF. The drug content was analyzed using UV Spectrophotometer at 270 nm against reference standard using simulated tear fluid as blank (Abdhul *et al*., 2011).

Viscosity

The relationship between contact time and the rheology was easily understood for viscosity enhanced ophthalmic solutions. It was noted from various literature that the formulations before gelling should have a viscosity of 5 to 1000 mPas and after gelling in the eye will have a viscosity from about 50-50,000 mPas. Rheological studies of the prepared formulations were carried out by Brookfield viscometer (DV-E) using spindle LV 62. The viscosity of the formulations were determined at different speed conditions (4,10,25,50,100 rpm) (Pandit *et al*., 2007).

Antimicrobial activity

Antimicrobial efficiency studies were carried out to ascertain the biological activity of sol-to-gel systems against microorganisms. This was determined in the agar diffusion medium employing “cup plate technique”. Sterile solution of marketed Lomefloxacin HCL eye drops was used as a standard. The standard solution and the developed formulations (test solution) were taken into separate cups bored into sterile Muller Hinton Agar (MHA) previously seeded with organisms (Staphylococcus aureus, E. Coli and Pseudomonas aeruginosa). After allowing diffusion of solutions for two hours, the plateswere incubated for 24 h at 37 °C. The zone of inhibition (ZOI) was compared with that of the standard (Naseem *et al*., 2003).

Sterility testing

IP method (1996) was followed for the sterility testing of eye drops. Sterility testing was carried out by incubating formulations for not less than 14 days at 30 to 35 ^{o}C in the fluid thioglycolate medium to find the growth of bacteria and at 20 to 25 ^{o}C in the soyabean-casein digest medium to find the growth of fungi in the formulations (Sindhu *et al*., 2009).

Accelerated stability studies

The ophthalmic formulations in amber colored vials were used for a short term accelerated stability studies by storing at 40 ±2 ^{o}C and 75±5% RH as per modified ICH guidelines. Samples were periodically evaluated for appearance, pH, gelling capacity and drug content during the study period (Satishkumar *et al*., 2008).

RESULT AND DISCUSSION

Preformulation studies

Every drug has intrinsic chemical and physical properties which has been consider before development of pharmaceutical formulation. This property provides the framework for drug's combination with ingredients in the fabrication of dosage form. Preformulation studies serve as an important establishment tool early in the development of both API and drug products. They generally influence:

- Selection of drug
- Selection of formulation components
- Drug development manufacturing process and
- Development of analytical methods

Description of drug

Description of Doxycyclin Hyclate found to be as per USP monograph. The Organoleptic properties of Doxycyclin Hyclate were found to be in the given table below (6.1).

Table No.5. Organoleptic properties of Doxycyclin hyclate

Drug	Test	Observation
Doxycyclin Hyclate	Color	Hygroscopic yellow crystalline powder
	Odor	Odorless
	Taste	Slightly bitter

Melting point determination

Melting point of Doxycyclin Hyclate was determined by melting apparatus and it was found to be 200 ^{o}C which is of the pure drug. Hence drug sample was free from any type of impurities.

Table No.6. Melting point of Doxycyclin hyclate by visual melting apparatus

Experimental value	Literature value
200 ºC	198-201˚C

Solubility study

Solubility was determined in different solvents systems such as distilled water, Methanol and simulated tear fluid pH 7.4 at 37˚ C.

Table No.7. Solubility data of Doxycyclin hyclate in different solvents

Sr. No.	Solvent	Solubility
1.	Distilled water	Soluble
2.	Methanol	Freely Soluble
3.	Simulated tear fluid	Soluble

FTIR

FTIR spectra of the drug with each excipient were recorded using fourier transform infrared spectrometer. Pellets of the drug sample in KBr were prepared on KBr press. The spectrum was scanned over a wave number range of 2000 cm^{-1} to 1 cm^{-1}.

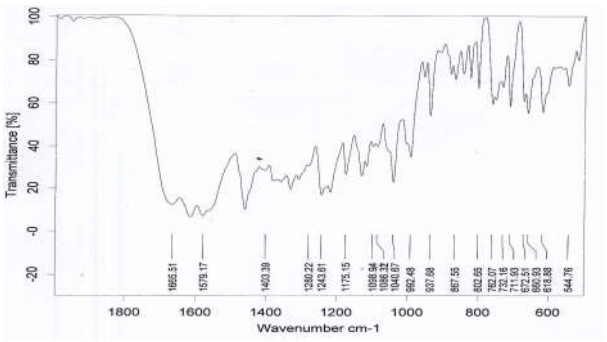

Figure 3. FTIR Plot of Doxycyclin Hyclate

The obtained spectra revealed that there was no interaction between the drug and the excipients.FTIR spectral studies: Doxycyclin Hyclate showed characteristics sharp peak FTIR spectra of Doxycyclin Hyclate with Carbopol 940 showed various characteristic peaks of Carbopol 940 without any significant shifting or deviation in characteristic peaks of drug. FTIR spectra of Doxycyclin Hyclate in combination with Carbopol 940 and hydroxypropyl methylcellulose(HPMC) showed various characteristic peaks of hydroxypropyl methylcellulose without any significant shifting or deviation in characteristic peaks of drug.

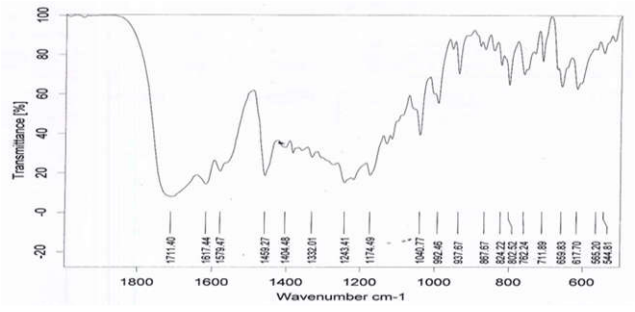

Figure 4. FTIR plot of Doxycyclin Hyclate + Carbopol 940

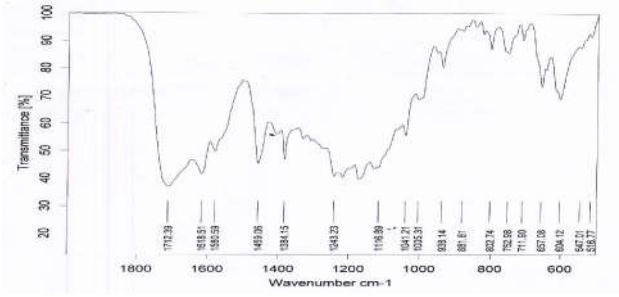

Figure 5. FTIR plot of Doxycyclin Hyclate + Carbopol 940 + HPMC

FTIR spectra of Doxycyclin Hyclate in combination with Carbopol 940 and Sodium alginate showed various characteristic peaks of Sodium alginate without any significant shifting or deviate on in characteristic peaks of drug.

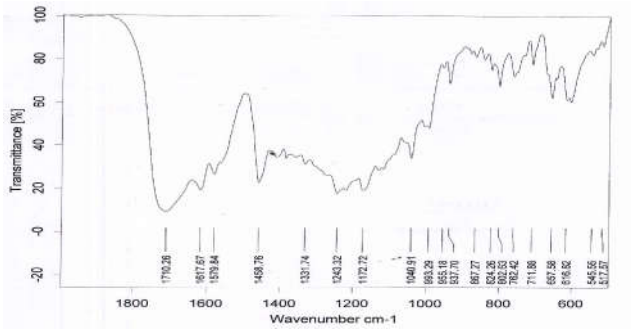

Figure 6. FTIR plot of Doxycyclin Hyclate +Carbopol 940 + Sodium alginate

Differential scanning calorimetry (DSC)

DSC thermogram (figure 6.5) of Doxycyclin Hyclate shows broad endothermic peak at 200.14℃, which is its melting point as Doxycyclin melt with decomposition over the range 198-201℃.

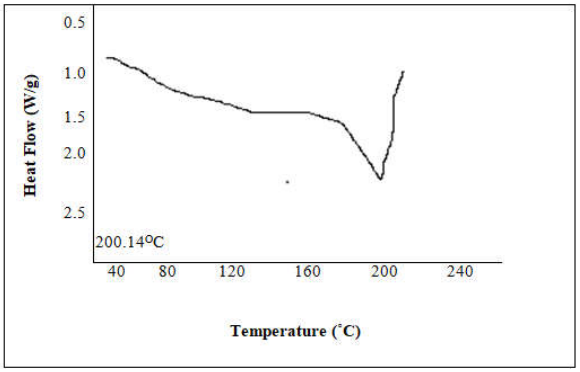

Figure 7. DSC Thermogram of Doxycyclin hyclate

λmax scanning

λmax scanning helps in identifying the drug's purity. The scanned graph was according to reported literature and hence confirmed that the obtained drug sample was Doxycyclin hyclate. λmax value of Doxycyclin hyclate was found to be 270 nm.

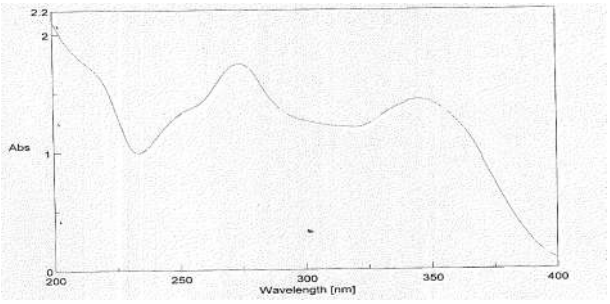

Figure 8. λmax scan for Doxycyclin hyclate

Calibration curve of Doxycyclin hyclate

The calibration data as shown in table 6, was collected according to the method mentioned in 5.2.2 and then the regression analysis was applied. The Beer's law was found to obey in the range of 5-25 µg/mL as revealed in figure 9. The obtained R^2 value is high (0.990), close to 1. From this study, it was concluded that there was a good correlation between the experimental and theoretical values.

Table No.8. Calibration data of Doxycyclin hyclate in 7.4 pH simulated tear fluid

S. No.	Concentration (µg/mL)	Absorbance
1	5	0.0886
2	10	0.1846
3	15	0.3142
4	20	0.3722
5	25	0.4839

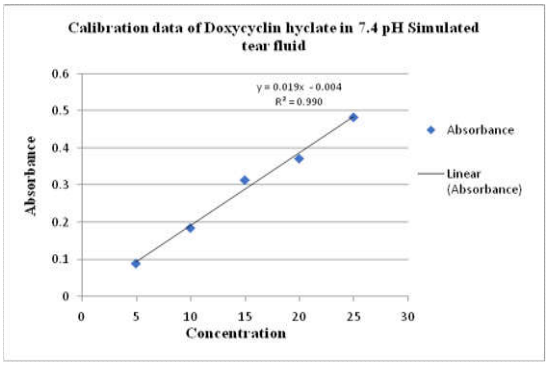

Figure 9. Calibration curve of Doxycyclin hyclate in pH 7.4 simulated tear fluid

The obtained R^2 value is high (0.996), close to 1. From this study, it was concluded that there was a good correlation between the experimental and theoretical values. Various calibration curves were prepared in different solvents for the determination of solubility of drug in a particular solvent. Calibration curve is also utilized for the determi- nation of unknown concentration of drug sample.

Table No.9. Calibration data of Doxycyclin hyclate in Water

S. No.	Concentration (µg/mL)	Absorbance
1	5	0.0724
2	10	0.1524
3	15	0.2671
4	20	0.3649
5	25	0.4472

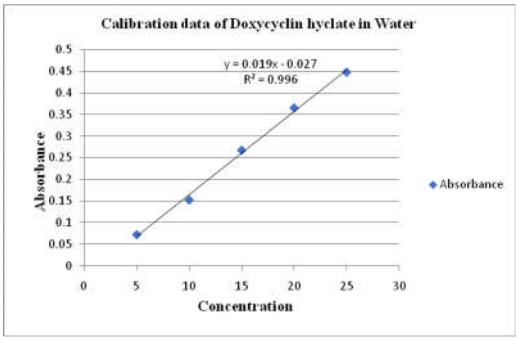

Figure 10. Calibration curve of Doxycyclin hyclate in Water

Evaluation of drug loaded gel

Appearance, clarity, pH and drug content

The clarity of all the formulations was found to be satisfactory, as shown in Table 2.

Table No.10. Clarity and visual appearance

Evaluation Steps	FI	F2	F3	F4	F5	F6	F7	F8
Visual Appearance	Light yellow Solution	Light yellow Solution	Light yellow Solution	Light yellow Solution	Light yellow Solution	Light yellow Solution	Light yellow Solution	Light yellow Solution
Clarity	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear

Table No.11 : pH & the percent drug content of insitu ocular solution (F1-F8)

Evaluation Steps	FI	F2	F3	F4	F5	F6	F7	F8
pH	5.01	5.06	5.11	4.99	5.00	5.06	5.00	5.05
% drug content	92.68	98.20	96.47	95.01	95.50	96.48	97.77	95.47

Moist heat sterilization by autoclaving had no effect on the clarity and other physicochemical properties of the formulations. The haziness that was observed after autoclaving (due to precipitation of HPMC at elevated temperature) was found to disapp-ear, the original clarity was regained after overnight standing. The pH of the formulations was found to be satisfactory and was in the range of 4.99 to 5.11 as shown in Table 2. The formulations were liquid at room temperature and at the pH formulated. Terminal sterilization by autoclaving had no effect on the pH. The drug content of the ophthalmic formulations of Doxycyclin in situ gel was found satisfactory (ranging between 92.68%-98.20%), indicating uniform distribution of the drug.

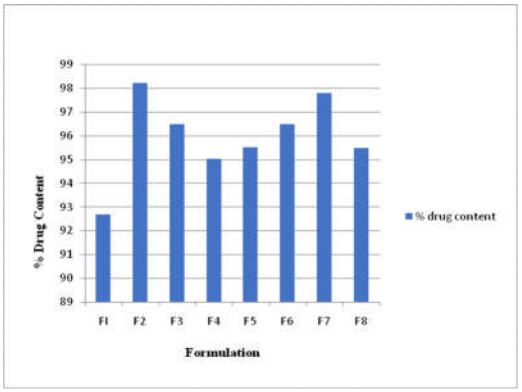

Figure 11. Percentage drug content of formulations (F1-F8)

Gelling capacity

The formulation should have an optimum viscosity, which would allow easy instillation into the eye as a liquid (drops), but would also allow the formulation to undergo rapid sol-to-gel transition. Additionally, to facilitate sustained release of the 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 11 shows the gelling capacity of all formulations, which is depicted as + (gel forms in 60 seconds and dissolves rapidly), ++ (gel forms within 60 seconds and remains stable for 3 hours) and +++ (gel forms within 60 seconds and remains for 6 hours). The gelling capacity increases with increasing concentration of gelling agent. Table 11 shows the gelling capacity of formulations F1 to F8. All the formulations, except F1, and F5, showed instantaneous gelation when contacted with artificial simulated tear fluid (STF). However, the nature of the gel formed depended on the concentration of the polymers used. The formation of instantaneous gels can be attributed to the buffering capacity of the simulated tear fluid. Formulation F1 & F5 showed gel formation within 60 seconds, which dissolved rapidly.

Formulations F2, F3 & F6 showed immediate gelation within 60 seconds and remained stable for a few hours, whereas formulations F4, F7 and F8 showed immediate gelation within 60 seconds and remained stable for an extended period.

Table No.12. Gelling Capacity of prepared In situ gel

Sr. No.	Formulation Code	Gelling Capacity
01	F1	+
02	F2	++
03	F3	++
04	F4	+++
05	F5	+
06	F6	++
07	F7	+++
08	F8	+++

+ gelation within 50-60 seconds, dissolves rapidly,
++ gelation within 60 seconds and remains stable for 3 hours,
+++ gelation within 60 seconds and remains stable for 6 hours

Viscosity

Viscoelastic fluid with a viscosity that is high under conditions of low shear rate and low under conditions of high shear rates are preferred. In order to evaluate the rheological behavior, the viscosity of the formulation before and after addition of simulated lacrimal fluid was evaluated by a Brookfield viscometer, using increased shear stress and varying the angular velocities or shear rate. All the selected formulations were shear thinning, exhibiting pseudo plastic behavior. All the formulations were liquid at room temperature and underwent rapid gelation upon contact with simulated tear fluid, as shown in Table 12. The results obtained from the rheological study of the prepared in situ gelling system (F1-F8) revealed that the viscosity decreased as the angular velocity or shear rate increased. The viscosity of formulations F1-F8 ranged from 12-467 cps at room temperature 25 °C. The viscosity of the formulations ranged from 91-2504 cps at 37 °C in gel. The rheological profile of the prepared in situ gelling systems of Doxycyclin hyclate before and after gelation is shown in Table no.12. To assess the rheological behavior, the viscosity of the formulation (F1- F8), before and after addition of simulated tear fluid, was evaluated using a Brookfield viscometer (Spindle no. 62), varying the angular velocities.

In vitro release studies

The in-vitro Doxycyclin hyclate release studies were carried out for various insitu ocular gel containing Doxycyclin hyclate in simulated tear fluid (pH 7.4) for 8 h.

Antimicrobial activity

The Zone of Inhibition was better with Staphylococcus aureus (gram positive micro organism) when compared to Pseudomonas aeruginosa for the formulations and Marketed

Table No. 13. Viscosity of prepared In situ gel

RPM	Stage	FI	F2	F3	F4	F5	F6	F7	F8
4	Before Gelation	78	148	222	412	81	172	310	467
	After Gelation	249	774	1237	1952	296	853	1487	2078
10	Before Gelation	61	129	184	378	64	153	275	428
	After Gelation	218	430	1013	1753	254	695	1259	1833
25	Before Gelation	48	98	157	314	51	124	230	340
	After Gelation	197	397	874	1372	203	482	967	1425
50	Before Gelation	31	66	106	227	36	81	152	249
	After Gelation	162	276	414	809	152	318	573	904
100	Before Gelation	13	34	68	132	15	43	86	143
	After Gelation	110	189	242	523	121	197	268	556

Table No.14. *In vitro* release studies of formulation (F1 to F8)

Time (h)	F1	F2	F3	F4	F5	F6	F7	F8
1	11.15	12.25	14.51	14.93	11.52	13.17	12.79	14.15
2	21.52	22.41	23.57	22.97	21.69	23.55	22.57	23.74
3	34.14	33.85	34.68	35.47	33.77	33.01	34.59	34.15
4	48.67	49.57	51.38	54.19	47.47	48.77	51.27	52.41
5	62.38	63.41	62.93	63.47	61.52	63.85	65.88	64.33
6	73.15	74.52	75.28	74.85	71.42	73.43	75.66	75.11
7	81.08	81.67	83.19	84.93	80.82	82.14	85.48	85.88
8	92.52	92.07	94.41	95.89	91.44	93.14	94.44	95.51

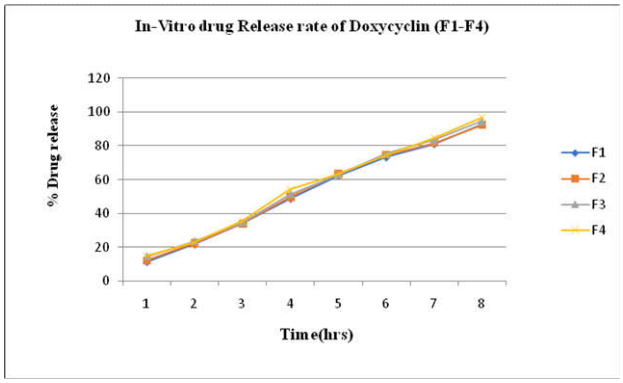

Figure 12. In-Vitro drug Release rate of Doxycyclin (F1-F4)

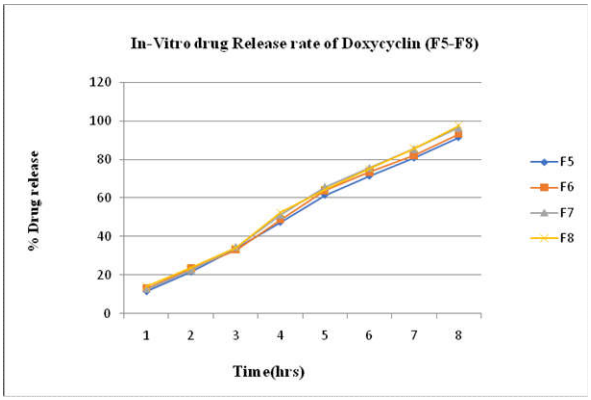

Figure 13. In-Vitro drug Release rate of Doxycyclin (F5-F8)

product. The zone of inhibition of marketed and prepared formulations was found to be almost similar. The present study results indicate that doxycycline hyclate retained its antimicrobial efficacy when formulated as an in situ gelling system.

Sterility test

All the formulation passed the test for sterility hence no evidence of microbial growth when incubated for not less than 14 days at 30-35ºC in case of fluid thioglycolate medium and at 20- 25 ºC in the case of soyabean casein digest medium.

Accelerated stability studies

From the results it has been observed that the formulations showed no change in appearance, clarity and pH. Further it was observed that the gelling capacity of the formulations was least affected.

Conclusion

The novel ophthalmic pH-monitered *in situ* gelling drug delivery was successfully formulated by using carbopol 940 and HPMC. The formulated *in situ* gelling systems were characterized for appearance, clarity, pH, gelling capacity, rheological character, in vitro release in simulated tear fluid, Antimicrobial activity, Sterility testing & Accelerated stability study. The formulation was liquid at the formulated pH (5.0) and underwent rapid gelation upon raising the pH to 7.4. The pH-triggered *in situ* gelling system showed sustained drug release over 8-h period of time. So, this formulation is an alternate to conventional eye drops to improve the bioavailability through its longer precorneal residence time and ability to sustain drug release. The patient compliance may be improved due to the decrease in frequency of drug administration.

Summary

Ophthalmic drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. The challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage. Ocular therapy would be significantly improved if the precorneal residence time of drugs could be increased. Systemic absorption of the drug drained through the nasolacrimal duct may result in some undesirable side effects. To overcome these problems ophthalmic in situ gels have been investigated in an attempt to extend the ocular residence time of medications for topical application to the eye. The doxycycline hyclate is a broad-spectrum antibiotic oxytetracycline synthetic derivative used in several countries.

It has been used to treat infectious diseases and as an additive in animal nutrition to facilitate growth. Doxycyclin inhibit bacterial protein synthesis through their link to the bacterial 30S ribosome, impeding access of aminoacyl-tRNA acceptor site in the mRNA-ribosome complex. Its therapeutic value has been ascribed to an ability to inhibit matrix metalloproteinase (MMP) activity and both MMP and IL-1 synthesis[41]. Doxycycline undergoes hepatic metabolism and its half-life is 18-22 hrs. It is an amphoteric compound with three pKa values. At 20˚C, pKa values of doxycycline are reported as 3.5(tricarbonyl system), 7.7 (ketophenolic system) and 9.5 (dimethyl ammonium group).

In-situ gel was prepared by using HPMC and Sodium alginate improves its adhesion property. The optimized formulation (F7) was transparent and clear in appearance with 97.77 % drug content. The sol gel transformation of in-situ gel was found at pH 7.4 with immediate gelation property. The in-vitro drug release of optimized formulation was found 94.44 % drug release in 5 hr. Viscosity of optimized formulation was measured by Brookfield viscometer. Viscosity of optimized formulation before gelation at 4, 10, 20, 50, 100 rpm was found 310, 275, 230,152,86 cps and after gelation 1487, 1259, 967, 573,268 cps respectively. Optimized formulation was analyzed for visual appearance, clarity, pH and drug remaining for 6 weeks of stability studies reveal that there was no change in visual appearance and clarity. All the formulations showed slight changes in pH, but it were in acceptable limits (±0.5).Study of % drug remaining in all formulations reveals that there were no definite changes observed to justify for drug degradation. The FTIR studies of formulation shows that no interaction between drug and excipient.

REFERENCES

Abdhul, H., Mohanambal, E. and Arun, K. 2011. Formulation and evaluation of pH-triggered insitugelling system of levofloxacin. *Ind J Pharm Edu Res,* 45(1):58-64.

Ana, C.K. 2012. Doxycycline hyclate a review of properties, applications and analytical methods, *Int J of life sci & pharm res*, 2(4):2250-0480

Aparna, V., Bhalerao and Sitanshu, S. 2011. *In situ* gelling ophthalmic drug delivery system for glaucoma. Int J Pharm and biosci. 2011;(7-14).

Bhoyar, B.S., Agnihotri, V.V. and Bodhankar, M.M. 2011. New method for sustained ocular drug delivery based on polymeric combination with thermosensitive gelling agents. *Int J Res Pharm and Biomed Sci*., 2(3):1151-1160.

Deshpande, J. 2013. Design and development of pH-monitered in situ gel of lomefloxacin. *JPSBR*., 3(1)10-15.

Eaga, C.M., Jagan, M.K. and Venkateshan, A. 2009. Preparation and evaluation of in-situ gels for Ocular Drug Delivery. *J Pharm Res*, 2(6):1089-1094.

Hong, R.L. and Sung, K.C. 2000. Carbopol/ pluronic phase change solutions for ophthalmic drug. *J of Cont Rel,* 69:379–388

Jantratid, E., Strauch, S., Becker, C. and Dressman, J.B. 2010. Biowaiver monographs for immediate release solid oral dosage forms doxycycline hyclate. *Int J pharm sci*, 99(4):639-1653.

Kanoujia, J. 2012. Formulation and characterization of a novel pH-triggered in-situ gelling ocular system containing gatifloxacin. *Int Curr Pharm J, 1(*3):43-49.

Kugular, G.P., Rangaswamy, M., Balasubramanyam, S.K. and Mirza, B.A. 2010. Formulation and evaluation of Ketorolac ocular pH triggered in-situ gel. *Int J Drug Dev & Res,* 2(2):379-387

Lalitkumar, Ravindra, P.S. Stuti, G. and Dhiraj, K. 2011. *In situ* gel a novel system for ocular drug delivery. *Int J pharm Sci Rev and Res*, 9(2):83-91.

Mitali, Bodhankar M., Bhushan S., Bhoya and Vinit, V.A. 2012. Environmentally responsive ophthalmic gel formulation based on combination with natural polycationic copolymer. *Int J Pharm Res Dev*, 3(11):212-222.

Mohima, T., Dewan, I., Islam, S.M.A, and Rana, S. 2015. Hossain. Encapsulation of zidovudin in different cellulosic acrylic and methacrylic polymers loaded microspheres: *in vitro* characterization and compatibility studies. *Int J of Pharma and Pharm Sci*, 7(1):487-495.

Nair, A., Vidhya, K.M., Saranya, T.R., Shreekakshmy, K.R. and Nair, S.C. 2014. Mucoadhesive buccal patch of cefixime trihydrate using biodegradable natural polymer. *Int J of Pharma and Pharm Sci*, 6(6):366-371.

Naseem, A., Charoo, Kanchan, K. and Asgar, A. 2003. Preparation of in situ forming ophthalmic gels of ciprofloxacin hydrochloride for the treatment of bacterial conjunctivitis in vitro and in vivo studies. *J Pharm Sci*, 92(2):407-415.

Pandit, J.K., Bharathi, D., Srinatha, A. Ridhurkar, N. and Singh, S. 2007. Long acting ophthalmic formulation of indomethacin: evaluation of alginate gel systems. *Ind J Pharm Sci,* 69(1):37-40.

Peng, Y. Deng. Z. and Wang, C. 2008. Preparation and prodrug studies of quercetin pentabenzensulfonate. *The Pharm Society of Japan*, 128(12):1845-1849.

Rajkumar, M. and Bhise, S.B. 2008. Effect of HPMC on solubility and dissolution of carbamazepine form III in simulated gastrointestinal fluids. *Asian J of Pharmace*, 2(1):38-42.

Sahoo, S., Chakraborti, C.K. and Mishra, S.C. 2011. Qualitative analysis of controlled release ciprofloxacin/ carbopol 940 mucoadhesive suspension. *J of Advan Pharm Tech and Res*, 2(3):195-205.

Satishkumar, P.J., Sejal, P.S., Namita, S.R., Singh, P. S. and Purnima, D. 2008. Amin. In situ ophthalmic gel of ciprofloxacin hydrochloride for once a day sustained delivery. Drug. *Dev Ind Pharm*, 34:445–452.

Sindhu, A., Sharon, F., Bharath, S., Basavaraj, B.V., Deveswaran, R. and Madhavan, N. 2009. Sustained ophthalmic delivery of ofloxacin from an ion-activated in situ gelling system. *Pak J Pharm Sci*, 22(2):175-179

Smith, V.A. and Cook, S.D. 2004. Doxycycline a role in ocular surface repair. *Br J Ophth*, 88(5):619-625.

Swati, G., Suresh, P.V. 2010. Carbopol/chitosan based pH triggered *in situ* gelling system for ocular delivery of timolol maleate. *Sci Pharm*, 78:959-976.

****