WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 7, 1236-1250. Research Article ISSN 2277– 7105

FORMULATION AND EVALUATION OF INSITU GEL CONTAINING PEFLOXACIN MESYLATE

Pentewar Ram Shankarrao*, Mali Supriya, Prof. R.V.Sugave, Dr. Bhushan Patil, Kore Priyanka

Channabasweshwar Pharmacy College, Latur, India.

Article Received on
06 May 2017,

Revised on 26 May 2017,
Accepted on 16 June. 2017

DOI: 10.20959/wjpr20177-8813

***Corresponding Author**
Pentewar Ram Shankarrao
Channabasweshwar Pharmacy College, Latur, India.

ABSTRACT

The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid precorneal elimination of the drug may be overcome by use of in–situ gel forming system that are instilled as a drops into the eye and it undergoes a sol-gel transition in the cul-de-sac. The present work describes the formulation and evaluation of an ophthalmic delivery system of an antibacterial agent, Pefloxacin, using xanthan gum as a gelling agent in combination with HPMC K15 as viscosity enhancing agent which is used in the treatment of eye infection such as, bacterial conjunctivitis, corneal ulceration and blepharitis, based on the concepts of pH-triggered *In -situ* gelation, thermo reversible gelation and Ion activated system. In situ gelling system of Pefloxacin Mesylate provides sustained release of drug based on polymeric carriers that undergo sol-to-gel transition upon change in temperature & PH. The formulations were evaluated for clarity, pH measurement, gelling capacity, drug content estimation, rheological study, in vitro release study. The optimized formulation F6 was stable and provided sustained release up to 92% at the end of 8^{th} hour and it is a viable alternative to conventional eye drops. The developed system is thus a viable alternative to conventional eye drops.

KEYWORDS: *In situ* gel, Gelling capacity, Rheological evaluation, *In vitro* diffusion study.

INTRODUCTION

From last 30 years greater attention has been given on development of controlled and sustained drug delivery systems. Ophthalmic drug delivery is a most challenging and interesting area for upcoming pharmacists and formulation chemists due to its unique anatomy and physiology. Ophthalmic solutions have poor bioavailability and therapeutic

response, because of high tear fluid turnover and dynamics that cause rapid precorneal elimination of the drug. A high frequency of ophthalmic solutions instillation is main cause of patient non-compliance. 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. 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.[1-4] The ocular bioavailability of the drugs can be improved by prolonging their residence time in the cul-de-sac and by increasing their corneal permeability. There are various new dosage forms like In situ gel, collagen shield, niosomes, liposomes, dendrimers and implants.[5]

In-Situ Gelling System, a more desirable dosage form would be one that can deliver drug in a solution form, create little to no problem of vision and need be dosed no more frequently than once or twice daily. In situ activated gel forming systems are those which are when exposed to physiological conditions will shift to a gel phase. This new concept of producing a gel in situ was suggested for the first time in the early 1980s. Gelation occurs via the cross linking of polymer chains that can be achieved by covalent bond formation (chemical cross-linking) or non-covalent bond formation (physical cross-linking). *In situ* gel forming systems can be described as low viscosity solutions that undergo phase transition in the conjunctival cul-de-sac to form viscoelastic gels due to conformational changes of polymers in response to the physiological environment. The rate of in situ gel formation is important because between instillation in the eye and before a strong gel is formed; the solution or weak gel is produced by the fluid mechanism of the eye. Both natural and synthetic polymers can be used for the production of in situ gels. *In-situ* ophthalmic drug delivery system based on the concept of pH triggered *In-situ* gelation, temperature dependent *In-situ* gelation and ion activated *In-situ* gelation by using different polymers.[6,7] The various approaches that have been attempted to increase the bioavailability and the duration of the therapeutic action of ocular drugs can be divided into two categories. The first one is based on the use of sustained drug delivery systems, which provide the controlled and continuous delivery of ophthalmic drugs. The second involves maximizing corneal drug absorption and minimizing precorneal drug loss.[8]

Pefloxacin Mesylate, 1-Ethyl-6-fluoro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3quinoline carboxylic acid mono methane sulfate dehydrate. The Flouroquinolone represent an expanding class of broad-spectrum antibacterial which cover a host of Gram-negative and

anaerobic species responsible for ocular infections. Fluoroquinolones were introduced as monotherapy for suspected bacterial keratitis owing to their broad spectrum of activity, low toxicity, good corneal penetration, and their efficacy at commercially available strength. It bacterial enzyme DNA gyrase, a type II and type IV topoisomerase enzymes, which are needed for the transcription and replication of bacterial DNA. The inhibition of the topoisomerase results in strand breakage, super coiling and releasing of the bacterial chromosome. Therefore, DNA replication and transcription is inhibited.[9,10,11]

MATERIALS

Pefloxacin was obtained from Smruthi Organics ltd Solapur, India as a gift sample. Xanthan Gum was obtained from Lucid Colloids Ltd., Mumbai. HPMCK15M obtained from fine-chem. Ltd, Mumbai. All other reagents used were of analytical grade.

Estimation of Pefloxacin by UV- Spectroscopy Method

Analytical methods for the estimation of Pefloxacin: - In order to a certain a wavelength of maximum absorption (λ max) of the drug in different solution of the drugs (2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml,10µg/ml) in simulated tear fluid [STF] was scanned using spectrophotometer within the wavelength region of 200-400 nm against double simulated tear fluid blank.

The composition of STF[10]

Sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride 0.008 g, distilled water q.s. 100 g.

Standard curve of Pefloxacin in Stimulated Tear Fluid PH 7.4

Pefloxacin (10 mg) was dissolved in 20 ml STF 7.4 and volume was made up to 100 ml in volumetric flask using STF 7.4. From this stock solution 0.2 ml solution was withdrawn and diluted up to 10 ml in volumetric flask (2µg/ml). Same way solution of 4,6,8,10µg/ml was prepared. Absorbance of each solution was measured at 272 nm using shimadzu UV-1800 UV/Visible double beam spectrophotometer and distilled water as a reference standard.

Method of formulation

Firstly, the required quantity of acetate buffer of pH 5 was prepared to this; HPMCK15M & xanthan gum were added and stirred slowly with magnetic stirrer. Care was taken to avoid lumps of HPMC during stirring. Required quantity of Pefloxacin mesylate was added to polymeric solution and stirred to get a final drug concentration of 0.3% w/v. Mannitol [5%

w/v] and Benzalkonium chloride [0.01% w/v] was added later which acts as isotonicity adjusting agent and preservative, respectively. The complete formulas of the formulations are given in the [table1]. The formulations were filled in sterile 20 ml glass vials, capped with rubber closures and sealed with aluminum caps. The formulations in their final pack were terminally sterilized by autoclaving at 121°C and 15 psi for 20min. The sterilized formulations were stored in refrigerator [4°C – 8°C] until further use.

1: Composition of Pefloxacin mesylate in situ gel

Ingredients [Gm]	**Formulation code**							
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Drug	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
HPMC K 15 M	0.2	0.2	0.4	0.6	0.4	0.2	0.2	0.4
XANTHAN GUM	0.2	0.4	0.4	0.4	0.8	0.6	0.8	1.0
MANNITOL	5	5	5	5	5	5	5	5
BENZOLKONIUM CHORIDE	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
ACETATE BUFFER	100	100	100	100	100	100	100	100

Drug - Excipients compatibility study:[11,12]

Differential Scanning Calorimetric (DSC) Analysis

DSC scans of powdered sample of Pefloxacin and mixture of excipients with drug. DSC analyses of powders were recorded using DSC- Shimadzu 60 with TDA trend line software. The pans were positioned on sample pan holder of a DSC 60. The thermal traces were obtained by heating from 50ºC to 300ºC at heating rate of 20ºC per minute. Thermograms were obtained by the DSC 60 thermal analyzer program and recorded chart speed of 1 inch/min. The thermogram, transition temperature range, the onset of peak transition and the maximum peak of transition were recorded.

FTIR analysis

The pure drug, Pefloxacin and a mixture of its excipients powder was mixed separately with IR grade KBr and corresponding pellets were prepared by applying 10 tons of pressure in the hydraulic press. The pellets were scanned over a wave number range of 400 to 4000cm-1 in FTIR 8400S model instrument.

EVALUATION AND CHARACTERIZATION OF IN-SITU OPHTHALMIC[15-24]

- **Physical parameter**

The formulated In-situ solution is tested for clarity, pH, gelling capacity, appearance.

- **Viscosity**

Viscosity can be calculated by using Brookfield viscometer, cone and plate viscometer. The *Insitu* gel formulation was placed in sampler tube. The samples are analyzed both at room temperature at 25°c and thermo stated at 37°c ± 0.5°c by a circulating bath connected to viscometer adaptor prior to each measurement.

- **Gelling Capacity**

Gelling capacity of prepared formulation is determined by placing the drop of formulation in vial containing 2.0 ml of freshly prepared simulated tear fluid and visually observed. The time taken for gelling was noted.

- **Isotonicity Evaluation**

Isotonicity is important characteristics of ophthalmic preparation. Isotonicity is maintained to prevent tissue damage or irritation of eye. All ophthalmic preparation are subjected to isotonicity testing, science they exhibited good release characteristics & gelling capacity & the requite velocity. Formulation mixed with few drops of blood & observed under microscope at 45x magnification & compared with standard marketed ophthalmic formulation.

- **Drug content**

It is determined by taking 1ml of the formulation and diluting it to 100ml with distilled water. 1 ml was withdrawn and further diluted to 10 ml with distilled water. Concentration was determined at 200-400nm by using UV visible spectroscopy.

- ***In Vitro* Drug Release Studies**

By using dialysis tube

This study is performed in the Dialysis tube containing 1 ml of the formulation, which is then suspended in beaker at 37 ± 0.50C containing 100 ml artificial simulated tear fluid (pH 7.4) under continuous stirring at 20 RPM to stimulate the blinking effect. Dialysis membrane (0.22 µm pore size), previously soaked overnight in simulated tear fluid.

- **Antibacterial activity**

An agar diffusion method was used for the determination of antibacterial activity of formulations. Standard Petri dishes (9 cm diameter) containing medium to a depth of 0.5 cm were used. The sterility of the lots was controlled before use. Suspension was prepared by suspending 1-2 colonies of Staphylococcus aureus & Pseudomonas aeruginosa from 24hr

cultures in Nutrient agar medium into tubes containing 10 mL of sterile saline. The tubes were diluted with saline. The inoculum (0.5mL) was spread over the surface of agar and the plates were dried at 35°C for 15 min prior to placing the formulation. The bores of 0.5 cm diameter were prepared and 2 drops of formulation (0.3% w/v) were added in the bores. After incubation at 35°C for 24 hrs, the zone of inhibition around the bores was measured.

- **Accelerated stability studies**

Formulations are placed in ambient colour vials and sealed with aluminum foil for a short term accelerated stability study at 40±2°C and 75±5% RH as per International Conference on Harmonization (ICH) states Guidelines. Samples are analyzed after 21 days for Clarity, pH, gelling capacity, drug content, rheological evaluation, and in vitro dissolution. The storage conditions and the lengths of studies chosen should be sufficient to cover storage, shipment, and subsequent use.

RESULTS AND DISCUSSION

Calibration Curve of Pefloxacin in STF

The calibration curve (Fig1.) was found to be linear in the concentration range of 2-10µg/mL (Table no. 2) having coefficient of regression value R^2 = 0.996 and Slope y = 0.071x.

Table no. 2

Sr. no	Concentration (ug/ml)	Absorbance
1	2	0.134
2	4	0.261
3	6	0.427
4	8	0.565
5	10	0.726

272

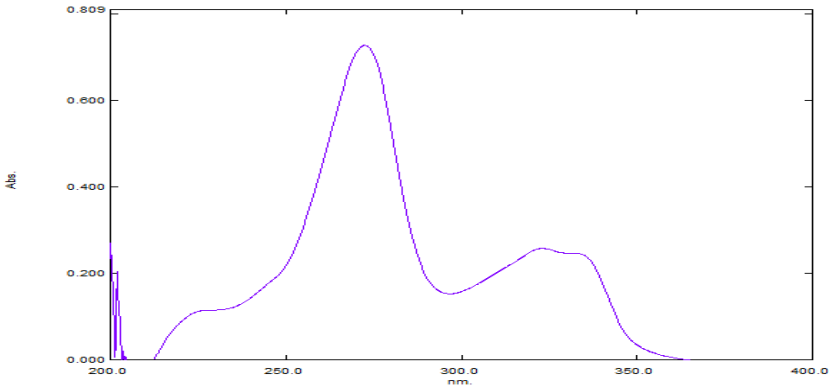

Fig 1: UV spectra of Pefloxacin in STF

Fig 2: Calibration curve of Pefloxacin in STF

Drug - Excipients compatibility study

- **Infra-Red Spectrum**

Infra- red spectrum of Pefloxacin is shown in Fig. The major peaks observed and corresponding functional groups. Infra-red spectrum shows peak characteristic of structure of Pefloxacin.

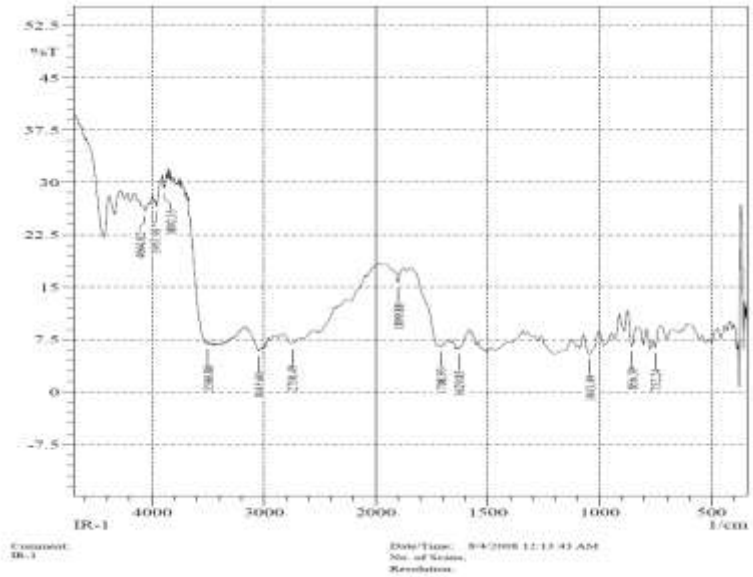

Fig 3: FTIR spectrum of Pefloxacin

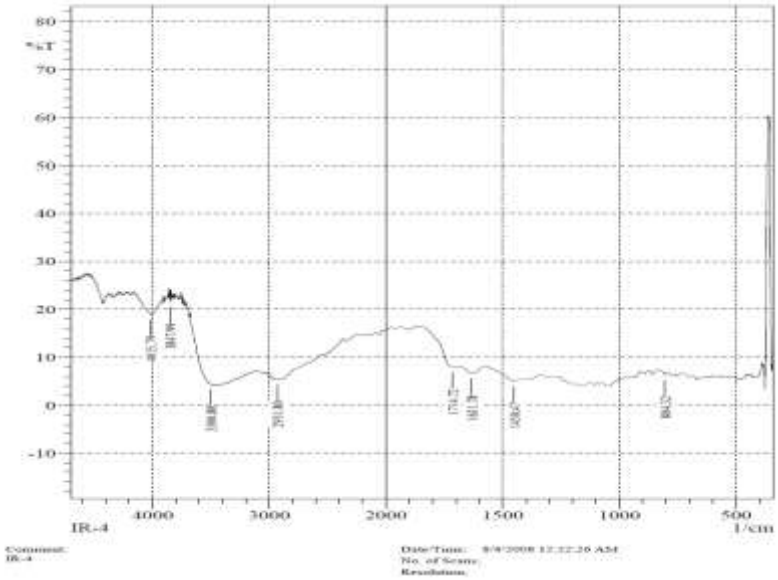

Fig 4: FTIR spectrum of formulation

Table 3: Interpretation of FTIR Spectra of Pefloxacin and the formulation

Functional group	Peak observed
−C=O	1708.93
O=C-O	1200
Aromatic C = C	1500
Aromatic C− H	3045.60
−N – H	1629.85
$-CH_2OH$	3500.80
C − F	1340
N − H	752.24
C − C	856.39
C-C stretching mode	1487.79
C-H stretch of the aromatic group	2976.52

- **Differential Scanning Calorimetry (DSC)**

Figure no. 4 and 5, compares the DSC thermo gram of Pefloxacin and physical mixture of Pefloxacin. Pefloxacin showed a long and sharp characteristic endothermic peak at 247.0C due to its phase transition system. The physical mixture of Pefloxacin with Xanthan Gum and HPMC shows characteristic peak at 190.840C. It showed the slight change in characteristic peak may be due to fusion of excipient present in the Physical mixture. From this result, it clears that there is no interaction in between Pefloxacin and excipients.

Fig 5: Thermal analysis of Pefloxacin

Fig 6: Thermal analysis of Pefloxacin & excipients

Evaluation of Ophthalmic *Insitu* Gel Formulation

Physical parameter

Clarity

On careful visual inspection against dark and white background, all the prepared ophthalmic gel formulations were found to be free from any suspended particulate matter.

Table no 4: pH, Gelling Capacity of Formulations

Formulation	PH	Gelling Capacity
F1	6.3±0.288	+
F2	6.1±0.121	++
F3	5.9±0.115	++
F4	6.1±0.1	+++
F5	5.96±0.05	+++
F6	6.4±0.057	+++
F7	6.53±0.115	+++
F8	6.16±0.057	+++

- No gelation; + Gels after few minute, dissolves rapidly; ++, Gelation immediate, remains for few hours; +++, Gelation immediate, remains for extended period.

Viscosity of Formulations

The viscosity was proportional to the concentration of the mucoadhesive polymer in the formulation shown in table number 5. All the formulations exhibited quite low viscosity at low temperature. However, upon increasing the temperature, a gel was formed in well-defined temperature and viscosity of the formulation was increased.

Table no 5: Viscosity of Formulations

FORMULATION	Viscosity [cp]
F1	991
F2	1037
F3	1160
F4	1269
F5	1456
F6	1513
F7	1663
F8	1811

Drug content of formulations

All the formulations reflected fairly uniform drug content ensuring adequacy in the method of preparation of the in situ gel. Drug content was found to be within the range of 93.58–98.85%.

Table no 6: Drug content of formulations

FORMULATION	% Drug Content
F1	95.64
F2	93.58
F3	97.88
F4	95.56
F5	95.49
F6	98.85
F7	97.53
F8	97.51

***In-Vitro* Release Studies**

The *in-vitro* release studies were carried out for all formulations using STF as the dissolution medium. The formulation F6 shows 92.25 % drug release at the end of 8 hours. The data of these studies are presented in Table No.7.

Table no 7: *In-vitro* release profile of Pefloxacin mesylate in situ gel formulations F1-F8

Sr. no	Time [hr]	% drug release							
		F1	F2	F3	F4	F5	F6	F7	F8
1	1	11.634	12.88	13.325	13.728	14.05	14.291	17.834	14.211
2	2	20.923	22.81	26.041	25.882	23.41	26.046	19.302	25.965
3	3	38.405	35.17	33.432	33.756	33.44	31.586	34.144	34.483
4	4	49.449	46.96	42.877	42.558	45.14	42.711	43.110	49.65
5	5	59.182	57.12	58.090	55.683	57.06	54.058	56.311	58.057
6	6	68.283	67.12	66.220	68.712	61.00	67.158	66.203	67.394
7	7	71.594	70.70	71.10	80.817	74.86	75.980	75.020	77.909
8	8	80.916	84.08	83.016	91.410	88.23	92.258	87.427	85.903

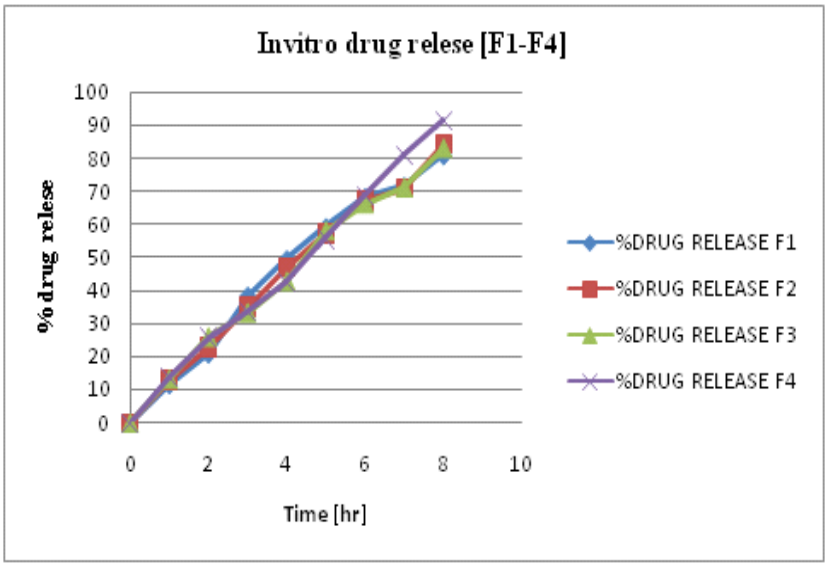

Fig 7: Invitro Drug Release [F1-F4]

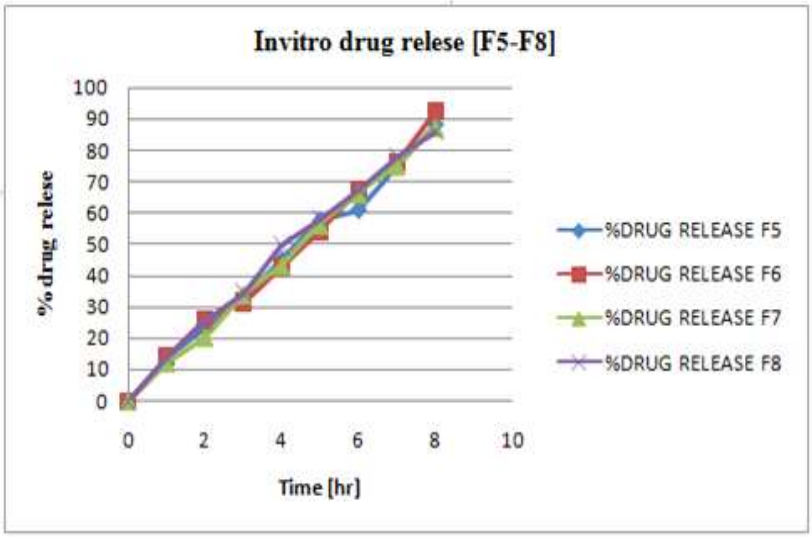

Fig 8: Invitro Drug Release [F5-F8]

Table no 8: Curve fitting analysis for different formulation

Formulation	Zero order (R)	First order (R)	Higuchis's (R)	Hix-crow(R)	Peppas	
					R	N
F1	0.9738	0.9918	0.9721	0.9967	0.9762	0.8791
F2	0.9893	0.9723	0.9647	0.9902	0.9926	0.7389
F3	0.9956	0.9720	0.9509	0.9906	0.9978	0.8821
F4	0.9950	0.8919	0.9332	0.9468	0.9953	0.8730
F5	0.9967	0.8968	0.9325	0.9514	0.9937	0.8826
F6	0.9956	0.9720	0.9509	0.9906	0.9978	0.8802
F7	0.9955	0.9439	0.9390	0.9753	0.9720	0.8326
F8	0.9954	0.9683	0.9544	0.9899	0.9987	0.8751

All values are expressed as mean ± SD, n =3, F= formulation codes.

Table no 9: Best fit model of formulation F1 to F8

Formulation code	Best fit model
F1	Peppas
F2	Peppas
F3	Peppas
F4	Hix- crow
F5	Zero order
F6	Peppas
F7	Zero order
F8	peppas

The release kinetics of all the formulations (F1-F8) is shown in Table 8. The release kinetics of the optimized formulation (F6) was well fitted to Peppas model based on the concepts of the highest regression coefficient (R) value. The 'n' value for the optimized formulation was found to be 0.9978. The 'n' value obtained from Peppas equation was greater than 0.5, which indicated that the formulation showed drug release by the non-fickian diffusion mechanism.

Table no 10

Diffusion Exponent (n)	Diffusion mechanism
0.45	Fickian diffusion
0.45 < n < 0.89	Anomalous diffusion (non Fickian)
0.89	Case II transport
n > 0.89	super Case II transport

Antibacterial Activity

Pefloxacin has been proved to exhibit superior antibacterial activity against Pseudomonas aeruginosa & Staphylococcus aureus which is a causative organism of bacterial conjunctivitis. Hence antimicrobial activity was done, using strain of gram negative bacteria, pseudomonas aeruginosa & Staphylococcus aureus. Microbiological studies were carried out

of the optimized formulation (F6) against microorganisms Staphylococcus aureus and Pseudomonas aeruginosa. Results of Antimicrobial study of optimized formulation (F6) are as shown in the table.

Table no 11: The result of antibacterial activity of optimizied formulations (F6)

Test micro organism	Diameter of the Zone of Inhibition Produced By In-situ Gels (mm)	
	Pure drug	formulation
Staphylococcus aureus	48	43
Pseudomonas aeruginosa	44	40

Accelerated stability studies

For the stability studies different tests were done. In Table 10 the results of accelerated stability studies are shown. The appearance of the gels remained clear and no significant variation in pH was observed after subjecting the formulations to stability stress for 21 days. Also there was no significant change in drug content was observed after 21 days period.

Table no 12: Accelerated stability studies

Sr. No.	Time (Hr.)	Cum. % Drug Released ± S.D. 1 St Day	Cum. % Drug Released ± S.D. 21 St Day
1	0	0.00	0.00
2	1	14.291	12.17
3	2	26.046	21.59
4	3	31.44	34.54
5	4	42.711	43.11
6	5	54.058	56.59
7	6	67.158	67.11
8	7	75.98	80.08
9	8	92.25	93.09

CONCLUSION

Pefloxacin was successfully formulated as in situ gel-forming eye drops using xanthan gum as a gelling agent and HPMC K15 as a viscolyzing agent. In situ gel produced the prolonged drug release and decreases the Nasolacrimal drainage. The present study was to improve the precorneal residence time, and sustain the drug release by utilizing the approach of in situ gelling systems. The formulation also promises to reduce the frequency of drug administration, thus improving patient compliance. Physicochemical characterization and *in vitro* drug release studies indicated that the developed formulation (F6) may prove to be a viable alternative to conventional eye drops and ointment in terms of ease of administration

with added benefits of sustained drug release which may ultimately result into improved 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. Finally it can be concluded that in-situ ophthalmic gel is alternative to conventional eye drops.

REFERENCES

1. Singh, B. N. and Kim, K. H., Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention. Journal of Control Release, 2000; 63: 235259.
2. Suryawanshi Sarika S, Kunjwani H K, Kawade Jayashree V, Alkunte Mohita A, Yadav Dattatraya J, Novel polymeric in-situ gels for ophthalmic drug delivery system, International journal of research in pharmacy and science, 2012; 2(1): 67-83.
3. Savita Gambhire, Karuna Bhalerao, Sushma Singh, In-situ hydrogel: different approaches to ocular drug delivery, International Journal Of pharmacy and pharmaceutical Sciences, 2013; 5(2).
4. Desi H.A, Bhalla, H.L. Preparation and Evaluation of new eye drops containing a combination of ciprofloxacin and dexamethasone, Indian drugs, 2000; 37(4).
5. Mali Mahesh N,Hajare Ashok A.In-situ gel forming systems for sustained ocular drug delivery system.European Industrial Pharmacy, 2010; 5: 17-20.
6. Patil Rajeshwari N,Kumar Rachana S.Insitu gelling system: Novel approach for ophthalmic drug delivery. World Journal of Pharmacy and Pharmaceutical Sciences, 2014; 3(7): 423-440.
7. Mitra AK. Ophthalmic drug delivery systems. 2nd ed., New York; Marcel Dekker, Inc., 2003.
8. Pandya TP, Modasiya MK, Patel VM. Ophthalmic In-Situ Gelling System. Int. J. of Pharm. & Life Sci., 2011; 2(5): 730-738.
9. Tuft SJ, Matheson M. In-vitro antibiotic resistance in bacterial keratitis in London. Br JOphthalmol, 2000; 84: 687–691.
10. Khurana AK. Ophthalmology. 2nd ed. New Delhi: New Age International (P) Ltd; 2000.

11. Balasubramaniam J, Kant S, Pandit JK. (In vitro and in vivo evaluation of the Gelrite gellan gum-based ocular delivery system for Indomethacin). Acta Pharm., 2003; 53: 251–261.
12. Kashyap N, Viswanad B, Sharma G, Bhardwaj V, Ramarao P, Kumar MNV. Design and evaluation of biodegradable, biosensitive in situ gelling systems for pulsatile delivery of insulin. Biomaterials, 2007; 28: 2051-60.
13. Sautou –Miranada V, Labret F, GrandBoyer A , Gellis C, Chopineau J. Impact of deep-freezing on the stability of 25mg/ml vancomycin ophthalmic solutions. Int J pharm., 2002; 234: 205-207.
14. Gupta A, Manocha N. Formulation and Evaluation of In-Situ Ophthalmic Drug Delivery System. International Journal of Pharmaceutical & Biological Archives, 2012; 3(4): 715- 718.
15. Mitan R, Gokulgandhi Jolly R, Parikh, Megha B, Dharmesh MM. A pH triggered insitu gel forming ophthalmic drug delivery system for Tropicamide. Drug Deliv Technol, 2007; 5: 44-49.
16. Pandit D, Bharathi, A, Srinatha, Ridhirkar, Singh S. Long acting ophthalmic formulation of indomethacin: Evaluation of alginate gel system. Indian J Pharm Sci., 2007; 69: 37-40.
17. Sudam Nagargoje, Atul Phatak, Chandrashekhar Bhingare, Shilpa Chaudhari, Formulation and evaluation of ophthalmic delivery of fluconazole from ion activated in situ gelling system, Der Pharmacia Lettre, 2012; 4(4): 1228-1235.
18. Katrina E, Johan C, Roger P. Rheological evaluation of Poloxamer as an in situ gel for ophthalmic use. Euro J pharm Sci., 1998; 6: 105-112.
19. Mitan R, Gokulgandhi Jolly R, Parikh, Megha B, Dharmesh MM. A pH triggered in situ forming opthalmic drug delivery system for tropicamide. Duug Deliv. Technol, 2007; 5: 44-49.
20. Sautou –Miranada V, Labret F, GrandBoyer A , Gellis C, Chopineau J. Impact of deep-freezing on the stability of 25mg/ml vancomycin ophthalmic solutions. Int J pharm., 2002; 234: 205-207.
21. Doijad RC, Manvi FV, Malleswara Rao VSN, Prajakta, Alsae. Sustained ophthalmic delivery of gatifloxacin from In-situ gelling, Indian J pharma Sci., 2006; 8: 814818.
22. Draize J, Woodward G, Calvery O. Method for the study of irritation and toxicity of substance applied topically to the skin and Mucous Membrane. J Pharmacol exp ther, 1994; 82: 377-390.

23. Gambhire Savita, Bhalerao Karuna, Singh Sushma. In-situ hydrogel: different approaches to ocular drug delivery. International Journal of Pharmacy and Pharmaceutical Sciences., 2013; 5(2): 27-36