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Thermo and PH Responsive Ocular *Insitu* Gels Formulation: Based on Combination with Natural Polymers

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ABSTRACT

To increase the bioavailability and short ocular residence time of Norfloxacin eye drops, aqueous solutions of drug in chitosan/ Pluronic (poloxamer) were prepared. Mixtures of solutions of Pluronic (5-17.5% w/w) with chitosan (0.1-0.6% w/w) were prepared. The formulations so prepared were in the liquid state at 4°C while turned into a gel at the temperature of the Cul-de-sac. Naturals polymers i.e., Poloxamer was used as the polymer which exhibited the phase transition behavior and chitosan was used to improve residence time. Norfloxacin release was determined using a membrane less dissolution model in artificial tear solution up to 8 hours and the samples were analyzed spectrophotometrically at 277nm. The rheological behavior of solutions in response to dilution or temperature changes and also the phase change temperature (PCT) were determined. Antimicrobial effect of the solutions was studied in nutrient agar in comparison to all formulations of Norfloxacin using *Pseudomonas aueroginosa, Staphylococcus aureus and E.coli* by the agar diffusion test using the cup-plate technique. The formulation consisted of 15% Pluronic and 0.5% chitosan, with the highest release efficiency (73.46 ± 0.876%) and an acceptable mean release time, is suggested as a suitable ophthalmic preparation for sustained release of Norfloxacin.

Keywords: Ocular drug delivery, *in situ* gels, chitosan, poloxamer, phase transition temperature.

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INTRODUCTION

Although various marketed formulations exist for ocular drug delivery, but are not able to provide highest bioavailability related to administered dose[1]. Whenever an ophthalmic drug is applied through an 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[2,3.]

Various problems encountered in poor bioavailability of the eye installed drugs are Binding by the lachrymal proteins, Drainage of the instilled solutions, Lachrimation and tear turnover, Limited corneal area and poor corneal, Metabolism, Non-productive absorption/adsorption, Tear evaporation and permeability[4]

In situ-forming systems are liquid aqueous solutions before administration, but gel under physiological conditions. There are several possible mechanisms that lead to *in situ* gel formation[5] ionic crosslinkage, pH change, and temperature modulation, solvent exchange and UV-irradiation. These approaches, which do not require organic solvents, copolymerization agents, or an externally applied trigger for gelation, have gained increasing attention, such as a thermosensitive approach for *in situ* hydrogel formation[6,7].

In order to reduce the total polymer content and improve the gelling properties[8], used the combination of polymers in the delivery system. Kumar and other workers[9] developed an ocular drug delivery system based on a combination of carbopol and ethylcellulose or hydroxypropylmethylcellulose[10,11]. For both systems, it was found that a reduction in the carbopol concentration without compromising the *in situ* gelling properties as well as overall rheological behaviors can be achieved by adding a suitable viscosity-enhancing polymer[9,12].

Poloxamer (Pluronic®), a block copolymer that consists of polyethylene oxide (PEO) and polypropylene oxide (PPO) units, is known for exhibiting the phenomenon of reverse thermal gelation under a certain concentration and temperature[13-14] and Chitosan is a biodegradable, thermosensitive, polycationic polymer obtained by alkaline deacetylation of chitin, a natural component of shrimp and crab shell. Chitosan is a biocompatible pH dependent cationic polymer, which remains dissolved in aqueous solutions up to a pH of 6.2[15].

Norfloxacin (1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1H-quinoline-3-carboxylic acid). is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, enzymes necessary to separate bacterial DNA, thereby inhibiting cell division. The present study aim was to develop the in-situ gelling ophthalmic delivery system of Norfloxacin, a third-generation Fluoroquinoline derivative used in external infections of eye such as conjunctivitis[16].

MATERIALS AND METHODS

Norfloxacin, Chitosan, Poloxamer 407 was PVA, Benzalkonium chloride are gifted samples from KAPL, Bangalore, Sodium chloride and deionised water from Rolex chem., Bangalore.

Preparation of Poloxamer-Chitosan and PVA ocular *insitu* gels

The formulations were prepared on a weight/weight basis using the cold method. Appropriate amounts of P407 was added to bidistilled water (4°C).The dispersions were stored in a refrigerator at 4°C over night results in clear solution. Chitosan was initially dissolved in a solution of acetic acid (0.5% v/v) and used as a solvent for the poloxamer dispersion was then kept in a refrigerator at 4°C over night results in clear solution. To the above solution PVA was dissolved in bidistilled water, stirred to make clear colorless solution and added to chitosan - Poloxamer polymeric solution. For preparation of Norfloxacin containing polymer solutions, 0.3% of Norfloxacin was added to the Polymeric solutions with continuous stirring until thoroughly mixed. Benzalkonium chloride (0.01%) and all the sample solutions were, sterilized at 121ºC and 15 psi for 20 min and then stored in the refrigerator prior to further evaluation.(Table.1)

Table.1 Formulation design of Poloxamer-Chitosan-PVA Norfloxacin *insitu* gel.

S.No	Ingredients	Concentration(%w/v)					
		F1	**F2**	**F3**	**F4**	**F5**	**F6**
1	Norfloxacin	0.3	0.3	0.3	0.3	0.3	0.3
2	Poloxamer 407	5	7.5	10	12.5	15	17.5
3	Chitosan	0.1	0.2	0.3	0.4	0.5	0.6
4	PVA	0.3	0.3	0.3	0.3	0.3	0.3
5	Benzalkonium chloride	0.01	0.01	0.01	0.01	0.01	0.01
6	Deionized water	100	100	100	100	100	100

pH

pH is one of the most important factors involved in the formulation process. Two areas of critical importance are the effects 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. The pH of the prepared formulations was checked by using pH meter.

Visual appearance and clarity

Visual appearance and clarity was checked under florescent light against a white and black back ground for presence of any particle matter.

Drug Content

Uniform distribution of active ingredient is important to achieve dose uniformity. The drug

content was determined by taking 1ml of the formulation and diluting it to 100 ml with distilled water. Aliquot of 5 ml was withdrawn and further diluted to 25 ml with distilled water. Norfloxacin concentration was determined λmax at 277nm by using UV-Vis spectrophotometer.

Measurement of Phase Change Temperature.

An aliquot of 2 ml of formulation was transferred to a test tube and sealed with a parafilm. The tube was maintained in a water bath at 4°C. The temperature of the water bath was increased gradually in increments of 3°C in the beginning of the experiment and then 1°C increments in the region of sol–gel transition temperature (25–34°C) and 0.1°C when it approaches gelation. The tested formulation was left to equilibrate for 10 min at each new setting. The gelation is considered to be occurred when the meniscus of the formula would no longer move upon tilting through angle 90°.

Rheological Studies

Viscosity of the instilled formulation is an important factor in determining residence time of drug in the eye. The prepared solutions were allowed to gel in the simulated tear fluid and then the viscosity determination were carried out by using Brooke field viscometer RVT model in spindle no S-34, angular velocity ran from 10-100 rpm. Viscosity of the formulations increased with increase in polymer concentration. The hierarchy of shear rate was reversed and average of two readings was used to calculate viscosity.

***In-vitro* release studies**

In-vitro release studies were carried out using bichambered donor receiver compartment model (Franz diffusion cell) using cellophane membrane soaked overnight in the receptor medium (simulated tear fluid, pH 7.4). The diffusion medium was 100ml of simulated tear fluid stirred at 50rpm at 37^0C ±0.5^0C. One end of the diffusion tube was covered by a cellophane membrane. The 1ml formulation were spread on the cellophane membrane was placed such that it just touches the diffusion medium (STF) present in receptor compartment. The drug samples were withdrawn at the interval of one hour for the period of 8hrs from diffusion medium and analyzed by a UV spectrophotometer Shimadzu (UV 1601) at 277nm using simulated tear fluid as blank.

Antimicrobial Efficacy Studies

The Antimicrobial efficacy studies were carried out to ascertain the biological activity of the optimized formulations. Staphylococcus aureus, Pseudomonas aeruginosa and E.coli were used as the test organisms. Anti microbial efficiency was determined by agar diffusion test employing Cup-Plate method. Sterile solutions of Norfloxacin (standard solution) and the developed formulations were diluted at different concentration (test solutions) these solutions were poured

in to cups bored into sterile nutrient agar previously seeded with test organisms (*Pseudomonas aeruginosa, E.coli and Staphylococcus aureus*), after allowing diffusion of the solutions for 2 hours, the agar plates were incubated at 37^{o}C for 24hrs. The zone of inhibition (ZOI) measured around each cup and was compared with that of control. The entire operation except the incubation was carried out in a laminar airflow unit. Both positive and negative controls were maintained during the study.

Sterility Testing

All the prepared *in situ* gelling systems were evaluated for the sterility. After 7 days of incubation the results showed no microbial growth in all formulations.

Accelerated stability studies

Stability is defined as the extent, to which a product retains with in specified limits and though out its period of storage and use i.e., shelf life. Stability studies were carried out on optimized formulations according to International Conference on Harmonization (ICH) guidelines.

A sufficient quantity of formulation in previously sterilized vials was stored in desiccators containing a saturated solution of sodium chloride, which gives a relative humidity of 75±5%. The desiccators were placed in a hot air oven maintained at a temperature 40^{o}c±0.5^{o}c and at room temperature. Samples were withdrawn at 7days interval for 42 days. Percent drug remaining was calculated and plotted against time in days.

RESULTS AND DISSCUSSION

Evaluation Studies of ocular insitu gel Formulations

All the evaluation parameters stated above were shown in Table 2.The appearance of all the gelling solutions and gel formed were transparent and clear except F6 is cloudy. So one may be concluded that concentration above formulation F5 (15% poloxamer w/v, 0.5% w/v chitosan and 0.3% PVA) restricts the gel formation. All the formulations shows percentage drug content within the range of 92.96% to 98.05%. pH of all the formulation were in at 7.11 ±0.29.(Table.2)

Table.2 Evaluation parameters

S.NO	Formulation Code	Clarity Solution	Clarity Gel	Appearance Solution	Appearance Gel	pH	Drug Content %	Gelation Capacity
1	**F1**	Clear	Clear	Transparent	Transparent	7.11	98.05	++
2	**F2**	Clear	Clear	Transparent	Transparent	7.25	97.56	+++
3	**F3**	Clear	Clear	Transparent	Transparent	7.26	96.12	+++
4	**F4**	Clear	Clear	Transparent	Transparent	7.24	95.30	+++
5	**F5**	Clear	Clear	Transparent	Transparent	7.32	93.79	+++
6	**F6**	Not clear	Not clear	Whitish	Whitish	7.39	92.96	++++

Estimation of Norfloxacin by Spectrophotometric method

A simple Spectrophotometric method for estimation of Norfloxacin was developed in Simulated Tear Fluid, which exhibited λ max at 277 nm in Beer's range of 1-6 µg/ml[17].

Rheological studies

The viscosity values obtained for all the formulations using Brookfield DV-111+ Rheometer. The formulations exhibited pseudo plastic rheology, as evidenced by shear thinning and an increase in shear stress with increased angular velocity. Since the ocular shear rate is very high ranging from 0.03 s^{-1}. During inter-blinking periods to 4250-28500 s^{-1} during blinking. Viscoelastic fluid with a viscosity that is high under low shear condition and low under high shear rate condition, which is called pseudo plastic fluid, is often preferred, so dynamic viscosity of formulations were measured as the change of shear rate before and after gelation. The viscosity was directly dependent on the polymeric content of the formulation. The viscosity increased with increasing concentration of chitosan and PVA. Dynamic viscosity of the formulations was measured as the change of the shear rate before and after gelation. (Table.3-4 and Fig.1-2)

Table.3 Rheological studies of *in situ* gels before gelation shear

	Viscosity of the formulation					
Shear Rate (RPM)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
10	560	830	1125	1375	1645	1865
20	385	645	800	870	1200	1315
50	260	425	445	490	660	845
100	185	235	275	285	325	490

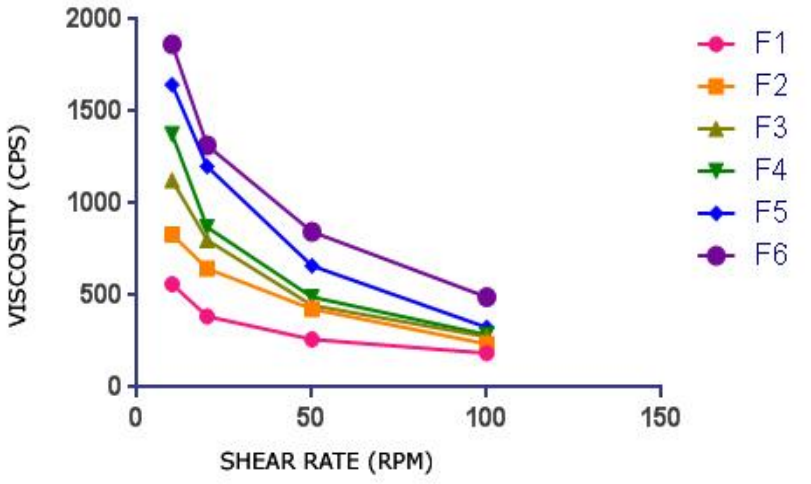

Figure.1 Rheological studies of Norfloxacin *in situ* gels before gelation shear

Table.4 Rheological studies of Norfloxacin *in situ* gels after gelation shear

	Viscosity of the formulation					
Shear Rate (RPM)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
10	1330	1420	2615	2025	3390	4270
20	720	1180	1350	1550	2205	2280
50	475	690	740	880	1090	1545
100	335	415	450	515	570	635

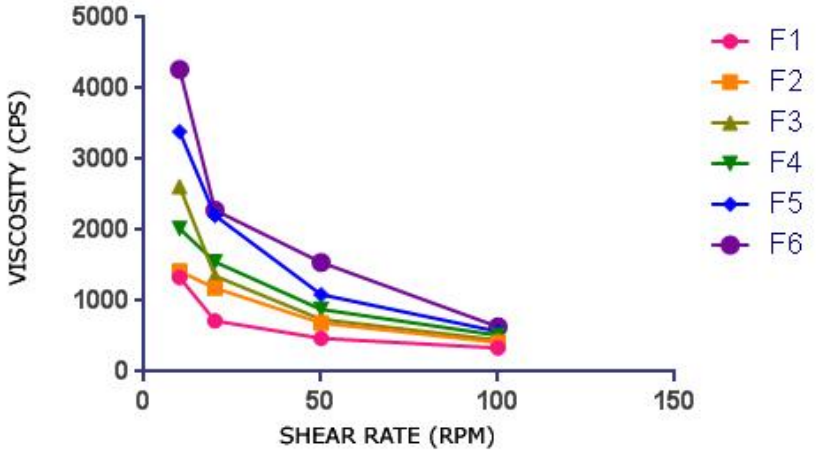

Figure.2 Rheological studies of Norfloxacin *in situ* gels after gelation shear

***In vitro* release studies**

The *in-vitro* release of Norfloxacin from the prepared formulations was studied through cellophane membrane of 213-219 microns using diffusion cell. The release studies of prepared *insitu* gelling systems were carried out up to 8 hrs.

In-vitro release studies of marketed eye drops (Norflox) was done though cellophane membrane using diffusion cell and the release marketed product was up to 3 hrs.

The results obtained *in vitro* release studies were plotted in different modes of data treatment as follows.

- Comparative *invitro* release data of *in situ* gels and marketed eye drops.
- Cumulative percent drug released Vs. time (Zero order rate kinetics).
- Log cumulative percent drug retained Vs. time (First order kinetics).
- Log cumulative percent drug released Vs. log time (Peppas exponential equation).
- Cumulative percent released Vs. square root of time (Higuchi's classical diffusion equation).

(Table.5 and Figure 3-7)

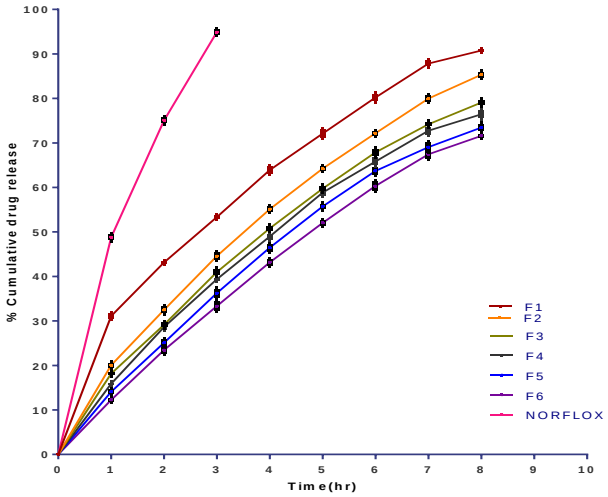

Figure.3 comparative *in-vitro* release profile of Norfloxacin *in situ* gels and marketed eye drop (NORFLOX).

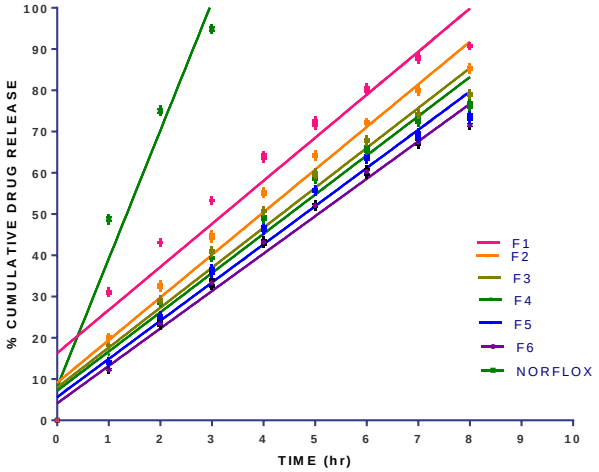

Figure.4 comparative zero order release kinetics profile of Norfloxacin *in situ* gels.

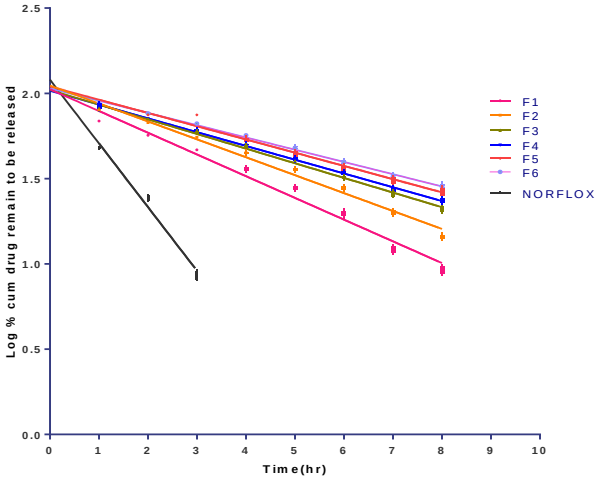

Figure.5 Comparative first order release kinetics profile of Norfloxacin *in situ* gels.

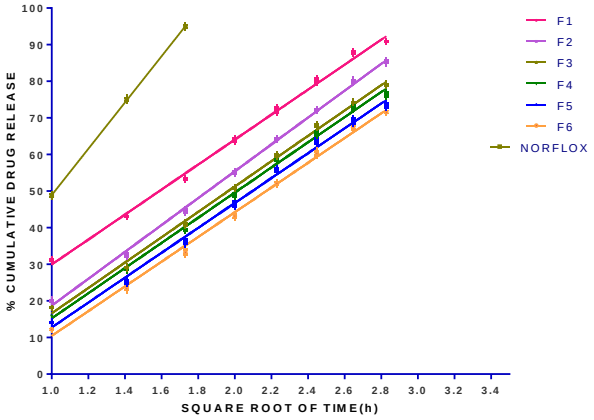

Figure.6 Comparative Higuchi release kinetics profile of Norfloxacin *in situ* gels.

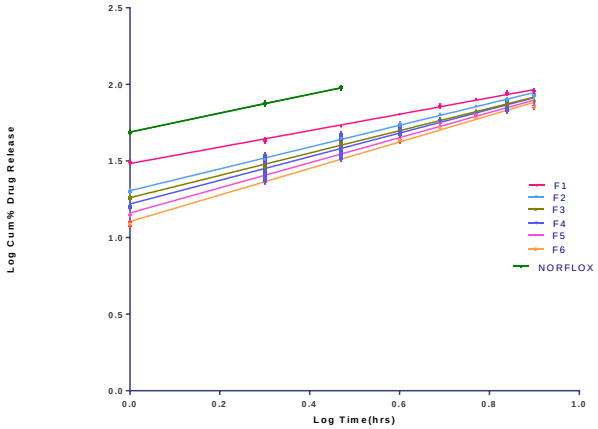

Figure.7 Comparative Peppas release kinetics profile of Norfloxacin *in situ* gels.

Antimicrobial Efficacy Studies

The optimized *in situ* gelling formulations showed antimicrobial activity when tested microbiologically by the Cup-Plate technique. Clear zones of inhibition were obtained in all the formulations. The diameter of zone of inhibition produced by formulations against all test microorganisms is given in Table.6

Table.6 Anti-microbial activity of Norfloxacin *insitu* gels

Test micro-organisms	Diameter of the zone of inhibition produced by *insitu* gels (mm)						
	F1	F2	F3	F4	F5	F6	NORFLOX
Staphylococcus aureus	23	24	25	24	26	25	19
Pseudomonas aeruginosa	29	30	27	31	32	31	26
E.coli	26	25	26	25	26	27	21

Sterility Testing

All the prepared *in situ* gelling systems were evaluated for the sterility. After 7 days of incubation the results showed no microbial growth in all formulations. (Table 7)

Table.7 Sterility testing of Norfloxacin *insitu* gels

Formulation code	Days of incubation						
	1	2	3	4	5	6	7
F1	-	-	-	-	-	-	-
F2	-	-	-	-	-	-	-
F3	-	-	-	-	-	-	-
F4	-	-	-	-	-	-	-
F5	-	-	-	-	-	-	-
F6	-	-	-	-	-	-	-

Where"–"sign indicate the no growth.

Accelerated stability studies

According to ICH guidelines, the accelerated stability studies were carried for prepared *in situ* gelling systems. All the formulations were analyzed for visual appearance, clarity, pH and drug remaining 6 weeks of stability studies reveal that there was no change in visual appearance and clarity. All the formulations showed remaining in all formulations revels that there were no definite changes observed to justify for drug deposition.(Figs 8,9)

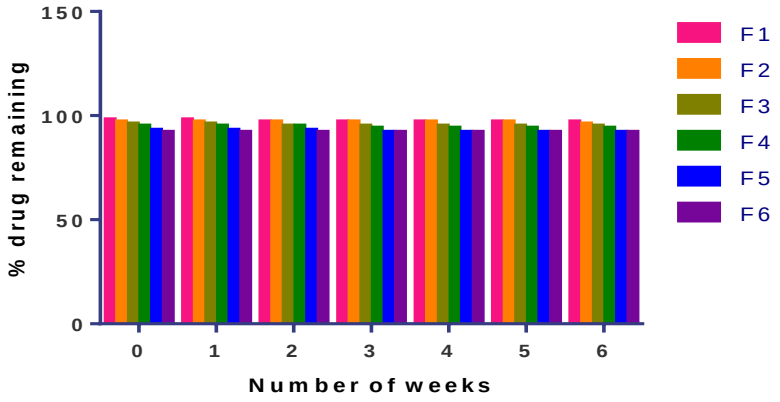

Figure.8 Stability studies of Norfloxacin *insitu* gels at room temperature.

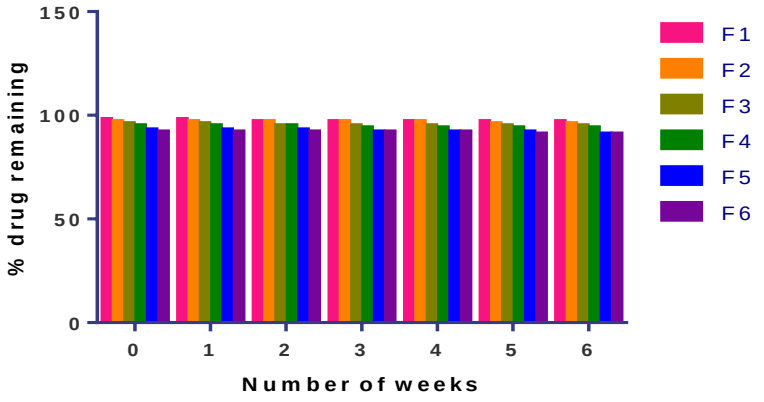

Figure.9 Stability studies of Norfloxacin *insitu* gels at 40^{O}C.

Hence from the above results we can conclude that it is possible to formulate *in situ* ophthalmic gels of Norfloxacin using the polymers chitosan and Poloxamer for treatment of various bacterial infections.

CONCLUSION

From the experimental results of the study *insitu* ophthalmic gel of Norfloxacin, Optimized formulation F5 (0.5 % chitosan, 15% poloxamer and PVA 0.3%) were liquid before instillation in to eye and underwent rapid gelation upon instillation in to eye, the formulations were found to be clear except F6 was not clear and whitish, all formulations having good *in situ* gelling capacity, having drug content 92-98%, all the prepared *in situ* gelling systems were found to be sterile. After 7 days of incubation the results showed no microbial growth in all formulations and sterile, showed sustained drug release over 8 hrs period as compared to marketed eye drop, release kinetic study showed that the formulations followed first order diffusion controlled and non-Fickian release mechanism, the optimized formulations was having good antibacterial efficacy, as per the Draize test protocol the ocular irritancy studies were carried out, results showed that formulations were non irritant. As per ICH guidelines the stability study of formulations were carried out results showed that formulations were stable (transparent and clear). Finally, systems that provide continuous, prolong drug release to the eye may in time find important uses in the treatment of ophthalmic diseases. The provision of continuous ocular drug delivery to the eyes fix the problem of frequent instillation and drug loss due to several factors like naso-lacrimal drainage etc, Thus. Insitu gel drug delivery system offers some hope for improving the epidemiological picture of severely debilitating eye diseases.

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