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FORMULATION AND EVALUATION OF IN SITU GELLING OCULAR SYSTEM OF OFLOXACIN

Taranbir Singh*, Dr. Rajeev Garg, Dr. G. D. Gupta

ASBASJSM College of Pharmacy, Bela, Ropar, Punjab (140111).

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***Correspondence for Author**
Taranbir Singh
ASBASJSM College of Pharmacy, Bela, Ropar, Punjab (140111)

ABSTRACT

Ocular bioavailability is always poor is always poor from conventional ophthalmic drops due to spillage and nasolachrymal drainage may be overcome by using In-situ gels nowadays. In situ gel formation has been extensively studied to enhance ocular bioavailability and the duration of drug activity. Purpose of the present research work deal with the formulation and evaluation of in situ gelling system based on sol-to-gel transition for ophthalmic delivery of an anti bacterial agent Ofloxacin. Chitosan was used as the viscosity increasing agent in combination with Poloxamer 407 act as emulsifying and solubilizing agent. The prepared formulation were evaluated for pH, clarity, drug content, gelling capacity, in vitro drug release. in vitro drug release data of optimized formulation 12 was treated according to zero, First, Korsmeyer Peppas, Higuchi Kinetic to access the mechanism of drug release. in vitro release study comparison with marketed eye drop (Ofloxacin) was done through cellophane membrane using diffusion cell. Such as visual appearance, clarity, pH and drug content of the formulation were found in range 6.8 to 7.4, drug content was in between 92-98%, these formulation were transparent and clear. These results suggest that thermosensitive in situ gel forming material for ocular drug delivery it may increase the bioavailability, efficacy and compliance of eye drugs.

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

INTRODUCTION

One of the most challenging and sensitive areas of topical drug delivery system is Ophthalmic drug dosage forms. The ocular formulations include various dosage forms which include solutions, suspensions, and semi- solids. The major problem encountered with the

ophthalmic solutions is the rapid pre-corneal loss caused by naso-lacrymal drainage and high tear fluid turnover which leads to only 10% drug concentrations available at the site of action.[1]

The frequent periodic instillation of eye drops becomes necessary to maintain a continuous sustained level of medication. This gives the eye a massive and unpredictable dose of medication, and unfortunately, the higher the drug concentration in the eye drop solution, the greater the amount of drug lost through lachrymal-nasal drainage system. Subsequent absorption of this drained drug, if it is high enough, may result in undesirable systemic side effects. The semisolid preparations or ointments though possess a higher bioavailability than liquid dosage forms due to lesser pre-corneal drainage but show disadvantages such as blurred vision and patient discomfort.[2]

Approaches to enhance the ocular bioavailability aim at increasing the corneal permeability by using penetration enhancers and prolonging the contact time with the ocular surface by using viscosity-enhancing or in-situ gelling polymers. The in-situ gelling polymers undergo sol-to-gel phase transition on exposure to the physiological conditions present in the eye. The major advantage of in-situ gel system is an accurate and reproducible administration of dose without disturbing the normal eye function.[3]

MATERIALS AND METHODS

1. MATERIALS

Ofloxacin was obtained as a gift from ranbaxy healthcare, jharmajri, baddi, H.P. Chitosan were obtained from Sigma-Aldrich chemie, Japan. Poloxamer were obtained from Yarrow chem. product, Mumbai. Benzalkonium chloride were obtained from Sigma-Aldrich chemie, Japan. Sodium bicarbonate were obtained from Nice chemical, Kerala. Sodium chloride were obtained from Fisher scientific, Mumbai. Calcium chloride and Acetic acid were obtained from Spectrochem, Mumbai.

2. METHODS.

2.1 Preparation of in situ gel of Ofloxacin

Table 2.1 Formulation formula of in situ gels.

Ingredient (gm)	Concentration w/v											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Ofloxacin	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Poloxamer	14	14	14	16	16	16	18	18	18	20	20	20
Chitosan	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
Benzalkonium Chloride	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Distilled water (q.s.)	100	100	100	100	100	100	100	100	100	100	100	100

2.2 Procedure for preparation of in situ gels of ofloxacin

The chitosan solutions (0.5-1.5% w/w), were prepared by dispersing the required amount in acetic acid solution (0.5% w/v) with continuous stirring until completely dissolved. For preparation of Pluronic solutions (14-20% w/w), the required amount of polymer (Poloxamer) was dispersed in distilled, deionized water with continuous stirring for 1 h at room temperature. The partially dissolved Poloxamer solutions were stored in the refrigerator (at 4ºC) until the entire polymer was completely dissolved (approximately 24 h). The chitosan/Poloxamer solutions were prepared by dispersing the required amount of Poloxamer in the desired concentration of chitosan with continuous stirring for 1 h. The partially dissolved solutions were then refrigerated until solutions were thoroughly mixed (approximately 24 h). The reported composition of chitosan/Poloxamer mixture was the final concentration of chitosan and Poloxamer content in the mixture. For preparation of Ofloxacin containing polymer solutions, 0.3% of Ofloxacin was added to the chitosan/Poloxamer solutions with continuous stirring until thoroughly mixed. Benzalkonium chloride solution was added 0.006% as preservative in all solutions. The prepared in situ gel were filled in glass vials closed with closures and sealed with aluminium caps and sterilized by autoclave at 121^0C 15 psi for 20 minutes.

2.3 Evaluation of in situ gels of Ofloxacin

2.3.1 Interaction studies

IR spectra was taken by using Fourier transformer infrared spectrophotometer(840, Shimadzu, Japan). FTIR study was carried on pure drug, physical mixture of drug and polymer, formulation to confirm the compatibility of drug with other excipients used in the preparation of in situ gels.[4]

2.3.2 Visual Appearance and Clarity

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

2.3.3 pH

The pH of the prepared in situ gelling system after addition of all ingredients was measured using pH meter.

2.3.4 In vitro gelation

Gelling capacity of formulation was evaluated in order to identify the formulation suitable for use as in situ gelling system. Gelling capacity was determine by mixing the formulation with simulated tear fluid in the examined visually.

The composition of simulated tear fluid was sodium chloride(0.670 g), sodium bicarbonate (0.200 g), calcium chloride dehydrate (0.008 g), distilled water quantity sufficient up to 100 ml. Physiological pH (7.4±0.2) was adjusted by adding the required amount of 0.1N HCL.[5]

2.3.5 Rheological studies

Viscosity of the instilled formulation is an impotent factor in determining residence time of drug in the eye. The prepared solution were allowed to gel in the simulated tear fliud then the viscosity determination were carried out by using Brook field viscometer in spindle no. 63, angular velocity ran from 10-100 rpm. Viscosity of the formulation increased with increase in polymer concentration.[6]

2.3.6 Measurement of Gelation Temperature (GT)

Ten millilitres of the sample solution and a magnetic bar were put into a transparent vial that was placed in a low temperature water bath. A thermometer with accuracy of 0.1 °C was immersed in the sample solution. The solution was heated at the rate of 2 °C/min with the continuous stirring of 500 rpm (Ruhromag, Germany). The temperature was determined as GT, at which the magnetic bar stopped moving due to gelation.

2.3.7 Sterility Testing

Sterility testing is intended for detecting the presence of viable form of micro-organisms and was performed for aerobic and anaerobic bacteria and fungi by using fluid thioglycate medium and soyabean casein digest medium, respectively as per the Indian pharmacopoeia.[7]

2.3.8 Preparation of media

Fluid thioglycate medium and soyabean casein digest medium were prepared by suspending all ingredients in 1000 ml of distilled water, separately boiled until it dissolved completely. Then it was sterilized by autoclaving at 15 lbs pressure, 121^0C for 15 minutes and cooled. After cooling 25 ml of both the medium were transferred to the Petri dish.[8]

2.3.9 Preparation of samples

The sterile formulation were taken into laminar airflow. Sterile formulation was removed from the vials by help of syringe. the solution was passed through the membrane filter of 0.45µm size with the help of vacuum pump. after filtration, the filter paper was removed from funnel and it was cut into two half. One half was dropped into bacterial media (Fluid thioglycolate) and other half was drooped in the fungal media (Soyabean casein digest). The media were kept for incubation for 14 days at 37^0C. Both the, media were observed every day for any microbial contamination and compared with a positive and negative control.[10]

2.4 Drug Content Analysis

2.4.1Estimation of Ofloxacin by spectrophotometric Method

0 A simple and rapid method for estimation of Ofloxacin by UV spectrophotometric method was developed in simulated tear fluid (STF). Ofloxacin in simulated tear fluid of pH 7.4shows λ_{max} at 296.5nm.[11]

2.4.2 Preparation of simulated tear fluid

Dissolve 0.670g of sodium chloride, 0.200g of sodium bicarbonate and 0.008g of calcium chloride di hydrate in 100ml of distilled water and adjust the pH 7.4 adding the required amount of 0.1N HCL.[12]

2.5 In vitro release studies

In vitro drug release from the formulation was studied by the diffusion cell. Here the pH of the Lacrimal fluid and the blinking rate of the eye were taken into consideration and were simulated. The procedure for standard calibration is same as mentioned under drug content determination.[13,14,15]

PROCEDURE

The in-vitro release of Ofloxacin as pure drug well as from the prepared formulations was studied through cellophane membrane using diffusion cell. The cellophane membrane was soaked overnight in the receptor medium.

Simulated Tear Fluid, pH 7.4). It was tied to one end of a glass diffusion cell. 50 ml of receptor medium was taken in the 200 ml beaker. The diffusion cell was filled with 2 ml of the formulation and suspended in 50 ml of receptor containing beaker by assuring that the membrane was just touched the receptor medium surface. The whole assembly was transferred on magnetic stirrer and was maintained at 37±2^{0}C and 20 rpm. The drug samples (1 ml) were withdrawn at the interval of one hour for the period of 8 hrs from receptor medium. The samples were diluted was done with 5 ml simulated tear fluid and analyzed by a UV-Visible spectrophotometer at 296.5 nm using receptor medium as a blank.

2.5.1 Comparative evaluation of marketed with prepared in situ gels

The in-vitro release studies of marketed formulation was studied through cellophane membrane using diffusion cell. The cellophane membrane was soaked overnight in the receptor medium (Simulated Tear Fluid, pH 7.4). It was tied to one end of a glass diffusion cell. 50 ml of receptor medium was taken in the 200 ml beaker. The diffusion cell was filled with 2 ml of the formulation and suspended in 50 ml of receptor containing beaker by assuring that the membrane was just touched the receptor medium surface. The whole assembly was transferred on magnetic stirrer and was maintained at 37±0.5^{0}C and 20 rpm. The drug samples (1 ml) were withdrawn at the interval of one hour for the period of 8 hrs from receptor medium. The samples were diluted was done with 5 ml simulated tear fluid and analyzed by a UV-Visible spectrophotometer at 296.5 nm using receptor medium as a blank.[16,17]

2.6 Antimicrobial Efficacy Studies

The antimicrobial efficacy studies were carried out to ascertain the biological activity of the optimized formulation. Staphylococcus aureus, E.coli were used as the test organisms. Anti microbial efficiency was determined by agar diffusion test employing Cup-Plate method. Sterile solution of Ofloxacin (standard solution) and the development formulation were dilute at different concentration (test solution) these solution were poured in to cups bored into sterile nutrient agar previously seeded with the test organisms (E.coli, Staphylococcus aureus), after allowing diffusion of the solution for 2 hours, the agar plate were incubated at 37^{0}C for 24 hours. The zone of incubation 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 negative and positive controls were maintained during the study.[18,19,20]

2.7 Accelerated Stability Studies

Stability is defined as the extent, to which a product retains with in specified limits and through out its period of storage and use i.e., shelf life. Stability studies were carried out on optimized formulation according to international conference on harmonization (ICH) guideline.[21,22,23]

A sufficient quantity of formulation in previously sterilized vials was stored in desiccators containing a saturated solution of sodium chloride, which give a relative humidity of 75±5 %. The desiccators were placed in a hot air oven maintained at a temperature 40^0C±0.5^0C and at room temperature. Sample were withdrawn at 7 days interval for 42 days.

RESULTS AND DISCUSSION

Twelve formulation of Ofloxacin in situ gelling system were prepared by using various concentration of chitosan with different concentration of poloxamer 407. All formulation had fixed drug concentration of (0.3%w/v) Ofloxacin.

Appearance, Clarity, Ph and Drug Content

All the prepared in situ gelling system were evaluated for preliminary steps such as visual appearance, clarity, pH and drug content. These formulations were transparent and clear. The pH of the formulation was found to be 6.8 to 7.4, and drug content was in between 90-98%

In vitro gelation

The prepared in situ gelling system were evaluated for the in vitro gelation capacity. All the formulation gave satisfactory results (Table 5.4)

Table 5.4 Evaluation of gelling capacity.

Formulation	Gelling Capacity
F1	+
F2	+
F3	++
F4	++
F5	+++
F6	+++
F7	++
F8	++
F9	++
F10	++
F11	+++
F12	+++

Note: + gel forms after few minutes, disperses rapidly, ++ gelation immediate and remain for few hours, +++ shows gelation immediate and remain for extended period

Rheological studies of in situ gels before gelation.

Shear Rate(RPM)	Viscosity of the formulation											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
10	153	142	184	120	140	162	125	138	153	162	165	174
20	70	50	73	85	74	76	87	86	103	118	119	126
50	32	35	69	50	52	53	63	68	72	75	79	83
100	27	26	34	28	30	41	43	44	46	51	52	53

Measurement of Gelation Temperature (GT)

Formulation	Poloxamer (% w/w)	Chitosan (% w/w)	$T_{Sol/gel}$ (^{0}C)
1	14	0.5	42
2	14	1.0	41
3	14	1.5	41
4	16	0.5	34
5	16	1.0	36
6	16	1.5	35
7	18	0.5	25
8	18	1.0	27
9	18	1.5	28
10	20	0.5	23
11	20	1.0	26
12	20	1.5	27

Sterility Testing

All the prepared in situ gelling system were evaluated for the sterility. After 14 days of incubation the result showed no microbial growth in all formulation.

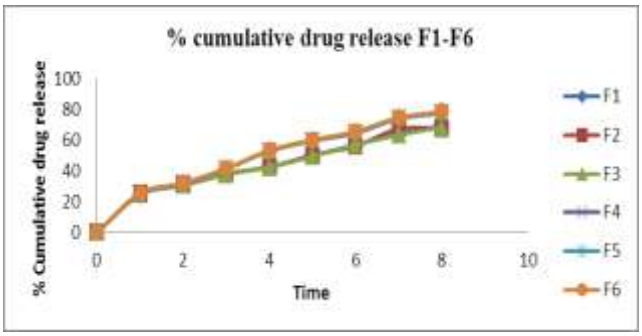
Table 5.7 Test of sterility.

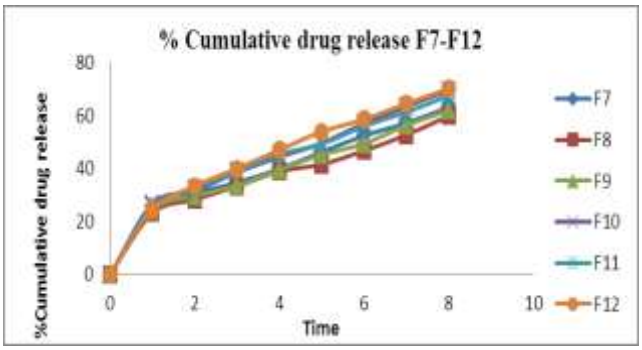
Formulation Coad	DAYS OF INCUBATION													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
F1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F11	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F12	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Where "-" sign indicate the no growth.

In vitro Release Studies

The in vitro release study of Ofloxacin from the prepared formulation was studied through cellophane membrane using diffusion cell. The release studies of prepared in situ gelling system were carried out up to 8 hours.





Kinetic Release data

The in vitro release profile were fitted to various kinetic models in order to find out the mechanism of drug release. The rate content were calculated from the slope of respective plote. correlation (R^2=0.992) was observed in Higuchi plot rather than first order (R^2=0.989) and zero order (R^2= 0.942) models. The drug release was proportional to square root of time, indicating that the drug release from in situ gel was diffusion controlled. The data obtained was also fit in Korsmeyer peppes medel in n=order to find out n value, which describe the drug release mechanism. the n value (0.5005) obtained from Korsmeyer Peppes was more than 0.5, which indicated that the mechanism of the drug release was Anomalous and Non Fickian diffusion controlled.

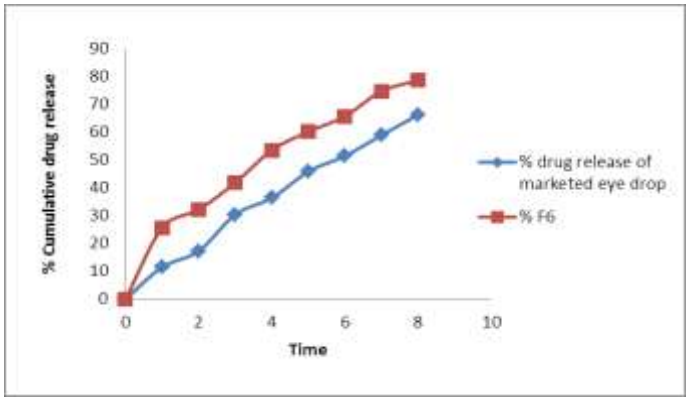
Release Kinetics of in situ gels F1-F12.

Formulation Code	Zero order 'R^2'	First Order 'R^2'	Higuchi Kinetic ' R^2'	Korsmeyer's peppas 'n'
F1	0.947	0.983	0.987	0.512
F2	0.934	0.969	0.982	0.493
F3	0.943	0.983	0.988	0.502
F4	0.952	0.989	0.987	0.571
F5	0.952	0.992	0.988	0.574

F6	0.953	0.997	0.990	0.575
F7	0.930	0.991	0.988	0.475
F8	0.917	0.961	0.985	0.444
F9	0.924	0.970	0.986	0.452
F10	0.932	0.977	0.987	0.470
F11	0.922	0.978	0.994	0.467
F12	0.931	0.987	0.997	0.507

Comparison of In vitro % cumulative drug Release and marketed drug release

In vitro release study of marketed eye drops (Ofloxacin) was done through cellophane membrane using diffusion cell and the release marketed product was up to 8 hours.



Antimicrobial Efficacy Studies

The optimized in situ gelling formulation showed antimicrobial activity when tested microbiologically by the Cup-Plate technique. Clear zone of inhibition were obtain in all 0the formulation. The diameter of zone of inhibition produced by formulation against all test micro-organisms.

Antimicrobial activity of in situ gels F1-F6.

Test Micro-Organisms	**Diameter of the Zone of Inhibition Produced By in situ Gels (mm)**						
	F1	F2	F3	F4	F5	F6	F7
Staphylococcus Aereus	24	25	25	24	23	25	23
Pseudomonas Aeruginosa	32	27	29	30	29	31	30
E.coli	25	24	26	24	24	26	25

Antimicrobial activity of in situ gels F7-F12.

Test Micro-Organisms	**Diameter of the Zone of Inhibition Produced By in situ Gels (mm)**					
	F8	**F9**	**F10**	**F11**	**F12**	**Ofloxacin**
Staphylococcus Aereus	25	23	24	25	24	26
Pseudomonas Aeruginosa	31	29	29	30	30	33
E.coli	25	24	25	24	24	27

Accelerated Stability Studies

According to ICH guideline, the accelerated stability studies were carried for prepared in situ gelling systems. All the formulation were analyzed for visual appearance, clarity, pH and drug remaining. 7 weeks of stability studies reveal that there was no change in visual appearance and clarity. All the formulation showed slight changes in pH, but it were in acceptable limits (±0.5). Study of % drug remaining in all formulation reveals that there were no definite changes observed to justify for drug degradation.

Stability study of F6.

Sr. No.	Number Of Days	Visual Appearance		Clarity		pH	
		RT	40^0C	RT	40^0C	RT	40^0C
1	0	Transparent	Transparent	Clear	Clear	7.24	7.24
2	7	Transparent	Transparent	Clear	Clear	7.24	7.24
3	14	Transparent	Transparent	Clear	Clear	7.24	7.25
4	21	Transparent	Transparent	Clear	Clear	7.25	7.26
5	28	Transparent	Transparent	Clear	Clear	7.24	7.25
6	42	Transparent	Transparent	Clear	Clear	7.23	7.24

Stability study of formulation 6 at room temperature.

Sr. No.	Number of Days	% Drug Remaining
		Formulation 6
1	0	97.20
2	7	97.20
3	14	97.20
4	21	97.21
5	28	97.20
6	42	97.22

Stability study of formulation 6 at 40^0C.

Sr. No.	Number of Days	% Drug Remaining
		Formulation 6
1	0	97.20
2	7	97.21
3	14	97.22
4	21	97.21
5	28	97.20
6	42	97.22

SUMMARY AND CONCLUSION

The Aim of the Present work was to prepare and evaluate in situ ophthalmic gel of an anti biotic drug for sustained ocular delivery for the treatment of various bacterial diseases of eye,

by providing comfortness, compliance to the patient and improve the therapeutic performance of the drug over conventional ocular dosage form.

Formulation containing chitosan (0.5,1.0,1.5% w/w) was prepared along with poloxamer (14%, 16%, 18% and 20% w/w), where chitosan was initially dissolved in a solution of acetic acid 0.5% w/v.

Optimized formulation F1(F1A 14%w/w Poloxamer and chitosan(0.5%w/w), F1B(14% w/w poloxamer and chitosan (1.0%w/w), F1C(14%w/w poloxamer and chitosan (1.5%w/w), F2(F2A 16% w/w poloxamer and chitosan 0.5%w/w), F2B(16%w/w Poloxamer and Chitosan (1.0%w/w), F2C(16%w/w Poloxamer and chitosan(1.5%w/w), F3(F3A 18%w/w Poloxamer and chitosan(0.5%w/w), (F3B 18%w/w Poloxamer and chitosan(1.0%w/w), (F3C 18%w/w Poloxamer and chitosan(1.5%w/w), F4(F4A 20%w/w Poloxamer and chitosan(0.5%w/w), (F4B 20%w/w Poloxamer and chitosan(1.0%w/w), (F4C 20%w/w Poloxamer and chitosan(1.5%w/w), Where liquid instillation to the eye and underwent rapid gelation upon instillation to the eye.

In the preformulation studies Ofloxacin was characterized by its physiochemical properties such as melting point, solubility, UV and FTIR studies. UV spectroscopic method was established for quantitative estimation of the drug and the absorption maxima was 296.5 nm. FTIR study of physical mixture of drug and polymer, prepare in situ gels was carried out and were compared with IR absorption spectra of pure drug. Study revealed that there were definite changes in bands observed with respect to pure drug. So it was confirmed that formulation do not have any drug polymer interaction.

Optimized in situ gels were subjected for preliminary evaluation such as visual appearance, clarity, pH, drug content. All formulation were found transparent and clear, pH of the formulation was within 6.8 to 7.4. All drug content was found within F1(86.98%), F2(87.54%), F3(88.12%), F4(97.04%), F5(97.12%), F6(97.20%), F7(95.23%), F8(95.31%), F9(95.36%), F10(94.10%), F11(94.38%), F12(94.89%) in all optimized in situ gelling system.

In order to evaluate Rheological Behaviour, Viscosity of the formulation before and after addition STF (Simulated Tear Fluids) was evaluated using Brook field Viscometer. It show

that viscosity of all formulation decreased as the share rate increase, which indicate the character of Pseudoplastic fluid.

Sterility test was done by using nutrient agar media and incubate 14 days under daily observation. The study show that formulation do not having any microbial contamination and was sterile.

In vitro release of ofloxacin from the selected formulation was studies through diffusion cell using cellophane membrane for 8 Hours. it was compared with the marketed eye drop. Antimicrobial efficacy study carried out by using Staphylococcus Aureus and E.coli as test micro-organisms after incubation up to 24 hours, It was found that all formulation were having effective anti microbial action.

The Stability study was carried for all optimized study up to 42 days. Results reveal thats no changes were found in visual appearance, clarity and pH. These study show that there were no definate change observed in the intactness of the drug after accelerated stability study of 42 days.

Hence form the all above results we can conclude that it is possible to formulate in situ gels of ofloxacin using chitosan for treatment of various Bacterial infection.

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