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Controlled release *In Situ* forming Ofloxacin Hydrogel for Ophthalmic Drug Delivery

S.N. Chaudhary[1]*, G.S. Rathore[1], C.S. Chauhan[1], R.K. Kamble[1]

1.Bhupal Nobels' College of Pharmacy, Udaipur, Rajsthan.

ABSTRACT

The objective of the present study was to prepare *in situ* hydrogel for controlled release of ofloxacin using various polymers such as Poloxamer P407, Sodium Alginate and Polyox. *In situ* were characterized for the Appearance, pH determination, *In vitro* Gelation studies and viscosity, Rheological studies, Drug Content, *In vitro* Drug release study, Sterility testing. Drug-excipient compatibility was determined by FTIR. Infrared spectroscopy studies of Ofloxacin, Polyox, Sodium alginate, Poloxamer and HPMC K4M alone and their physical mixture revealed that, Ofloxacin is compatible with all the polymers used. The clarity of the prepared formulations was found satisfactory. The pH of all formulations was found to be satisfactory in the range. The drug content of the prepared formulation was within the acceptable range, and ensures dose uniformity. The curve fitting data revealed that the release followed zero order kinetics.

Keywords: *In situ* Hydrogel, Ofloxacin, Controlled Release, Polyox, Poloxamer.

*Corresponding Author Email: samirchaudhary06@yahoo.com

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INTRODUCTION

Eye is most interesting organ due to its drug disposition characteristics. Generally, topical application of drugs is the method of choice under most circumstances because of its convenience and safety for ophthalmic chemotherapy. Conventional ophthalmic formulations like solution, suspension, and ointment have many disadvantages which result into poor bioavailability of drug in the ocular cavity. The specific aim of designing a therapeutic system is to achieve an optimal concentration of a drug at the active site for the appropriate duration. Ideal ophthalmic drug delivery must be able to sustain the drug release and to remain in the vicinity of front of the eye for prolong period of time. Consequently it is imperative to optimize ophthalmic drug delivery; one of the ways to do so is by addition of polymers of various grades, development of in situ gel or colloidal suspension or using erodible or non-erodible insert to prolong the precorneal drug retention.[1,2,3]

Ofloxacin is a broad spectrum antibiotic and its half-life in plasma has been reported to be 4-5 hours (Pongpaibul Y, *et al.* 1986; URL: rxlist). The shorter biological half-life and frequent dosing in wide varities of bacterial infections make it as an ideal candidate for controlled drug delivery system.[4] Therefore the objective of the work is to provide a controlled action pharmaceutical composition containing ofloxacin in a controlled release formulation.

Hydrogels are one of the upcoming classes of polymer-based controlled release drug delivery systems. Hydrogels are polymeric networks that absorb large quantities of water while remaining insoluble in aqueous solutions due to chemical or physical crosslinking of individual polymer chains. Polymeric drug delivery systems have been extensively studied in order to solve the potential problems associated with drugs or bioactive molecules including toxicity, site dependence, low effectiveness, poor solubility, short half-life, rapid degeneration and rapid clearance from the body. Considering various properties such as flexibility, structure, biocompatibility, and hydrophilicity, three dimensional matrices, hydrogels, are being extensively used as drug delivery carriers. [5] *In situ* hydrogels can be instilled as eye drops and undergo an immediate gelation when in contact with the eye. *In situ*-forming hydrogels are liquid upon instillation and undergo phase transition in the ocular cul-de-sac to form viscoelastic gel and this provides a response to environmental changes. [6] Three methods have been employed to cause phase transition on the surface: change in temperature, pH, and electrolyte composition. Hydrogels have a unique combination of characteristics that make them useful in drug delivery applications. Due to their hydrophilicity, hydrogels can imbibe large amounts of water (N90 wt.

%). Therefore the molecule release mechanisms from hydrogels are very different from hydrophobic polymers. [7, 8]

Rationale for development of *In situ* Hydrogel [9, 10]

1. Reduce toxic effects on the healthy tissue and reach sites that are conventionally Inaccessible due to the presence of various barriers 9 by targeted drug delivery.
2. Increase the half-life of drugs, preventing their rapid degradation, and reduce the rate of elimination, thus maintaining drug concentration within a therapeutically effective window.
3. Reduce the amount of drug required to achieve therapeutic efficacy.
4. Cut down the number of repeated invasive dosage required for certain conditions and thus helps to improve patient's compliance and offers better living.

MATERIALS AND METHODS

Materials:

Ofloxacin was obtained as gift sample from Dr. Reddy's, Polymers Polyox, Poloxamer was obtained from Johnson & Johnson Mumbai and HPMC was obtained from Colorcon Pharma.

Experimental design

A 2 level 2 factors factorial design (2^2) was employed to design ocular controlled in situ forming Ofloxacin hydrogel. The design was employed for formulations containing three different gelling agents Polyox, Sodium alginate & Poloxamer separately having a common viscofying agent HPMC K4 M.[11, 12, 13] The independent and dependent variables selected are common for all the polymers and are as follows:

Independent Variables

a) Concentration of Gelling agent (X1), b) Concentration of Viscofying agent HPMC K4M (X2)

Dependent Variables

a) Viscosity (Y1),b) Gelling capacity (Y2)

Quantities were taken on the considering the rheological properties of the polymers so that the formulations prepared showed not be too much viscous. Preliminary studies for the polymers were done to decide the quantities of the polymers.

Optimized method for in situ hydrogel preparation

Polyox in situ ophthalmic hydrogel

For the preparation of Polyox containing in situ hydrogel, a viscofying agent, HPMC K4M (0, 0.5 %w/v) was first added to 75 ml of citrophosphate buffer pH 6 and allowed to hydrate. Then polyox was sprinkled over this solution and allowed to hydrate overnight. Ofloxacin was

dissolved in 25 ml of buffer solution separately and then added to polymer solution under constant stirring until a uniform solution was obtained. The formulations, in their final pack were subjected to terminal sterilization by autoclaving at 121ºC and 15 psi for 20 min. [14, 15, 16]

Table 1: Composition of Ofloxacin in situ hydrogel prepared as per 2^2 factorial design

Gelling Agent	Sr.no	Formulation Code	Variable Levels		Actual Units	
			X_1	X_2	$X_{1(\%W/V)}$	$X_{2(\%W/V)}$
Polyox	1.	OF_1	-1	+1	0.3	0.5
	2.	OF_2	+1	-1	0.5	0
	3.	OF_3	-1	-1	0.3	0
	4.	OF_4	+1	+1	0.5	0.5
Sodium	5.	OF_5	-1	+1	0.5	0.5
alginate	6.	OF_6	+1	-1	1	0
	7.	OF_7	-1	-1	0.5	0
	8	OF_8	+1	+1	1	0.5
Poloxamer	9.	OF_9	-1	+1	18	0.5
	10.	OF_{10}	+1	-1	25	0
	11.	OF_{11}	-1	-1	18	0
	12.	OF_{12}	+1	+1	25	0.5

Sodium alginate in situ ophthalmic hydrogel

The sodium alginate in situ hydrogel was prepared by first dispersing the required amount of sodium alginate in 75ml distilled deionized water with continuous stirring until completely dissolved. Then the required amount of HPMC (0, 0.5 %w/v) was added to alginate solution with continuous stirring until completely dissolved. Ofloxacin was dissolved in hydrochloric acid and the pH was adjusted to 6.3 using sodium hydroxide. The drug solution was added to the polymer solution under constant stirring until uniform, clear solution was obtained. Distilled, deionized water was then added to make the volume up to 100 ml. [14, 15, 16]

Poloxamer in situ ophthalmic hydrogel

Poloxamer in situ forming gels were prepared by the modified cold method. Briefly, poloxamer (P407) and required amount of HPMC (0, 0.5 %w/v) was slowly added to the calculated amount of cold acetate buffer (pH 6.5) with continuous mixing using a thermostatically controlled magnetic stirrer. The partially dissolved poloxamer solutions were stored in a refrigerator and stirred periodically until clear homogenous solutions were obtained (approximately 24 h).The ophthalmic formulations were prepared by dissolving the appropriate amount of Ofloxacin, 0.5% (w/v), in the calculated amount of acetate buffer during the mixing step. [14, 15, 16]

EVALUATION PARAMETERS

Appearance

Clarity is one of the most important characteristic features of ophthalmic preparations. All

developed formulations were evaluated for clarity by visual observation against a black and white background. [17]

pH

pH is one of the most important parameter involved in the ophthalmic formulation. The two areas of critical importance are the effect 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. Ophthalmic formulations should have pH range in between 5 to 7.4. The developed formulations were evaluated for pH by using Elico India Systronics digital pH meter. [17]

Drug Content

Uniform distribution of active ingredient is important to achieve dose uniformity. The drug content was determined by diluting 1 ml of the formulation to 50 ml with phosphate buffer solution pH 7.4. Aliquot of 5 ml was withdrawn and further diluted to 50 ml with PBS. Ofloxacin concentration was then determined at 296 nm by using UV-Vis spectrophotometer. [18]

In vitro Gelation Studies

All prepared formulations were evaluated for gelling capacity and viscosity in order to identify the compositions suitable for use as in situ gelling systems. The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of artificial tear fluid freshly prepared and equilibrated at 37^0 C and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve. The composition of artificial tear fluid used was sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride 2H2O 0.008 g, purified water Q.S. 100.0 g. The viscosity was measured using a Brookfield Synchrolectric viscometer (RVT model) in the small volume adapter. The viscosity measured at 20 rpm was used for purposes of comparative evaluation. [19, 20]

Rheological Studies

Viscosity of instilled formulation is an important factor in determining residence time of drug in the eye. The developed formulations were poured into the small sample adaptor of the Brookfield Synchrolectric viscometer and the angular velocity increased gradually from 0.5 to 50 rpm. The hierarchy of the angular velocity was reversed. The average of the two readings was used to calculate the viscosity. [21, 22. 23]

***In vitro* Release Studies**

The *in vitro* release of Ofloxacin from the formulations was studied through cellophane membrane using a modified USP XXIII dissolution testing apparatus. The dissolution medium

used was artificial tear fluid freshly prepared (pH 7.4). Cellophane membrane, previously soaked overnight in the dissolution medium, was tied to one end of a specifically designed glass cylinder (open at both ends and of 5 cm diameter). A 1-ml volume of the formulation was accurately pipetted into this assembly. The cylinder was attached to the metallic driveshaft and suspended in 50 ml of dissolution medium maintained at 37±1 ^{0}C so that the membrane just touched the receptor medium surface. The dissolution medium was stirred at 50 rpm using magnetic stirrer. Aliquots, each of 1-ml volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with the receptor medium and analyzed by UV-Vis spectrophotometer at 296 nm. [24, 25, 26]

Sterility

All ophthalmic preparations should be sterile therefore the test for sterility is very important evaluation parameter. The sterility test was performed according to Indian Pharmacopoeia. Direct inoculation method was used. 2 ml of liquid from test container was removed with a sterile pipette or with a sterile syringe or a needle. The test liquid was aseptically transferred to fluid thioglycolate medium (20 ml) and soyabean-casein digest medium (20 ml) separately. The liquid was mixed with the media. The inoculated media were incubated for not less than 14 days at 30 ^{0}C to 35 ^{0}C in the case of fluid thioglycolate medium and 20 ^{0}C to 25 ^{0}C in the case of soyabean-casein digest medium. [27, 28, 29]

RESULT AND DISCUSSION

Appearance

Clarity of all the formulations was found to be satisfactory. Terminal 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 disappear and the original clarity was regained after overnight standing.

pH

The pH of the formulations was found to be satisfactory and was in the range of 6-7.4. The formulations were liquid at room temperature and at the pH formulated. Terminal sterilization by autoclaving had no effect on the pH.

***In-vitro* Gelation Studies and Viscosity:**

The two main prerequisites of an *in situ* gelling system are viscosity and gelling capacity (speed and extent of gelation). The formulation should have an optimum viscosity that will allow easy

instillation into the eye as a liquid (drops), which would undergo a rapid sol-to-gel transition. Additionally, to facilitate sustained release of 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 3 shows the gelling capacity of all formulations and is depicted as + (gels after few minutes and dissolves rapidly), ++ (gelation immediate, remains for few hours) and +++ (gelation immediate, remains for extended period). Table 2 shows the gelling capacity of formulations from OF_1 to OF_{12}. All the formulations except OF_3, OF_7 and OF_{11} showed instantaneous gelation when contacted with simulated tear fluid (STF). However, the nature of the gel formed depended on the concentration of polymers used. The formation of instantaneous gels can be attributed to the buffering capacity of the simulated tear fluid.

Formulation OF_3, OF_7 and OF_{11} showed the formation of gel after a few minutes which dissolved rapidly. Formulation OF_1, OF_2, OF_6, OF_{10} and OF_{12} showed immediate gelation and remained for few hours, whereas the formulation OF_4, OF_5, OF_8 and OF_9 showed immediate gelation and remained for extended period.

Table 2: Results of response variables for 2^2 factorial design

Gelling Agent	Sr. No.	Formulation Code	Variable levels in coded form				Viscosity (20rpm) (Y_1)	Gelling Capacity (Y_2)
			X_1	Gelling Agent (%w/v)	X_2	Viscofying Agent (%w/v)		
Polyox	1.	OF_1	-1	0.3	+1	0.5	1342.7	++
	2.	OF_2	+1	0.5	-1	0	908.1	++
	3.	OF_3	-1	0.3	-1	0	719.4	+
	4.	OF_4	+1	0.5	+1	0.5	1572.4	+++
SodiumAlginate	5.	OF_5	-1	0.5	+1	0.5	1610.8	+++
	6.	OF_6	+1	1	-1	0	2082.5	++
	7.	OF_7	-1	0.5	-1	0	1107.2	+
	8.	OF_8	+1	1	+1	0.5	2348.6	+++
Poloxamer	9.	OF_9	-1	18	+1	0.5	1521.9	+++
	10.	OF_{10}	+1	25	-1	0	1946.6	++
	11.	OF_{11}	-1	18	-1	0	1042.1	+
	12.	OF_{12}	+1	25	+1	0.5	2157.9	++

+: Gels after few minutes, dissolves rapidly.
++: Immediate gelation, remains for few hours.
+++: Immediate gelation, remains for extended period.

Table 2 also shows the viscosity (cp) of formulations from OF_1 to OF_{12} at 20 rpm. The viscosity measured at 20 rpm was used for purpose of comparative evaluation. In case of Polyox and poloxamer based hydrogels, the viscosity increased in proportion with viscofying agent both at lower and higher concentration of gelling agent, i.e. gelling agent had a little effect on viscosity.

While in case of sodium alginate based hydrogel, the viscosity increased in proportion with viscofying agent but gelling agent had more effect on viscosity than viscofying agent. This may be attributed to the higher viscosity of sodium alginate than other two gelling agents.

On the basis of gelling capacity and viscosity, formulations OF_4, OF_5 and OF_9 showed optimum results within the desired range. Hence, these three formulations were subjected for further evaluation parameters.

Rheological Studies:

Table 3 shows the viscosity values obtained for formulations OF_4, OF_5 and OF_9 using Brookfield DV-111+ rheomoter at different angular velocity. Formulations were shear thinning and an increase in shear stress was observed with increase in angular velocity (pseudoplastic rheology). The results obtained from the rheological study of prepared in situ gelling system OF_4, OF_5 and OF_9 revealed that the viscosity decreases as the angular velocity increases.

Table 3: Rheological profile of the In-situ gelling system.

Angular Velocity (rpm)	Viscosity (x 100cP)		
	OF_4	OF_5	OF_9
0.5	83.245	91.214	90.553
1.5	75.562	78.231	73.117
2.5	62.879	66.735	60.324
5	41.451	42.892	39.173
10	26.815	29.764	25.431
20	15.724	16.108	15.213
30	12.821	13.917	12.543
40	11.249	12.155	11.103
50	11.118	11.254	10.986

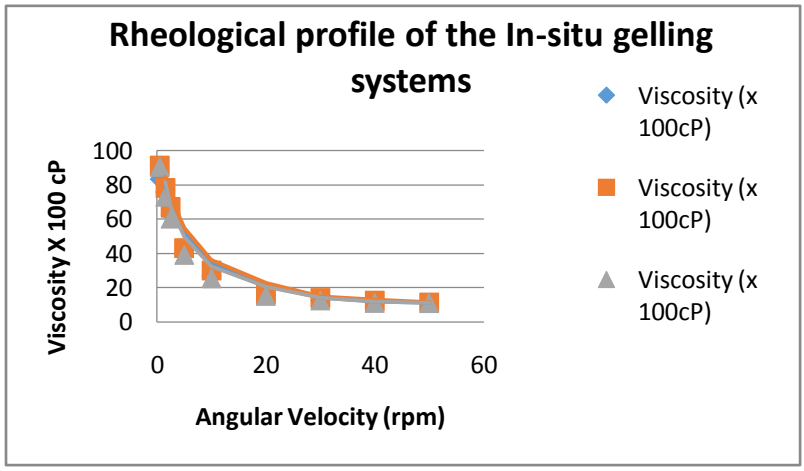

Figure 1: Rheological profile of the In-situ gelling systems

Generally viscosity values in the range of 15-50 cps significantly improve the contact time in the eye. Higher viscosity values offer no significant advantage and have a tendency to leave a

noticeable residue on the lid margin. The administration of ophthalmic preparation should influence as little as possible the pseudoplastic character of the precorneal tear film. Since the ocular shear rate is very large ranging from 0.03 s^{-1} during interblinking periods to 4250 – 28,500 s^{-1} during blinking, viscoelastic fluids with a viscosity that is high under conditions of low shear rate and low under conditions of high shear are preferred. The rheological profile of prepared *in situ* gelling systems of Ofloxacin is shown in Figure 1.

Drug Content:

Table 5 shows the percent drug content for formulations OF_4, OF_5 and OF_9. The drug content was found to be in acceptable range for all the formulations. Percent drug content of formulations OF_4, OF_5 and OF_9 was found to be 98.2%, 98.76% and 99.43% respectively, indicating uniform distribution of drug.

Table 5: Drug Optimization formulation

Formulation	Drug Content (%)
OF4	98.20
OF5	98.76
OF6	99.43

***In Vitro* Release Studies:**

The release profile of a drug predicts how a delivery system might function and gives valuable insight into its *in vivo* behaviour. The three *in situ gelling* formulations of Ofloxacin, OF_4, OF_5 and OF_9, were subjected to *in vitro* release studies. These *in vitro* release studies were carried out using simulated tear fluid (STF) of pH 7.4 as the dissolution medium.

The drug release data obtained for formulations OF_4, OF_5 and OF_9 is tabulated in Table 6, 7 and 8. Figure 2 shows the plot of cumulative percent drug released as a function of time for formulation OF_4, OF_5 and OF_9. It was found that cumulative percent drug release was 75.36%, 73.39% and 71.74% for formulation OF_4, OF_5 and OF_9 respectively after 8 hours. The ***in vitro*** release data indicated that the formulation OF_9 showed better sustained effect than other two formulations.

All the three formulations showed an initial burst release. The prolonged release in the later stage can be attributed to the slow diffusion of the drug through polymer matrix. The initial burst release of the drug can be explained by the fact that, the *in situ* gelling system is formulated in water and hence the polymer was completely hydrated. When they come in contact with STF, gelation occurs and a prehydrated matrix is formed in which hydration and water penetration no longer limit drug release, leading to an apparent diffusion-controlled release.

The *in vitro* drug release conditions may be very different from those likely to be encountered in

the eye. However, the results clearly show that the gels have the ability to retain drug for prolonged period of time (8 hour) and that premature drug release will not occur. In the cul-de-sac, the gels will probably undergo faster dissolution due to the shearing action of the eyelid and eyeball movement. It is also observed that the dissolution in the cul-de-sac will proceed more slowly than that seen in the *in vitro* experiments, as the normal resident volume of the lachrymal fluid in the human eye is 7.5-10 µl.

The gels on visual inspection at periodic intervals during the *in vitro* drug release experiments showed a gradual swelling after 6 hour that resulted in an increase in volume of most gels. No discernible relationship between the extent of swelling and gel composition could be established. Also, no apparent changes or disruptions in the integrity of the gels were noticed during the course of experiment. The only evidence to suggest a gradual dissolution of the polymers comprising the gels was that, the filtration of the aliquot of release medium became increasingly difficult after each successive withdrawal. This shows *in vitro* release of drug from the in situ formulation follows diffusion mechanism.

Table . 6: In vitro Drug Release of Ofloxacin from In-situ Hydrogel Formulation OF_4

Time	Root time	Log time	Absorbance	CDR	%CDR OF_1	%CDR OF_5	%CDR OF_9
1.	1	0	0.1264	1.48	30.142	29.614	27.911
2.	1.414	0.3010	0.1459	1.7596	35.837	36.089	32.136
3.	1.732	0.4771	0.1658	2.0442	41.633	42.283	37.597
4.	2	0.6020	0.1908	2.4039	48.959	48.407	42.350
5.	2.236	0.6989	0.2076	2.67	54.378	54.707	49.720
6.	2.449	0.7781	0.2349	3.0704	62.533	61.334	56.680
7.	2.645	0.8450	0.2551	3.3878	68.997	68.158	63.661
8.	2.828	0.9030	0.2746	3.7004	75.364	74.081	71.752

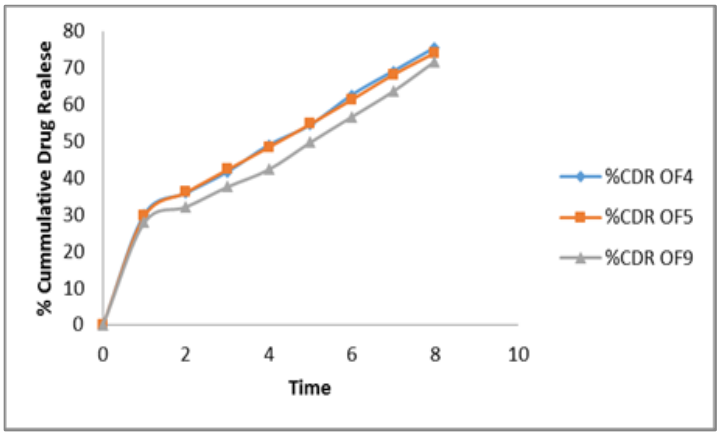

Figure 2*: In Vitro* release profile of Ofloxacin from selected hydrogel Formulation. (Zero Order)

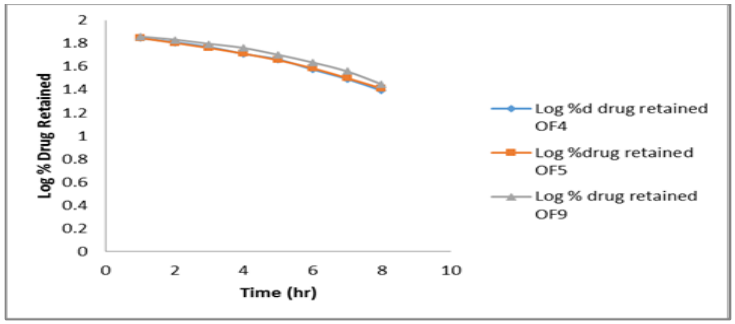

Figure 3: *In Vitro* release profile of Ofloxacin from selected hydrogel Formulation. (First Order)

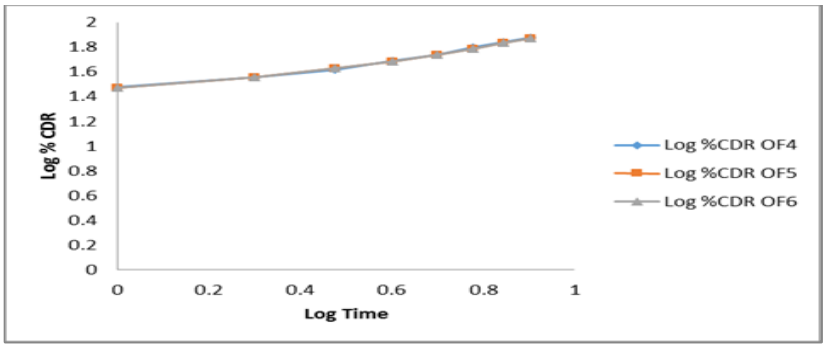

Figure 4*: In Vitro* release profile of Ofloxacin from selected hydrogel Formulation. (Korsmeyer Peppas)

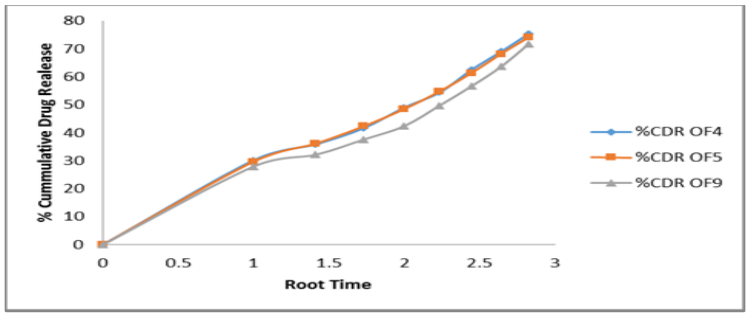

Figure 5: In Vitro release profile of Ofloxacin from selected hydrogel Formulation. (Higuchi Matrix)

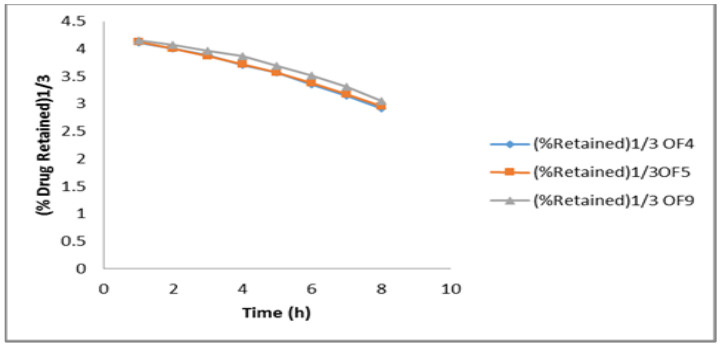

Figure 6: In *Vitro* release profile of Ofloxacin from selected hydrogel Formulation. (Hixon - Crowell)

Table 9: Model Fitting for the Release Profile of Formulation by using 5 Different Models

Formulation Code	Zero Order	First Order	Higuchi Matrix	Korsmeyer-Peppas		Hixon-crowell	Best Fit Model
	R	R	R	R	n	R	
OF4	0.998	0.971	0.971	0.960	0.449	0.985	Zero
OF5	0.999	0.978	0.979	0.972	0.446	0.989	Zero
OF9	0.990	0.951	0.946	0.934	0.457	0.967	Zero

Sterility Test:

There was no appearance of turbidity and hence no evidence of microbial growth when the formulations were incubated for not less than 14 days at 30 ^{0}C to 35 ^{0}C in case of fluid thio glycolate medium and at 20 ^{0}C to 25 ^{0}C in the case of soyabean-casein digest medium. The preparations being examined therefore passed the test for sterility.

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