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Design and Evaluation of Chloramphenicol Thermoreversible *Insitu* Gels for Occular Drug Delivery

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ABSTRACT

Conventional ophthalmic formulations like solution, suspension, and ointment have many disadvantages which result into poor bioavailability of drug in the ocular cavity. The poor bioavailability and therapeutic response exhibited due to pre corneal elimination of the drug may be overcome by the use of mucoadhesive *in situ* gel forming systems that are instilled as drops into the eye and undergo a sol-gel transition in the cul-de-sac and have good mucoadhesion with ocular mucus layers. The objective of this study was to formulate ophthalmic mucoadhesive system of chloramphenicol and to evaluate its *in vitro* antibacterial potential against, *Staphylococcus aureus* and *Escherichia coli*. Mucoadhesive systems were prepared by using polaxamer 188 combined with chitosan to enhance the gel bio adhesion properties. Increase in the concentration of mucoadhesive agent enhanced the mucoadhesive force significantly. *In vitro* release of chloramphenicol from the mucoadhesive system in simulated tear fluid was influenced significantly by the properties and concentration of chitosan, carbapol 934 showed to enhance bioavailability through its longer pre corneal residence time and ability to sustain the release of the drug. Significant reduction in the total bacterial count was observed between drug solution (control) and mucoadhesive batches against both tested organisms.

Keywords: Mucoadhesive, Chlorampjenicol, Polaxamer, Chitosan, Thermoreversible *in situ* gel, Occular drug delivery.

INTRODUCTION

A significant challenge to formulate ocular products is to bypass the protective barriers of the eye without causing permanent tissue damage. Development of newer, more sensitive diagnostic techniques and novel therapeutic agents continue to provide ocular delivery systems with high therapeutic efficacy. 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.

Ocular disposition and elimination of a therapeutic agent is dependent upon its physicochemical properties as well as the relevant ocular anatomy and physiology. A successful design of a drug delivery system, therefore, requires an integrated knowledge of the drug molecule and the constraints offered by the ocular route of administration. (Mundada AS *et al.*, 2008) 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 pre corneal drug loss. 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 way 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 pre corneal drug retention. (Wagh VD *et al*., 2008).

In this regard many polymers are very useful which undergo reversible sol to gel phase transition in response to physiological stimuli. (Khurana AK *et al*., 2007) *In situ* gels are conveniently dropped as a solution into the conjunctival sac, where they undergo a transition into a gel with its favorable residence time. The sol-gel transition occurs as a result of a chemical/ physical change induced by physiological environment. This type of gel combines the advantage of a solution being patient convenient with the favorable residence time of a gel for

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enhancing the ocular bioavailability (Snell RS *et al*., 2004).

The sol-gel transition can be induced by a shift in the pH as for cellulose acetate phthalate, a shift in temperature as for the thermo gelling Poloxamer 188 or by presence of cations as for deacetylated gellan gum and alginates. Thus, the *in situ* gelling systems for ophthalmic use can be classified as pH sensitive, temperature sensitive and ion-activated systems. The rate of gel formation *in situ*, is important since when dropped in the eye, before a strong gel is formed, a solution or a weak gel is prone to elimination by the fluid mechanics of the eye (Frietas M N *et al*., 2006).

MATERIAL AND METHODS

Chloramphenicol palmitate and polaxamer were purchased from Arrow Chem. Pvt. Ltd., New Delhi, India, chitosan was purchased from Dr. Reddy's laboratories, Hyderabad, India. Benzalkonium chloride from RFCL Ltd. New Delhi, India. Mucin type II and Cellulose membrane were purchased from Sigma-Aldrich Chemicals Pvt. Ltd., New Delhi. Diethanolamine was purchased from Merk. Mumbai, India. All other chemicals were purchased from SD fine chemical Ltd. Mumbai, India.

METHODS

Preformulation studies

Determination of melting point

Melting point of chloramphenicol was determined by capillary method.

Compatability studies

Compatability studies was carried out in order to establish, that there would be no interaction between the drug and excipients used in the formulation.(Noveon *et al*., 2005) These studies were carried out by FTIR studies.

Standard calibration curve of chloramphenicol using simulated tear fluid (STF)

STF having the composition of sodium chloride 0.67 gm, sodium bicarbonate0.20 gm, calcium chloride 0.008 gm in 100 ml distilled water was prepared. Accurately weighed 100 mg g chloramphenecol was dissolved in minimum amount of 0.2M NaoH solution and volume was made up to 100 ml with STF to get the stock solution of 100 µg/ml. From this stock solution, aliquots of 0.2, 0.4, 0.6, 0.8, 1.0 ml were withdrawn and diluted to 10 ml with STF to get concentrations in the range of 2 to 10 µg/ml. The absorbance of these solutions was measured at 285 nm by UV-Vis spectrophotometer (Martindale *et al.,* 2002).

Preparation of *In Situ* Gelling System of Chloramphenicol

Preparation of Phosphate buffer pH 6.8

50 ml of potassium dihydrogen phosphate (0.2 M) and 22.4 ml of sodium hydroxide (0.2 M) were mixed and volume was made up to 200 ml with water (Washington DC *et al*., 1986).

Preparation of *in situ* gelling system

Different formulations were prepared with various ratios of polaxmer 188 and carbopol 934 according to table 1. The slow addition of polaxmer 188 and methyl paraben were solubilized in required quantity of cold distilled water. Required quantities of carbopol 934 were kept overnight for swelling and required quantity of chitosan was dissolved in 15% acetic acid solution. The polymer solution taken in a beaker with continuous stirring (magnetic stir) until uniform solution obtained followed by the addition of a small amount of trienthanolamine to adjust the pH 7. An appropriate amount of drug solubilized in physiologically compatible solvent such as sodium chloride was dissolved in phosphate buffer solution pH 6.8 with continues stirring until uniform drug solution was obtained. Thermo reversible gels were prepared using cold technique (Choulis N H *et al*., 1976). Drug solution was added to this polymer solution. The developed formulations were filled in amber glass vials, closed with sterilized rubber closures and aluminum caps and were subjected to crimping. The formulations in their final pack were subjected to terminal sterilization by autoclaving at 121°C and 15 psi for 20 mins (Perez-Marcos B *et al*., 1991).

EVALUATION

Appearance and Determination of pH

The appearance of the formulation was observed which included clarity, color of solution visually and pH was measured using pH meter (Suh H *et al*., 1996).

Drug Content

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. Chloramphenicol concentration was determined at 243 nm by using UV-Vis spectrophotometer form the standard graph of r^2=0.997(Shimadzu, Japan).

Gelation Studies

To mimic the situation where, *in situ* chloramphenicol system, upon ocular instillation, is diluted with the available tear fluid and the gelation is induced by a limited supply of electrolytes, the *in situ* gelling system was mixed with STF in the proportion 25: 7 (application volume 25 µl, normal volume of tear fluid in the eye 7 µl). Gelation was assessed by visual examination.

Spreadability

For the determination of Spreadability (Maezaki, Y *et al*., 1993, Razdan A *et al*., 1994), excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000g weight for 5 min. weight (50 g) was added to the pan. The time in which the upper glass slide moves over to the lower plate was taken as measure of Spreadability.

$$S = ML/T$$

Where, M = weight tide to upper slide, L = length moved on the glass slide, T = time taken.

Measurement of Gel Strength

A sample of 50 gm of gel was placed in a 100 ml graduated cylinder and gelled in a thermostat at 37^0c. The apparatus for measuring gel strength was allowed to penetrate in ocular gel. The gels strength, which means the viscosity of the gels at physiological temperature, was determined by the time (seconds), the apparatus took to

sink 5cm down through the prepared gel (Waugh A *et al*., 2001).

Determination of mucoadhesive Force

The mucoadhesive force of all the optimized batches was determined as follows. A section of corneal membrane was obtained from eye of a goat and instantly fixed with mucosal side out onto each glass vial using rubber band. (Hecht G *et al*., 200) The vial with membrane was connected to the balance in inverted position while first vial was placed on a height adjustable pan. Occular gel was added onto the ocular membrane of first vial. Before applying the gel, 150µL of simulated tear solution was evenly spread on the surface of the test membrane. Then the height of second vial was so adjusted that the mucosal surfaces of both vials come in intimate contact. Two minutes time of contact was given. Then weight was kept rising in the pan until vials get detached. Mucoadhesive force was the minimum weight required to detach two vials. The ocular mucosa was changed for each measurement.

Detachment stress (dynes/cm^2) = mg/A

Where m is the weight added to the balance in grams, g is the acceleration due to gravity taken as 980 cm/s^2, A is the area of tissue exposed, i.e. 2.5 cm^2.

Rheology

Viscosity of formulation is an important factor in determining residence time of drug in the eye. The determination of viscosity of prepared formulations was carried out using Brookfield DV-111+ Rheometer with spindle LV-3. The prepared sol was allowed to gel in the STF and then viscosity was measured. Viscosity of samples was measured at different angular velocities. A typical run comprised changing angular velocity from 10 to 100 rpm with equal weight for each rpm. The hierarchy of angular velocity was reversed (100 to10 rpm) with similar weight. The average of two readings was used to calculate the viscosity. (Wanka G *et al*., 1994).

***In vitro* release studies**

The in vitro release of Chloramphenicol from the formulation was studied through Goat ocular membrane using modified apparatus. The diffusion medium used was freshly prepared STF, (pH 7.4). The ocular membrane previously soaked overnight in the dissolution medium was tied to one end of specially designed glass cylinder (opened at both cylinders). 1 ml of formulation (equal to 2 mg of chloramphenicol) was accurately placed in to this assembly.(Balasubramaniam J *et al*., 2003) The cylinder was attached to a stand and suspended in 50 ml of dissolution medium maintained at 37 ± 1^0c so that the membrane just touched the receptor medium surface. The diffusion medium was stirred at low speed using magnetic stirrer. Aliquots, each of 5 ml volume were withdrawn at hourly intervals and replaced by an equal volume of receptor medium. The aliquots were suitably diluted with the receptor medium and analyzed by UV- Vis spectrophotometry at 243 nm. The values had been subjected to the calculation of release profiles of the drug fitting the best model.

Sterility

All ophthalmic preparations should be sterile, therefore the test for sterility is very important evaluation parameter. 2 ml of the formulation from test container was removed with a sterile pipette or with a sterile syringe or a needle. The test formulation was aseptically transferred to fluid thioglycolate medium (20 ml) and soya bean-casein digest medium (20 ml) separately. The inoculated media were incubated for not less than 14 days at 30°C to 35°C in the case of fluid thioglycolate medium and 20°C to 25°C in the case of soya bean-casein digest medium.(Cohen S *et al*., 1997).

***In vitro* antimicrobial Efficacy**

Antimicrobial efficacy studies were carried out to ascertain the biological activity of sol-to-gel systems against micro-organisms. This was determined by agar diffusion test employing “cup plate technique”. Sterile solution of chloramphenicol as a standard was used and the developed formulations (test solutions) were poured into cups bored into sterile Muller Hinton Agar (MHA) previously seeded with test organisms (*Pseudomonas aeruginosa* and *Staphylococcus aureus*). After allowing diffusion of solutions for 2 hr, the plates were incubated for 24 hrs at 37°C. The zone of inhibition (ZOI) measured around each cup was compared with that of standard. Both positive and negative controls were maintained throughout the study. (Aikawa K *et al*., 1998, Lin HR *et al*., 2000).

RESULSTS AND DISCUSSION

Determination of Melting Point

Melting point of chloramphenicol was found to be in the range of 91°C to 93°C as reported in literature, thus indicating purity of the drug sample as any impurity if present will cause variations in the melting point of a given drug substance.

Compatibility

FTIR of pure drug Chitosan, Pure drug, polaxamer 188 and combination of drug with the polymers were obtained which are shown in fig 1, 2, 3, 4 and 5 respectively. All the characteristic peaks of Chloramphenicol were present in the formulation graph thus indicating compatibility between drug and the polymers. The spectrum confirmed that there is no significant change in chemical integrity of the drug.

Appearance

Clarity of all formulations were found to be satisfactory. The formulations were light yellow in color. Terminal sterilization with autoclaving had no effect on the physico chemical properties of the formulations. The pH values for all the formulations are given in table 2. The pH was within acceptable range and hence would not cause any irritation upon administration of the formulation.

Drug Content

Table 2 shows the result of percent drug content for all the formulations. The drug content was found to be in acceptable range for all the formulations. % Drug content in all eight formulations was in the range 96.57 – 101.382% indicating uniform distribution of drug.

Table.1 Formulation design for *in situ* gelling systems of chloramphenicol

Ingredients	F1	F2	F3	F4
Chloramphenicol (%w/v)	0.2	0.2	0.2	0.2
Polaxamer 188 (% w/v)	18	18	18	18
Carbapol 934 (%w/v)	0.01	0.02	0.03	0.04
Chitosan (%w/v)	-	-	0.01	0.05
Methyl paeraben(%w/v)	0.01	0.01	0.01	0.01
Sodium chloride(%w/v)	0.9	0.9	0.9	0.9

Phosphate buffer pH 6.8 added in a quantity to make 100 ml

Fig.1 FTIR spectra of a) Chitosan, b) Pure drug, c) Polaxamer, d) Formulation

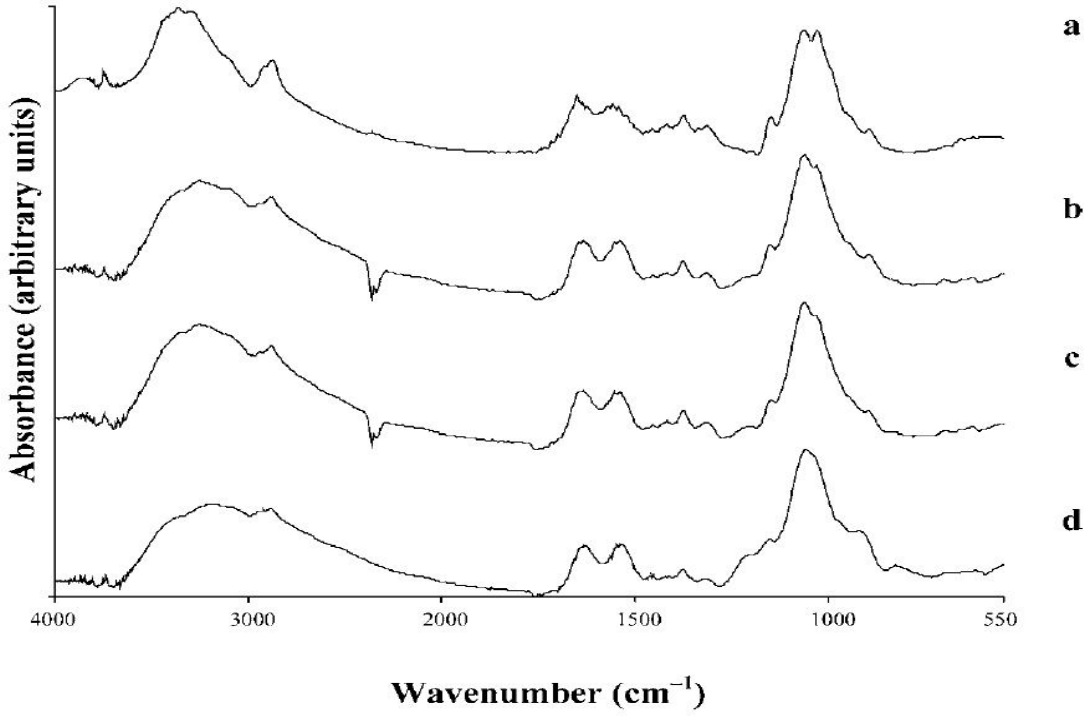

Table.2 pH and drug content of *insitu* gelling system of chloramphenicol

Formulation	pH	Drug content %
F1	6.8	96.57
F2	6.8	101.28
F3	6.8	100.92
F4	6.8	101.382

Table.3 Physico chemical evaluation of prepared formulations

Formulation	Mucoadhesive force(dynes/cm2)	Gel strength(sec)	Spreadability gms/sec
F1	13578	100	22.7
F2	17744	109	25.5
F3	18694	117	30.2
F4	18960	119	31.7

Table.4 Viscosity of prepared *in situ* gelling system of chloramphenicol

Angular velocity(rpm)	Viscosity			
	F1	F2	F3	F4
10	62.2	71	92	99
20	29.3	32	38	47
30	24.7	25	24	28.7
40	18.9	19.5	19.5	24.2
50	13.2	19.5	17.2	22.7
60	10.7	14.2	13.4	18.4
70	8.5	12.8	12	17.3
80	8	9.5	9.5	14.4
90	7.4	8.14	8.2	14.1
100	6.5	7.61	7.8	13.4

Table.5. *In vitro* percentage release of formulations

Time (hr)	F1	F2	F3	F4
0	0	0	0	0
0.5	10.28	9.26	8.378	7.23
1	25.98	24.72	22.43	21.73
1.5	35.82	36.76	32.65	31.65
2	47.78	48.24	45.12	43.63
3	55.69	56.74	53.06	51.83
4	60.59	61.222	57.16	56.32
5	65.48	66.32	62.04	60.42
6	71.22	69.56	66.29	63.22

Table.6 Drug release kinetics of all formulations

F.no	Zero order		First order		Higuchi		Korsmeyer	
	R^2	m	R^2	m	R^2	m	R^2	m
F1	0.968	22.51	0.679	0.273	0.927	58.68	0.679	1.056
F2	0.975	21.89	0.616	0.256	0.952	57.60	0.517	1.382
F3	0.978	20.57	0.653	0.261	0.949	53.95	0.535	1.391
F4	0.976	19.98	0.660	0.263	0.948	52.43	0.544	1.407

Table.7 In *vitro* efficacy of prepared in situ gels of chloramphenicol

	Standard ZOI (mm)	Formulations (ZOI) mm				Percent efficiency
		F1	F2	F3	F4	
Pseudomonas aeruginosa	26	26	26	26	26	100
Staphylococcus aureus	20	20	20	20	20	100

Fig.2 *In vitro* % drug release of prepared *in situ* gelling system of Chloramphenicol

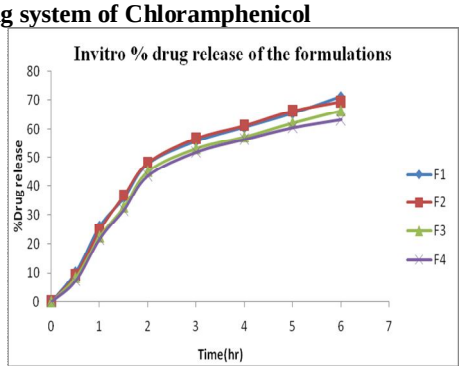

Fig.3 Zero order release of the formulations

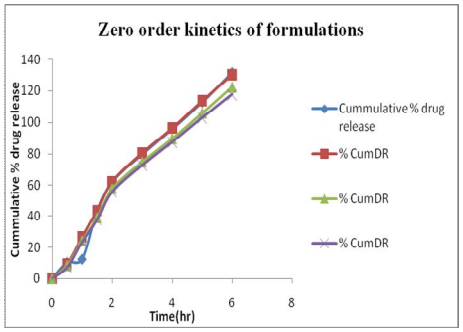

Fig.4 First order release of the formulations

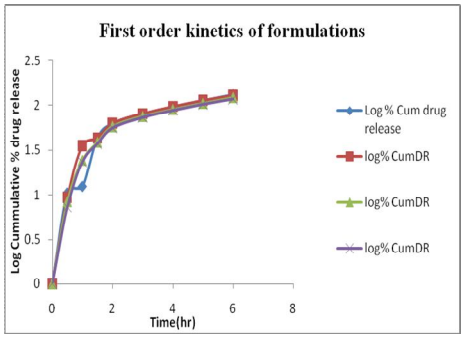

Fig.5 Peppas release of the formulations

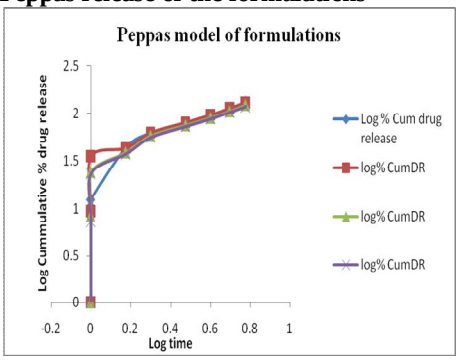

Fig.6 Higuchi release of the formulations

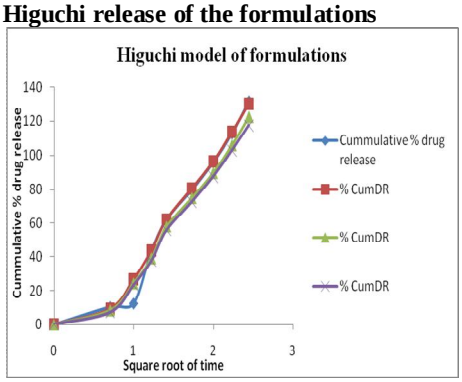

Fig.8 *in vitro* efficacy of formulation F1, F2, F3, F4 of chloramphenicol

Fig.7 Sterility test for Chloramphenicol *in-situ* gel in Soybean casein medium and Thioglycolate medium

Gelation studies

The two main prerequisites of gelling system are viscosity and gelling capacity (speed and extent of gelation). The formulation should have an optimum viscosity, which will allow its easy instillation into the eye as a liquid (drops), which will then undergo rapid sol to gel transition due to change in temperature. Moreover, to facilitate sustained release of drug to the ocular tissue, the *in situ* formed gel should preserve its integrity without dissolving or eroding for a prolonged period of time. All the formulations gelled instantaneously (less than a minute) on contact with STF in the body temperature. By visual inspection, the formulations formed a translucent matrix on addition to the STF. The gelation may be due change in temperature.

Gel Strength

All three formulations (F2, F3 and F4) exhibited good gel strength. This may be due to increase in concentration of carbapol 934and chitosan along with constant ratio of polaxamer 188.

Mucoadhesive Force

The mucoadhesive force is an important physicochemical parameters for prolonging ocular retention time and there by better therapeutic effects. Detachment stress of F4 was found to more in comparison with F1 formulation. The formulation F2, F3, F4 showed more mucoadhesive force than F1. This may be due to increased concentration of chitosan along with Carbopol 934 in the formulation. The thermo reverse formulation showed more muco adhesion force compared with the previous formulations.

Spreadability

F4 showed good Spreadability as compared with the any other formulation. Comparing F3 and F4 showed good Spreadability by comparing with F1 and F2. The formulation from thermo reverse gelation showed good Spreadability comparing with other formulations.

Rheological Studies

Table 4 shows the viscosity values obtained for all the formulations using Brookfield DV-111+ rheomoter. The formulations exhibited pseudo plastic rheology, as evidenced by shear thinning and an increase in shear stress with increased angular velocity. The viscosity was directly dependent on the polymeric content of the formulation. The viscosity increased with increasing concentration of carbapol and chitosan. F4 showed the maximum viscosity of 47 cps at 20 rpm whereas the minimum viscosity at 20 rpm was shown by F1. This indicated that addition of carbapol 934 and chitosan led to increase in viscosity.

***In vitro* release studies**

The in vitro release kinetics was carried out for all formulations using STF as the diffusion medium. The data of these studies are presented in tables. It was found that drug release was for the formulations F1-F4 respectively after 6 hr. these values indicated that F4 showed better sustaining effect among all formulations. This may due to the higher concentrations of carbapol, chitosan and polaxamer in F4. Figure shows the plot of in vitro release studies for all the formulations.

The drug release pattern obtained for the gelled samples is characteristic for hydrophilic matrices. The initial fact the release of chloramphenicol can be explained by the fact that polaxamer eye drops are formulated in water and hence the polymer was completely hydrated. When they exposed to body temperature with STF and gelation occurs, a pre hydrated matrix is formed in which hydration and water penetration no longer limit drug release leading to an apparent diffusion-controlled release.

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

• 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).

The kinetic values obtained for different formulations are indicated in Tables 6.

A brief overview of the table it can be inferred that all the prepared formulation follows zero order kinetics, wherein the drug release is independent to it concentration in the medium. The polymers slowly degrade and release the drug in sustained manner which is an advantageous feature for a successful ophthalmic formulation.

Sterility test

There was no appearance of turbidity and hence no evidence of microbial growth when all the formulations were incubated for not less than 14 days at 30°C to 35°C in case of fluid thioglycolate medium and at 20°C to 25°C in the case of soya bean-casein digest medium. The preparations being examined therefore passed the test for sterility.

***In vitro* efficacy**

The results of *in vitro* efficacy studies are shown in table 7. The study indicated that Chloramphenicol retained its antimicrobial efficacy when formulated as an *in situ* gelling system and the drug was active against the selected strains of micro-organisms. The zone of inhibition observed for selected micro-organisms is shown in figures 8.

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CONCLUSION

The Thermoreveresible *in situ* gelling systems of chloramphenicol had been prepared and evaluated.. Results proved the *in vitro* efficacy of the formulations as well as the sustained release had also been achieved. Taking this into consideration, many poorly bioavailable drugs intended for ocular delivery can be formulated as *in situ* gelling systems so can their bioavailability and efficacy are enhanced. The advantage that more residence time should be taken into account when formulating drug with narrow therapeutic index. The thermo reversible *in situ* gelling system is a promising approach for the ocular delivery of the drugs overcoming the hurdles of bioavailability and wash off.

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