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PREPARATION AND EVALUATION OF IN-SITU OPHTHALMIC GEL OF CHLORAMPHENICOL

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ABSTRACT

Objective: To develop a hydrogel to increase the retention time of a drug in an ophthalmic drug delivery system. **Methods:** The experiment was conducted on the preparation and evaluation of chloramphenicol ophthalmic hydrogel with various concentrations of sodium alginate, F1 (0.3% sodium alginate), F2 (0.4% sodium alginate), F3 (0.5% sodium alginate), F4 (0.6% sodium alginate). The organoleptic safety, pH, viscocity, sterility and in-vitro drug release were evaluated. **Result:** F3 formualtion shows gelling capacity over 6 hours and corneal drug release 98.28% for 8hrs. **Coclusion:** The chloramphenicol hydrogel ophthalmic preparations formulated with HPMC have shown a good characteristic, and acceptable sustained released profile that may extend absorption of the drug for an optimum bioavailability at the site of action.

KEYWORDS: chloramphenicol, hydrogel, hydroxypropyl methylcellulose, ophthalmic.

1. INTRODUCTION

Eye is the accessible site for topical administration of a medication. Ocular administration of drug is primarily associated with the need to treat ophthalmic diseases. The most commonly used conventional ocular product is eye drops, when the eye drop is introduced in the cul-de-sac, it is immediately diluted in the lachrymal fluid, therefore drug concentration decreases substantially. As soon as the eye drops are instilled, a part of the product is drained out due to blinking of the eye. The detailed description of each eye part is given below.[3,5]

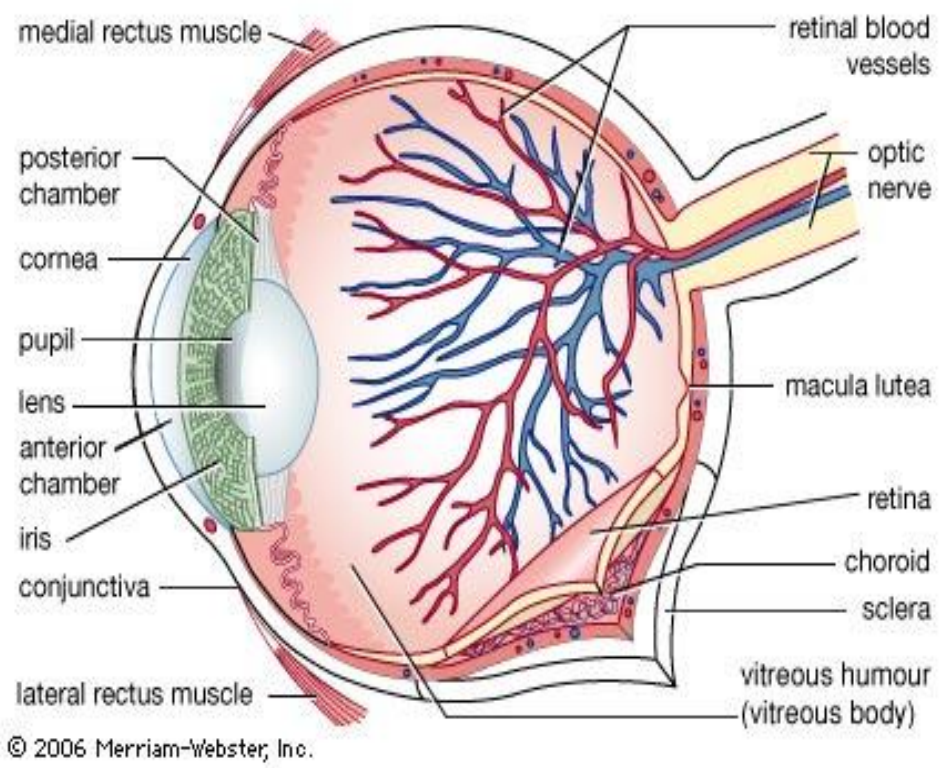


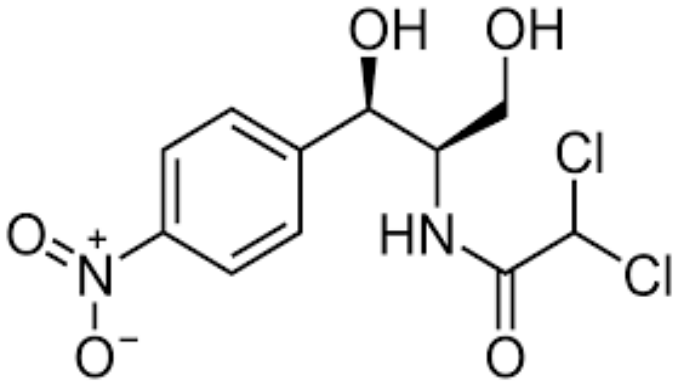
Fig. no. 1: Structure of eye.

The 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.

The newest dosage forms for ophthalmic drug delivery are gels, gel-forming solutions, ocular inserts, intravitreal injections and implants. Several, in-situ gelling system have been developed to prolong the precorneal residence time of a drug, improve patient compliance, and enhance ocular bioavailability. These are liquid dosage forms, which get transform to a gel or solid phase, when instilled into cul-de-sac. This happens because of change in the environmental stimuli. This gelation can be caused by change in the temperature, pH or ions, when the product is instilled in to the eye.[8, 9]

1.1.Chloramphenicol[2,20]

Structural formula: $C_{11}H_{12}CL_2N_2O_5$



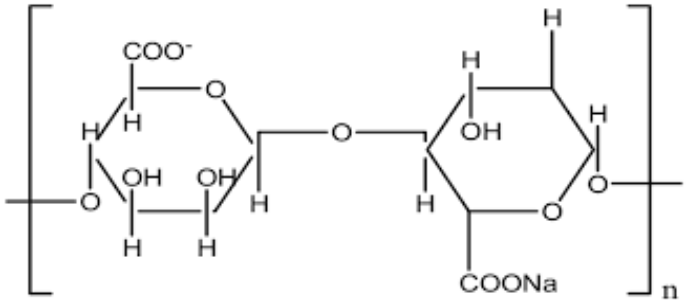
Chloramphenicol is 2,2-dichloro-*N*-[(1*R*,2*R*)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl] acetamide and fine white to greyish yellow white elongated plates/ needle like crystals; colourless crystals, intensity bitter taste.Chloramphenicol is a bacteriostatic by inhibiting protein synthesis.Melting point is 150.50^0C.

Mechanism of action of the drug as it inhibits protein synthesis in bacteria and, to a lesser extent, in eukaryotic cells. The drug readily penetrates bacterial cells, probably by facillated diffusion. Chloramphenicol acts primarily by binding reversibly to the 50S riobosomal subunits. Although binding of tRNA at the codon recognition site on the 30S ribosomal subunit is thus undisturbed, the drug appears to prevent the binding of the amino acid containing end of the aminoacyltRNA to the acceptor site on the 50S ribosomal subunit. The interaction between peptidyltransferase and its amino acid substrate cannot occur, and peptide bond formation is inhibited. It can also inhibit mitochondrial protein synthesis I mammalian cells, perhaps because mitochondrial ribosomes resemble bacterial ribosomes (both are 70S) more than they do the 80S cytoplasmic ribosomes of mammalian cells. The peptidyltransferase of bovine mitochondrial ribosomes, but not cytoplasmic ribosomes is susceptible to the inhibitory action of chloramphenicol. Mammalian erythropoietic cells seem to be particularly sensitive to the drug.

1.2.Excipients profile

- **Sodium Alginate**[18]

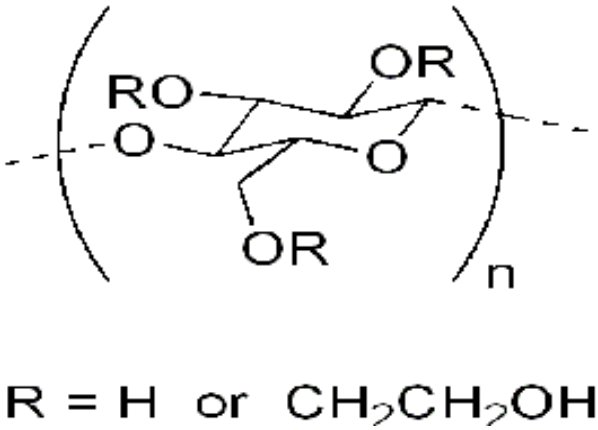
Structural Formula: (C6H8O6) n



Sodium Alginate is sodium; 3, 4, 5, 6-tetrahydroxyoxane-2-carboxylate. Sodium alginate occurs as an odorless and tasteless, white to pale yellowish-brown colored powder. Stabilizing agent, suspending agent, sustained release adjuvant, tablet binder, table disintegrent, and viscosity modifier. Melting point is>300^{0}C.

- **Hydroxypropyl Methylcellulose**[19]

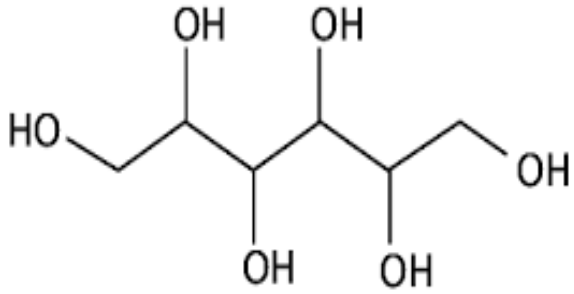
Structural formula



Hydroxypropyl Methylcellulose is (2*R*,3*R*,4*S*,5*R*,6*R*)-2,3,4-trimethoxy-6-(methoxymethyl)-5-[(2*S*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trimethoxy-6-(methoxymethyl)oxan-2-yl]oxyoxane;1-[[(2*R*,3*R*,4*S*,5*R*,6*S*)-3,4,5-tris(2-hydroxypropoxy)-6-[(2*R*,3*R*,4*S*,5*R*,6*R*)-4,5,6-tris(2-hydroxypropoxy)-2-(2-hydroxypropoxymethyl)oxan-3-yl]oxyoxan-2-yl]methoxy]propan-2-ol. It is an odorless, tasteless, white or creamy white fibrous or granular powder. Suspending and/ or viscosity increasing agent; tablet binder; coating agent; adhesive anhydrous ointment ingredient; film former; emulsion stabilize. Melting point is 172-174^{0}C.

- **Mannitol**

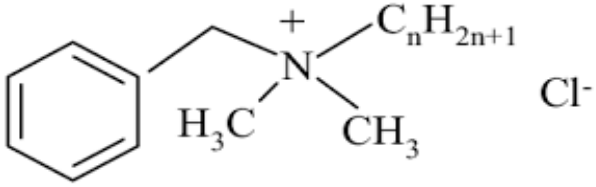
Structural formula: $C_6H_{14}O_6$



Mannitol is (2*R*,3*R*,4*R*,5*R*)-hexane-1,2,3,4,5,6-hexol. Mannitol is a naturally occurring alcohol found in fruits and vegetables and used as an osmotic diuretic. D-mannitol appears as odourless white crystalline powder or free flowing granules, sweet in taste. It is a type of sugar alcohol used as a sweetner and medication. Melting point is 165^0C

- **Benzalkonium chloride**

Structural formula: Chchn (CH) 2RCl, R= CH-CH



n= 8,10,12,14,16,18

Benzalkonium chloride is benzyl-dimethyl-[2-[2-[4-(2, 4, 4-trimethylpentan-2-yl)phenoxy]ethoxy]ethyl]azanium; chloride. Benzalkonium chloride ranges from colourless to pale yellow. Concentrated solution have a bitter taste and a faint almond like odour. Melting point is 29-34^0C.

The objective of the present work to prepare and evaluate the in situ ophthalmic gel of chloramphenicol, an antibiotic used to treat various bacterial infection of an eye, such as conjunctivitis.

2. Experiment

2.1. MATERIAL AND METHODS

Chloramphenicol, Sodium alginate, HPMC E 15LV, Mannitol and Benzalkonium chloride were obtained from Yarrow Chem. Pvt. Ltd, Mumbai, India.

2.2. Preparation of the formulation

The required amount of sodium alginate and HPMC were soaked in distilled water. Then, chloramphenicol was added to the above soaked polymeric solution under constant stirring until uniform, clear solution was obtained.

Mannitol and benzalkonium chloride were added to all formulations as thickening agent and preservative, respectively. Distilled water was then added to make the volume up to 100 ml. Ophthalmic formulations were autoclaved at 15psi pressure for 20min. sterilized formulation was transformed to glass vials.

Table no 1: chloramphenicol ophthalmic in-situ gel formulas.

Sr. no	Ingredients	Formula			
		F1	F2	F3	F4
1	Chloramphenicol	0.5	0.5	0.5	0.5
2	Sodium Alginate	0.3	0.4	0.5	0.6
3	HPMC	0.2	0.2	0.2	0.2
4	Mannitol	0.1	0.1	0.1	0.1
5	Benzalkonium Chloride	0.01	0.01	0.01	0.01
6	Distilled Water	100ml	100ml	100ml	100ml

2.3.Evaluation of formulations

2.3.1.1. Physicochemical characterisation

The ophthalmic formulations were evaluated for various characteristic as follows:

i. Appearance and clarity

The ophthalmic formulations were observed carefully for colour, odour and presence of suspended particulate matter if any. The clarity of solutions was further assessed by observing them against a dark and white background.

Formulations were graded as follows:

Turbid -

Slightly turbid +

Clear solution ++

Clear and transparent +++

ii. pH

The pH of ophthalmic formulations was determined by using pH meter. The pH meter was calibrated before each use with standard pH 4, 7 and 9.2 buffer solutions. The formulation temperature was maintained at 25±3^{0}C. The pH meter electrode was immersed in formulation and the pH was recorded.[15]

iii. Gelling ability

The individual ophthalmic formulations (50µl) were added into 2ml of STF (37±1^{0}C) contained in glass vials. The transition of solution to viscous gel was observed visually and numerical scores assigned depending upon

- Quickness of gel formation
- Time taken for collapse of gel structure on shaking the vials

The formulations were graded as shown in table

Table no 2: Composition of Simulated tear fluid.

Sr. no.	Grade	Observation
1	-	No phase transition
2	+	Formation of gel after 60 sec and gel collapsed within 1 h
3	++	Formation of gel after 60 sec and gel collapsed within 3 h
4	+++	Formation of gel within 60 sec and gel remained stable for more than 6-7 h

2.3.1.2. Rheological behaviour of ophthalmic formulations after and before gelation[11]

The rheological studies of the samples were carried out with Brookfield viscometer (LVDV-II model). Rheological measurements were done before and after gelation, gels were prepared using simulated tear fluid (STF) as solvent. All examined gel samples contained drug and were equilibrated at 34^{o}C ± 0.1^{o}C prior to each measurement. Viscosity was measured by Brook-field viscometer LVDV-II Pro using Spindle LV 2. A typical run comprised of changing the angular velocity from 0.5rpm, 2.5rpm, 5rpm, 10rpm, 20rpm, 50rpm and 100 rpm at a controlled ramp speed. All measurements were made in triplicate.

2.3.1.3. Isotonicity[16]

Formulations were mixed with few drops of blood and observe under microscope at 45X magnification and compared with standard marketed ophthalmic formulation.

2.3.1.4. Antimicrobial efficacy study[12, 16]

Antimicrobial efficacy was determined by the agar diffusion test employing ‘Boar well method’. Sterile solutions of chloramphenicol (marketed eye drop solution as standard

solution) and the developed formulations (test solutions) were poured in to wells bored into sterile nutrient agar previously seeded with test organisms (Staphylococcus aureus, and E. coli.) after allowing diffusion of the solutions for 2 hr. agar plates were incubated at 37^{o}C for 24 hr. The zone of inhibition (ZOI) measured around each well was compared with that of control. The entire operation except the incubation was carried out laminar airflow unit. Each solution was tested intriplicate.

2.3.1.5 In vitro drug release studies[12, 16]

The in vitro release studies were carried out by using a modified USP dissolution testing apparatus. The dissolution medium used was freshly prepared simulated tear fluid (pH 7.4). cellulose membrane (spectra/por analysis membrane 12,000 14,000 MW cut off), previously soaked overnight in the dissolution medium, was tied to one end of specifically designed glass cylinder (open at both ends and of 2.0 cm diameter). An accurately weighed amount of the formulation (1g) was transferred to the glass tube. The glass cylinder were attached to the metallic driveshaft to the dissolution apparatus and suspended in 100 ml of dissolution medium maintained at temperature of 37±1^{0}C. The shaft were allowed to rotate at a constant speed (50 rpm). At predetermined time intervals for 8 hrs, aliquots were withdrawn and replaced by an equal volume of the receptor medium. The formulations were optimized on the basis of viscosity and in vitro release studies.

3. RESULT AND DISCUSSION

3.1. Result of melting point of chloramphenicol

The Melting Point of given chloramphenicol was found to be 145^{o}C.

3.2. Standard curve of chloramphenicol

Chloramphenicol standard curve data by using ultraviolet spectrophotometry presented in Table 3, Using the least square equation and was obtained straight line equation as follows:

$y = 0.0513x – 0.0077$

Table no 3: Chloramphenicol standard curve data.

Concentration(ppm)	Absorbance
6	0.2916
8	0.3860
10	0.5168
12	0.6170
14	0.7086

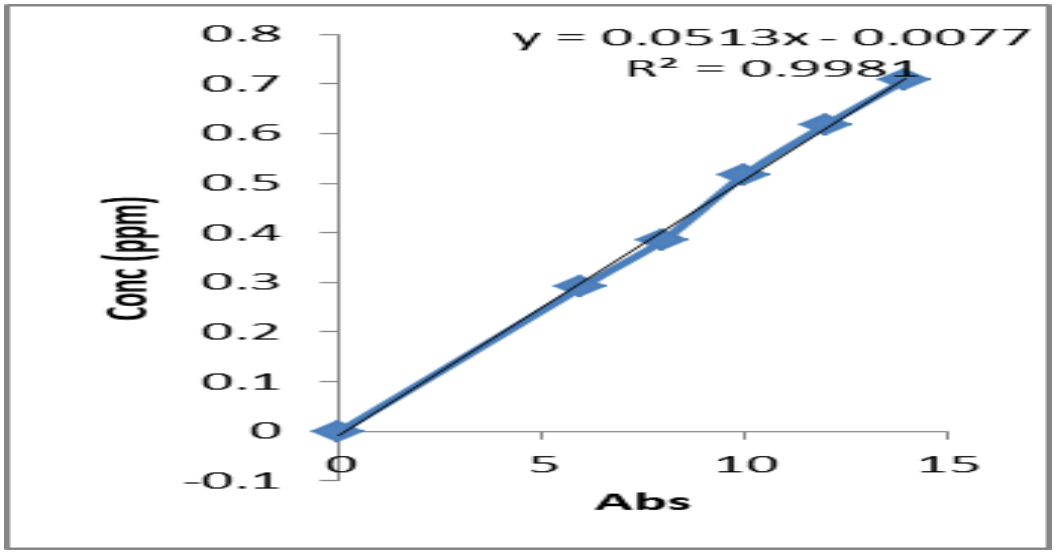


Fig. no 2: Chloramphenicol standard curve.

3.3. Observation result of organoleptic properties

Based on the observation result for 28days, the four hydrogel formulations of chloramphenicol had not changed in organoleptic where the preparation remains colorless, clear and odorless as shown in table 4.

Table no 4: Appearance of formulations.

Formula	Observation	Observation of organoleptic day to day					
		1	3	7	14	21	28
F1	Clarity	Clear	Clear	Clear	Clear	Clear	Clear
	Colour	C	C	C	C	C	C
	Odour	O	O	O	O	O	O
F2	Clarity	Clear	Clear	Clear	Clear	Clear	Clear
	Colour	C	C	C	C	C	C
	Odour	O	O	O	O	O	O
F3	Clarity	Clear	Clear	Clear	Clear	Clear	Clear
	Colour	C	C	C	C	C	C
	Odour	O	O	O	O	O	O
F4	Clarity	Clear	Clear	Clear	Clear	Clear	Clear
	Colour	C	C	C	C	C	C
	Odour	O	O	O	O	O	O

Description

F1: Hydrogel with sodium alginate 0.3%

F2: Hydrogel with sodium alginate 0.4%

F3: Hydrogel with sodium alginate 0.5%

F4: Hydrogel with sodium alginate 0.6%

C = Colourless

O = Odourless

3.4. pH and Gelling Capacity

The observation result of the pH of hydrogel ophthalmic present in table 5.

Table no 5: pH and gelling capacity.

Formula	pH	Gelation capacity
F1	4.7	+
F2	4.9	++
F3	5.0	+++
F4	5.2	++

3.5. Determination of viscosity

In order to evaluate the rheological behavior viscosity of the formulation before and after addition of simulated lacrimal fluid was evaluated using Brookfield rheometer. The selected formulation was shear thinning exhibiting pseudoplastic behaviour.

The rheological properties of in situ gel formulation F3 is shown in table 6.

Table no 6: Viscosity of sodium alginate formulation.

Sr. no.	Code	Viscosity of Sodium alginate formulations at different rpm at 37^{o}C						
		0.5	2.5	5	10	20	50	100
1	F3	220000	132000	69569	43780	16945	7896	4567

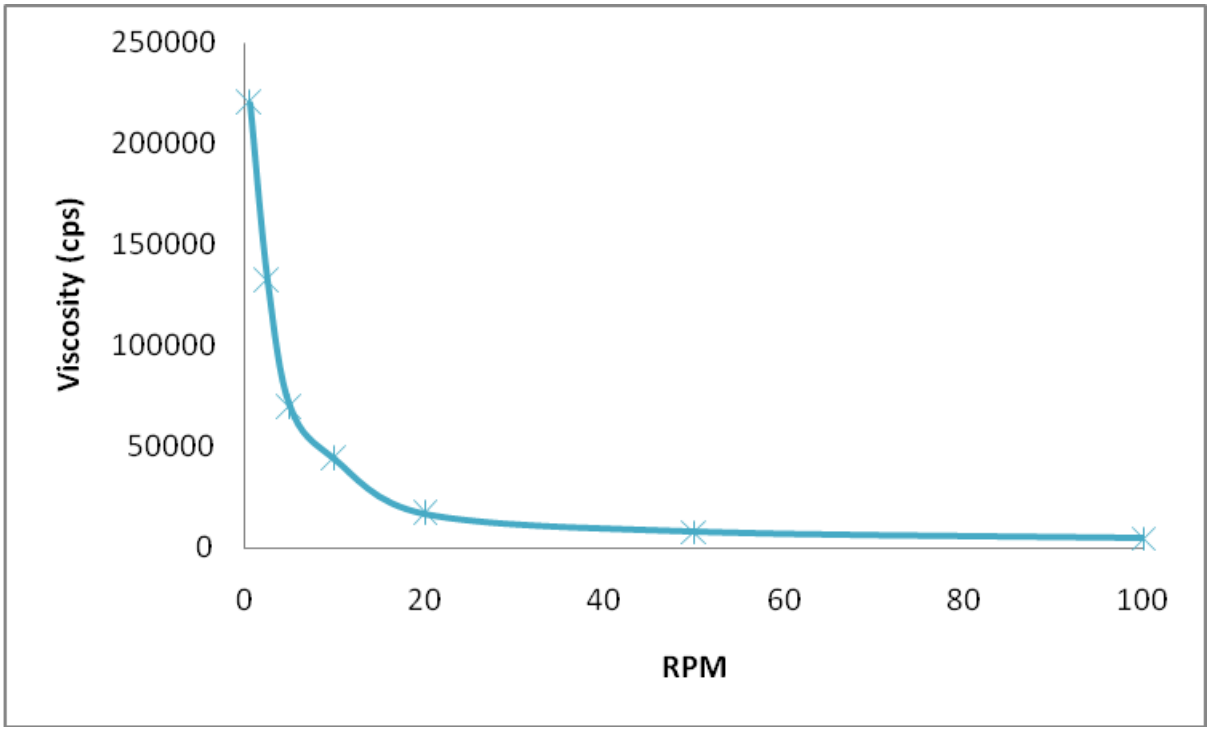


Fig no 3: Rheological properties of in-gel formulation at different pH conditions.

3.6. Isotonicity

The observation result of the isotonicity of hydrogel ophthalmic chloramphenicol present in figure 4.

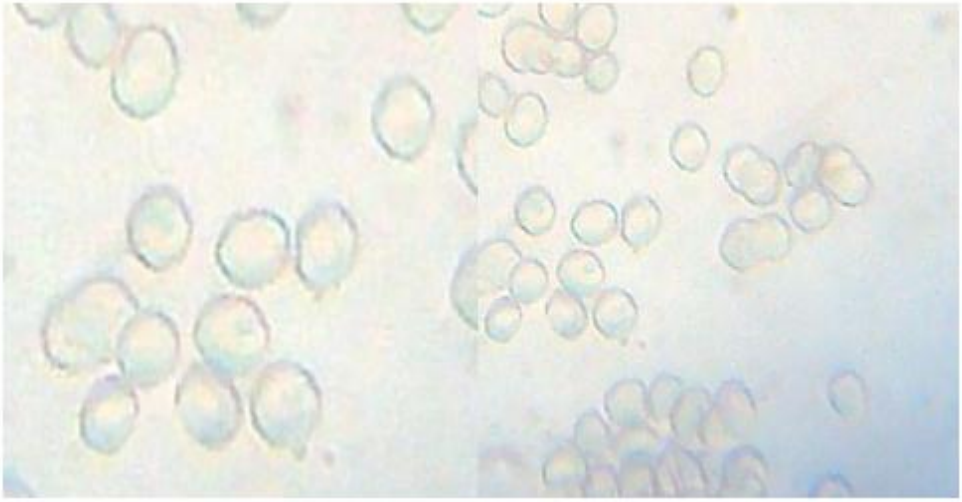


Fig no 4: Red blood cells with formulation remained isotonic.

3.7. Determination of antimicrobial efficacy

Antimicrobial efficacy studies were determined by the agar diffusion test employing "cup plate technique". Marketed and developed formulations poured into cups bored into sterile nutrient agar. These formualations are diluted at different concentration and test organisms used were (E. coli and Staphylococcus aureus). The results of the antimicrobial efficacy tests are represented in table 7.

Table no 7: Antimicrobial efficacy.

Sr. No.	Formulations	E. Coli		Staphylococcus aureus	
		Zone of Inhibition (mm)	% Efficacy	Zone of Inhibition(mm)	% Efficacy
1	Standard	35	100	38	100
2	F1	34	97.14	35	92.10
3	F2	34	97.14	34	89.47
4	F3	33	94.28	33	86.84
5	F4	33	94.28	35	92.10

3.8.Drug release

The chloramphenicol released profile test of the preparation can be seen in table8.

Table no 8: Drug release.

Sr. no.	Time (hours)	F3	Marketed eye drops
1	0	0	0
2	1	48.83	61.82
3	2	55.97	69.12
4	3	65.3	74.21
5	4	83.6	86.08
6	5	90.98	95.04
7	6	95.66	
8	7	96.22	
9	8	98.28	

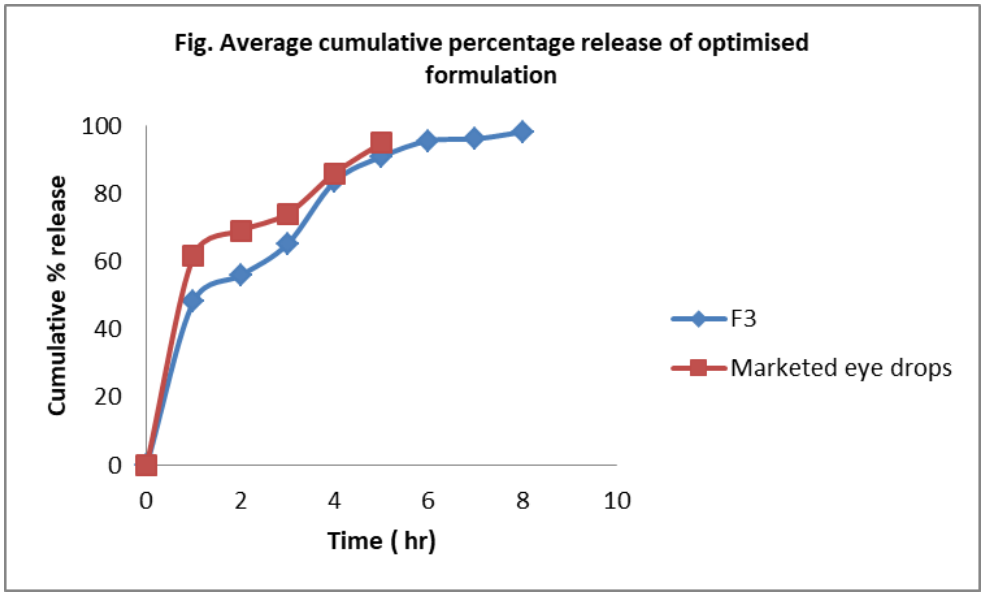


Fig. 5: average cumulative percentage release of optimized formulation.

4. CONCLUSION

The chloramphenicol, act as an antibiotic in ophthalmic in situ gel was formulated using mucoadhesive polymer such as sodium alginate with different concentrations and it is evaluated. On the basis of result, F3 formulation is optimized, which shows gelling capacity over 6hrs and corneal drug release 98.28% for 8hrs, which was done by dissolution apparatus.

As we have used mucoadhesive polymer with combination viscosity modifier, which will have more binding with drug molecule and show release of drug. Formulations were compared with marketed eye drops which last only 1hrs to overcome this disadvantage in situ hydrogel has been prepared.

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