WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 15, 1011-1043. Research Article ISSN 2277– 7105

FORMULATION AND EVALUATION OF OPHTHALMIC DRUG DELIVERY SYSTEM FOR LEVOFLOXACIN HEMIHYDRATE AND KETOROLAC TROMETHAMINE

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Article Received on
26 Sept. 2020,

Revised on 16 October 2020,
Accepted on 06 Nov. 2020

DOI: 10.20959/wjpr202015-19238

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ABSTRACT

Eye is a very sensitive organ and can get infected from bacteria, fungi, or viruses. Common eye infection are conjunctivitis and corneal ulcers. Conjunctivitis is a swelling (inflammation) or infection of the membrane lining the eyelids. Corneal ulcers are characterized by corneal edema, cellular infiltration and ciliary congestion. Currently available antimicrobials used in the prevention and treatment of these infections include antivirals, antifungals and antibacterials. Common topical antibacterial used in treatment of ocular infectious diseases include sulfonamides, aminoglycosides, polymyxin based combinations and fluoroquinolones.[10] Levofloxacin is a third generation fluoroquinolone derivative used to treat acute and sub-acute infections of the eye viz. conjunctivitis, bacterial keratitits and ketorolac conjunctivitis. Levofloxacin represent an expanding class of broad spectrum antibacterial which are effective against most of the gram negative and anaerobic species responsible for ocular infection. Ketorolac is a non steroidal anti inflammatory drug (NSAID) used to treat inflammation and sub acute inflammation of the eye. This can be achieved by using in situ gel forming ophthalmic drug delivery systems prepared from polymers that exhibit reversible phase transitions (sol-gel-sol) and pseudoplastic behavior. Out of various ophthalmic dosage forms, ophthalmic solutions are the most preferred dosage form due to the obvious reasons of patient convenience. 90% of the currently available ophthalmic formulations are solutions. However, they suffer from shorter residence time at the site of action and faster nasolacrimal drainage which subsequently leads to the need for frequent instillation.[48] This situation is

more common when the patient is suffering from acute infections caused by pathogenesis bacteria and is undergoing treatment with potent antibacterial agents like Levofloxacin Hemihydrate. In the present study, an attempt has been made to develop and evaluate in situ gel forming ophthalmic drug delivery system of Levofloxacin Hemihydrate and Ketorolac Tromethamine by using various polymers like Poloxamer 407, Carbopol 974, HEC, HPMC. In the present study, various in-situ gelling opthalamic formulations of poloxamer 407 in concentration of 16-20% w/w (P1-P5). Further, different formulations of poloxamer 407 (18%w/w) were prepared by combining with HEC in concentration of 0.2, 0.4, 0.6%w/w (P12-P14) and HPMC E50 LV in concentration of 0.2, 0.4, 0.6%w/w (P15-P17). All above are prepared with Levofloxacin Hemihydrate. So, with Ketorolac Tromethamine three batches also prepared i.e poloxamer 407(18%w/w), poloxamer (18%w/w) and HEC (0.4%w/w) last one poloxamer407 (18%w/w) and HPMC E50 LV (0.4%w/w). All these opthalamic formulations were evaluated for appearance, clarity, pH, gelling ability, viscosity and percent drug release. The formulations showing transparent and clear appearance, quick and stable gelation, shear thinning properties, excellent sustained drug release upto 75-85% after 8hrs were selected for further characterization. The optimized formulations P21, P22 and P23 showed excellent mucoadhesion and drug release. These formulations were found to be stable to autoclaving, at ambient conditions and non-irritant to eye.

KEYWORDS: Levofloxacin Hemihydrate, Ketorolac Tromethamine, poloxamer 407, HPMC E50 LV, Carbopol 974 etc.

INTRODUCTION

Ophthalmic drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. The topically applied ocular drugs have to reach inner parts of the eye to elicit responses. The transcorneal penetration is believed to be the major route for drug absorption. Most ocular drugs seem to penetrate the cornea by diffusion. Currently available antimicrobials used in the prevention and treatment of these infections include antivirals, antifungals and antibacterials. Levofloxacin is a third generation fluoroquinolone derivative used to treat acute and sub-acute infections of the eye viz. conjunctivitis, bacterial keratitits and keratoconjunctivitis. Ketorolac is a non steroidal anti inflammatory drug (NSAID) used to treat inflammation and sub acute inflammation of the eye. Ketorolac gives Lower incidence of side effects.[47] Rapid penetration through corneal epithelium. Longer half life 6-8 hrs which is greater than other formulatons.

MATERIAL AND METHOD

Material

Levofloxacin (Gift sample from Emcure Pharmaceutical Ltd.), Ketorolac (Gift sample from Symed Lab Limited), Poloxamer 407 (BASF, Mumbai), Carbopol 974 (Noveon, Mumbai.), Polycarbophil (Noveon, Mumbai.), HPMC E50 LV (Colorcon Asia Pvt. Ltd., Verna-Goa), HEC (Natrosol 250) (Research Lab., Carbopol-974 (Noveon, Mumbai), Mannitol (Research Lab Fine Chemicals, Mumbai), Benzalkonium Chloride (Research Lab Fine Chemicals, Mumbai), Propylene Glycol (Loba Chem), all the reagent were used of analytical grade.

Method

Formulation of in situ gelling ophthalmic solution by using different gelling agents

The in situ gelling ophthalmic solutions of Levofloxacin were prepared using Levofloxacin equivalent to 0.3%w/w of Levofloxacin, suitable amount of different gelling agents. tonicity adjusting agents and antimicrobial preservative. The in situ gelling ophthalmic solutions of Ketorolac were prepared using Ketorolac equivalent to 0.5%w/w of Ketorolac, suitable amount of different gelling agents. tonicity adjusting agents and antimicrobial preservative. The in situ gelling ophthalmic solutions were prepared by using combination of drug i.e Levofloxacin (0.3%) and Ketorolac (0.5%) suitable amount of different gelling agents. Tonicity adjusting agents and antimicrobial preservative.

The formulation were prepared by following ways

1) **Development of sterile ophthalmic in situ gels of levofloxacin**

A. Using individual polymer

I. Poloxamer 407

B. Using combination polymers

I. Poloxamer 407 with carbopol 974
II. Poloxamer 407 with polycarbophil
III. Poloxamer 407 with HEC
IV. Poloxamer 407 with HPMC E50 LV

2) **Development of sterile ophthalmic in situ gels of ketorolac**

A. Using individual polymer

I. Poloxamer 407

B. Using combination of polymers

I. Poloxamer 407 with HEC

II. Poloxamer 407 with HPMC E50 LV

3) Development of sterile ophthalmic in situ gels in combination of drug

A. Using individual polymer

I. Poloxamer 407

B. Using combination of polymers

I. Poloxamer 407 with HEC

II. Poloxamer 407 with HPMC E50 LV

Table 1: Composition of in situ gels of Levofloxacin using poloxamer 407.

Code	Poloxamer 407(%w/w)	Carbopol 974(%w/w)	Polycarbophil (%w/w)	HEC (%w/w)	HPMC E50LV(%w/w)
P1	16	-	-	-	-
P2	17	-	-	-	-
P3	18	-	-	-	-
P4	19	-	-	-	-
P5	20	-	-	-	-
P6	18	0.2	-	-	-
P7	18	0.4	-	-	-
P8	18	0.6	-	-	-
P9	18	-	0.2	-	-
P10	18	-	0.4	-	-
P11	18	-	0.6	-	-
P12	18	-	-	0.2	-
P13	18	-	-	0.4	-
P14	18	-	-	0.6	-
P15	18	-	-	-	0.2
P16	18	-	-	-	0.4
P17	18	-	-	-	0.6

Table 2: Composition of in situ gels of ketorolac using poloxamer 407.

Code	Poloxamer 407 (%w/w)	HEC(%w/w)	HPMC E50 LV(%w/w)
P18	18	-	-
P19	18	0.4	-
P20	18	-	0.4

Table 3: Composition of in situ gels of Levofloxacin + Ketorolac using poloxamer 407.

Code	Poloxamer 407 (%w/w)	HEC (%w/w)	HPMC E50 LV (%w/w)
P21	18	-	-
P22	18	0.4	-
P23	18	-	0.4

All above formulations contained Levofloxacin equivalent to 0.3%w/w, Ketorolac equivalent to 0.5%w/w, 15%w/w propylene glycol, 5%w/w Mannitol, 0.01%w/w benzalkonium chloride and distilled water to make 20ml of solution.

Evaluation of in situ gelling ophthalmic formulations

The ophthalmic formulations were evaluated for various characteristics as follows:

1. Test for 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 +++

2. Determination of pH values of ophthalmic formulations

The pH of ophthalmic formulation 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 formulations temperature maintained at 25±30C. The pH meter electrode was immersed in formulation and the PH was recorded.

3. Test for gelling ability[27]

The test for gelling ability was conducted using a STF, composition of which is shown in table 4.

Table 4: Composition of simulated tear fluids.

Sr. no.	Ingredients	Quantity
1	Sodium Chloride	0.67g
2	Sodium bicarbonate	0.2g
3	Calcium chloride dehydrate	0.008g
4	Distilled water	q.s to 100ml

*The pH was adjusted to 7.4 using 0.1N HCL.

The individual ophthalmic formulations (100µl) were added into 1ml of STF (37±10C) contained in glass vials. The transition of solution to viscous gel was observed visually and numerical scores were assigned depending on

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

The formulations were graded as shown in table 5

Table 5: Gradation of gelling ability.

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

4. Determination of viscosity of ophthalmic formulations

The viscosity of the gel was determined using programmable viscometer (Brookfield LVDV-II) with T-bar spindle code S95 and it was operated under following conditions-

The spindle was attached to the lower shaft of the viscometer. The motor was turned on and spindle was rotated within the container containing 20ml of performed gel. The helipath movement was controlled to avoid touching of the spindle to any part of the sample holder especially the bottom. A typical run involved changing the angular velocity from 0.5 to 100rpm at a controlled speed which was changed after every 10sec (0.5……..100rpm). The viscosity values at each rpm were noted from the display window.

5. Estimation of Drug presence in ophthalmic solutions by UV spectrophotometric method

1ml of each of the ophthalmic formulation was taken and diluted upto 100ml with distilled water. Again 1ml of this solution was taken and diluted upto 10ml with distilled water. The solutions were analyzed UV spectrophotometrically at respective maximum wavelength

keeping distilled water as a blank. The concentration of drug in sample was determined from a previously prepared calibration curve.

6. In vitro release of performed ophthalmic gels formulation

Modified dissolution apparatus for ophthalmic gels[27,51]

The USP dissolution apparatus I was modified for release studies on gels. The details of dissolution test of the gel are as shown in table 6.

Table 6: Details for dissolution test of in situ gel.

Sr. no.	Specification	Standard Value
1	Apparatus	Modified USP tablet dissolution test apparatus I with glass cylinder tied to central shaft
2	Speed	50rpm
3	Volume of dissolution media	50ml of PBS 7.4
4	Quantity of formulation	1ml
5	Membrane	Dialysis membrane110(Himedia Lab.)
6	Dimension of membrane	Diameter-1.75cm
7	Temperature	37±10C
8	Sampling interval	Every 1hr
9	Total test time	8hr
10	Sampling volume	1ml

The in vitro release of formulation was studied through dialysis membrane using a modified USP XXIII dissolution testing apparatus. The dissolution medium used was PBS 7.4 freshly prepared. Dialysis 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 1.75 cm diameter). 1 ml volume of the formulation (solution) was accurately added into this assembly. The cylinder was attached to the metallic driveshaft and suspended in 50 ml of dissolution medium maintained at 37 ±1 °C so that the membrane just touched the receptor medium surface. The shaft was rotated at 50 rpm. Aliquots, each of 1 ml volume, were withdrawn at half hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with receptor medium and analyzed by UV spectrophotometry at respected maximum wavelength.

7. Mucoadhesive strength study by texture analyzer

For mucoadhesive strength measurement Brookfield texture analyzer apparatus were used. In this the cornea were used for strength measurement.

8. Determination of effect of autoclaving on the properties of the selected formulations of gels

As the ophthalmic formulations must be sterile before dispensing, the method of sterilization selected was the moist heat sterilization by autoclave at pressure 15 psi for 20min. To check the effect of heat due to autoclaving on the formulations, selected formulations were sterilized by autoclaving. The evaluation parameters like appearance, PH, Gelling ability, viscosity, drug content and drug release were studied before and after autoclaving.

9. Microbiological study

Weighed 12.5 gm of sabouraud dextrose agar was transferred in a 500 ml of conical flask and 250 ml of purified water and some amount of heat is applied to dissolve it completely. Sterilized for 15 min at 121°C at 15 lb pressure in autoclave for about 20 min. Then cooled it at room temperature and the bacterial strain (staphylococcus aureus) was dispersed in the medium(1 ml) and then the medium(0.1 ml) was poured it in to the three petridish and allowed it cool it for sometime at room temperature until it forms solidifies at room temperature and then the three cups are bored in petridish with the help of sterile steel bore of 6 mm and in the bores and incubated the petri plates for 72 h at 37°C in incubators. Then the zone of inhibition was observed and calculated the radius of the zone of inhibition.

10. Iso-osmotic effect study

Optimized preparation are subjected to isotonicity testing, since they exhibit good release characteristics and gelling capacity and the requisite viscosity. Formulation is mixed with few drops of blood and observed under microscope at 45x magnification.

11. Sterility testing study

Membrane filtration method was used for optimized batches study. In this 2ml of liquid from test container was removed with a sterile pipette or with a sterile syringe or a needle and filter through membrane. Then this filtered test liquid was aseptically transferred to fluid thioglycolate medium (20ml). The liquid was mixed with the media. The inoculated media were incubated for 48hrs at 37^0C and observed.

12. Test for stability of selected in situ gelling ophthalmic formulations

Test for stability of selected in situ gelling ophthalmic formulations at ambient environmental condition in depicted in table 6.9 and at refrigerator conditions in depicted in table 7.

Table 7: Details of stability test at ambient environmental conditions.

Sr. No.	Specifications	Standard values
1	Duration of study	30 days
2	Temperature condition	30±20C
3	Relative humidity condition	65±5%RH
4	Frequency of testing	Day 0, day 15, day 30

To assess the shelf life of optimized gel formulations, the stability tests were conducted (environmental zone IV) for the period of 30 days in stability chamber. The formulations were filled in glass containers and were stored at ambient environmental conditions (30±20C/65±5 %RH) and at refrigerator conditions (5±30C). The samples were tested initially and then at 15 days and 30 days intervals for the following parameter.

I. Appearance/Clarity
II. PH
III. Gelling ability
IV. Viscosity
V. Drug content by UV spectrophotometric method
VI. In vitro drug release of ophthalmic formulations using dialysis membrane.

RESULT AND DISCUSSION

Appearance and clarity

All the formulations of poloxamer 407 alone and with HEC and HPMC E50 LV were found to be very clear without any precipitation. All the formulations containing polycarbophil and carbopol 974 were found to be turbid, which might be because precipitation as a result of the incompatibility of Levofloxacin Hemihydrate and Ketorolac Tromethamine with these polymers. Hence these formulations were not considered for further study.

pH of ophthalmic formulations

The pH of ophthalmic formulations formulated using poloxamer 407 was found to be in the range 6.7-7.4 and that of HEC and HPMC E50 LV formulations were in the range of 4.5-7.4.These pH values were considered to be acceptable since the ophthalmic pH ranges between 4.5-7.4. Hence no discomfort or excessive tear fluid might occur on instillation.

In situ gelling ability of formulations

An ideal in situ gelling system should be a free flowing liquid with low viscosity at non-physiological conditions (pH 4.0-250C) to allow reproducible administration into the eye as

drops. It should also undergo in situ phase transition to form strong gel capable of withstanding shear forces in the cul-de-sac and sustain drug release at physiological condition in PBS (pH 7.4 and 370C). Hence, formulations of poloxamer 407, HEC and HPMC E50 LV were evaluated for in situ gelling ability in PBS 7.4 at physiological pH 7.4 and temperature 370C.

Table 8: Physical characteristics of in situ gelling opthalamic formulation of poloxamer 407 + Levofloxacin Hemihydrate.

Code	Poloxamer 407	Carbopol 974	Poly-carbophil	HEC	HPMC E50 LV	Appearance and Clarity	pH	Gelling ability	Gelation Temp.
P1	16	-	-	-	-	+++	7.1	-	32.3±0.58
P2	17	-	-	-	-	+++	7.15	+	27±0.00
P3	18	-	-	-	-	+++	7.2	+++	23.8±1.18
P4	19	-	-	-	-	+++	7.2	+++	23±0.00
P5	20	-	-	-	-	+++	7.4	+++	22.1±0.56
P6	18	0.2	-	-	-	-	-	-	17.3±0.19
P7	18	0.4	-	-	-	-	-	-	14.9±0.71
P8	18	0.6	-	-	-	-	-	-	12.7±1.18
P9	18	-	0.2	-	-	-	-	-	-
P10	18	-	0.4	-	-	-	-	-	-
P11	18	-	0.6	-	-	-	-	-	-
P12	18	-	-	0.2	-	+++	6.8	+++	24±0.00
P13	18	-	-	0.4	-	+++	7.2	+++	23±0.5
P14	18	-	-	0.6	-	+++	7.4	+++	21±0.92
P15	18	-	-	-	0.2	+++	6.8	+++	23.8±0.64
P16	18	-	-	-	0.4	+++	7.0	+++	23±0.00
P17	18	-	-	-	0.6	+++	7.0	+++	22.1±0.29

Table 9: Physical characteristics of in situ gelling ophthalmic formulation of poloxamer 407 + Ketorolac Tromethamine.

Code	Poloxamer 407	HEC	HPMC E50 LV	Appearance and clarity	pH	Gelling ability	Gelation Temp.
P18	18	-	-	+++	7.2	+++	24±0.59
P19	-	0.4	-	+++	7.2	+++	23.7±0.2
P20	-	-	0.4	+++	7.0	+++	22.8±0.00

Table 10: Physical characteristics of in situ gelling ophthalmic formulation of poloxamer 407 + Levofloxacin Hemihydrate + Ketorolac Tromethamine.

Code	Poloxamer 407	HEC	HPMC E50 LV	Appearance and clarity	pH	Gelling ability	Gelation Temp.
P21	18	-	-	+++	7.2	+++	23.8±0.02
P22	-	0.4	-	+++	7.2	+++	23±0.00
P23	-	-	0.4	+++	7.0	+++	22±0.5

*Appearance and Clarity:

Turbid (-), slightly turbid (+), Clear (++), clear and transparent (+++)

**Gelling ability

(-) No phase transition

(+) Formation of gel after 60sec and gel collapsed within 1hr

(++) Formation of gel after 60sec and gel collapsed within 3hr

(+++) Formation of gel within 60sec and gel remained stable for more than 6-7hr

Spreadability of the formulations:

Table 11: Spreadability of in situ gelling opthalamic formulations.

Code	Distance (Cm)	Code	Distance (Cm)
P1	2.2	P14	0.9
P2	2	P15	2.5
P3	1.8	P16	2
P4	1.5	P17	1.8
P5	1.1	P18	1.9
P6	2.3	P19	2.1
P7	2	P20	2
P8	1.5	P21	2
P12	2	P22	2.2
P13	1.2	P23	2.3

Percent drug content

Table 12: Percent drug content of in situ gelling ophthalmic formulations.

Code	% Drug content
P1	99.15±0.75
P2	98.67±0.56
P3	99.62±0.98
P4	99.15±0.03
P5	100.56±0.25
P6	98.2±0.26
P7	100.09±0.29
P8	101.51±0.64
P12	99.15±0.72
P13	101.03±0.73
P14	100.56±0.59
P15	100.09±0.48
P16	99.15±0.43
P17	101.03±0.09
P18	100.99±0.07
P19	98.61±0.04
P20	99.8±0.18
P21	100.56±0.5

P22	100.75±0.25
P23	99.5±0.50

Viscosity of ophthalmic formulations

There are no specification as such for the viscosity of in situ gelling systems. These systems are expected to undergo shear thinning (decreasing in viscosity at increasing shear rate) in gel state due to the pseudo behavior of the gels formed.

The viscosities of ophthalmic formulation were determined using Brookfield viscometer.

Table 13: Viscosity of ophthalmic formulations of poloxamer 407 + Levofloxacin Hemihydrate.

Rpm	Code				
	P1	P2	P3	P4	P5
0.5	960000	990000	1220000	2320000	2370000
1	590000	422000	1050000	1850000	1950000
2	310000	390000	470000	730000	738000
4	160000	228000	251000	370000	409000
5	125000	185000	210000	290000	301000
10	60000	99000	109000	157000	186000
20	3200	52000	55750	81500	106000
50	1330	22800	25400	37500	46100
100	1250	12400	14000	19400	24500

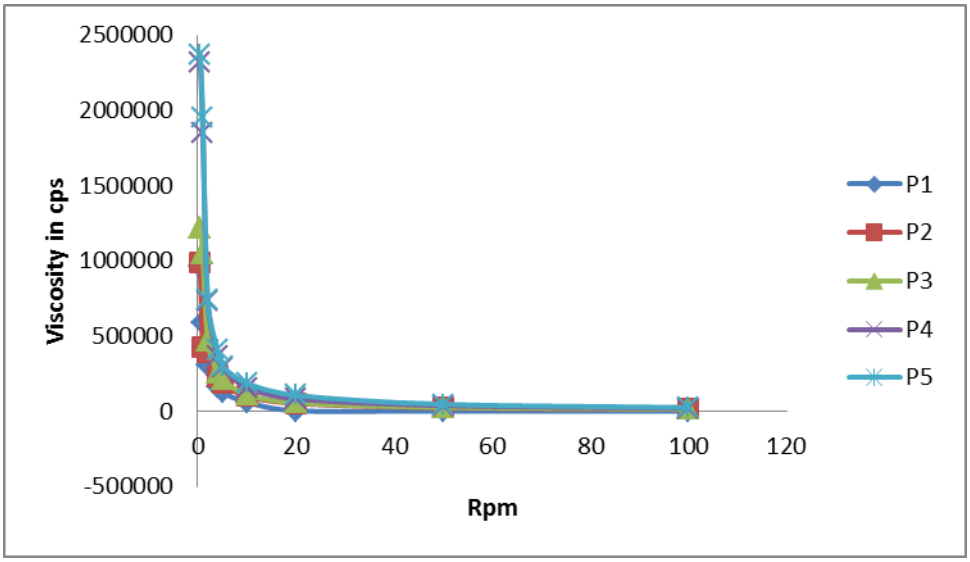

Fig. 1: Rheogram of ophthalmic gels.

Table 14: Viscosity of opthalamic formulations of poloxamer 407 + Levofloxacin Hemihydrate + Carbopol 974.

Rpm	Code		
	P6	P7	P8
0.5	6140000	15300000	15500000
1	3320000	5150000	5600000
2	1690000	2460000	2500000
4	890000	1310000	1450000
5	726000	1080000	1150000
10	390000	588000	595000
20	210000	312000	320000
50	93600	105000	110000
100	52300	67000	72000

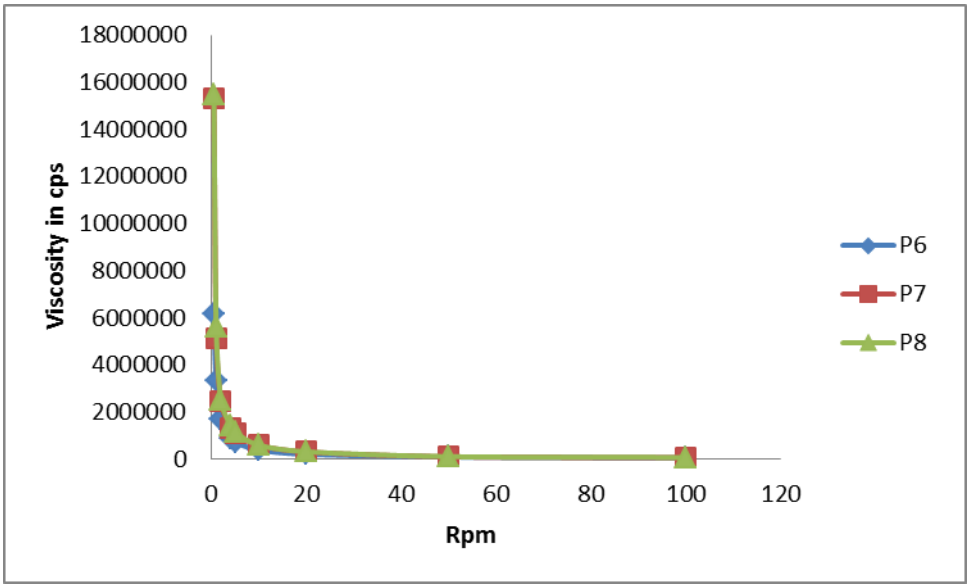

Fig. 2: Rheogram of ophthalmic gels.

Table 15: Viscosity of ophthalmic formulations of poloxamer 407 + Levofloxacin Hemihydrate + HEC (Natrosol 250).

Rpm	Code		
	P12	P13	P14
0.5	1590000	1640000	2300000
1	1020000	1240000	1290000
2	563000	660000	670000
4	291000	344000	350000
5	235000	260000	267000
10	125000	130000	141000
20	67000	70000	76700
50	29100	31500	34500
100	19200	21500	22250

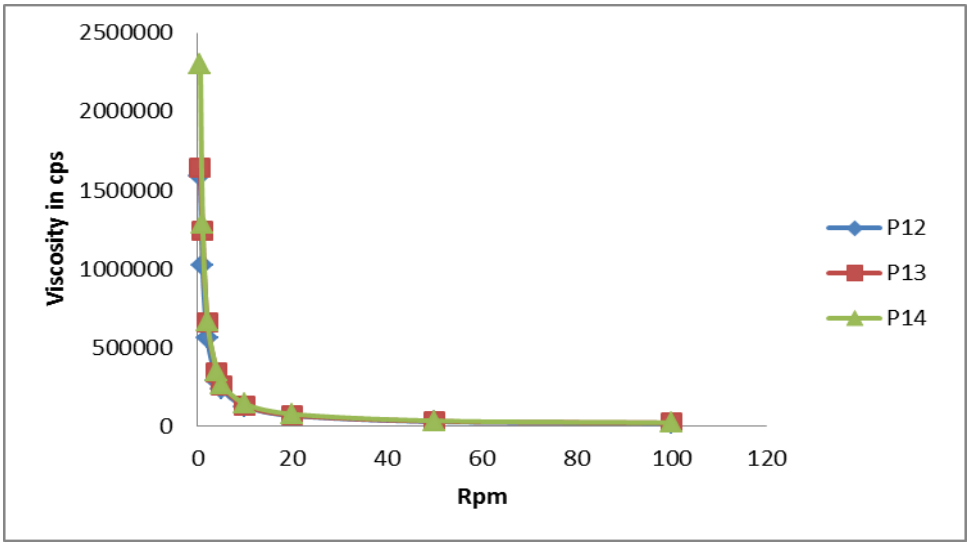

Fig. 3: Rheogram of ophthalmic gels.

Table 16: Viscosity of ophthalmic formulations of poloxamer 407 + Levofloxacin Hemihydrate + HPMC E50 LV.

Rpm	Code		
	P15	P16	P17
0.5	2000000	2150000	2250000
1	1160000	1250000	1310000
2	595000	610000	625000
4	306000	315000	320000
5	248000	260000	279000
10	132000	140000	156000
20	69500	72000	74500
50	30500	32500	33200
100	16500	19000	21500

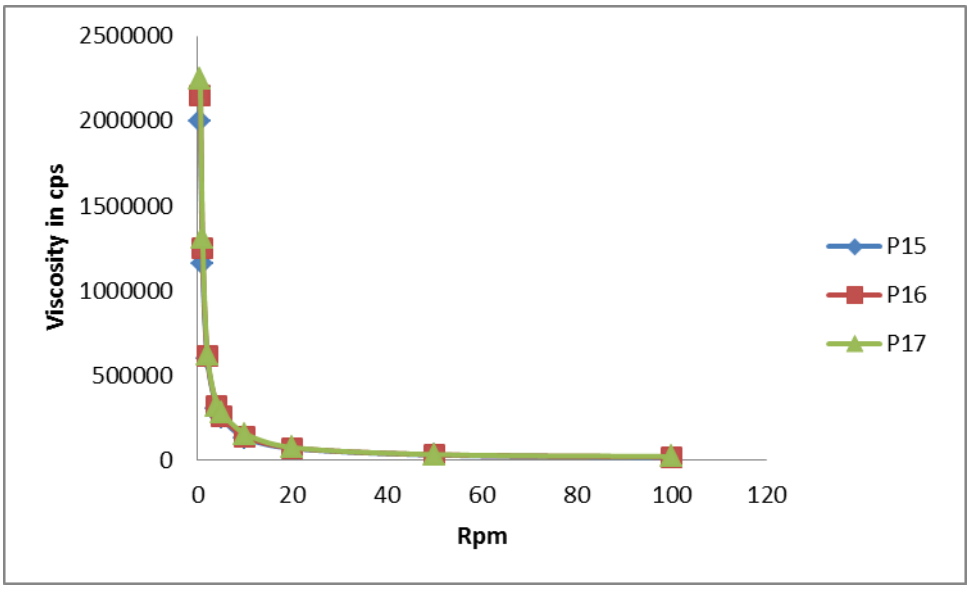

Fig. 4: Rheogram of ophthalmic gels.

Table 17: Viscosity of ophthalmic formulations of ketorolac tromethamine.

Rpm	Code		
	P18	P19	P20
0.5	1250000	1750000	2250000
1	1080000	1250000	1350000
2	590000	790000	690000
4	398000	595000	325000
5	251000	345000	285000
10	125000	210000	165000
20	60500	105000	101000
50	30500	65000	80500
100	21500	30500	42000

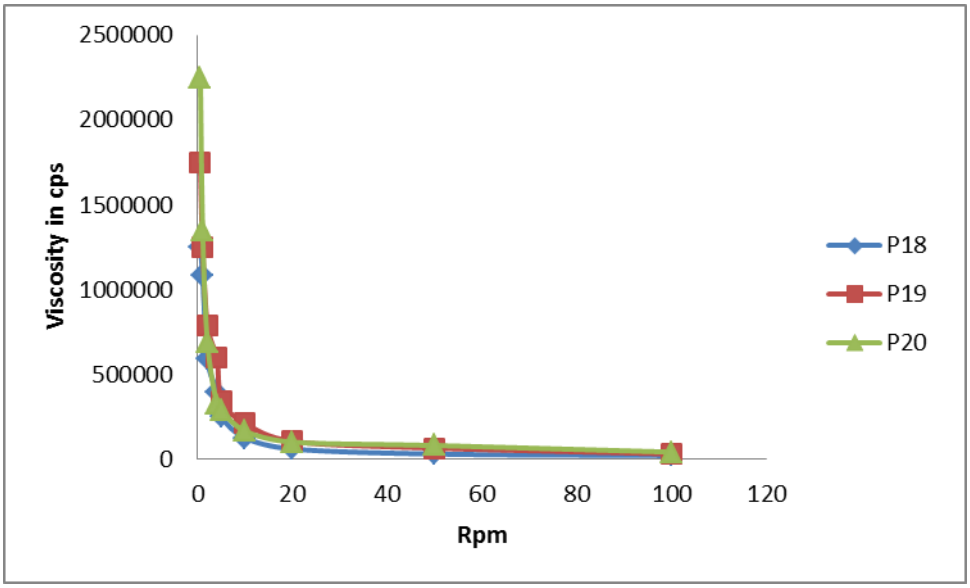

Fig. 5: Rheogram of ophthalmic gels.

Table 18: Viscosity of optimized ophthalmic formulations.

Rpm	Code		
	P21	P22	P23
0.5	1840000	1980000	1900000
1	1240000	970000	990000
2	630000	495000	522000
4	322000	260000	274000
5	257000	212000	223000
10	135000	111000	121000
20	70500	62000	60500
50	31400	27900	26700
100	16900	16500	17000

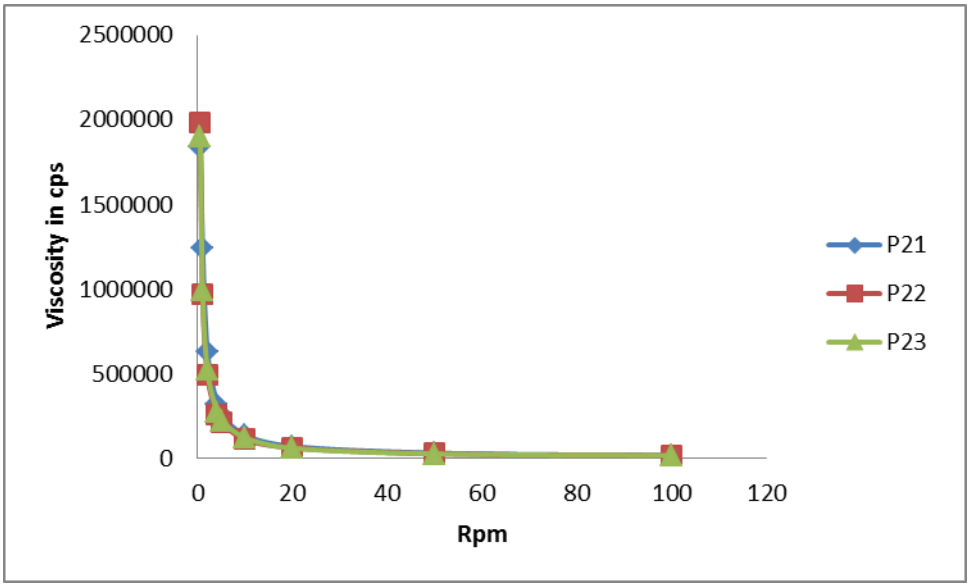

Fig. 6: Rheogram of ophthalmic gels.

Release of drug from ophthalmic formulations

Table 18: Average (±SD) cumulative percentage release of ophthalmic formulations.

Time (hrs)	P1	P2	P3	P4	P5
0	0	0	0	0	0
0.5	11.94±0.24	9.88±0.36	8.38±0.59	4.291±0.59	6.48±0.76
1	12.87±0.63	15.11±0.63	14.79±0.54	7.681±0.77	13.57±0.47
2	24.87±0.77	28.53±0.75	20.19±0.86	24.5±0.87	22.39±1.38
3	37.06±0.97	38.25±0.26	28.72±0.52	28.25±0.14	27.57±0.49
4	44.20±0.4	51.48±0.38	34.62±0.4	32.32±0.75	34.01±0.5
5	54.56±0.79	62.23±0.78	48.05±1.21	47.68±0.68	45.16±0.51
6	69.18±0.78	70.39±0.53	57.99±0.54	61.24±1.13	55.04±0.54
7	78.23±1.24	75.42±1.24	68.46±0.55	69.9±0.67	66.79±0.53
8	83.83±0.81	81.18±0.69	79.48±0.67	76.68±1.24	74.56±0.9

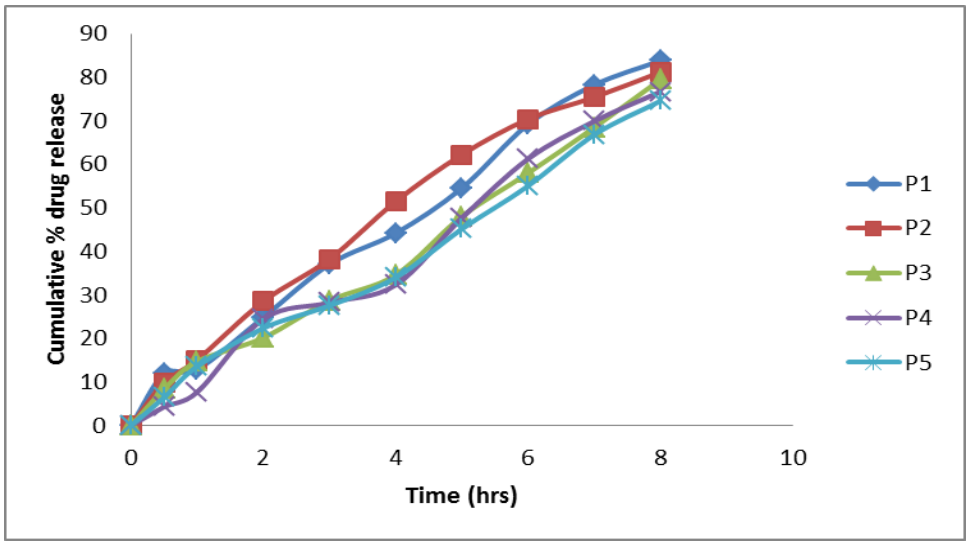

Fig. 7: Average (±SD) cumulative percentage release of ophthalmic formulations.

As the concentration of poloxamer 407 was increased from 16% to 20% w/v the drug release was found to be decreased and fig. 7. represent the dissolution profiles of the formulations.

Table 19: Average (±SD) cumulative percentage release of ophthalmic formulations.

Time (hrs)	P12	P13	P14
0	0	0	0
0.5	26.13±0.36	19.54±0.24	13.17±0.21
1	32.81±0.24	23.76±0.6	18.11±0.43
2	36.86±0.42	27.41±0.51	21.07±0.33
3	54.99±0.6	30.7±0.39	24.39±0.54
4	59.03±0.52	35.75±0.52	26.69±0.35
5	65.68±0.65	38.77±0.64	34.75±0.45
6	77.53±0.72	56.48±0.65	38.812±0.56
7	79.05±0.65	64.98±0.55	50.37±0.36
8	83.01±0.56	73.63±1.43	60.02±0.81

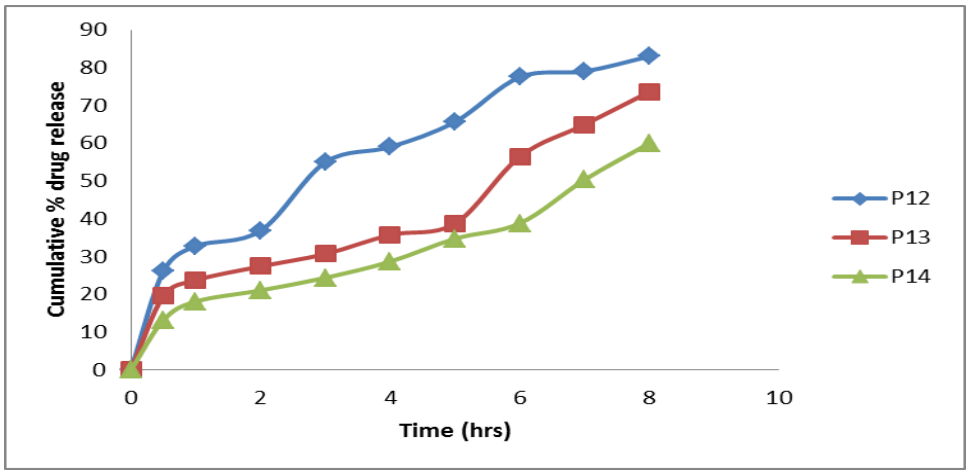

Fig. 8: Average (±SD) cumulative percentage release of opthalamic formulations.

As the concentration of HEC (Natrosol 250) was increased from 0.2% to 0.6% w/v the drug release was found to be decreased and fig. 8. represent the dissolution profiles of the formulations.

Table 20: Average (±SD) cumulative percentage release of ophthalmic formulations.

Time (hrs)	P15	P16	P17
0	0	0	0
0.5	26.13±0.32	20.67±0.56	17.71±0.24
1	34.3±0.56	25.44±0.44	22.74±0.24
2	40.5±0.44	28.24±0.75	27.87±0.6
3	46.6±1.08	33.15±0.35	35.79±0.48
4	53.86±0.57	43.2±0.48	44.11±0.38
5	61.04±0.48	48.62±0.47	49.38±0.52
6	65.59±0.49	54.13±0.47	56.71±0.43
7	71.05±0.58	64.55±0.59	63.43±0.64
8	75.53±0.51	72.41±0.5	69.96±0.54

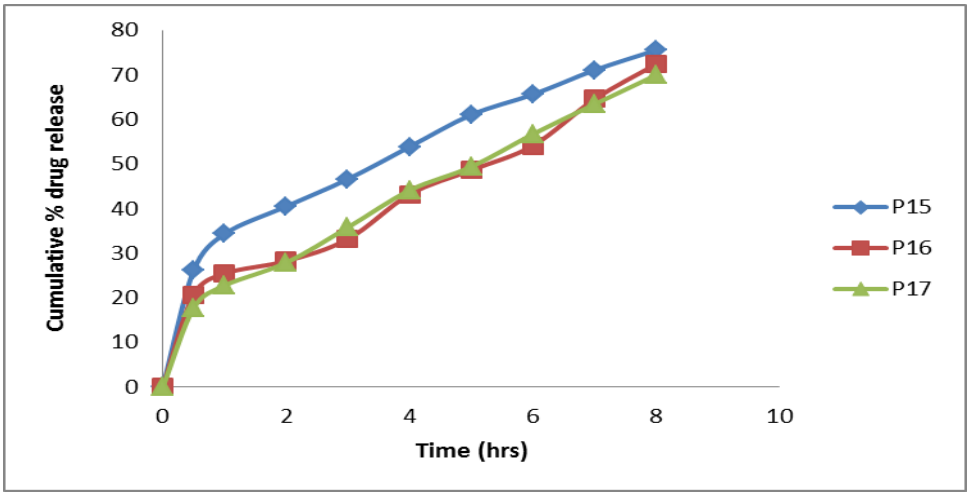

Fig. 9: Average (±SD) cumulative percentage release of ophthalmic formulations.

As the concentration of HPMC E50 LV was increased from 0.2% to 0.4% w/v the drug release was found to be decreased and fig. 9. represent the dissolution profiles of the formulations.

Table 21: Average (±SD) cumulative percentage release of ophthalmic formulations.

Time (hrs)	P18	P19	P20
0	0	0	0
0.5	17.46±0.3	14.96±0.36	11.22±0.27
1	26.18±0.31	23.82±0.19	20.71±0.54
2	36.15±0.31	31.42±0.36	29.85±0.28
3	45.23±0.31	40.23±0.29	38.1±0.2
4	53.94±0.43	49.38±0.48	44.9±0.12
5	61.39±0.44	56.2±0.3	52.89±0.2
6	70.38±0.45	62.79±0.4	58.7±0.3
7	74.19±0.35	68.24±0.31	64.79±0.39
8	80.37±1.04	72.53±0.5	70.44±0.21

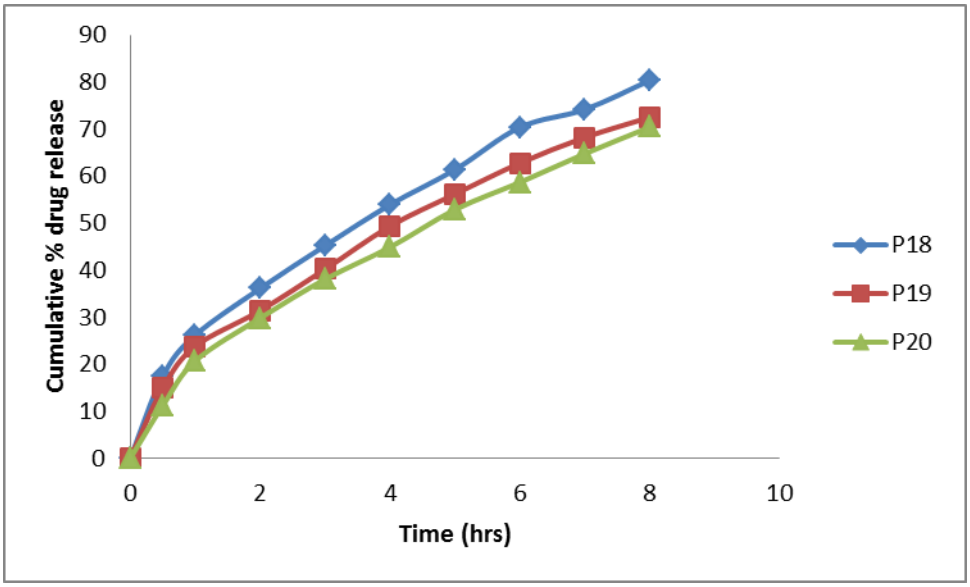

Fig 10: Average (±SD) cumulative percentage release of ophthalmic formulations.

As the concentration of poloxamer 407(18%w/v), HEC (0.4%w/v), HPMC (0.4%w/v) were increased the drug release was found to be decreased and fig.10. represent the dissolution profiles of the formulations.

Table 22: Average (±SD) cumulative percentage release of ophthalmic formulations.

Time	P21		P22		P23	
	Levo	Keto	Levo	Keto	Levo	Keto
0	0	0	0	0	0	0
0.5	9.348±0.44	7.48±0.27	10.4±0.56	17.46±0.94	11.47±0.56	9.26±0.45
1	15.48±0.45	12.98±0.64	15.29±0.98	27.43±0.96	15.31±0.69	13.19±0.44
2	28.11±0.77	24.1±0.74	22.6±1.1	34.03±1.31	24.74±0.98	22.54±0.8
3	41.62±1.02	35.8±0.93	33.66±0.61	39.68±0.86	35.21±1.08	31.71±0.74
4	56.45±0.98	48.98±0.77	42.81±1.23	46.86±0.88	47.37±0.59	42.49±1.01
5	60.73±0.24	58.83±0.61	52.35±1.04	54.70±1.62	55.72±0.6	50.43±0.92
6	67.41±1.49	66.55±0.5	62.69±1.03	60.37±0.93	64.005±0.52	57.81±0.79
7	72.93±0.71	70.66±0.55	68.13±1.77	67.73±0.37	68.61±0.65	64.78±1.26
8	80.23±0.15	80.17±0.56	72.8±0.71	73.43±1.32	72.01±1.16	70.62±1.35

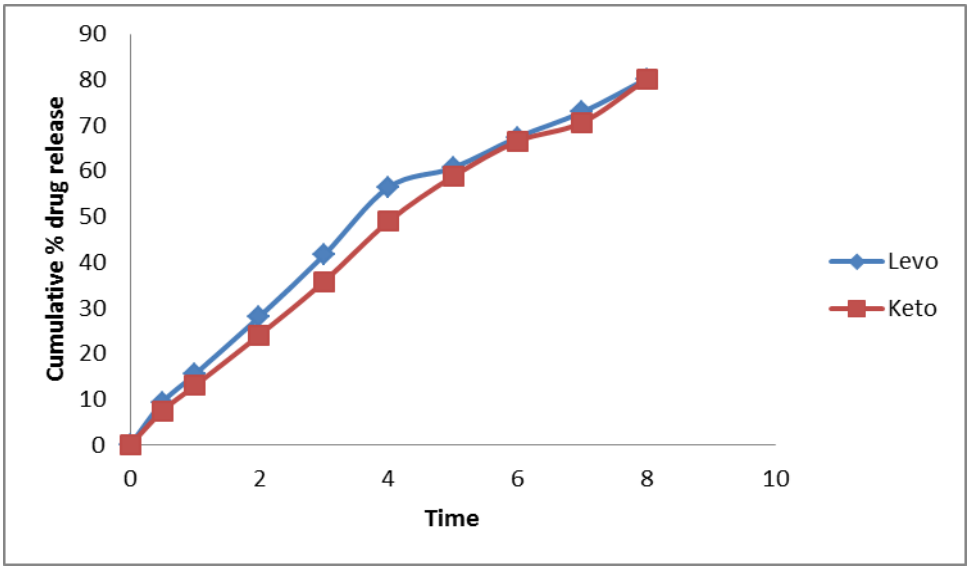

Fig. 11: Average (±SD) cumulative percentage release of P21 ophthalmic formulations.

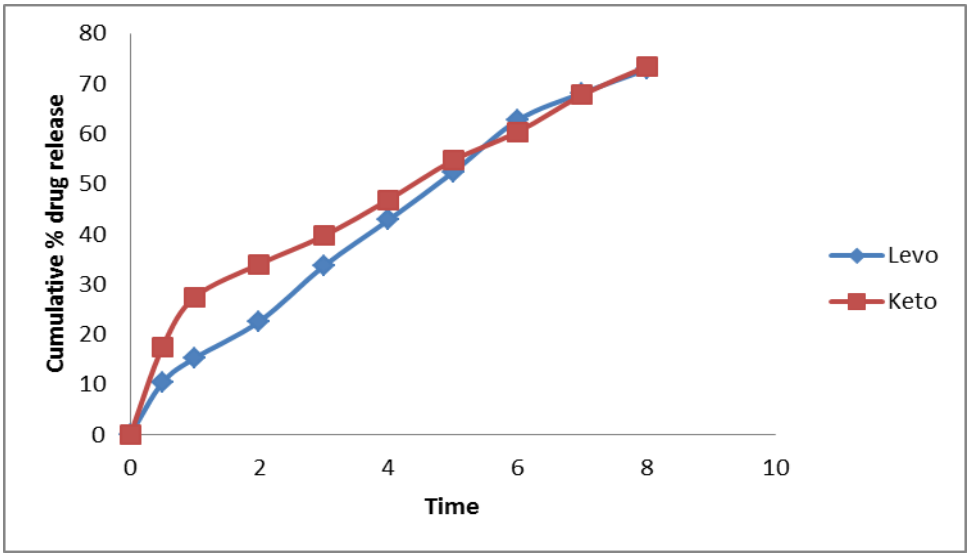

Fig. 12: Average (±SD) cumulative percentage release of P22 ophthalmic formulations.

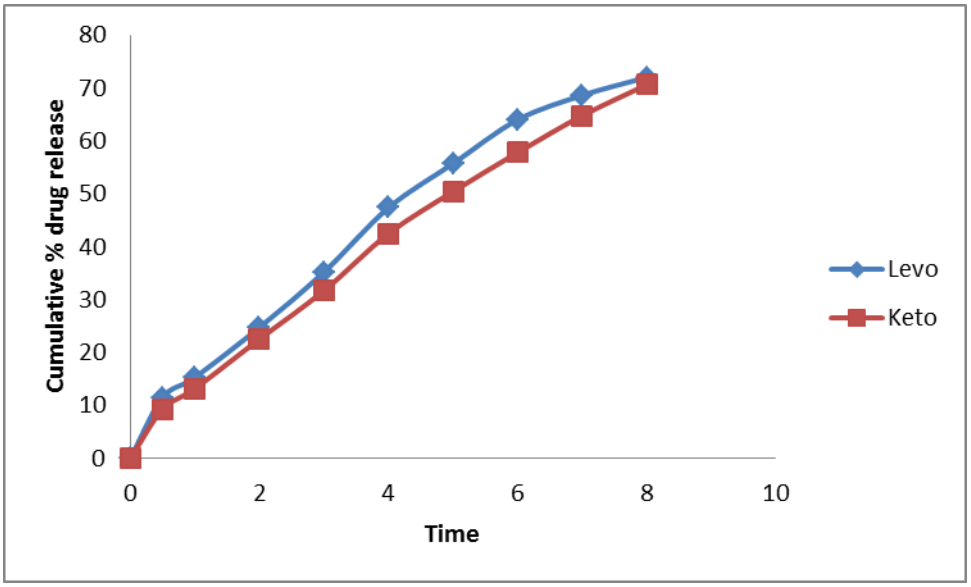

Fig. 13: Average (±SD) cumulative percentage release of P23 ophthalmic formulations.

Kinetics of drug release from ophthalmic formulations

The cumulative amount of drug released from selected ophthalmic formulations at different time intervals was fitted to zero order, first order, higuchi matrix and korsemayer peppas model to find out the mechanism of drug release. The coefficient between the time and cumulative amount of drug release was also calculated to find the fit to appropriate kinetics.

The result are depicted in table 23.

Code	Zero order	First order	Matrix		Korsmeyer peppas	
	R2	R2	R2	K	R2	N
P1	0.9913	0.9826	0.957	24.8746	0.9825	0.7557
P2	0.9737	0.9959	0.9741	28.017	0.9966	0.7717
P3	0.995	0.954	0.9374	23.84	0.9893	0.7852
P4	0.9924	0.9686	0.9272	23.0712	0.9892	1.0746
P5	0.995	0.9364	0.9441	26.62	0.9943	0.8388
P12	0.873	0.9837	0.9934	33.47	0.9828	0.4484
P13	0.9347	0.9417	0.9484	24.27	0.9247	0.4554
P14	0.9498	0.9591	0.9538	17.11	0.9555	0.5022
P15	0.7947	0.9596	0.9889	27.0846	0.9915	0.3793
P16	0.9239	0.9722	0.9784	21.07	0.958	0.4782
P17	0.9347	0.9861	0.9906	21.83	0.9852	0.5018
P18	0.926	0.9925	0.997	30.48	0.9991	0.5519
P19	0.9343	0.9937	0.9954	24.83	0.9979	0.5713
P20	0.9542	0.9961	0.9909	23.42	0.9975	0.6483

Table 23: Release kinetics of ophthalmic formulations.

	L	K	L	K	L	K	L	K	L	K	L	K
P21	0.972	0.9898	0.9965	0.9903	0.9743	0.9599	26.51	25.22	0.996	0.998	0.813	0.8922
P22	0.987	0.9079	0.9945	0.9843	0.9668	0.9956	23.43	24.86	0.994	0.992	0.757	0.4982
P23	0.9764	0.9892	0.9975	0.9966	0.9742	0.9667	24.12	22.38	0.994	0.996	0.732	0.7592

The mechanism of drug release from gel matrix is complex and is based on diffusion of drug through hydrated portion of the gel matrix and erosion of the outer fully hydrated polymer on the surface of the matrix. Due to permeation of more water into core of gel matrix, the hydration of gel matrix increases and provides a diffusion barrier to drug release. As gel matrix becomes fully hydrated, the polymer chains become completely relaxed and can no longer maintain the integrity of the gel leading to disentanglement and erosion from the surface of the gel matrix.

Mucoadhesive strength of ophthalmic formulations

Ocular mucoadhesion relies on the interaction of a polymer and the mucin coat covering the conjunctiva and corneal surface of the eye. This mucus layer is secreted by goblet cells of the conjunctiva. Structurally, mucin consists of a protein or polypeptide core with carbohydrate side chains branching off the core. The polymer with many hydrophilic functional groups (eg. Carboxyl group, hydroxyl group and sulphate) can establish electrostatic interactions and hydrogen bond with the underlying surface. Of these non-covalent forces, hydrogen bonding appears to be the most important.

The mucoadhesive potential of all gel formulations was evaluated by using goat corneal membrane. Weight (gm) required to detach gel formulation from the excised cornea was measured and force was calculated.

$$\text{Force of adhesion (N)} = \frac{\text{Bioadhesive strength} \times 9.81}{100}$$

The mucoadhesive strength of various gel formulations is shown in table 23.

Table 14: Mucoadhesive force of ophthalmic formulations.

Formulation	Mucoadhesive strength (gms)	Force of adhesion (N)
P21	4.9	0.4806
P22	6.9	0.6768
P23	7.0	0.6867

Fig. 14: Mucoadhesion strength measurement graph of P21 ophthalmic gel.

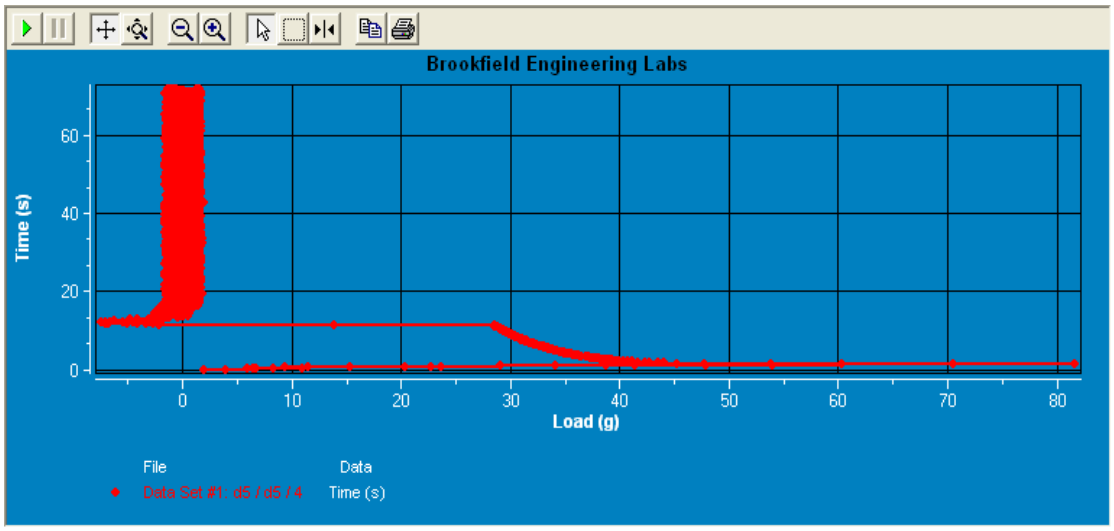

Fig. 15: Mucoadhesion strength measurement graph of P22 ophthalmic gel.

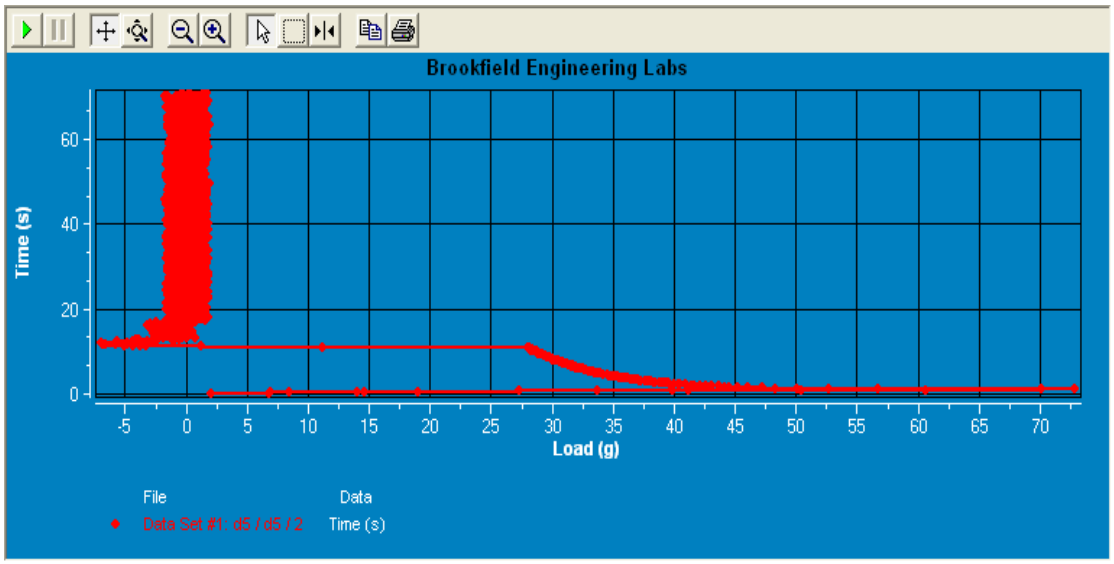

Fig. 16: Mucoadhesion strength measurement graph of P23 ophthalmic gel.

Effect of autoclaving on the ophthalmic formulations

As the ophthalmic formulations must be sterile before dispensing, the method of sterilization selected was the moist heat sterilization by autoclave. To check the effect of heat due to autoclaving on the formulations of Levofloxacin Hemihydrate and Ketorolac Tromethamine, selected formulation were sterilized by autoclave. Evaluation parameters like appearance, pH, gelling ability, viscosity and drug content were studied before and after autoclaving.

The effect of heat for the selected formulation was estimated by evaluating appearance, pH, gelling ability, viscosity and drug content are shown in table 15.

Table 15: Evaluation of selected ophthalmic formulations of Levofloxacin Hemihydrate and Ketorolac Tromethamine before and after autoclaving.

Evaluation parameter	P21		P22		P23	
	Before	After	Before	After	Before	After
Appearance/Clarity	+++	+++	+++	+++	+++	+++
pH	7.2	7	7.2	7.08	7	7.12
Gelling Ability	+++	+++	+++	+++	+++	+++
Viscosity at 100 rpm	16900	16525	16500	16250	17000	16725
Percent drug content	100.56±0.5	99.5±0.5	100.75±0.25	100±0.25	99.5±0.5	100±0.5

All the formulations did not show any change in appearance after autoclaving. The formulations containing HEC and HPMC were found to be slightly turbid after autoclaving, but turbidity disappeared after storage for overnight. It was observed that there was no change in the pH and gelation property of the prepared in situ gelling formulations. It was found that drug content decreases slightly after autoclaving but changes were within the acceptable limits. The viscosity of formulation was found to be decreased. The change in viscosity was very negligible.

Sterility testing of optimized batches

All the optimized formulations were sterilized by membrane filtration method and observed visually that shows no growth of microorganism so the formulations were sterilized.

The images taken of the sterilized batches are shown in fig 17,18,19 below:

Fig. 17: Poloxamer batch.

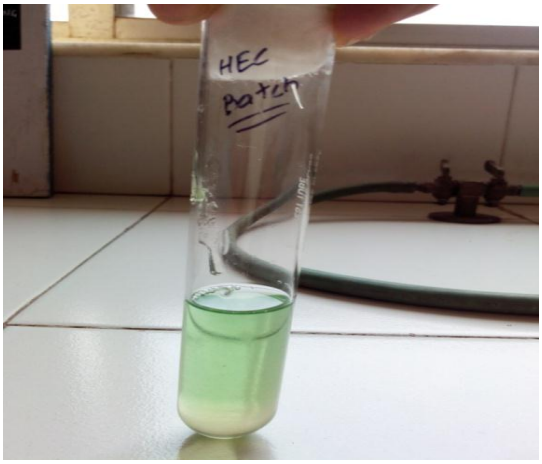

Fig. 18: HEC batch.

Fig. 19: HPMC batch.

Iso-osmotic effect study

All the optimized formulations were studied for iso-osmotic effect. In this, the formulations were mixed with blood sample and the diameter of RBC was measured with the help of eyepiece at 45X magnification. So, from the observation it is clear that there is no change in the diameter of the RBC before and after addition of formulation. From this, it is clear that the formulation were iso-osmotic.

Microbiological study

In these studies, the diameter of the zone of inhibition of optimized batches were compared with the standard drug and it shows more diameter of zone of inhibition shown by the optimized formulations.

The below figure shows the zone of inhibition of optimized formulations

Fig. 20: Zone of inhibition of poloxamer batch.Fig. 21: Zone of inhibition of HEC batch.

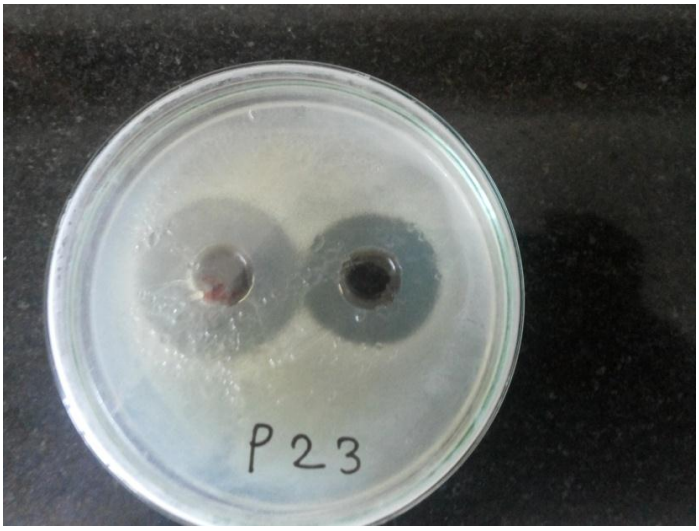

Fig. 22: Zone of inhibition of HPMC batch.

Test for stability of selected in situ gelling ophthalmic formulations of Levofloxacin Hemihydrate and Ketorolac Tromethamine

The optimized ophthalmic formulations stored for 30 days in stability chamber (Temp: 30°C, RH: 65%). Effect of temperature on their stability during storage was assessed by evaluating there appearance, gelling ability, pH, viscosity, uniformity in drug content and in vitro drug release characteristics. The results are shown 16 in Table below:

Table 16: Evaluation of selected ophthalmic formulations of levofloxacin Hemihydrate and Ketorolac tromethamine before and after stability.

Evaluation parameter	P21		P22		P23	
	Before	After	Before	After	Before	After
Appearance/Clarity	+++	+++	+++	+++	+++	+++
pH	7.2	7	7.2	7.08	7	7.12
Gelling Ability	+++	+++	+++	+++	+++	+++
Viscosity at 100 rpm	16900	16525	16500	16250	17000	16725
Percent drug content	100.56±0.5	99.5±0.5	100.75±0.25	100±0.25	99.5±0.5	100±0.5

Table 17: Cumulative percent release from ophthalmic formulations stored for 30 days at ambient environmental conditions.

Time (hrs)	P21				P22				P23			
	Before		After		Before		After		Before		After	
	L	K	L	K	L	K	L	K	L	K	L	K
0	0	0	0	0	0	0	0	0	0	0	0	0
0.5	9.348 ±0.44	7.48± 0.27	9.25±0 .38	7.5±0. 25	10.4± 0.56	17.46± 0.94	10.25 ±0.5	16.5± 0.85	11.47± 0.56	9.26± 0.45	11.45 ±0.4	9.5±0. 5
1	15.48 ±0.45	12.98 ±0.64	15.2±0 .4	13.2± 0.5	15.29 ±0.98	27.43± 0.96	15±1. 0	26.45 ±1.17	15.31± 0.69	13.19 ±0.44	15.7± 0.65	14.2± 0.25
2	28.11 ±0.77	24.1± 0.74	28.01± 0.75	23.5± 0.48	22.6± 1.1	34.03± 1.31	22.5± 0.9	33.5± 1.15	24.74± 0.98	22.54 ±0.8	23.8± 1.0	23.5± 0.98
3	41.62 ±1.02	35.8± 0.93	40.8±1 .05	33.9± 0.45	33.66 ±0.61	39.68± 0.86	34.6± 0.42	40.21 ±0.69	35.21± 1.08	31.71 ±0.74	34.25 ±1.42	32.5± 0.56
4	56.45 ±0.98	48.98 ±0.77	55.5±0 .78	49.62 ±0.32	42.81 ±1.23	46.86± 0.88	43.5± 0.42	45.68 ±0.85	47.37± 0.59	42.49 ±1.01	48.3± 0.62	43.5± 1.45
5	60.73 ±0.24	58.83 ±0.61	61.5±0 .25	59.6± 0.35	52.35 ±1.04	54.70± 1.62	51.5± 1.25	55.9± 1.62	55.72± 0.6	50.43 ±0.92	54.6± 0.53	51.3± 0.92
6	67.41 ±1.49	66.55 ±0.5	68.5±1 .42	67.2± 0.35	62.69 ±1.03	60.37± 0.93	63.2± 1.02	61.3± 0.5	64.005 ±0.52	57.81 ±0.79	63.9± 0.62	58.9± 0.85
7	72.93 ±0.71	70.66 ±0.55	73.5±0 .82	71.2± 0.42	68.13 ±1.77	67.73± 0.37	69.3± 1.22	68.9± 0.62	68.61± 0.65	64.78 ±1.26	69.5± 0.5	64.5± 1.2
8	80.23 ±0.15	80.17 ±0.56	81.2±0 .25	81.25 ±0.6	72.8± 0.71	73.43± 1.32	73.2± 0.42	74.2± 0.52	72.01± 1.16	70.62 ±1.35	73.2± 1.12	71.5± 1.32

All the formulations did not show any change in appearance, clarity and colour of the formulations at any of the storage conditions. It was also observed that there was no change in the gelation properties of the prepared in situ gelling formulations at any storage conditions. Variations were observed in the pH values at ambient storage conditions. There was no change in drug content of the formulations. The viscosity of the formulations found to be decreased with time when stored at ambient conditions. The change in viscosity was very negligible. Normally, the drug release was higher in all the formulations stored at ambient conditions because change in the viscosity of formulations at ambient temperature conditions.

SUMMARY AND CONCLUSION

An eye infection is a condition caused by bacteria or a virus. While there are many different types of eye infection with different causes and treatments, the most common is *bacterial conjunctivitis.* Another well known infection is corneal ulcer which has ulcerative lesions in the central area of eye ball. Eye infections can occur in any age group of patients and since relatively benign infections can develop into serious disorders. People who have undergone eye surgery or experienced trauma to the eye are at greater risk of infection. Levofloxacin

Hemihydrate is a third generation fluoroquinolone which is optically active levo-isomer of ofloxacin. It has excellent activity against gram positive, gram negative as well as anaerobic bacteria. It acts by inhibiting bacterial DNA gyrase enzyme which is required for DNA replication, transcription, repair or recombination and thus causing bacterial lysis. Ketorolac is a non steroidal anti inflammatory drug (NSAID) used to treat inflammation and sub acute inflammation of the eye. It has also excellent activity against gram positive, gram negative as well as anaerobic bacteria. The mechanism of action of ketorolac like that of other NSAID is not completely understood but may be related to prostaglandin synthetase inhibition. The biological activity of ketorolac tromethamine is associated with the S-form. Ketorolac tromethamine possesses no sedative or anxiolytic properties. The peak analgesic effect of ketorolac tromethamine occurs within 2-3 hrs and is not statistically significantally different over the recommended dosage range of ketorolac tromethamine. The greatest difference between large and small doses of ketorolac tromethamine by either route is in the duration of analgesia. Most ocular diseases are treated with topical application of eye drops. The major trouble encountered with eye drops is quick precorneal drug loss. To achieve the desired therapeutic drug concentration at the site, repeated administration of the drug formulation is needed. As ocular efficiency of topically applied drugs is influenced by the corneal contact time, most common method of improving ocular availability of drugs is to increase precorneal residence time by using delivery systems based on the concept of in-situ gel formation which exhibit sol to gel phase transitions due to a change in a specific physiochemical parameter (temperature, pH of specific ion).[52] In the present study, various in-situ gelling ophthalmic formulations of poloxamer 407 in concentration of 16-20% w/w (P1-P5). Further, different formulations of poloxamer 407 (18%w/w) were prepared by combining with HEC in concentration of 0.2, 0.4, 0.6%w/w (P12-P14) and HPMC E50 LV in concentration of 0.2, 0.4, 0.6%w/w (P15-P17). All above are prepared with Levofloxacin Hemihydrate. So, with Ketorolac Tromethamine three batches also prepared i.e poloxamer 407(18%w/w), poloxamer(18%w/w) and HEC(0.4%w/w) last one poloxamer407(18%w/w) and HPMC E50 LV(0.4%w/w). All these opthalamic formulations were evaluated for appearance, clarity, pH, gelling ability, viscosity and percent drug release. The formulations showing transparent and clear appearance, quick and stable gelation, shear thinning properties, excellent sustained drug release upto 75-85% after 8hrs were selected for further characterization. The selected formulations were then subjected for studying the stability to autoclaving and stability at ambient (30 ± 2^{0}C/65±5% RH). The optimized formulations P21 (18%w/w poloxamer, levofloxacin, ketorolac), P22 (18% w/w poloxamer, 0.4%w/w HEC,

levofloxacin, ketorolac) and P23 (18% w/w poloxamer, 0.4%w/w HPMC E50 LV, levofloxacin, ketorolac) were found to be very clear, transparent, forming quick and stable gels with shear thinning behavior. The optimized formulations P21, P22 and P23 showed excellent mucoadhesion and drug release. These formulations were found to be stable to autoclaving, at ambient conditions and non-irritant to eye.

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