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pH-induced In Situ Gelling System of Lomefloxacin for Sustained Ocular Delivery

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ABSTRACT

In the present work, an attempt has been made to formulate pH-induced in-situ gel as an ophthalmic drug delivery system. In situ gels are made from polymers that exhibit phase transition due to physicochemical change in the environment. They can be conveniently dropped as a solution into the conjuctival sac in the eye. Upon contact with the lacrimal fluid, the polymer changes its conformation to form a gel. This delivery system has the ease of administration similar to an opthalmic solution and has a long retention time because of the gel formation. Different polymers for preparing pH induced in-situ gels have been evaluated. The topical application of ophthalmically active drugs to the eye is the most prescribed route of administration for the treatment of various ocular diseases. It is generally agreed that the intraocular bioavailability of topically applied drugs is extremely poor. Upon instillation of an ophthalmic solution; most of the instilled volume is eliminated from the pre-corneal area. This loss is mainly due to drainage of the excess fluid by the nasolacrimal duct or elimination of the solution by tear turnover, which will results in poor ocular bioavailability. Lomefloxacin is member of the fluoroquinolone class of antimicrobial drugs. It is active against a wide range of gram positive and gram negative organisms.

Keywords: Lomefloxacin, Bioavailability, Antimicrobial drugs, Ophthalmic Drug Delivery System, *In situ* gels.

INTRODUCTION

Today, topical ophthalmic application is considered the preferred way to achieve therapeutic levels of drug agents used to treat ocular diseases. The conventional preparations for this route fall in to several categories: solutions, suspensions, semisolids, and others. From a biopharmaceutical standpoint, their use has met some criticism over their efficiency as drug delivery systems. Bioavailability, particularly for ocular solutions, ranges from 1 to 10% of the total administered dose. This is due in part to the rapid precorneal clearance kinetics resulting from reflex tearing and blinking, where half-life times of instilled isotonic solutions approximate only 15 seconds in the human. [1-2] The topical application of ophthalmically active drugs to the eye is the most prescribed route of administration for the treatment of various ocular diseases. It is generally agreed that the intraocular bioavailability of topically applied drugs is extremely poor. [3] Upon instillation of an ophthalmic solution; most of the instilled volume is eliminated from the pre-corneal area. This loss is mainly due to drainage of the excess fluid by the nasolacrimal duct or elimination of the solution by tear turnover, which will results in poor ocular bioavailability. [3]

Drug administered by instillation must penetrate the eyes and do so primarily through the cornea. Corneal absorption is much more effective than scleral or

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conjuctival absorption, in which removal by blood vessels into the general circulation occurs. Lomefloxacin is member of the fluoroquinolone class of antimicrobial drugs. It is active against a wide range of gram positive and gram negative organisms. Lomefloxacin known to exert their antibacterial action by antagonism of the enzyme DNA gyrase, also known as bacterial topoisomerase II. Lomefloxacin acts on this enzyme and cause inhibition of DNA synthesis, antagonism of RNA and protein synthesis and ultimately cell death. [4-5]

Table 1: Contents of Formulations

S. No.	Ingredients	Concentrations (% w/v)			
		CHL 11	CHL 15	CHL 21	CHL24
1.	Lomefloxacin HCl	0.3	0.3	0.3	0.3
2.	Carbopol 940	0.3	0.4	0.2	0.3
3.	HPMC E50LV	1.5	1.5	—	—
4.	HPMC E4M	—	—	0.4	0.4
5.	Sodium chloride	0.9	0.9	0.9	0.9
6.	Benzalkonium chloride	0.01	0.01	0.01	0.01
7.	Acetate buffer (pH 5) up to	100	100	100	100

MATERIALS AND METHODS

Lomefloxacin HCl (Ipca Limited, Ratlam, Madhya Pradesh), HPMC E50LV & HPMC E4M (Color cone Asia Ltd., Verna, Goa), carbopol 940, sodium acetate, glacial acetic acid, sodium hydroxide, sodium chloride, sodium bi carbonate (S.D. Fine Chemicals Ltd., Mumbai), UV-Visible Spectrophotometer (UV-1700), Rheometer (DV-E).

Preparation of pH Induced In-Situ Gelling System

As we know that the pH sensitive polymers like carbomer derivatives, carbopol, forms aqueous solution in the unionized form. These polymers undergo reversible ionization at pH 7.4, physiological condition, to form a stiff gel network, which swells and forms large aqueous pores. Hence, carbopol 940 was selected for preparation of pH induced in-situ gelling system. For this, optimization of polymer ratio is necessary, so intended work was divided into the two parts. [6]

- Optimization of polymer ratio
- Incorporation of active ingredients in optimized polymer ratio.

Optimization of Polymer Ratio for Preparation of In-Situ Gelling System [7-8]

Method 1: By taking the benefit of polymer like, carbopol 940, formulations containing different concentration of carbopol 940 (0.1-0.5%) were prepared in different nonphysiological condition (like, pH 4 & pH 5), in order to identify the pH, clarity and Gelling capacity.

Procedure: Firstly, the acetate buffer of pH 4 and 5 were prepared. Two sets of, each with five beakers was filled with acetate buffer of pH 4 and 5, respectively. Different concentration of carbopol 940 from 0.1% to 0.5% was sprinkled to the beakers containing buffer solution of respective pH and allows hydrating overnight. Later the solution containing in the beakers were stirred with an overhead stirrer. These polymers were evaluated for clarity, gelling capacity and viscosity at physiological (pH 7.4) as well as non physiological (pH 4 & 5) condition by using Brook Field viscometer (DV-E Rheometer). These were carried out to get optimum polymer concentration as shown in Table 1.

Method 2: As observed during practical work that carbopol solution with lower concentration of it, viscosity of gels formed after gellation at pH 7.4 was very low and at higher concentration of it, viscosity of gels formed was very high. One more problem was found that as carbopol concentration increase, the pH of the formulation became acidic. Another problem was observed that as carbopol concentration increases, the solution became translucent at pH 4 but at pH 5 it was clear. Hence for further procedure, formulation pH was selected as pH 5. Form the above observations; carbopol cannot be used alone for preparation of in-situ gels. Hence it was decided to take the help of polymer, which increases the viscosity without compromising the gelling capacity of carbopol 940.

Method 3: In this method the high viscosity grade was taken to reduce the concentration of HPMC without compromising the viscosity increasing capacity. So that, that the HPMC E50LV (40-60 cps) was replaced by the HPMC E4M (4000-5600 cps) as shown in Table 1.

Incorporation of Lomefloxacin HCl in Optimized Formulation

Results from method 2 reveal that the formulations CH 11 (0.3% carbopol 940 and 1.5% HPMC E50LV) and CH 15 (0.4% carbopol 940 and 1.5% HPMC E50LV) showed good gelling capacity and desire viscosity.

Results from method 3 reveal that the formulations CH 21 (0.2% carbopol 940 and 0.4% HPMC E4M) and CH 24 (0.3% carbopol 940 and 0.4% HPMC E4M) showed good gelling capacity and desire viscosity.

These four formulations were selected as those had satisfactory attributes of gelling capacity and desired viscosity at physiological (pH 7.4) as well as non-physiological (pH 5) conditions. The detail data of the prepared formulations for pH induced in-situ gelling system of Lomefloxacin HCl are given in Table 1.

Procedure: The buffer salts were dissolved in 50 ml of purified water; HPMC (E50LV / E4M) was added to hydrate. Carbopol 940 was sprinkled over this solution and allowed to hydrate overnight. The solution was stirred with an overhead stirrer. Lomefloxacin hydrochloride was dissolved in small quantity of water, benzalkonium chloride (Preservative) and sodium chloride (Isotonicity adjusting agent) was added to this solution; the drug solution was added to the polymer solution under constant stirring until a uniform solution was obtained. Purified water was then added to make up the volume to 100 ml. Formulation pH was adjusted to pH 5 with the help of 0.5M sodium hydroxide. This solution was filtered through 0.2µm filter paper. The optimized formulations

were sterilized in an autoclave at 121°C and 15 psi for 15 minutes. [9]

Evaluation of Prepared In-Situ Gelling System

Visual Appearance and Clarity

Visual appearance and Clarity was done under fluoroscent light against a white and black back ground for presence of any particulate matter as shown in Table 2.

pH

The pH of the prepared in-situ gelling system after addition of all the ingredients was measured using pH meter as shown in Table 2.

Drug Content Analysis

The assay of these formulations was carried out by pipetting 0.1 ml of all four optimized formulations, and it was diluted up to 100 ml of simulated tear fluid (pH 7.4). The absorbance was measured at 281.5 nm using UV-Visible spectrophotometer. The results are shown in Table 2.

In-Vitro Gellation

The gelling capacity of the formulations containing different ratio of carbopol 940 and HPMC (E50LV / E4M) was evaluated. It was performed by placing a drop of polymeric solution in vials containing 1 ml of simulated tear fluid, freshly prepared and equilibrated at 34°C, and visually assessed the gel formed and time for gellation as well as time taken for the gel formed to dissolve. The results are shown in Table 3. The Composition of simulated tear fluid was sodium chloride (0.670 g), sodium bi carbonate (0.2 g), calcium chloride dihydrate and bi-distilled water quantity sufficient up to 100 g. Physiological pH (7.4±0.2) was adjusted by adding the required amount of 0.1 N HCl. [10]

Interaction studies

Prepared in-situ gel formulations were tested for the intactness of drug in the various formulations by comparing with pure drug. These were done to ensure that the therapeutically active drug has not undergone any change after it has been subjected to processing steps during preparation of in-situ gelling systems. Taking IR spectra using KBr method did these studies. The spectra are shown in Figures 1-3.

Sterility Testing

Sterility testing were intended for detecting the presence of viable form of microorganisms and were performed for aerobic and anaerobic bacteria and fungi by using fluid thioglycolate medium and soyaban casein digest medium, respectively as per the Indian Pharmacopoeia.

Rheological Studies

Rheological properties of the prepared in-situ gelling systems under the different Shear rates (2, 4, 6, 10, 20, and 30 rpm) were measured at non physiological (pH 5 and 25°C) and physiological condition (pH 7.4 and 34°C), respectively. The hierarchy of the shear rate was reversed, and the average of two reading was used to calculate the viscosity. The results are shown in Tables 4-5 and Figures 4-5.

Table 2: Preliminary evaluation of Visual appearance, Clarity, pH, and Drug Content

Evaluation steps	CHL 11	CHL 15	CHL21	CHL 24
Visual Appearance	Transparent	Transparent	Transparent	Transparent
Clarity	Clear	Clear	Clear	Clear
pH	4.99	4.97	5.01	4.98
Drug Content	99.71 %	99.38 %	99.43 %	99.67 %

Table 3: Evaluation of Gelling capacity

Formulations	Gelling Capacity
CHL 11	++
CHL 15	++
CHL 21	++
CHL 24	++

Table 4: Rheological studies of Formulations at Nonphysiological Condition (pH 5) and physiological Condition (pH 7.4)

Shear Rate (rpm)	Non physiological Condition (pH 5) Viscosity (cps) of Formulations				Physiological Condition (pH 7.4) Viscosity (cps) of Formulations			
	CHL 11	CHL 15	CHL 21	CHL 24	CHL 11	CHL 15	CHL 21	CHL 24
2	102.3	109.4	98.4	101.7	221.0	234	242.1	254.5
4	90.1	92.3	86.7	89.2	132.5	125.1	133.9	156
6	78.2	80.4	71.5	74.3	113.5	103	101.3	109.4
10	60.0	56.1	53.9	58.1	105.8	92.3	88.1	85.6
20	35.2	40.3	45.3	50.7	87.9	83.9	70.9	68.2
30	26.9	22.9	27.1	22.8	72.6	63.8	51.3	57.5

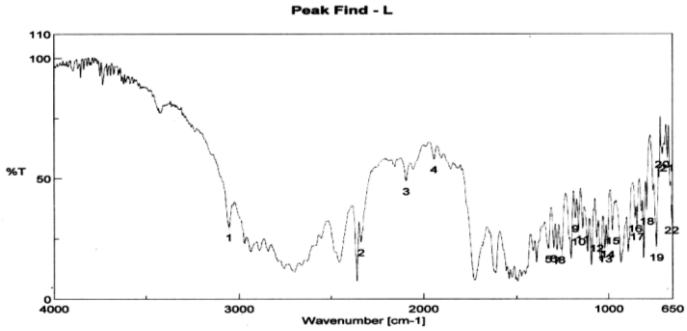

Fig. 1: IR spectra of pure drug

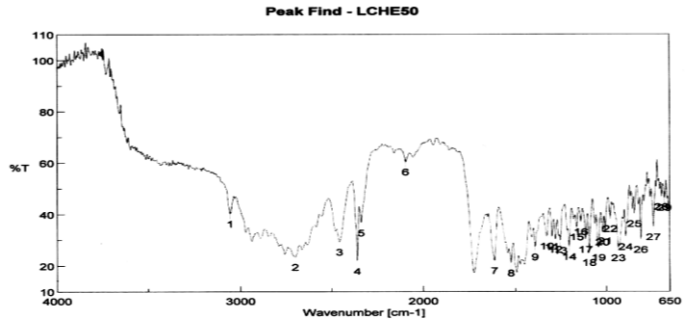

Fig. 2: IR spectra of Formulation LCHE50LV

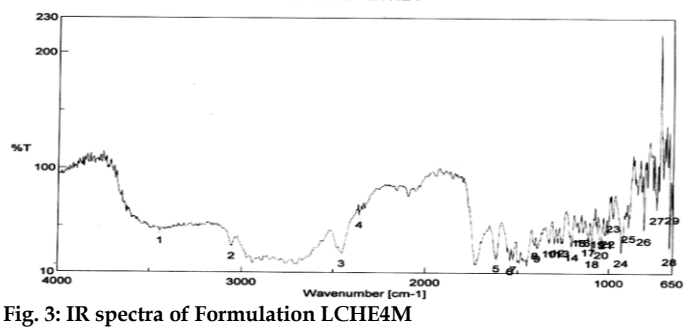

Fig. 3: IR spectra of Formulation LCHE4M

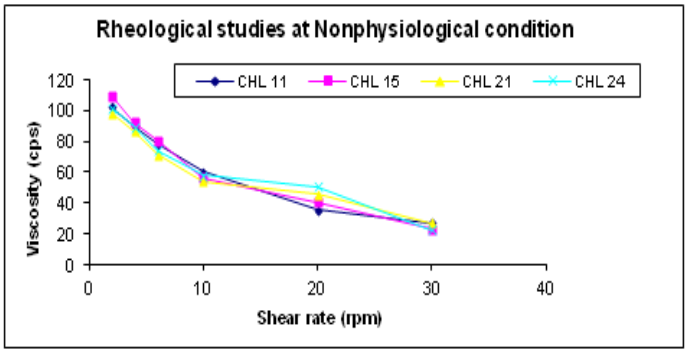

Fig. 4: Rheological studies of Formulations at Nonphysiological Condition (pH 5)

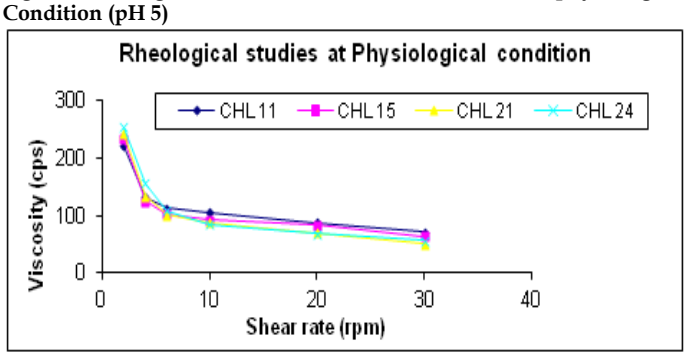

Fig. 5: Rheological studies of Formulations at physiological Condition (pH 7.4)

Table 6: Comparative In-Vitro Release Profile of In-Situ Gel Formulations

Sr. No.	Time (min.)	% CDR of CHL 11	% CDR of CHL 15	% CDR of CHL 21	% CDR of CHL 24
1	30	12.33	11.33	11.66	10.83
2	60	16.45	14.93	15.45	15.26
3	90	20.31	19.08	21.60	19.58
4	120	25.70	23.61	25.65	22.45
5	150	30.08	27.50	31.18	27.00
6	180	35.03	33.11	35.76	32.10
7	210	40.06	37.76	41.08	36.90
8	240	44.90	41.96	44.31	42.43
9	270	50.35	48.03	50.9	47.83
10	300	55.33	52.28	54.56	51.30
1	330	61.36	58.65	60.91	57.78
12	360	67.28	64.71	68.65	63.16
13	390	72.25	69.65	72.96	68.60
14	420	78.26	76.65	79.81	78.25
15	450	86.50	81.70	87.56	84.15
16	480	94.63	88.96	95.55	90.00

Table 7: Comparative In-Vitro Release of Marketed Eye Drop and Prepared In-Situ Gels

Sr. No.	Time (min.)	% CDR of CHL 11	% CDR of CHL 15	% CDR of CHL 21	% CDR of CHL 24	% CDR of MED
1	30	12.33	11.33	11.66	10.83	22.50
2	60	16.45	14.93	15.45	15.26	49.88
3	90	20.31	19.08	21.60	19.58	62.55
4	120	25.70	23.61	25.65	22.45	79.16
5	150	30.08	27.50	31.18	27.00	90.28
6	180	35.03	33.11	35.76	32.10	96.88
7	210	40.06	37.76	41.08	36.90	
8	240	44.90	41.96	44.31	42.43	
9	270	50.35	48.03	50.9	47.83	
10	300	55.33	52.28	54.56	51.30	
1	330	61.36	58.65	60.91	57.78	
12	360	67.28	64.71	68.65	63.16	
13	390	72.25	69.65	72.96	68.60	
14	420	78.26	76.65	79.81	78.25	
15	450	86.50	81.70	87.56	84.15	
16	480	94.63	88.96	95.55	90.00	

In-vitro Release Studies

The in-vitro release of Lomefloxacin HCl as pure drug well as from the prepared formulations was studied through cellophane membrane using diffusion cell. The cellophane membrane was soaked overnight in the receptor medium (simulated tear fluid, pH 7.4). It was tied to one end of a glass diffusion cell. 100 ml of receptor medium was taken in the 200 ml beaker. The diffusion cell was filled with 2 ml of the formulation and suspended in 100 ml of receptor containing beaker by assuring that the membrane was just touched the receptor medium surface. The whole assembly was transferred on magnetic stirrer and was maintained at 34°C ± 1°C and 22 rpm. The drug samples (1 ml) were withdrawn at the interval of 30 minutes from receptor medium and replaced by equal volumes of the receptor medium. The samples were diluted with appropriate receptor medium and analyzed by a UV-Visible spectrophotometer at 281.5 nm using receptor medium as a blank. The results are shown in Table 6 and Figure 6.

Comparative Evaluation of Marketed Products with prepared In-Situ Gels

The drug samples (1 ml) were withdrawn at the interval of 30 minutes from receptor medium and replaced by equal volumes of the receptor medium. The samples were diluted with appropriate receptor medium and analyzed by a UV-Visible spectrophotometer at 281.5 nm using receptor medium as a blank. [11] Shown in Tables 7 and Figures 7.

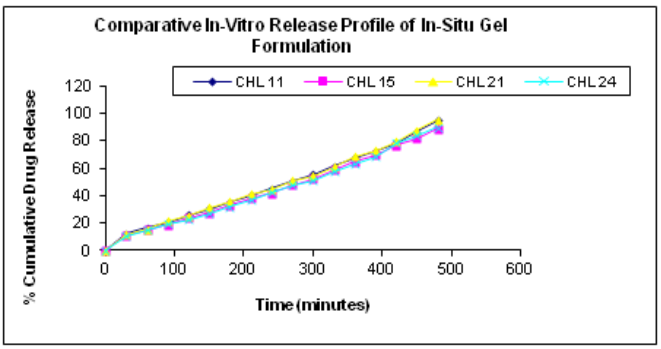

Fig. 6: Comparative In-Vitro Release Profile of In-Situ Gel Formulations.

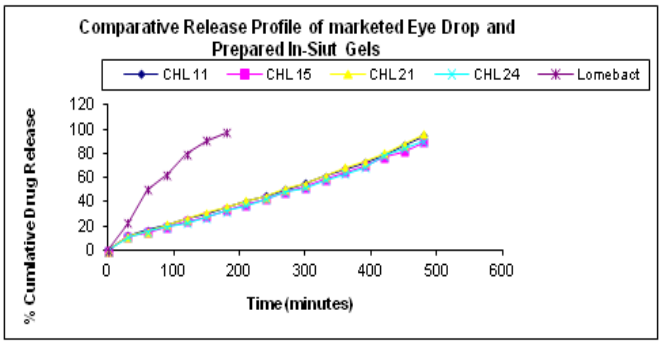

Fig. 7: Comparative In-Vitro Release of Marketed Eye Drop and Prepared In-Situ Gels.

Pharmacokinetic Release Studies

All the optimized formulations were subjected to study the release kinetics of it. So the best fit kinetic model

was determined for the optimized formulations using analysis software PCP – Disso V2. The results are shown in Table 8.

Antimicrobial Efficacy Studies

The antimicrobial efficacy studies were carried out to ascertain the biological activity of the optimized formulations. *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli* were used as the test organisms. These were determined by agar diffusion test employing cup-plate method. A layer of nutrient agar (20 ml) seeded with the test microorganisms (0.2 ml) were allowed to solidify in the petri dishes. Cups were made on the solidified agar layer with the help of sterile borer. Then the volume of the formulations containing equivalent amount of drug was poured into the cups. After keeping the Petri dishes at room temperature for 4 hours, the plates were incubated at 37°C for 24 hours. The zones of inhibitions were measured around the cups. The entire operation except the incubation was carried out in a laminar airflow unit. [12] Shown in Table 9.

Ocular Irritancy Studies

According to the Draize test, the amount of the test substance applied to the eye is normally 100µl placed into the lower cul-de-sac with observation of the various criteria made at a designated required time interval of 1, 24, 48, 72 hours, and 1 week after administration. [13] A total four albino rabbits (male) weighing 1.5-2 kg was used for the present study. The sterile formulations were instilled twice a day for a period of 7 days. Rabbits were observed periodically for redness, swelling, watering of the eye. The evaluation was made according to the Draize test protocol. The results are shown in Table 10.

Table 8: Release Kinetics of Optimized Formulations.

Release type	Parameters	CHL 11	CHL 15	CHL 21	CHL 24
Zero order	R	0.9917	0.9936	0.9907	0.9939
	k	0.1973	0.1890	0.1986	0.1878
	t-test	29.881	34.017	28.207	34.990
First order	R	0.8581	0.9135	0.8528	0.9105
	k	-0.0041	-0.0036	-0.0042	-0.0036
	t-test	6.473	8.695	6.325	8.526
Matrix	R	0.9444	0.9355	0.9426	0.9307
	k	3.5462	3.3862	3.5689	3.3603
	t-test	11.127	10.258	10.933	9.857
peppas	R	0.9879	0.9834	0.9889	0.9825
	k	24.691	0.8180	0.9286	0.7778
	t-test	0.9635	21.019	25.792	20.446
Hixoncrowell	R	0.9422	0.9598	0.9376	0.9570
	k	-0.0010	-0.0009	-0.0010	-0.0009
	t-test	10.891	13.249	10.444	12.770
t-table at p 0.05 (two tails) DF =n-2		2.131	2.131	2.131	2.131

Table 9: Antimicrobial Activity of In-situ gels

Test Microorganisms	Diameter of the Zone of Inhibition Produced By In-situ Gels (mm)			
	CHL 11	CHL 15	CHL 21	CHL 24
Staphylococcus aureus	26	25	26	23
Pseudomonas aeruginosa	30	28	29	28
E. coli	26	24	25	24

Table 10: Ocular Irritancy Profile

Eye Tissues	Cornea	Iris	Conjuctiva	Total
Score point	1.5	2	1.5	5

Table 11: Stabilities Studies of Formulation CHL 11, CHL 15, CHL 21 and CHL 24.

Sr. No.	Number of Days	% Drug Remaining							
		CHL 11		CHL 15		CHL 21		CHL 24	
		RT	40°C	RT	40°C	RT	40°C	RT	40°C
1	0	99.79	99.79	99.43	99.42	99.47	99.46	99.65	99.65
2	7	99.72	99.63	99.41	99.37	99.42	99.41	99.64	99.62
3	14	99.68	99.52	99.46	99.34	99.40	99.38	99.56	99.59
4	21	99.53	99.46	99.35	99.31	99.43	99.35	99.55	99.54
5	28	99.49	99.31	99.29	99.26	99.39	99.28	99.55	99.52
6	35	99.48	99.28	99.23	99.14	99.36	99.12	99.52	99.46
7	42	99.42	98.97	99.24	99.01	99.23	98.94	99.53	99.41

RT- Room Temperature

Accelerated Stabilities Studies

A sufficient quantity of formulations in previously sterilized vials was stored in desiccators containing a saturated solution of sodium chloride, which gives a relative humidity of 75±5%. The desiccators were placed in a hot air oven maintained at a temperature 40°C±0.5°C and at room temperature. Samples were withdrawn at 7 days interval for 42 days. The logarithms of percent drug remaining were calculated and plotted against time in days. [14-15] Shown in Tables 33-34 and Figures 8-9. It was also analyzed for visual appearance, clarity, and pH. The results are shown in Table 11.

RESULTS AND DISCUSSION

Ocular Irritancy Studies

Prepared in-situ gelling systems were subjected for ocular irritancy studies. A total four albino rabbits (male) weighing 1.5-2 kg was used for the present study. The sterile formulations were instilled twice a day for a period of 7 days. Rabbits were observed periodically for redness, swelling, watering of the eye. The evaluation was made according to the Draize test protocol. All the formulations were found to be non-irritating with no ocular damage or abnormal clinical signs to the cornea, iris, and conjuctiva observed. The results are shown in Table 10.

The aim of the present work envisaged formulating a "pH induced in-Situ gelling system of antibacterial agent, Lomefloxacin HCl" for the treatment of various bacterial diseases of eye, by providing comfortness, compliance to the patients and improved therapeutic

performance of the drug over conventional ocular dosage forms. From the 25 prepared formulations best 4 formulations were selected on the bases of Viscosity and gelling capacity. Drug was incorporated in these optimized formulations with other excipients. Results from method 2 reveal that the formulations CH 11 (0.3 % carbopol 940 and 1.5 % HPMC E50LV) and CH 15 (0.4 % carbopol 940 and 1.5 % HPMC E50LV) showed good gelling capacity and desire viscosity. Results from method 3 reveal that the formulations CH 21 (0.2 % carbopol 940 and 0.4 % HPMC E4M) and CH 24 (0.3 % carbopol 940 and 0.4 % HPMC E4M) showed good gelling capacity and desire viscosity.

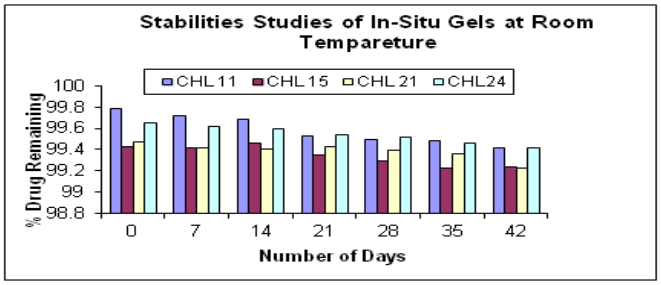

Fig. 8: Stabilities Studies of In-Situ Gels at Room Temperature

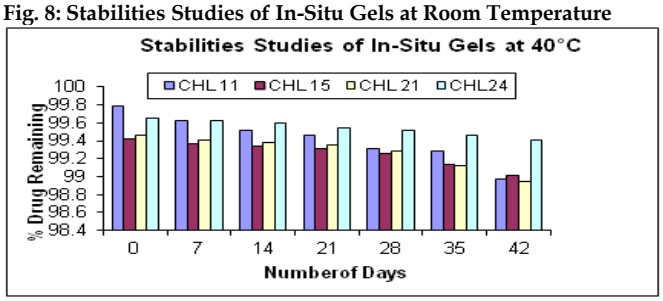

Fig. 9: Stabilities Studies of In-Situ Gels at 40°C

In-vitro release of Lomefloxacin HCl from the selected formulations was studied through diffusion cell using cellophane membrane for 8 hours. It was compared with the pure drug as well as marketed eye drop. Results reveal that all formulations exhibited sustained release of the drug (above 88 %) from the carbopol 940 and HPMC E50LV /E4M network over 8-hours. Cellulose derivatives like, HPMC E50LV / E4M dissolve in water and yield much more viscous solution compared to carbopol 940 solution. Thus, the increase in viscosity might have contributed to the decrease in rate of drug release from these formulations. The best fit kinetic model for the optimized formulation were the zero order and peppas model, which suggest that the drug release was independent of the concentration and occurred by diffusion mechanism. Antimicrobial efficacy study carried out by using *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli* as test microorganisms.

Optimized formulations CH 11 (0.3 % carbopol 940 and 1.5 % HPMC E50LV), CH 15 (0.4 % carbopol 940 and 1.5 % HPMC E50LV), CH 21 (0.2 % carbopol 940 and 0.4 % HPMC E4M) and CH 24 (0.3 % carbopol 940 and 0.4 % HPMC E4M) were liquid at the formulation pH and underwent rapid gellation upon raising the pH to 7.4. Also, the formulations were found to be clear, having good in-situ gelling capacity and sustained drug release over 8 hours periods as compared to pure drug and marketed eye drop. The best fit kinetic models for the optimized formulation were the zero order and peppas model. All optimized formulations were sterile, having good antibacterial efficacy and non-irritant as per the Draize test protocol. As per the stability study formulations were stable (transparent and clear) at room temperature as well as at 40°C.

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