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TO PREPARE AND EVALUATE SUSTAINED RELEASE OPHTHALMIC IN-SITU GEL OF LEVOFLOXACIN

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ABSTRACT

Ophthalmic drug delivery is one of the maximum widely used drug delivery systems for the delivery drug to eye phase. In situ gels are a form of formulations in which drug is ingested in solution form and while coming in touch with frame fluids or temperature, it receives or get converted to gel form in an effort to produce a sustained release components. The goal of present work is to expand in situ gel of Levofloxacin which is an antibiotic for bacterial infections. It is a kind of extensive spectrum antibiotic. It has a low molecular weight of 361.368gm/mol. it is one of the maximum appropriate applicants for ophthalmic drug transport. Levofloxacin is an antibiotic this is used to treat bacterial infections; it stops the multiplication of bacteria by way of inhibiting the reproduction and restore in their genetic material (DNA).marketed eye drop solution is cleared very rapidly from the corneal area while, in-situ gelling structures, it become found that clearing time is slow and retention of drug is for longer length at the corneal surface for a longer period Levofloxacin may be focused in treatment of the bacterial contamination and also reduce dosing frequency, growth bioavailability of Levofloxacin to be able to bring about better patient compliance with minimum facet results.

Keywords: Ophthalmic Drug delivery system, In situ gel, gel form, gelling state, Levofloxacin

Introduction:

Ophthalmic drug transport gadget is one of the maximum broadly used areas for round several years. It's far one of the most broadly researched regions inside the field of medicines. The young technology of scientist has a totally eager hobby in this ophthalmic field nowadays. The principle motive of non-stop robust hobby of scientists on this drug delivery system is the trouble of a low bioavailability of drug after the software to the eyeball. [1]

Gelling potential and gelling temperature each play a crucial function in formula of In-situ gel. Both the parameters are determined to check the potential of the system to shape a gel because of interaction with different environmental situations like temperature, pH, humidity etc.

The method should go through speedy conversion among sol to gel transition on the web site of software due to exchange in pH and temperature through retaining its integrity without erosion or dissolution. On experimentation it changed into located that as the awareness of polymers will increase, the integrity of shaped gel additionally increases. The viscosities of all the formulations at cold temperature and at 37°C were in the range between 980 to 1839 and 6032 to 8907 centipoises respectively. [2] These *in situ* solutions are liquid at room temperature but undergo gelation when it comes in contact with body fluids or change in pH. [3]

The aim of any drug delivery system is to offer adequate quantity of drug at the focused site of motion to perform its required pharmacological response. That Spatial placement pertains to concentrated on a drug to a selected organ or tissue, even as temporal shipping refers to controlling the fee of drug shipping to the target tissue. A sustained launch drug transport gadget can be a prime development in curing ophthalmic illnesses. The main aim is to design in the sort of manner so that a small quantity of dose can provide required pharmacological reaction for long period of time commonly 18 to 24hrs. [4] The formulations of *in situ* gels possibly possess characteristics of a pseudo plastic behavior. The developed formulations were therapeutically efficacious, stable, non -irritant and provide sustained release of the drug up to eight hours. [5]

Materials and methods:

Drug levofloxacin was obtained as a gift sample from Cipla, Indore. Sodium alginate polymer, Carbopol 934, Hydroxy propyl methyl cellulose, Chitosan was obtained from Oxford Laboratory Mumbai. Glacial Acetic Acid was obtained from Sara Fine Chemical, Vadodara. Calcium chloride ware obtained from Qualigens fine chemicals.

Preformulation Parameters

Organoleptic evaluation

Colour / Odour

A small quantity of drug was taken on a butter paper and it was analyzed for identification of colour and odour by visual inspection in light.

Melting Point

Melting point is defined as a temperature at which drug gets converted from solid form to liquid form. It is determined mainly by two methods: Capillary tube Method (Fusion Method) and through Melting

point apparatus. The melting point was determined by fusion method. A capillary tube was taken which was sealed at one end, and then filled with small amount of drug sample. The capillary tube was inserted into melting point apparatus along with thermometer. Switch on the apparatus and wait till drug sample gets melted. Record the temperature at which it was recorded.

Solubility Analysis

Solubility parameter is the property of a solid, liquid or gaseous chemical substance called solute to dissolve in a solid, liquid or gaseous solvent. The solubility of a substance mainly depends on the physical and chemical properties of the solute and solvent as well as on temperature and pressure of the solution.

Table 1: Solubility Profile according to BP

S.NO.	Description term	Part of solvent required for part of solute
1.	Very Soluble	Less than 1ml
2.	Freely Soluble	From 1ml to 10ml
3.	Soluble	From 10ml to 30ml
4.	Sparingly Soluble	From 30ml to 100ml
5.	Slightly Soluble	From 100ml to 1000ml
6.	Very Slightly Soluble	From 1000 to 10000
7.	Practically in soluble	From 10000 or more

Procedure:

Take a small amount of drug in a test tube. Then check its solubility in different solvents like distilled water, 0.1N HCl, 0.1N NaOH, ethanol, methanol, phosphate buffer pH 7.2 & 6.8.

Determination of Absorption Maxima of Levofloxacin

Preparation of Phosphate buffer pH 7.2:

34 gm of potassium dihydrogen phosphate were dissolved in 1000ml to produce phosphate buffer of pH 7.4.

Spectrophotometric estimation of Levofloxacin

Levofloxacin was analyzed quantitatively by UV spectrophotometer in Phosphate buffer pH 7.2. Standard calibration curve was plotted between concentration and absorbance.

Preparation of standard curve of Levofloxacin in phosphate buffer pH 7.2

Weigh 100 mg of drug and dissolve in 100ml of phosphate buffer solution pH 7.2. Pipette out 1ml of stock solution and dilute to 100ml of phosphate buffer solution (Sub stock solution). Then pipette out 0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml from sub stock solution and dilute up to 10ml to prepare 1 µg/ml, 2 µg/ml, 3 µg/ml, 4 µg/ml and 5 µg/ml solution. Then absorbance is recorded using UV spectrophotometer at ƛ max 286nm.

Determination of Drug Excipients Incompatibility by FT-IR Spectroscopy

Infra red spectra were recorded by mixing powdered drug with dry powder potassium bromide. FT-IR spectra of the selected formulations were taken and compared with the spectrum of pure drug. The characteristic peaks of drug were obtained by scanning in the range of 400-4000 cm-1 by using the

spectrometer (Bruker-a-T, Germany). FT-IR is a technique used to determine the chemical interaction between drug and polymers.

Formulation Batch In situ gel:

Table 2 Formulation Batch In situ gel

Formulation batch	Drug	Carbopol 934 (%)	HPMC (%)	Chitosan (%)	Sodium alginate (%)
F1	0.3	1	-	-	-
F2	0.3	-	2	-	-
F3	0.3	-	-	0.25	-
F4	0.3	-	-	-	6

Formulation of in situ gel

Carbopol 934 - The weighed quantities of polymers were kept for swelling overnight in distilled water and dissolved using a magnetic stirrer. Aqueous solution of Levofloxacin was added in to the polymeric solution with continuous agitation for 20 min. Benzalkonium chloride was added to the resulting solution. The pH of the formulation was adjusted to 7.2 using 0.1 N NaoH/0.1 N HCl. Dispersion was allowed to hydrate for 60 min. Keep the gel for 24 hrs at room temperature.

HPMC (E15) - The weighed quantities of polymers were kept for swelling overnight in distilled water and dissolved using a magnetic stirrer. Aqueous solution of Levofloxacin was added in to the polymeric solution with continuous agitation for 20 min. Benzalkonium chloride was added to the resulting solution. The pH of the formulation was adjusted to 7.2 using 0.1 N NaoH/0.1 N HCl. Dispersion was allowed to hydrate for 60 min. Keep the gel for 24 hrs at room temperature.

Chitosan - The weighed quantities of polymers were kept for swelling overnight in mix of 0.25% glacial acetic acid and distilled water and dissolved using a magnetic stirrer. Aqueous solution of Levofloxacin was added in to the polymeric solution with continuous agitation for 20 min. Benzalkonium chloride was added to the resulting solution. The pH of the formulation was adjusted to 7.2 using 0.1 N NaoH/0.1 N HCl. Dispersion was allowed to hydrate for 60 min. Keep the gel for 24 hrs at room temperature.

Sodium Alginate - The weighed quantities of polymers were kept for swelling overnight in distilled water and dissolved using a magnetic stirrer. Aqueous solution of Levofloxacin was added in to the polymeric solution with continuous agitation for 20 min. Benzalkonium chloride was added to the resulting solution. The pH of the formulation was adjusted to 7.2 using 0.1 N NaoH/0.1 N HCl. Dispersion was allowed to hydrate for 60 min. Keep the gel for 24 hrs at room temperature.

Evaluation Parameter

Clarity Test:

In this method in situ gel was observed by visual inspection under a good light, viewed against a black and white background. It was also analyzed for formation of turbidity or any unwanted particles dispersed in the solution [6].

Determination of pH:

pH of each formulation was determined immediately after preparation by using digital pH meter which was previously calibrated by pH 4 and pH 7 standard buffers.[7]

Gelling Capacity:

The gelling capacity of the prepared formulation was determined by placing a drop of the formulation in a beaker containing 50 ml of freshly prepared concentrated calcium chloride solution and was visually observed for gelling time. [8].

Measurement of Gelation Temperature:

About 10 ml of the formulation was transferred into a 50 ml beaker with a magnetic bead and placed on a magnetic stirrer with thermostatically controlled heater. The temperature of the stirrer was increased in increments of 1°C and the temperature of the formulation was recorded using a thermometer. The rotation of bead gradually slowed down as the viscosity increased. The temperature at which the magnetic bead stopped rotating was taken as gelation temperature [9].

Viscosity Measurement:

The viscosity was measured using a Brookfield viscometer and the angular velocity increased gradually from 2 to 50 rpm. The studies were performed using spindle no. 96 for gels at physiological temperature (37°C) and for sols at normal room temperature (28°C) [10].

In-Vitro Release Studies:

The in-vitro drug release was studied by using a USP rotating paddle apparatus. Phosphate buffer 7.2 maintained at 37oC was used as the medium. The paddle speed was set to 50 rpm. 3ml of the formulation was placed in a dialysis tube with cellophane membrane covered cells and it was placed such that it just touches the diffusion medium. The drug samples were withdrawn at the interval of one hour for a period of ten hours from the medium and were analyzed by U.V spectrophotometer at their respective wavelength using Phosphate buffer pH 7.2 as blank. The cumulative percentage drug release was evaluated. [11]

Results and discussion:

Organolaptic evaluation

The drug was found to be creamish amorphous powder. The melting point of drug was found to be 225^0C – 227^0C. The drug does not have any obnoxious odour. Levofloxacin is soluble in phosphate buffer pH 7.2, it is slightly soluble in water, ethanol, and methanol. It is very slightly soluble in 0.1N HCl. It is sparingly soluble in 0.1 N NaOH.

Determination of Absorption Maxima of Levofloxacin

Calibration curve of Levofloxacin in phosphate buffer pH 7.2:

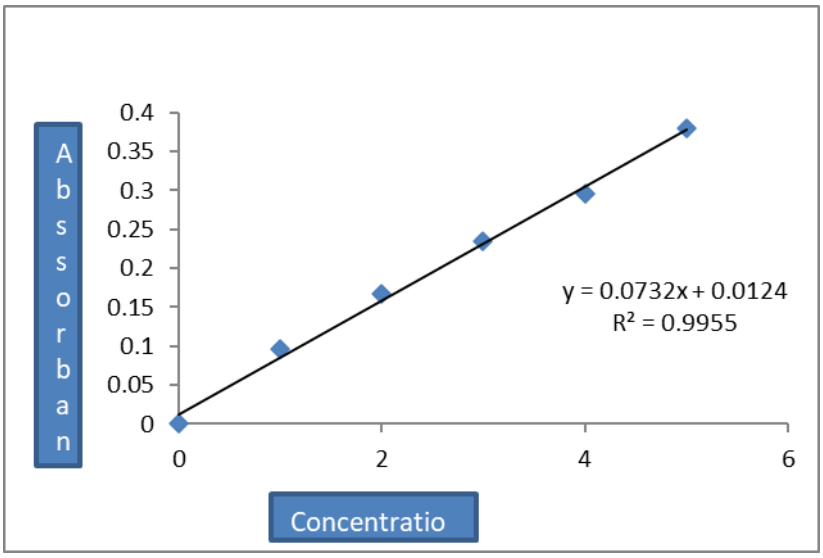


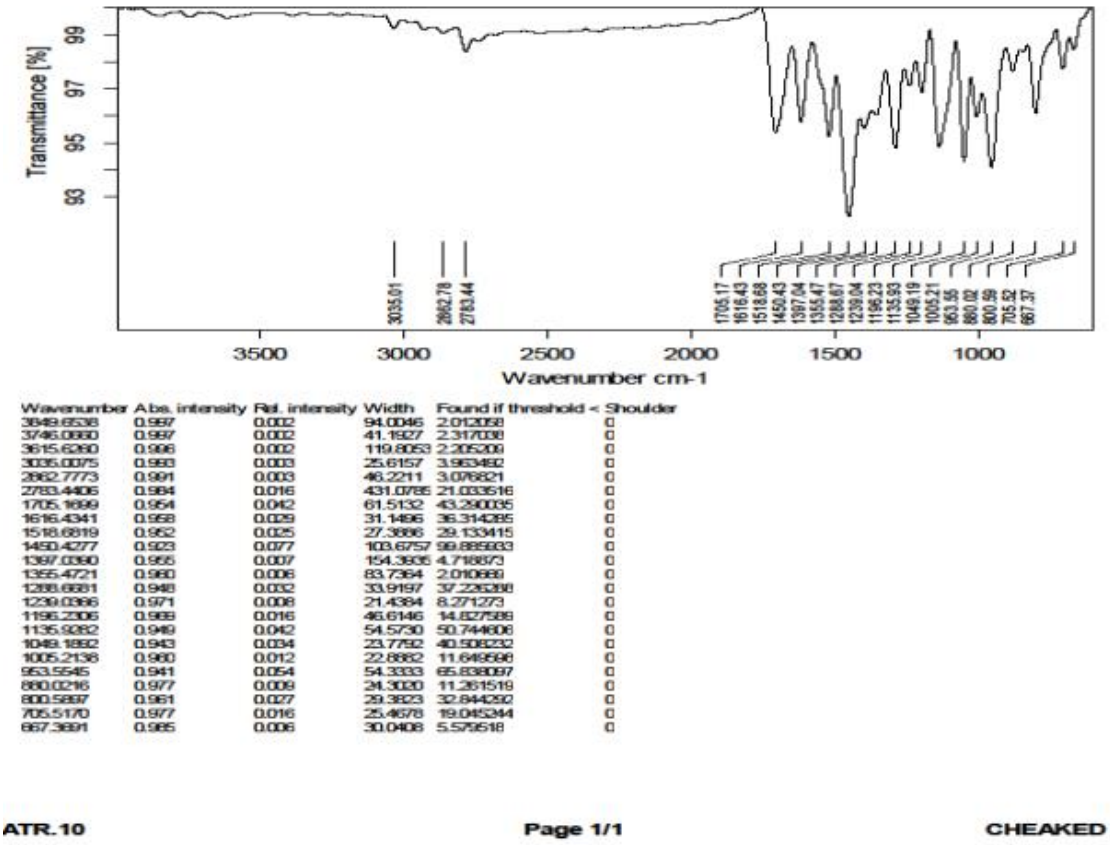
Fig 1: Calibration curve of ofloxacin

Table 3: Concentration and absorbance data for calibration curve of ofloxacin

S. No	Concentration (µg/ml)	Absorbance (at 286nm)
1	0	0
2	1	0.092
3	2	0.150
4	3	0.225
5	4	0.276
6	5	0.360

FT-IR studies

Drug-excipients compatibility study was performed by FTIR technique. The IR spectra of the solution were taken, which indicate no interaction between Levofloxacin HCl and polymers was observed [12,13].



Wavenumber	Abs. intensity	Rel. intensity	Width	Found if threshold <	Shoulder
3849.6538	0.997	0.002	94.0046	2.012058	0
3746.0860	0.997	0.002	41.1927	2.317038	0
3615.6260	0.996	0.002	119.8053	2.205209	0
3035.0075	0.988	0.003	25.6157	3.963462	0
2862.7773	0.991	0.003	46.2211	3.076821	0
2783.4406	0.984	0.016	431.0785	21.033516	0
1705.1699	0.954	0.042	61.5132	43.290035	0
1616.4341	0.958	0.029	31.1486	36.314285	0
1518.6819	0.952	0.025	27.3886	29.133415	0
1450.4277	0.923	0.077	103.6757	99.888683	0
1397.0360	0.955	0.007	154.3605	4.716873	0
1355.4721	0.980	0.006	83.7364	2.010888	0
1288.6881	0.948	0.032	33.9197	37.226398	0
1239.0366	0.971	0.008	21.4384	8.271273	0
1196.2306	0.968	0.016	46.6146	14.827588	0
1135.9282	0.949	0.042	54.5730	50.744606	0
1049.1862	0.943	0.034	23.7752	40.508232	0
1005.2136	0.980	0.012	22.8882	11.648598	0
953.5545	0.941	0.054	54.3333	65.838097	0
880.0216	0.977	0.009	24.3020	11.261519	0
800.5897	0.961	0.027	29.3823	32.844252	0
705.5170	0.977	0.016	25.4678	19.045244	0
667.3891	0.985	0.006	30.0408	5.575518	0

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Fig 2: FT-IR Studies

Evaluation Parameter

Clarity:

The formulations (F1–F4) were prepared by using various concentrations of sodium alginate along with HPMC in different ratios. All the formulations prepared were clear without any turbidity and suspended particles or impurities.

Determination of pH:

The pH of in situ gel solution was found to be around 6.42 to 7.20 for all the formulations. The pH of all formulations is in acceptable range. So they can be easily used in eyes for proper treatment.

Table 4: Result for pH, Clarity Test, Gelling capacity, Gelation temperature

S.NO.	Formulation code	Clarity	pH	Gelling capacity	Gelation temperature	Viscosity (at 50 rpm)
1.	F1	Transparent	7.13 ± 0.10	+ + +	25 ± 0.59	75
2.	F2	Transparent	7.20 ± 0.122	+ +	26.5 ± 0.7	50
3.	F3	Transparent	7.29 ± 0.166	-	31 ± 0.12	42
4.	F4	Transparent	6.29 ± 0.62	+ +	28.5 ± 0.31	70

Table 5: Gelling Capacity

S. No	Gelling Capacity	Observation
1	No gelation	-

2	Gelation occurred in few minutes and remained for few hour	+
3	Gelation immediate, remained for few hour	+ +
4	Gelation immediate, and for extended period	+ + +
5	Very stiff gel	+ + + +

Gelling capacity

The gelling capacity of batches was found to be in the range of immediate gelation for extended period to gelation for few hours.

Gelation temperature

The gelation temperature of formulations was found to be in the range 25 to 31.

In-vitro release studies:

Table 6: In-vitro release studies

Time (hrs)	Cumulative % drug release			
	F1	F2	F3	F4
0.5	21 ± 0.84	17 ± 0.62	18 ± 0.98	16 ± 0.30
1	33 ± 0.61	24 ± 0.30	18 ± 0.58	22 ± 0.28
2	42 ± 0.89	33 ± 0.64	28 ± 0.97	30 ± 0.57
3	50 ± 0.96	44 ± 0.94	37 ± 0.87	40 ± 0.53
4	71 ± 0.96	57 ± 0.88	49 ± 0.92	52 ± 0.26
5	82 ± 0.98	62 ± 0.93	53 ± 0.45	57 ± 0.95

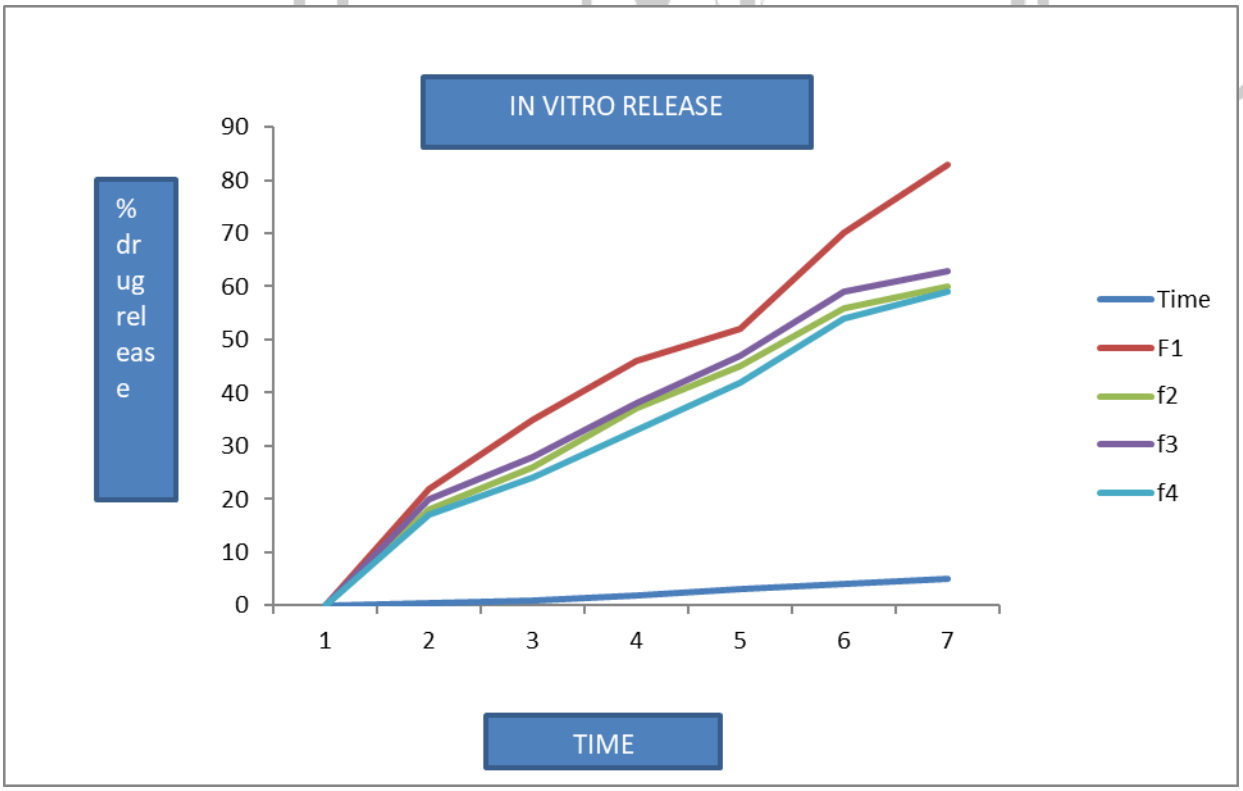

Fig 3: In vitro release of drug Ofloxacin

Summary:

The real challenge in the development of a controlled drug delivery system is not just to sustain the release but also to prolong the presence of the dosage form in the eye until all the drug is completely released in the desired period of time. Various approaches for preparation of in situ ophthalmic gels were designed.

The aim of the present investigation was to formulate and study ophthalmic in – situ gel of ofloxacin. Carbopol, Hydroxy Propyl methyl cellulose (HPMC), Chitosan, and Sodium alginate were used as polymers for the preparation of ophthalmic in-situ gel. All prepared formulations were evaluated for viscosity, determination of pH, clarity test, gelling capacity, measurement of gelation temperature, and in-vitro release studies etc.

FTIR spectra of the selected formulations were taken and compared with the spectrum of pure drug. The characteristic peaks of drug were obtained by scanning in the range of 400-4000 cm-1 by using the spectrometer (Bruker-a-T, Germany). It was found that no incompatibility between drug and excipients was obtained.

Solubility is the property of a solid, liquid or gaseous chemical substance called solute to dissolve in a solid liquid or gaseous solvent. The drug was freely soluble in phosphate buffer pH 7.2.

The melting point was determined by the capillary method using melting point apparatus. The melting point of drug was found to be in the range 225^{0}C to 227^{0}C.

The viscosity of gel was determined by Brookfield viscometer. It was obtained in the range 42 to 75cps.

The pH of the in situ gel was found to be in the neutral range 6.42 to 7.20. The pH range of gel shows that any of the formulation can be used easily because it will not produce any irritation in eyes.

The formulations (F1–F4) were prepared by using various concentrations of sodium alginate along with HPMC in different ratios. All the formulations prepared were clear without any turbidity and suspended particles or impurities.

In in-vitro gelation study the formulations were evaluated for their in-vitro gelling capacity, accurately measured 10 mL of formulation was added to 100 mL of 0.1N (HCl, pH 7.2-7.8) at 37^{0}C in a beaker with mild agitation that avoids breaking of formed gel. The in vitro gelling capacity was graded in three categories on the basis of stiffness of formed gel, gelation time and time period for which the formed gel remains as such.(+) Gels after few minutes, dispersed rapidly, (++) Gelation immediate remains for few hours, (+++) Gelation immediate remains for an extended period

Conclusion:

The aim the present study is to develop Levofloxacin *in-situ* gel for sustained ophthalmic preparation. Levofloxacin is an antibiotic that is used to treat bacterial infections; it stops the multiplication of bacteria by inhibiting the reproduction and repair of their genetic material (DNA).Marketed eye drop solution cleared very rapidly from the corneal region whereas, both in-situ gelling systems were cleared at a slow rate and retained at the corneal surface for a longer duration Levofloxacin can be targeted in treatment of the bacterial infection and also reduce dosing

frequency, increase bioavailability of Levofloxacin that will result in better patient compliance with minimum side effects.

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