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Ocular Novel Delivery *In-Situ* Gel: Development and Characterization of Gentamicin Sulfate and Dexamethasone

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ABSTRACT

The main goal of this investigation study ocular novel delivery in-situ gel development and characterization of gentamicin sulfate and dexamethasone is achieved novel ocular prolonged delivery system under the specific manner of formulation The ingenious design of in-situ gel dosage regimen is important of executing this goal and the drug achieved a steady-state in an ocular specific side that is therapeutically effective, nontoxic and sterilized for an extended period. The whole entity development of polymeric in-situ gel done with Pluronic F127, carbopol 934 and HPMC, solution of 0.5% and 0.1% of drugs was prepared in acetate buffer 5.0 than cooled in an ice bath and pluronic F127 was added slowly with continuous stirring carbopol 934 and HPMC 15cps were added. Dexamethasone used topical ocular corticosteroids long-lasting role in anti-inflammatory, anti-allergy, and anti-shock activities Dexamethasone reduces the intraocular inflammation as well as the breakdown of the blood ocular barrier in proliferative vitreoretinopathy Gentamicin (GT) inhibits bacterial protein synthesis mainly through binding with the 30S ribosomal subunit. The ocular in-situ gel entrapment of gentamicin sulfate and dexamethasone site-specific prolonged absorption developed a different formulation using polymeric condition with formulation numbering F1 to F6. In the all formulation determination of active dosage with FT-IR study under the interaction between drug and polymers and purity of drug All the six formulations stability study done according to ICH guidelines were subjected to stability studies at ambient humidity conditions at 20°C to 80°C, ambient temperature and 40±10ºC for one month under day-wise interval. All the formulations except F1, F6 and F5 showed instantaneous gelation when contacted with simulated tear fluid (STF), formulation F4 showed best gelation property amongst all other formulation F4 showed sustained drug release for 5 hours The results of the ocular irritation studies indicate that the formulations F4 were non-irritant and excellent ocular tolerance was noticed.

INTRODUCTION:

The conventional ophthalmic delivery systems often result in poor bioavailability therapeutic response and also drug waste overcome drug characterization also responsible for that. The present innovative study full-fill this problem in-situ gel having the capability to treat the drug slow-release pattern release it provide sustained release. Ophthalmic In-situ gels are viscous polymer-based liquids that exhibit sol-to-gel phase transition on the ocular surface due to change in a specific physicochemical parameter like ionic strength, pH or temperature Gel dosage forms are successfully used as drug delivery systems considering their ability to prolong the drug release. The In situ formulation exhibited well, viscosity, drug content, and sustained drug release.[1] Conventional liquid ophthalmic formulations demonstrate low bioavailability because of constant lacrimal drainage in the eye. The normal drainage of an instilled drug dose commences immediately upon installation and is essentially completed within 5 min. typically ophthalmic bioavailabilities of only 1–10% are achieved due to the short precorneal residence time of ophthalmic solutions.[2] Each system has its advantages and drawbacks. The choice of a particular hydrogel depends on its intrinsic properties and envisaged therapeutic use. This research includes temperature and pH, induced in situ-forming polymeric systems used to achieve prolonged contact time of drugs with the cornea and increase their bioavailability.[3]

Merits of *In-situ* ocular drug delivery systems[4, 5, 6, 7]

✓ Increased accurate dosing. To overcome the side effects of pulsed dosing produced by conventional systems.

✓ To provide sustained and controlled drug delivery.

✓ To increase the ocular bioavailability of drugs by increasing the corneal contact time. This can be achieved by effective adherence to the corneal surface.

✓ To provide targeting within the ocular globe to prevent the loss to other ocular tissues.

✓ To circumvent the protective barriers like drainage, lacrimation, and conjunctival absorption.

✓ To provide comfort, better compliance to the patient, and to improve the therapeutic performance of the drug.

✓ To provide better housing of the delivery system.

MATERIALS AND METHODS:

Pluronic F-68[8, 9, 10]

S. D. Fine Chem. Ltd., Mumbai

Pluronic® F-68 is a copolymer of ethylene and propylene oxide. It is a non-ionic detergent and is used as an anti-foaming agent in cell culture. It helps in stabilizing the cell membranes against the shear forces exerted during batch culture processes. It is suitable for insect cell culture.

Carbopol[11]

Rankem Pvt. Ltd., Mumbai

Carbomers are white-colored, 'fluffy', acidic, hygroscopic powders with a characteristic slight odor. A granular carbomer is also available (Carbopol 71G). Bioadhesive material, controlled-release agent, emulsifying agent, stabilizing agent, rheology modifier, tablet binder, suspending agent.

Hydroxypropyl methyl cellulose[12]

Rankem Pvt. Ltd., Mumbai

Hypromellose, short for hydroxypropyl methylcellulose (HPMC), is a semisynthetic, dormant, viscoelastic polymer utilized as an ophthalmic oil, and additionally an excipient and controlled-conveyance segment in oral medicaments, found in an assortment of business items. As a nourishment added substance, hypromellose is an emulsifier, thickening and suspending operator, and another option to creature gelatin. Its Codex Alimentarius code (E number) is E464. It is by and large perceived as sheltered by the FDA.

Gentamicin[13]

Gift sample obtained from Bioplus Life Sciences Pvt. Ltd. Bangalore.

Gentamicin is a broad-spectrum aminoglycoside antibiotic. Aminoglycosides work by binding to the bacterial 30S ribosomal subunit, causing misreading of t-RNA, leaving the

bacterium unable to synthesize proteins vital to its growth. Aminoglycosides are useful primarily in infections involving aerobic, Gram-negative bacteria, such as Pseudomonas, Acinetobacter, and Enterobacter. Also, some mycobacteria, including the bacteria that cause tuberculosis, are susceptible to aminoglycosides. Infections caused by Gram-positive bacteria can also be treated with aminoglycosides, but other types of antibiotics are more potent and less damaging to the host. In the past, the aminoglycosides have been used in conjunction with penicillin-related antibiotics in streptococcal infections for their synergistic effects, particularly in endocarditis. Aminoglycosides are mostly ineffective against anaerobic bacteria, fungi, and viruses.

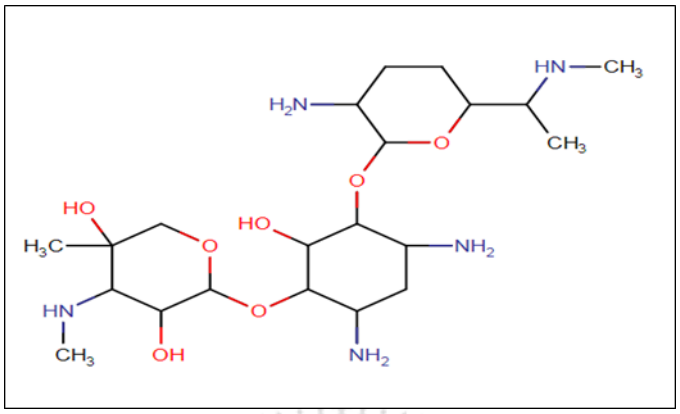

Figure No. 1: Gentamicin Structure

Dexamethasone [14, 15]

Gift sample obtained from Bioplus Life Sciences Pvt. Ltd. Bangalore.

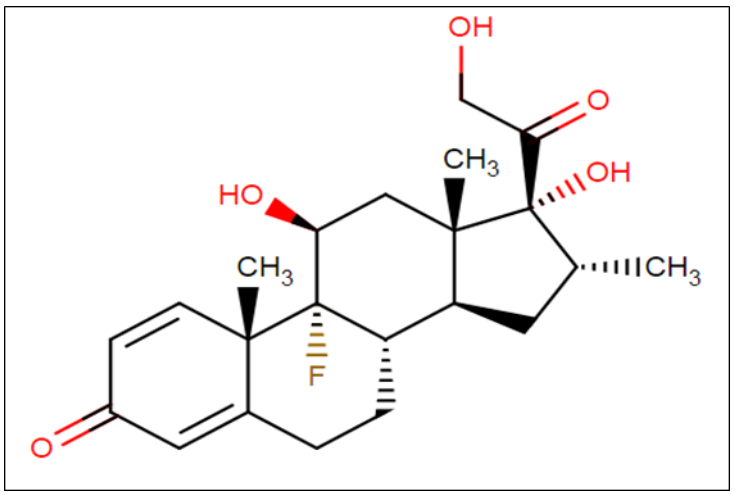

Figure No. 2: Dexamethasone Structure

Dexamethasone and its derivatives, dexamethasone sodium phosphate and dexamethasone acetate, are synthetic glucocorticoids. Used for its anti-inflammatory or immunosuppressive properties and ability to penetrate the CNS, dexamethasone is used alone to manage cerebral edema and with tobramycin to treat corticosteroid-responsive inflammatory ocular conditions Dexamethasone is a glucocorticoid agonist. Unbound dexamethasone crosses cell membranes and binds with high affinity to specific cytoplasmic glucocorticoid receptors. This complex binds to DNA elements (glucocorticoid response elements) which results in a modification of transcription and, hence, protein synthesis to achieve inhibition of leukocyte infiltration at the site of inflammation, interference in the function of mediators of the inflammatory response, suppression of humoral immune responses, and reduction in edema or scar tissue. The anti-inflammatory actions of dexamethasone are thought to involve phospholipase A2 inhibitory proteins, lipocortins, which control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes.

Identification Test using FTIR Spectroscopy

The infrared spectrum is an important record that gives sufficient information about the structure of a compound. This technique provides a spectrum containing a large number of absorption bands from which a wealth of information can be derived about the structure of an organic compound. The region from 0.8 μ to 2.5 μ is called Near Infra-red and that from 15 μ to 200 μ is called Far infra-red region. Identification of Gentamicin and Dexamethasone was done by FTIR Spectroscopy concerning marker compound. Gentamicin and Dexamethasone were obtained as white amorphous powder and white to off-white crystalline powder respectively. It was identified from the result of the IR spectrum as per specification.

Loss on drying

The moisture in a solid can be expressed on a wet weight or dry wet basis. On a wet weight basis, the water content of a material is calculated as a percentage of the weight solid. The term loss on drying is an expression of moisture content on a wet weight basis Loss on drying is directly measured by IR moisture balance. Firstly calibrated the instrument by knob then taken 5 gm sample (powder) and set the temp at 100°C to 105°C for 15 minutes and constant reading set the knob and check % moisture.

Determination of λ max of Gentamicin and Dexamethasone

For the preparation of Gentamicin solution of different concentrations from 10-50µg/ml were prepared in simulated tear fluid pH 7.4, 5 ml solution of these concentrations was taken into 10 ml volumetric flask and add 0.5ml Ninhydrin reagent as a derivatizing agent. The resulting solution was heating on a water bath on 950C for 15 minutes, after cooling the solution filter it and taking a reading at 400 to 800nm. Accurately weighed 10 mg of drug was dissolved in 10 ml of simulated tear fluid (pH 7.4) in 10 ml of volumetric flask. The resulted solution 1000µg/ml and from this solution 0.1 ml pipette out and transfer into 10 ml volumetric flask and volume make up with simulated tear fluid (7.4) solution prepare suitable dilution to make it to a concentration of 10µg/ml for Dexamethasone. The spectrum of this solution was run in the 200-400 nm range in the U.V. spectrophotometer (Labindia-3000+).

Calibration curve of Gentamicin and Dexamethasone

Preparation of Standard Stock Solution

10mg of drugs were weighed accurately and transferred to a 10 ml volumetric flask, and the volume was adjusted to the mark with methanol to give a stock solution of 1000 ppm or µg/ml.

Preparation of Working Standard Solution

From stock solutions of Gentamicin 1 ml was taken and diluted up to 10 ml. from this solution 1.0, 2.0, 3.0, 4.0 and 5.0 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with simulated tear fluid (pH 7.4), gives standard drug solution of 10, 20, 30, 40, 50µg/ml concentration and add 0.5 ml Ninhydrin reagent as a derivatizing agent. The resulting solution was heating on a water bath on 950C for 15 minutes, after cooling the solution filter it and taking absorbance.

From stock solutions of Dexamethasone 1 ml was taken and diluted up to 10 ml. from this solution 1.0, 2.0, 3.0, 4.0 and 5.0 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with simulated tear fluid (pH 7.4), gives standard drug solution of 10, 20, 30, 40, 50µg/ml concentration.

Formulation development of *In-situ* gel

1. For the preparation of Pluronic F127 based ocular in-situ gel, all the ingredients were sieved from sieve no 44.

2. Then a solution of 0.5% and 0.1% of drugs were prepared in acetate buffer 5.0 I.P.

3. The solution was cooled in an ice bath and pluronic F127 was added slowly with continuous stirring.

4. Then the resulting solution was kept in a refrigerator under 40C for 24h. this storage was help in dissolving the Pluronic F 127 completely.

5. After 24h carbopol 934 and HPMC 15cps were added slowly along with other excipients with continuous stirring. The stirring should be continued to 2-3 hours for proper mixing and avoid slug formation.

6. The resulting formulation kept on probe sonicator to remove the air bubble. All formulations were stored in LDPE (Low-Density Polyethelene) bottles for further use. All the containers stored in refrigerator.

Table No. 1: Composition of *In-situ* gel

Sr. No.	Ingredient (%)	Formulations								
1.	Gentamicin	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
2.	Dexamethasone	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%
2.	Pluronic F127	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
3.	Carbopol 934	18	16	14	18	16	14	18	16	14
4.	HPMC 15cps	0.2	0.2	0.2	0.3	0.3	0.3	0.4	0.4	0.4
5.	EDTA	1.0	1.0	1.0	0.75	0.75	0.75	0.5	0.5	0.5
6.	Benzalkonium Chloride	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
7.	NaCl	0.013 %	0.01 3%	0.013 %	0.013 %	0.013 %	0.013 %	0.013 %	0.013 %	0.013 %
8.	Poly ethylene glycol	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
9.	Acetate Buffer (pH 5.0)	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%

Evaluations of formulations[16-20]

Appearance

Clarity is one of the most important characteristic features of ophthalmic preparations. All developed formulations were evaluated for clarity by visual observation against a black and white background.

Drug content

The assay of drug Gentamicin and Dexamethasone was performed by the UV method. The calculation was based on the calibration curve method using the regression equation (Y=mx+c)[40].

pH

pH is one of the most important parameters involved in the ophthalmic formulation. The two areas of critical importance are the effect of pH on solubility and stability. The pH of the ophthalmic formulation should be such that the formulation will be stable at that pH and at the same time there would be no irritation to the patient upon administration of the formulation. Ophthalmic formulations should have a pH range between 5 to 7.4. The developed formulations were evaluated for pH by using calibrated digital pH meter.

In-Situ gelling capacity

In situ gelling capacity determined by visual inspection. The formulation has been exposed to the physiological conditions of temperature and pH. Simulated tear fluid (STF) was prepared and warm up to 370C. Formulations were introduced into STF in a ratio of 1:2 Change in consistency of Formulations were visually inspected.

Composition of Simulated Tear Fluid (STF)

Sodium chloride: 0.670 gm

Sodium bicarbonate: 0.2 gm

Calcium chloride dihydrate: 8 mg

Water up to 100ml

pH was adjusted by 0.5 N NaOH to 7.4.

The gelling capacity of all formulations is depicted as + (gels after five minutes and dissolves rapidly), ++ (gelation immediate, remains for few hours), and +++ (gelation immediate, remains for an extended period up to 8 hours).

Viscosity study

At pH 5.0 and temperature less than 16ºC the developed formulations were in the liquid state and show low viscosity. For viscosity studies, the pH of formulations was raised from pH 5.0 to pH 7.4 and the temperature was raised to 37ºC. pH was raised to 7.4 by the addition of 0.5M NaOH43. The resulting gel studied for viscosity on Brookfield Synchrolectric Viscometer using Spindle No. 7 at 50 RPM for comparative study. The angular viscosity was measured by gradually increase the RPM from 10 to 70.

Sterility testing

The test for sterility is applied to pharmacopoeial articles that are required according to the Pharmacopoeia to be sterile. However, a satisfactory result only indicates that no contaminating viable micro-organisms have been found in the sample examined in the conditions of the test. The test must be carried out under aseptic conditions designed to avoid accidental contamination of the product during testing. For achieving these conditions, a grade-A laminar airflow cabinet or an isolator is recommended. The test environment has to be adapted to how the tests are performed. Precautions taken for this purpose should not adversely affect any micro-organisms, which are to be revealed in the tests. The working conditions in which the tests are carried out should be monitored regularly by appropriate sampling of the air and surfaces of the working area and by carrying out control tests.

Culture Media

The following culture media are suitable for the test. Fluid thioglycollate medium is primarily intended for the culture of anaerobic bacteria; however, it will also detect aerobic bacteria. Soybean-casein digest medium is suitable or the culture of both fungi and aerobic bacteria.

Table No. 2: Composition of Fluid Thioglycollate Medium

Ingredients	Quantity(gm)
L-Cystine	0.5 g
Sodium chloride	2.5 g
Dextrose monohydrate/anhydrous	5.5 g/5.0 g
Granular agar	0.75 g
Yeast extract (water-soluble)	5.0 g
Pancreatic digest of casein	15.0 g
Sodium thioglycollate or	0.5 g
Thioglycolic acid	0.3 ml
Resazurin sodium solution (0.1 percent), freshly prepared	1.0 ml
Distilled water to	1000 ml
a pH of the medium after sterilization	7.1 ± 0.2

Method A: Membrane filtration.

Method B: Direct inoculation

Method B is recommended for clear aqueous preparation.

Method B - Direct Inoculation

Quantities of Sample to be used

The quantity of the substance or preparation under examination to be used for inoculation in the culture media varies according to the quantity in each container. The sample that was used was not less than 200 mg.

Method of Test

For aqueous solutions: Remove the liquid from the test containers with a sterile pipette or with a sterile syringe or a needle. Transfer the quantity of the preparation under examination into the culture medium so that the volume of the preparation under examination is not more than 10 percent of the volume of the medium unless otherwise prescribed. If the preparation under examination has antimicrobial activity, carry out the test after neutralizing this with a suitable neutralizing substance or by dilution in a sufficient quantity of culture medium.

When it is necessary to use a large volume of the product it may be preferable to use a concentrated culture medium prepared in such a way that it takes account of the subsequent dilution. Where appropriate, the concentrated medium may be added directly to the product in its container. Incubate the inoculated media for not less than 14 days. Observe the cultures several times during the incubation period. Observe the containers of media periodically during the 14 days of incubation. If the test specimen is positive before 14 days of incubation, further incubation is not necessary. For products terminally sterilized by a validated moist heat process, incubate the test specimen for not less than 7 days.

***In-vitro* drug release**

***In-vitro* drug diffusion study**

The *in-vitro* release of drugs from the formulations was studied through the cellophane membrane. The dissolution medium used was artificial tear fluid freshly prepared (pH 7.4). The cellophane 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 5 cm diameter). A 1-ml volume of the formulation was accurately pipetted 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 dissolution medium was stirred at 50 rpm using a magnetic stirrer. Methodology Aliquots, each of 1-ml volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium.

Stability studies

Stability is defined as the extent to which a product retains, within specified limits, and throughout its period of storage and use (i.e. its shelf life), the same properties and characteristics that it possessed at the time of its manufacture. Stability testing is performed to ensure that drug products retain their fitness for use until the end of their expiration dates. All the five formulations were subjected to stability studies at ambient humidity conditions at 2°C to 8°C, ambient temperature and 40±1°C for one month. The samples were withdrawn after 7, 15, and 30 days and were evaluated for the following parameters. Packs that are to some degree permeable to moisture (as are most plastics) will lose or gain moisture according to whether they are exposed to a high or low relative humidity respectively. 40°C/75% RH may be particularly severe on a fully exposed blister pack and give an artificially low shelf-

life prediction. The same condition may offer little challenge to moisture loss as the vapor pressure inside the pack may virtually be at equilibrium with the external atmosphere (plastic containers). The formulations were further evaluated for evaluation parameters after each sampling period.

Table No. 3: Storage conditions for Stability Studies according to ICH guidelines

Study	Storage Condition	Minimum Time Period
Long term	25ºC±2ºC, 60%±5%RH Or 30ºC±2ºC, 65%±5%RH	12 Months
Intermediate	30ºC±2ºC, 65%±5%RH	6 Months
Accelerated	40ºC±2ºC, 75%±5%RH	6 Months

RESULTS AND DISCUSSION:

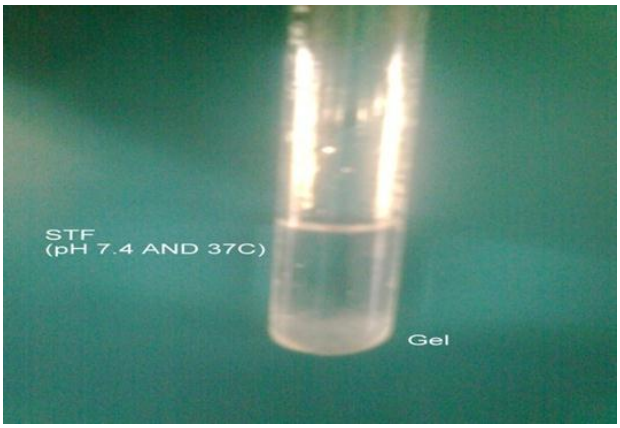

Figure No. 3: *In-situ* Gel formation in STF

Identification Test using FTIR Spectroscopy

Sample of pure Gentamicin and Dexamethasone

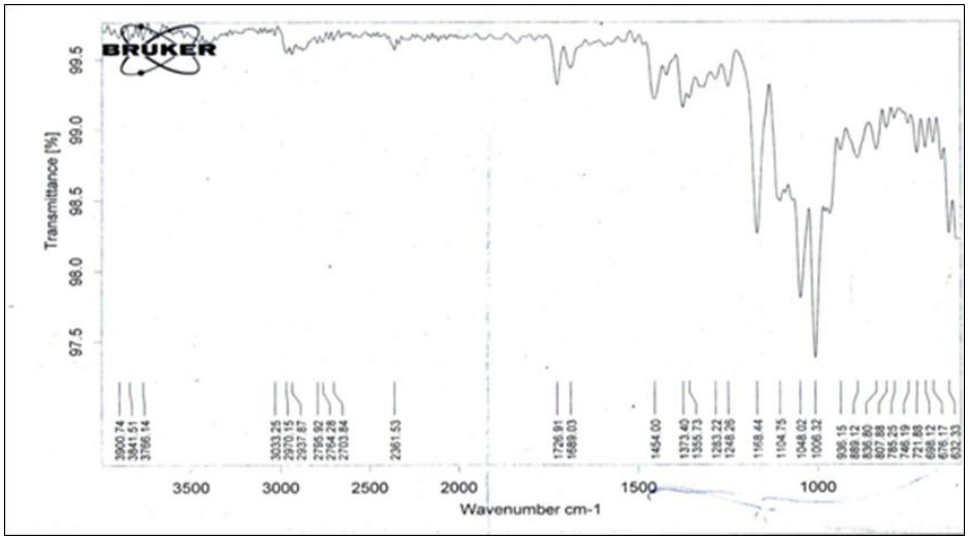

Figure No. 4: FT-IR Spectrum of Pure Drug (Gentamicin)

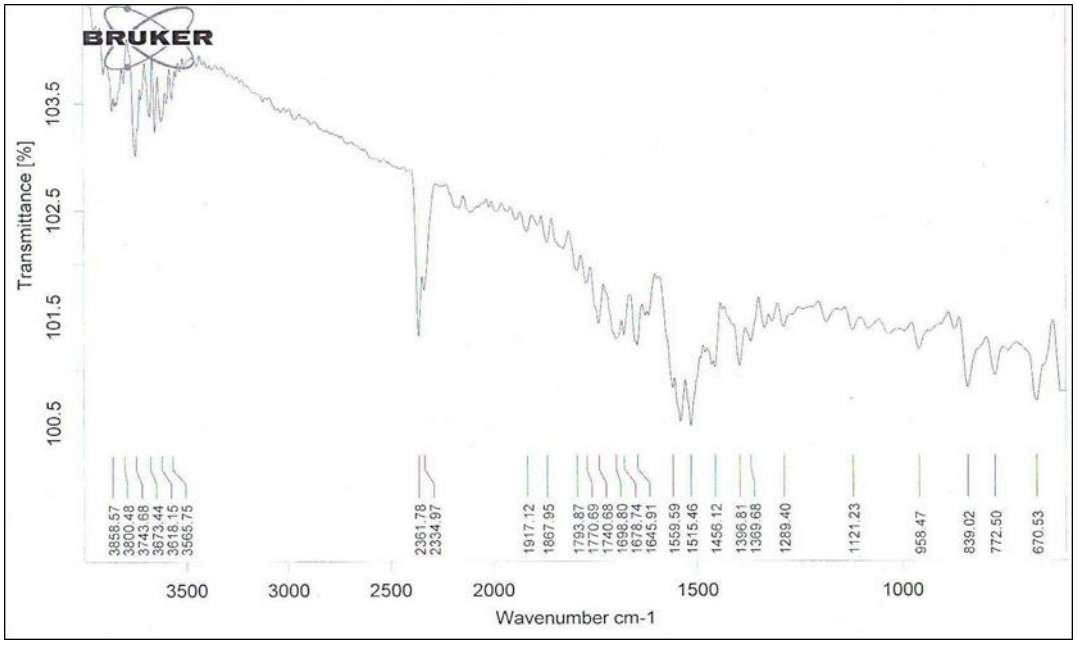

Figure No. 5: FT-IR Spectrum (Dexamethasone)

Results of Loss on drying

Table No. 4: Loss on drying of Gentamicin sample

Sr. No.	Initial weight	Final weight after 15 minutes	% loss of drying	Avg. % loss of drying
1.	5gm	4.88 gm	2.4 %	
2.	5gm	4.86 gm	2.8 %	2.33±0.41
3.	5gm	4.91 gm	1.8 %	

Table No. 5: Loss on drying of Dexamethasone sample

Sr. No.	Initial weight	Final weight after 15 minutes	% loss of drying	Avg. % loss of drying
1.	5gm	4.92 gm	1.6 %	
2.	5gm	4.91 gm	1.82 %	1.67±0.103
3.	5gm	4.92 gm	1.6 %	

Determination of λ max of Gentamicin and Dexamethasone

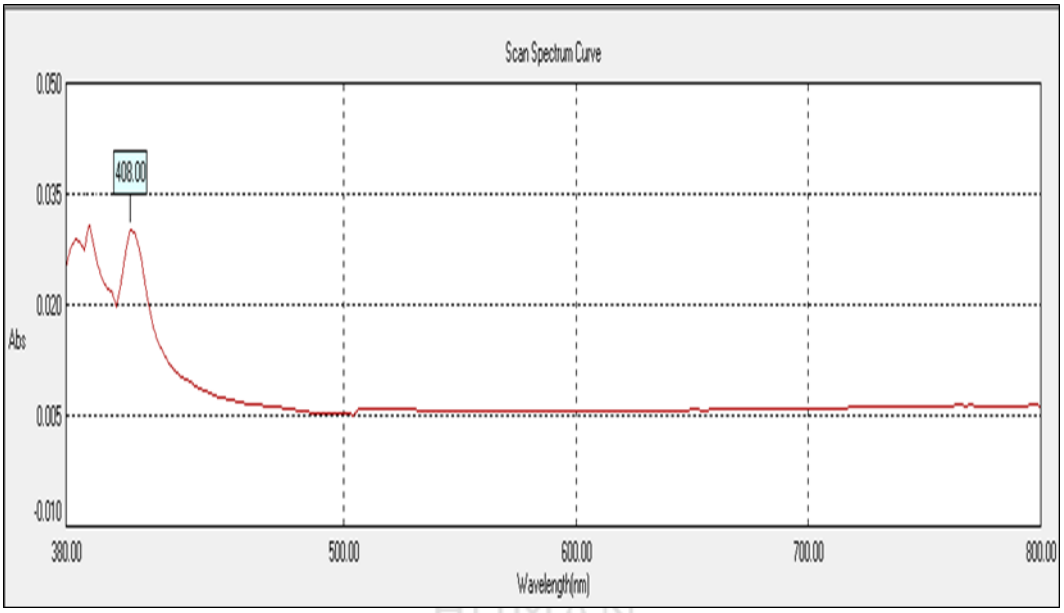

Figure No. 6: Determination of λmax of Gentamicin

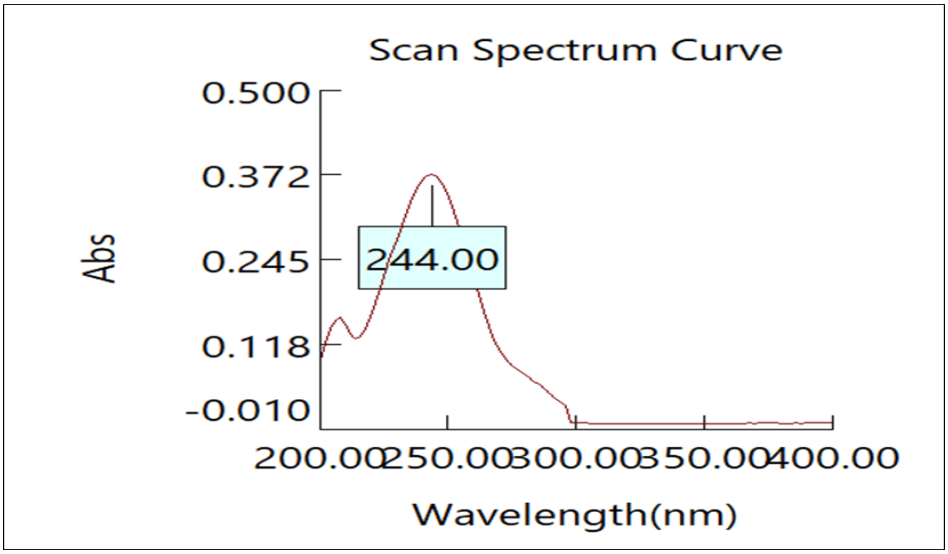

Figure No. 7: Determination of λmax of Dexamethasone

Calibration curve of Gentamicin and Dexamethasone

Table No. 6: Readings for Calibration curve of Gentamicin

Sr. No.	Concentration (µg/ml)	Mean Absorbance*
1	10	0.267±0.001
2	20	0.477±0.002
3	30	0.719±0.001
4	40	0.928±0.002
5	50	1.168±0.001

*Average of three determinations (n=3)

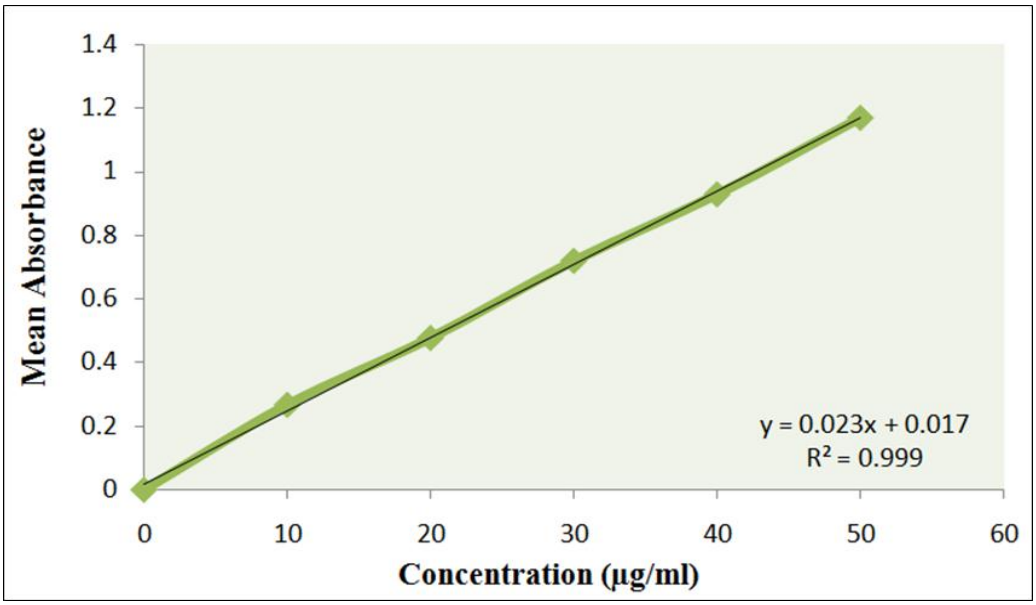

Figure No. 8: Calibration Curve of Gentamicin at 408 nm

Table No. 7: Readings for Calibration curve of Dexamethasone

Sr. No.	Concentration (µg/ml)	Mean Absorbance*
1	10	0.194±0.002
2	20	0.422±0.001
3	30	0.637±0.002
4	40	0.848±0.001
5	50	1.035±0.001

*Average of three determinations (n=3)

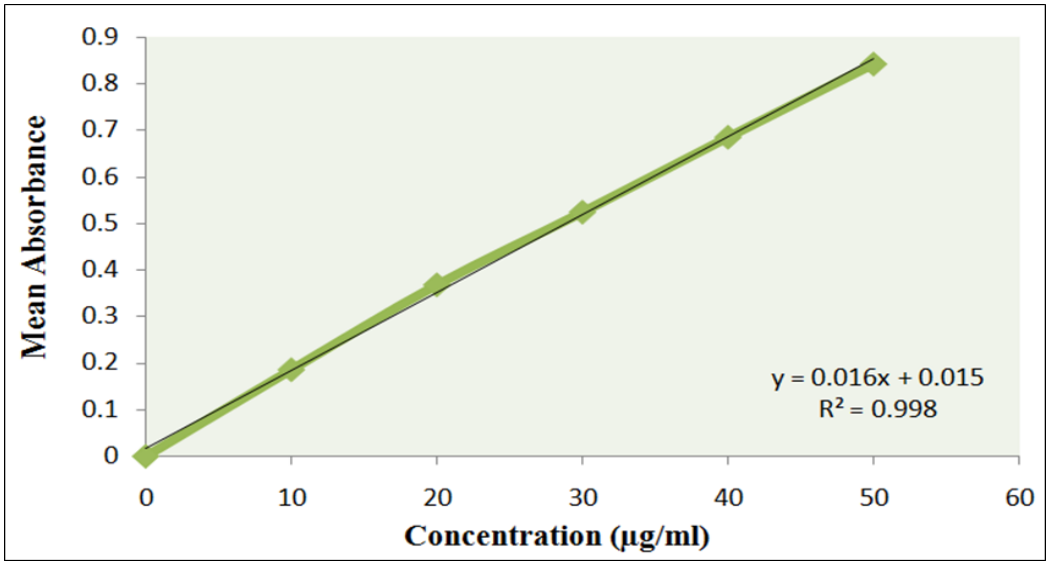

Figure No. 9: Calibration curve of Dexamethasone at 244nm

Table No. 8: Statistical Data For Linearity

Sr. No.	Parameter	Gentamicin Remark	Dexamethasone Remark
1.	Linearty Range	10-50 µg/ml	10-50 µg/ml
2.	Regression Equation	0.023x+0.017	0.016 x+0.015
3.	Correlation Cofficient	0.999	0.998

Results of evaluation parameter

Clarity test

Formulations were evaluated for clarity by visual observation against a black and white background. Some formulations had the problem of precipitation of carbopol during storage, the problem overcome by increasing the stirring time up to 2-3 hours during formulation.

Table No. 9: Clarity test of In situ gel formulations

Formulation code	Clarity
F1	Clear
F2	Clear
F3	Clear
F4	Clear
F5	Clear
F6	Clear
F7	Precipitate observed
F8	Turbid
F9	Turbid

Drug Content

The drug content of both the drug in formulations was determined by the UV method.

Table No. 10: Drug content analysis

Formulation	Drug Content (%)*	
F1	**Gentamicin**	**Dexamethasone**
F2	98.22±0.12	96.65±0.65
F3	99.14±0.25	99.89±0.62
F4	97.22±0.32	98.56±0.41
F5	98.65±0.14	97.85±0.32
F6	95.51±0.15	97.65±0.14
F7	95.56±0.32	98.12±0.52
F8	96.69±0.56	95.65±0.14
F9	97.89±0.14	98.85±0.32

*Average of three determinations (n=3)

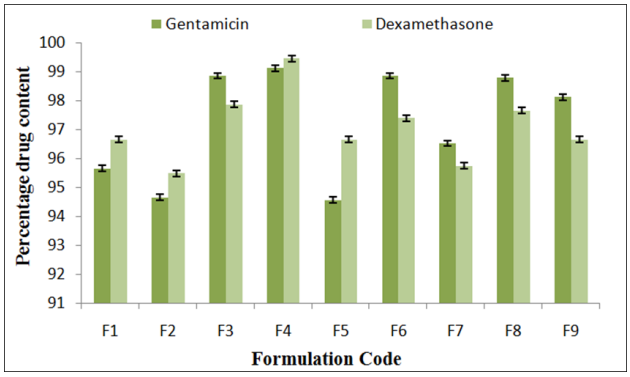

Figure No. 10: Graph of Drug content analysis

pH Determination

The developed formulations were evaluated for pH by using digital pH meter. The pH of formulations was decreased from buffer pH 5.0 because of acidic groups of carbopol so that the pH was adjusted to 5.0 by using 0.5N NaOH.

Table No. 11: pH Determination

Formulation	pH	Adjust to
F1	4.1	5.0 ±0.1
F2	4.3	5.0 ±0.1
F3	4.2	5.0 ±0.1
F4	3.9	5.0 ±0.1
F5	3.8	5.0 ±0.1
F6	4.1	5.0 ±0.1
F7	3.9	5.0 ±0.1
F8	4.0	5.0 ±0.1
F9	4.2	5.0 ±0.1

***In-situ* gelling capacity**

In-situ gelling capacity determined by visual inspection. The formulation has been exposed to the physiological conditions of temperature and pH. Simulated tear fluid (STF) was prepared and warm up to 370C. The solution was introduced into STF in a ratio of 1:2 Change in consistency of solution visually inspected. Formulation F3, F6 and F9 show poor gelling capacity in simulated physiological conditions of pH and temperature because of comparatively less concentration of pluronic F127 in IG6 and F9 and due to low concentration of carbopol in F1. F4 and F5 formulation show a better gelling capacity.

Table No. 12: Formulation *In-situ* gelling capacity

Formulation code	*In situ* gelling capacity
F1	"++"
F2	"++"
F3	"+"
F4	"+++"
F5	"+++"
F6	"+"
F7	"++"
F8	"++"
F9	"+"

"+" gelation after five minutes and dissolves rapidly

"++" gelation immediate, remains for few hours

"+++" gelation immediate, remains for extended period 8 hours

Viscosity study

The viscosity of formulation was determined before and after gelation by using Brookfield's viscometer in the small volume adaptor and the angular velocity was increased gradually from 10, 20, 40, 50, 60, and 70 RPM. The comparative study of viscosity was done at 50 RPM. F4, F5, and F7 show comparatively better viscosity and good consistency gel.

Table No. 13: Comparative viscosity* of *in-situ* formulation

Formulation code	% of Pluronic F 127	Viscosity of solution (in cps)	Viscosity after gelation
F1	18	987	2423
F2	16	881	2205
F3	14	811	2150
F4	18	1053	2671
F5	16	933	3056
F6	14	613	2330
F7	18	741	2685
F8	16	771	2535
F9	14	668	2831

* Spindle no.7

Rpm 50

Sterility testing

All the formulations terminally sterilized by autoclaving. The process was executed by placing the formulations in borosilicate conical flask these flasks are placed in a preheated autoclave. Then the autoclave well closed by clamps. Formulations were sterile by heating at 1210C for 15 minutes under pressure.

For sterility testing formulations were diluted ten times by sterile distilled water. From this dilution remove quantity and placed in culture media, this quantity should be equivalent to more than 200 mg of the formulation. Petri dishes.

Table No. 14: Sterility testing of formulations

Formulation code	Observation
F1	No growth
F2	No growth
F3	No growth
F4	No growth
F5	No growth
F6	No growth
F7	No growth
F8	No growth
F9	No growth

***In-vitro* drug release study of optimized formulation**

Table No. 15: In vitro drug release profile of Gentamicin from in situ Formulation F4

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	23.25	1.366	76.75	1.885
1	1.000	0.000	36.65	1.564	63.35	1.802
1.5	1.225	0.176	48.85	1.689	51.15	1.709
2	1.414	0.301	59.98	1.778	40.02	1.602
2.5	1.581	0.398	67.25	1.828	32.75	1.515
3	1.732	0.477	85.65	1.933	14.35	1.157
4	2.000	0.602	92.32	1.965	7.68	0.885
5	2.236	0.699	98.78	1.995	1.22	0.086

Release Kinetics of drugs loaded optimized formulation F-4

Zero-order release kinetics of optimized formulation

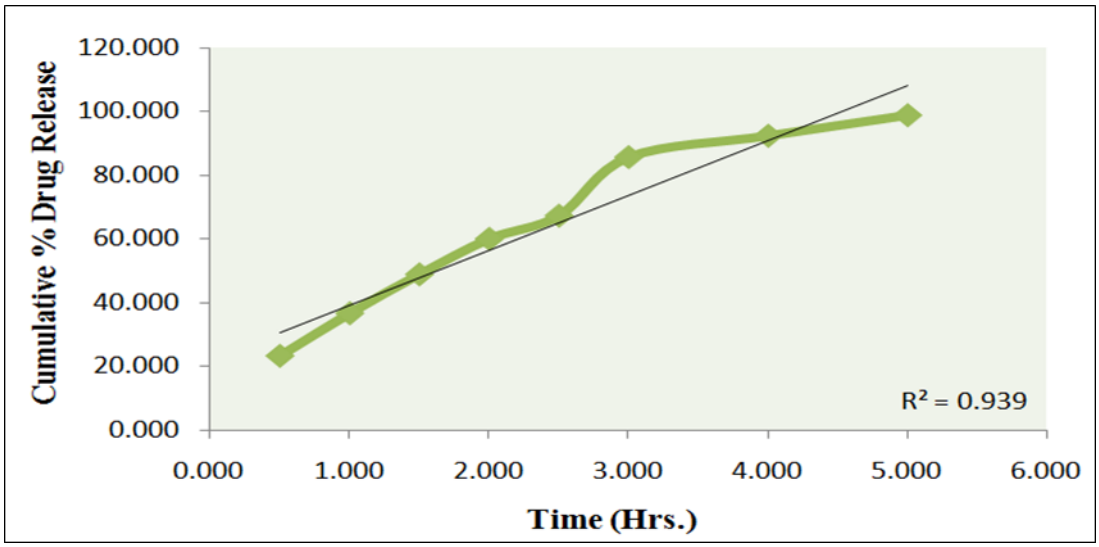

Figure No. 11: Zero-order release Kinetics (Cumulative % drug released Vs Time)

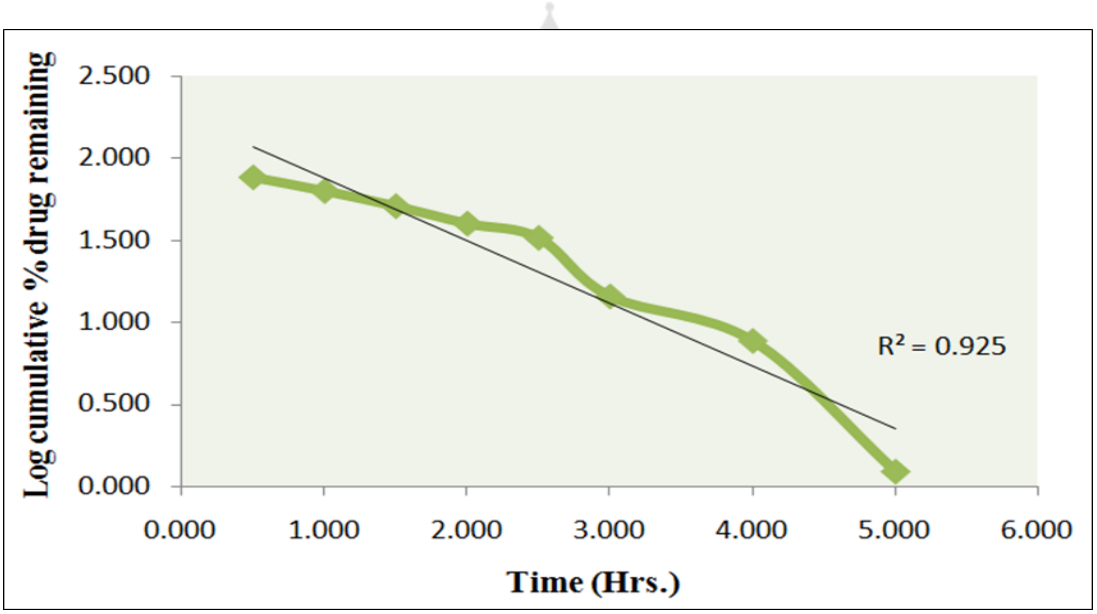

Figure No. 12: First order release kinetics (Log cumulative % drug remaining Vs Time)

Table No. 16: *In-vitro* drug release profile of dexamethasone from in Situ Formulation F4

Time (h)	Square Root of Time $(h)^{1/2}$	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	28.98	1.462	71.02	1.851
1	1.000	0.000	42.32	1.627	57.68	1.761
1.5	1.225	0.176	55.65	1.745	44.35	1.647
2	1.414	0.301	63.32	1.802	36.68	1.564
2.5	1.581	0.398	75.65	1.879	24.35	1.386
3	1.732	0.477	89.98	1.954	10.02	1.001
4	2.000	0.602	94.48	1.975	5.52	0.742
5	2.236	0.699	98.12	1.992	1.88	0.274

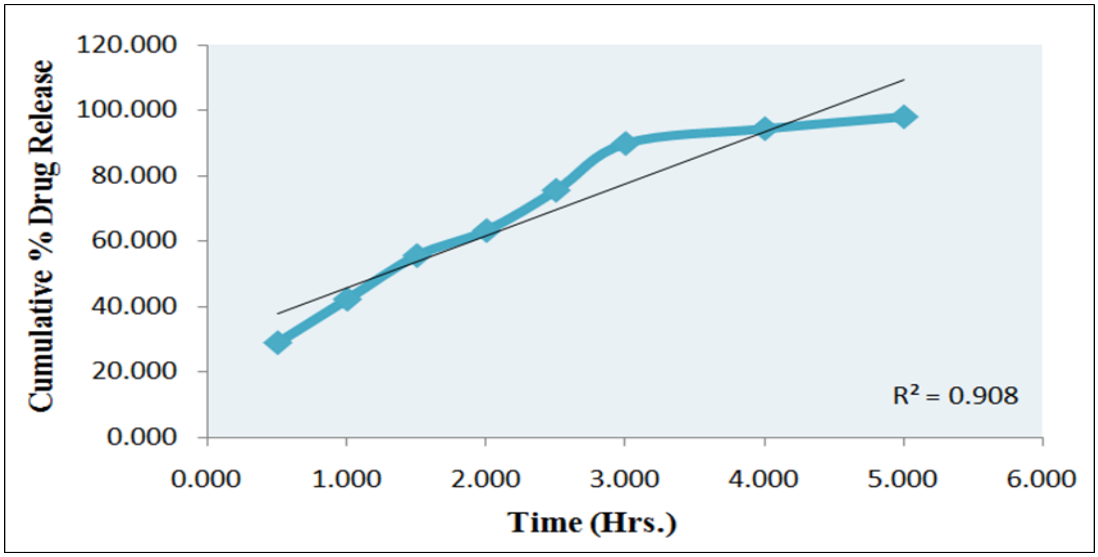

Figure No. 13: Zero-order release Kinetics (Cumulative % drug released Vs Time)

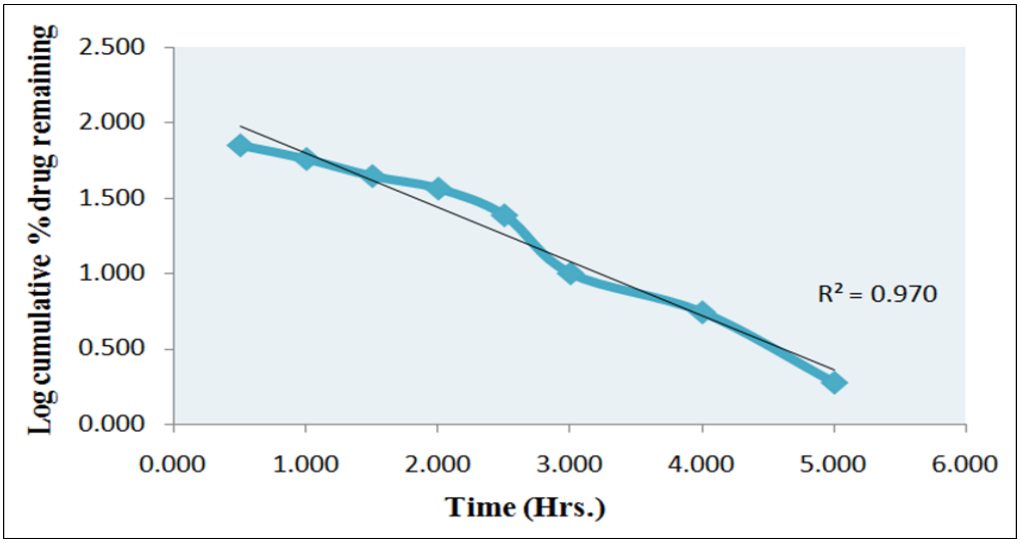

Figure No. 14: First order release kinetics (Log cumulative % drug remaining Vs Time)

Table No. 17: Comparative study of the regression coefficient for selection of optimized Formulation F5

Drug	Zero-order	First-order
Gentamicin	$R^2 = 0.938$	$R^2 = 0.925$
Dexamethasone	$R^2 = 0.908$	$R^2 = 0.970$

The *In-vitro* drug release data of the optimized formulation was subjected to the goodness of fit test by linear regression analysis according to zero-order and first-order kinetic equation to determine the mechanism of drug release. When the regression coefficient values were compared, it was observed that 'r' values of First-order were maximum hence indicating drug release from formulations was found to follow first-order release kinetics.

Stability studies

Table No. 18: Stability datasheet

F. code	Parameters evaluated														
	7 days					15days					30days				
	Drug content	pH	Clarity	Viscosity	In-situ gelling capacity	Drug content	pH	Clarity	Viscosity	In-situ gelling capacity	Drug content	pH	Clarity	Viscosity	In-situ gelling capacity
F1	*98.22*	*5.05*	*Clear*	*2330*	++	*94.18*	*5.06*	*clear*	*2333*	++	*91.54*	*5.11*	*Clear*	*2340*	++
F2	*99.14*	*5.01*	*Clear*	*2190*	++	*96.88*	*5.01*	*Clear*	*2186*	++	*93.87*	*5.09*	*Clear*	*2181*	++
F4	*97.22*	*5.0*	*Clear*	*2623*	+++	*94.38*	*5.04*	*Clear*	*2610*	+++	*90.45*	*5.08*	*Clear*	*2640*	+++
F5	*98.65*	*5.0*	*Clear*	*3080*	+++	*96.22*	*5.02*	*Clear*	*3086*	+++	*94.61*	*5.07*	*Clear*	*3098*	+++
F6	*95.51*	*5.1*	*Clear*	*2525*	++	*91.88*	*5.11*	*Clear*	*2546*	++	*88.78*	*5.15*	*Clear*	*2563*	++

CONCLUSION:

Ophthalmic in situ gelling system of Gentamicin and Dexamethasone was successfully formulated using a polymeric combination of gelling agents Pluronic F127, Carbopol 934 as, temperature-sensitive and pH-sensitive respectively along with HPMC 15cps as a viscosity-enhancing agent. The clarity of the prepared formulations was found satisfactory but precipitate observed in the formulation during storage. The pH of all formulations was found at 5.0. The drug content of the prepared formulation was within the acceptable range and ensures dose uniformity. The formulation F4 showed maximum drug content. All the formulations except F1, F6, and F5 showed instantaneous gelation when contacted with simulated tear fluid (STF), formulation F4 showed the best gelation property amongst all other. Formulation F4 showed sustained drug release for 5 hours. Results of the sterility test confirmed that all the formulations were sterile.The In vitro drug release data of the optimized formulation was subjected to the goodness of fit test by linear regression analysis according to zero-order and first-order kinetic equation to determine the mechanism of drug release. When the regression coefficient values were compared, it was observed that 'r' values of First-order were maximum hence indicating drug release from formulations was found to follow first-order release kinetics. The results of the ocular irritation studies indicate that the formulations F4 were non-irritant and excellent ocular tolerance was noticed.The stability of in situ gelling formulations was observed at 40±1°C and 4°C (significant decrease in drug content). Formulation F4 was more stable than other formulation. In Conclusion, Evaluation of in situ gel is determined to ensure that the prepared preparation meets the standard and is safe. In the chemical evaluation in situ gel determined the diffusion of the active substance of a compound by measuring its concentration. In microbiology, evaluation determines if the preparations are contaminated or not, also be effective and safe.

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