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FORMULATION AND EVALUATION OF OPTHALMIC NOVEL IN-SITU GEL CONTAINING GANCYCLOVIR FOR THE TREATMENT OF HERPES SIMPLEX KERATITIS BY USING NATURAL GELLING AGENT

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Abstract: The present research work deals with the formulation and evaluation of in-situ gelling system based on sol-to-gel transition for ophthalmic delivery of an antiviral agent Ganciclovir, to overcome the problems of poor bioavailability and therapeutic response exhibited by conventional formulation Ganciclovir is an antiviral medication primarily used to treat infections caused by certain types of viruses, particularly cytomegalovirus (CMV). It was formulated as ocular in situ gel forming eye drop are using polymers such as Guar gum as a gelling agent in combination of suitable preservatives. It is a newer approach to improve easy to eye instillation residence time and enhance bioavailability, prolonged and sustained drug release. Also important in the ease of administration afforded and the decrease frequency of administration resulting in the better patient compliance and acceptance

Keywords: ocular in situ gel, Ganciclovir, Guar gum, Drug Formulation, bioavailability, sustained drug release

1. Introduction

The main objective of any pharmaceutical delivery system is to have a beneficial impact on the pharmacokinetic characteristics and tissue distribution of the drug. During the past sixty years, there has been significant attention given to the advancement of pharmaceutical delivery systems that can control and maintain the release of drugs over time. The in situ gel drug delivery system has emerged as a very efficient and innovative method for administering drugs. The in-situ gel drug delivery system possesses a distinctive capability to transform from a liquid to a gel state, enabling the controlled and delayed release of medications. This enhances patient compliance and comfort. An in situ gelling system is a formulation that initially exists as a solution but undergoes a transformation into a gel state when exposed to different physiological circumstances. Several factors, such as alterations in pH, temperature, and solvent substitution, affect the transformation of a solution into a gel. Several experiments have been conducted using different methods of administration, such as oral, ocular, nasal, rectal, vaginal, injectable, parenteral, and intraperitoneal routes. Various

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polymeric systems have been created that possess the capability to release medicines. Upon exposure to physiological stimuli, these polymers undergo a sol-gel transition. In situ gel drug delivery systems are made from a variety of natural and synthetic polymers.[1] Insitu gel formulations provide an appealing alternative to parenteral routes, which can be cumbersome, and oral routes, which can result in insufficient absorption and processing in the liver, particularly for proteins and peptides, in order to produce a systemic pharmacological effect. This unique drug delivery approach improves the crucial elements of easy and convenient administration, exact dosage delivery, and long-term medicine interaction with the mucosa. These are common issues with semi-solid dosage formulations. pH changes, temperature fluctuations, and solvent replacement are among the stimuli that might cause the formation of an in situ gel. When it comes to treating eye diseases, liquid drops are the best option because patients can tolerate them.

2. Method and Material: Drug Ganciclovir was a gift sample provided by Odon life Science Private Limited Dehradun (U.K) and other excipients were procured from institute

3. Preformulation Studies

Physical Appearance: Through visual inspection of physical appearance of pure drug was carried out as per I.P

Melting point: Melting Point determination is one of the preformulation property in which the temperature at which it changes state from solid to liquid at atmospheric pressure. At the melting process the solid and liquid can exist equilibrium. The Melting point of Ganciclovir pure drug is determine by using two types of method one is Conventional method and another is Digital method**.**

Determination of λ_{max}

100 mg drug dissolve in100 ml dm water it's a obtained stock solution of 1000 microgram/ml .then stock solution are removed 10 ml and volume make up the 100 ml with dm water and scanned of UV was 200 to 400 nm wavelength**.**[28]

Preparation of Calibration Curve

Preparation of calibration curve of drug and dm water

Calibration curve is determined by using UV spectrophotometric methods. In which 10 mg of drug dissolve in 100 ml dm water .to prepare different dilution (2, 4, 6, 8, and 10) of above solution. Take absorbance at 304 nm / 276 nm respectively**.**

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Preparation of calibration curve of drug with phosphate buffer(pH7.4)

The calibration curve for the drug was established using UV spectrophotometric methods by dissolving 10 mg of the drug in 100 ml of pH 7.4 phosphate buffer solution. Dilutions ranging from 2 to 10 times were prepared from this solution, and absorbance measurements were taken at 304 nm and 276 nm wavelengths. Solubility studies involved preparing solutions with water and pH 7.4 phosphate buffer, adding the drug until a supersaturated state was achieved, and incubating on an orbital shaker for 24 hours followed by filtration and absorbance measurement of the filtrate. For drug and excipient interaction studies, a 1:1 ratio of drug and excipient was incubated in ampules in a stability chamber for one week. After incubation, Thin Layer Chromatography (TLC) using silica gel plates and a mobile phase of toluene acetate acetic acid (20:10:0.2 ratio) evaluated compatibility. This comprehensive approach ensures accurate drug quantification, assesses solubility under varying conditions, and evaluates the compatibility of drug-excipient combinations for pharmaceutical applications.

4. Experimental design for Formulation

Table 1: Composition of ocular *in situ* gel

S.NO.	INGREDIENTS	F1	F2	F3	F4
2	Ganciclovir	10 mg	10 mg	10 mg	_
3	Guar gum	0.5 mg	0.75 mg	1.0 mg	0.5 mg
4	Benzolkonium chloride	0.1 ml	0.1 ml	0.1 ml	0.1 ml
5	DM-water	10 ml	10 ml	10 ml	10ml

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5. Evaluation of Formulation

The physico-chemical characterization of the in-situ gels involved several assessments. Visual inspection under fluorescent light against white and black backgrounds was conducted to detect any particulate matter and ensure clarity and uniformity of visual appearance. pH determination was performed using a digital pH meter after all ingredients were added to the gels. Drug content analysis was carried out by diluting 1 ml of the formulation in 100 ml of distilled water, withdrawing 5 ml of the solution, further diluting it to 25 ml, and measuring ganciclovir concentration at 304 nm using a UV-visible spectrophotometer. Gelling capacity assessments involved placing drops of the formulations into vials containing 2 ml of pH 7.4 artificial tear fluid equilibrated at 37°C. Gel formation time and dissolution time were observed visually to identify compositions suitable for in-situ gelling systems based on their ability to form and maintain gel structure.

Gelling capacity parameter

parameter	Gelling capacity
+	Gelation within 50-60 second
++	Gelation within 60 second for 3hours
+++	Gelation within 60 second for 6 hours

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Composition of artificial tear fluid

Table Composition of tear fluid

S.NO.	COMPOSITION
1	Sodium chloride
2	Sodium bicarbonate
3	Calcium chloride dihydrate
4	water

Sterility studies: Sterility studies conducted on ocular preparations are of utmost importance in order to guarantee patient safety and prevent the occurrence of severe infections that may result in rapid vision deterioration. Ophthalmic goods, such as eye drops and ointments, need to maintain sterility from the time they are made until they are given to patients. If any living germs are present in these preparations, they have the potential to induce severe infections of the cornea, resulting in irreparable damage and possible full loss of eye sight within a time frame of 24 to 48 hours.

The process of ensuring sterility commences with stringent production processes in a controlled environment, such as a cleanroom facility that adheres to international standards for cleanliness and air quality. Sterilisation processes like as filtration, autoclaving, and aseptic processing are used to remove microorganisms from both the product and its packing materials.

Sterility testing is the collection of samples from the completed product batches and subjecting them to microbiological analysis. The purpose of these tests is to identify the existence of bacteria, fungus, and other microorganisms that have the potential to contaminate the product during the process of manufacturing or packing. The procedures employed for sterility testing encompass membrane filtration, direct injection into culture media, and additional validated methods as stipulated by regulatory criteria.

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The US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), which are regulatory authorities, have strict criteria for testing the sterility of ophthalmic solutions. These rules guarantee that products adhere to strict safety.
If a product batch fails sterility testing, investigations are carried out to determine the origin of contamination and adopt corrective measures to prevent it from happening again. To ensure the integrity of the manufacturing process and safeguard patient safety, methods such as root cause analysis, environmental monitoring, and validation of cleaning procedures are implemented. Sterility studies are essential for ensuring the quality of ophthalmic preparations and protecting patients from severe infections that could harm their vision and overall well-being. Pharmaceutical businesses and healthcare practitioners maintain the safety, effectiveness, and reliability of ophthalmic products in clinical practice by following tight regulatory criteria and using rigors testing methods.

In vitro Release Studies

In vitro release studies are essential for assessing the controlled release and dissolution behaviour of pharmaceutical formulations under simulated physiological conditions outside the body. These studies provide valuable insights into how drugs are released from their dosage forms and how their release profiles may affect therapeutic outcomes.Typically, in vitro release studies involve placing the dosage form, such as tablets, capsules, or gels, into a dissolution apparatus filled with a suitable medium that mimics the physiological environment where the drug is intended to act. The apparatus maintains constant agitation and temperature to simulate physiological conditions, ensuring consistency and reproducibility of results.

Samples of the dissolution medium are withdrawn at specified time intervals, and the concentration of the drug released into the medium is measured using analytical techniques such as UV-Vis spectrophotometry, high-performance liquid chromatography (HPLC), or other validated methods. These measurements allow researchers to construct dissolution profiles that depict the rate and extent of drug release over time.The data obtained from in vitro release studies are crucial for several purposes, including formulation development,

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quality control, and regulatory submissions. They help optimize formulations by assessing factors like excipient composition, drug release kinetics, and the influence of pH and dissolution media on release behavior. Moreover, these studies provide critical information for establishing bioequivalence between generic and branded formulations, ensuring consistency in drug absorption and efficacy.in vitro release studies are integral to pharmaceutical research and development, enabling scientists and manufacturers to understand and refine the performance of drug formulations before advancing to in vivo studies and clinical trials. By simulating drug release under controlled conditions, these studies support the development of safe, effective, and reliable pharmaceutical products for patient use.

6. Result & Discussion:

Physical appearance: Through visual inspection of physical appearance of pure drug was carried out as per I.P

RESULT:

Ganciclovir	The sample drug was found to be in powdered form, white to off white in colour which is accordance to standard.

Melting point: The melting point of drug was found to be in the range which accordance with standard

RESULT:

Ganciclovir	250*C

Determination of ʎ max by UV Spectrophotometer in Ganciclovir

Ganciclovir 10 µg/ml stock solution showed maxima at 340 nm which match with the Spectrum of Ganciclovir as reported in literature. So this wavelength was used for the analysis of Ganciclovir solution throughout the studies.

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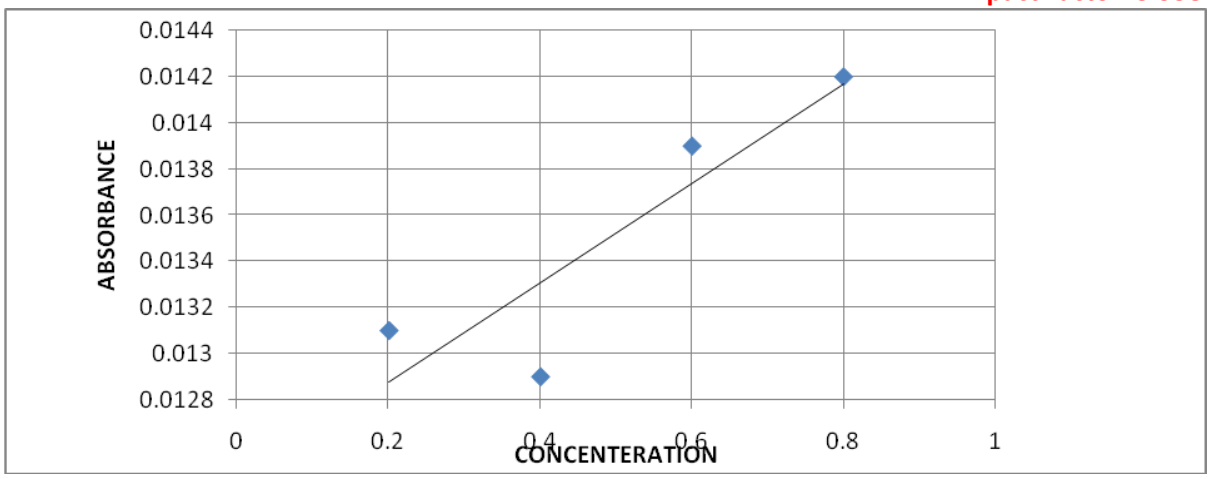

UV Spectrum of Ganciclovir:

Table-2 Absorbance data of Ganciclovir with Calibration curve at

S.no.	concentration	Absorbance
1	0	0
2	0.2	0.0131
3	0.4	0.0129
4	0.6	0.0139
5	0.8	0.0142

Solubility studies

Table no. 3 Solubility data of Ganciclovir.

RESULT: Solubility studies of Ganciclovir.

s.no.	solvents	Solubility (mg/ml)	inference
1	DM water	2.6mg/ml	Slightly soluble

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2	PBS (PH 7.4)	3.415mg/ml	Slightly soluble

Ganciclovir was found to be slightly soluble in water, and phosphate buffer (pH 7.4).

Drug-Excipients interaction studies

Table no. 4: Drug and Excipients interaction studies

RESULT: • Ganciclovir

s.no.	**Drug – Excipient**	**Initial physical state**	**observation (In days)**	
			1st	**7th**
1	Ganciclovir	OWP	N	N
2	Ganciclovir +Ganciclovir	OWP	N	N
3	Ganciclovir + BKC	OWP	N	N

• OWP – off white powder

• N - No change in color and physical state

The stability of the several formulation excipients, the drug substance, and their physical blends was assessed under refrigerated circumstances and at room temperature. During the testing period, there were no detected alterations in their physical attributes. These observations led to the conclusion that the chosen excipients are compatible with the medication.

The compatibility assessment is essential to guarantee the stability and effectiveness of the formulation over its shelf life. It also highlights the appropriateness of the selected excipients for the intended pharmaceutical use.

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Evaluation Parameter

Physico chemical charecterisation.

Table no. 5: Clarity and pH determination

S.no.	Formulation	Clarity	pH
1	F1	CLEAR	7.4
2	F2	CLEAR	7.4
3	F3	CLEAR	7.4
4	F4	CLEAR	7.4

Table no. : 6 Drug content determination

s.no.	Formulation	Concentration of Ganciclovir
1	F1	76.93 +/- 3.24
2	F2	73.01 +/- 0.86
3	F3	83.50 +/- 1.43
4	F4	78.03 +/- 2.41

The result of drug content are (shown in table).

Out of the different batches (F1-F4) of in situ gel formulations that were examined, batch F3 exhibited the highest drug concentration in comparison to the other formulations. The discovery suggests that batch F3 has a higher level of medication content retention, indicating that it may be the most efficient formulation out of the ones that were assessed. This review highlights the significance of conducting batch-specific testing to ensure the consistent and dependable distribution of drugs in pharmaceutical formulations.

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Gelling Capacity: The developed 4 formulation of ocular *in situ* gel formulation resulted in gel formation instantaneously in simulated lacrimal fluid. F1 and F3 shown good gelling capacity then rest formulation.

Table no. 7: gelling capacity

RESULT

s.no.	Formulation	Gelling Capacity
1	F1	++
2	F2	+
3	F3	++
4	F4	+

RESULT:

Table: 8
In vitro permeation studies of different concentrations of Ganciclovir in aqueous solution through excised goat, corneas.

Conc. % (w/v) cm/sec 10^6	Amount permeated (mg) (120 min) hydration * Goat	Permeation (%) (120 min) Papp cm/sec $\times 10^6$ Goat	Goat	Corneal Goat
0.1	**1.006 ± 0.005**	**98**	**80.2 ± 0.582**	**13.81 ± 0.2**
0.2	**1.8±90 0.0012**	**90**	**80.5 ± 0.021**	**11.55 ± 0.1**
0.3	**2.05 ± 0.004**	**68.33**	**80.1 ± 0.187**	**9.15 ± 0.02**
0.4	**2.43±60.75**	**0.006**	**80.4 ± 0.456**	**7.89 ± 0.8**
0.5	**2.73±54.6**	**0.001**	**79.2 ± 0.213**	**7.24 ± 0.12**

The presented values indicate the average ± standard error (SE) calculated from three corneas in each experimental group. Statistical significance ($P < 0.05$) was established through the

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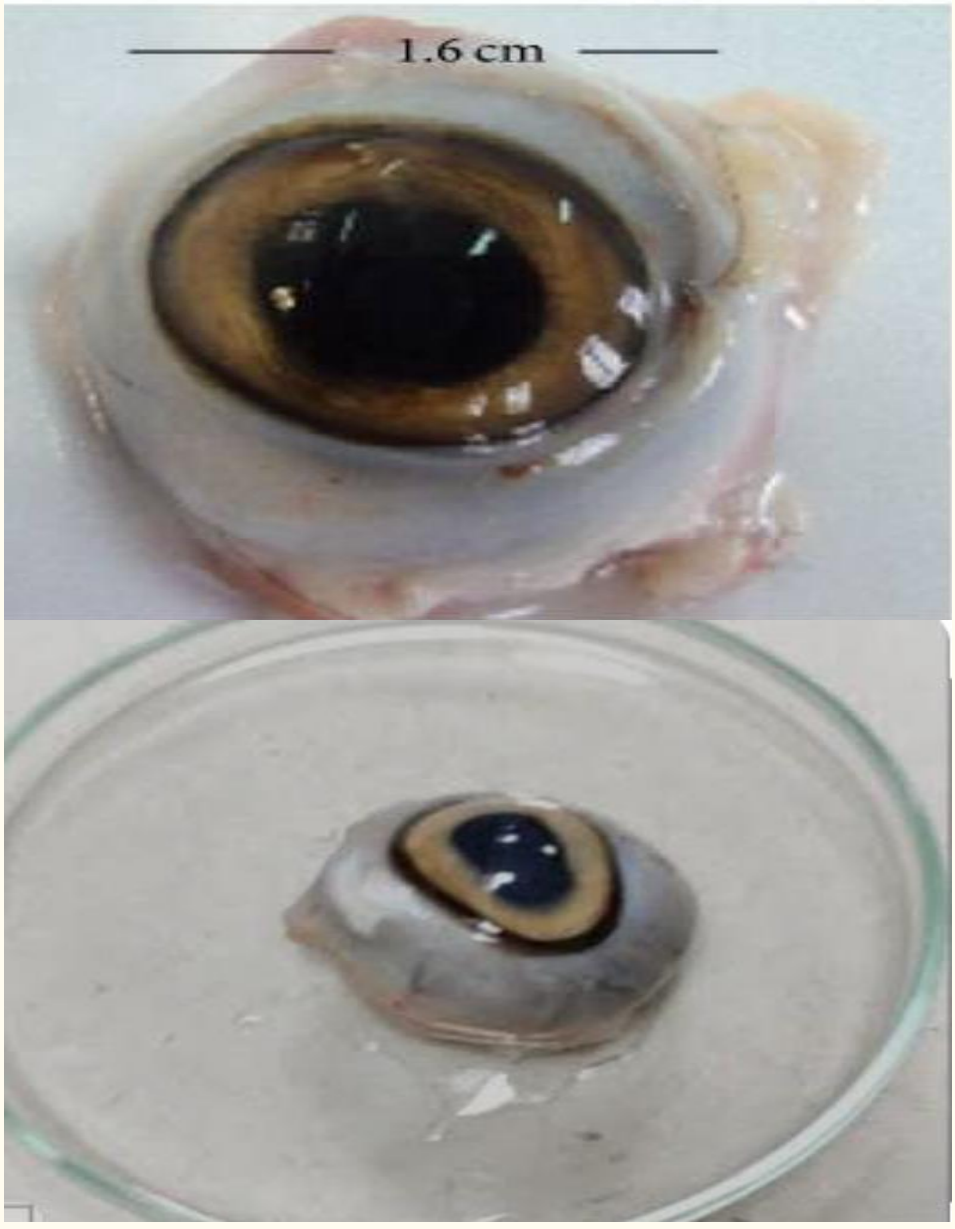
utilisation of one-way analysis of variance (ANOVA), followed by Dunnett's test for subsequent comparisons. All experiments were completed in triplicate to ensure the results' This was done to maintain consistency over numerous trials and enhance the validity of the statistical analyses undertaken. This methodology guarantees that any detected disparities between experimental conditions are statistically valid and capable of being replicated robustness and reproducibility.



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GOAT EYE	GOAT EYE IN PETRIDISH WITH SOLUTION

Table: 8
In vitro permeation studies of 0.5% aqueous solution of GANCICLOVIR from with different preservatives through excised goat cornea.

EX - VIVO DIFFUSION STUDIES

7. Summary &Conclusion: Herpes Simplex Keratitis (HSK), which is a major reason of corneal infection. The virus (Herpes simplex virus) enters into a latent phase. It presents primary infection as conjunctiva and eyelids swelling and mild inflammation. According to global research of disease, it is around 1.4 million, including 38,000 new cases of visual impairment or blindness every year Ganciclovir is an antiviral medication primarily used to treat infections caused by certain types of viruses, particularly cytomegalovirus (CMV). .it was formulated as ocular in situ gel forming eye drop are using polymers such as Guar gum

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