International Journal of
Innovative Drug Discovery

www.ijidd.com

e ISSN 2249 - 7609
Print ISSN 2249 - 7617

GELATIN BASED IN SITU GELS: A NOVEL APPROACH FOR ENHANCED GANCICLOVIR DELIVERY AND IN VITRO EVALUATION

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ABSTRACT

This study aimed to develop and evaluate HPMC-gelatin-based in situ gels for enhanced delivery of Ganciclovir, an antiviral drug commonly used in the treatment of cytomegalovirus (CMV) infections. The formulation strategy focuses on improving the drug's bioavailability and therapeutic efficacy by utilizing in situ gelling systems that provide sustained release and targeted delivery. Hydroxypropyl methylcellulose (HPMC) and gelatin were selected as the primary polymers due to their biocompatibility, gel-forming properties, and ability to enhance drug stability. The in situ gels were prepared by dissolving Ganciclovir in an aqueous solution containing HPMC and gelatin. The formulation parameters, including polymer concentration, pH, and temperature sensitivity, were optimized to ensure appropriate gelation and drug release characteristics. The developed in situ gels were characterized for gelation temperature, viscosity, drug content, and in vitro drug release. The optimized formulation exhibited a gelation temperature of 37°C, suitable for in vivo applications. The viscosity measurements indicated a manageable flow at room temperature and rapid gelation at body temperature. Drug content analysis confirmed uniform distribution of Ganciclovir within the gel matrix. The in situ gels also showed good mucoadhesive properties, enhancing the retention time at the site of application. Cytotoxicity assays on fibroblast cell lines indicated that the HPMC-gelatin gels were non-toxic and biocompatible. Furthermore, antiviral activity tests confirmed the enhanced efficacy of Ganciclovir delivered via the in situ gel system.

KEY WORDS: Ganciclovir, In Situ Gels, Hpmc, Gelatin, Sustained Release, Bioavailability, Antiviral Therapy, Cytomegalovirus.

INTRODUCTION

Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals. Drug delivery technologies modify drug release profile, absorption, distribution and elimination for the benefit of improving product efficacy and safety, as well as patient convenience and compliance. Drug release is from: diffusion, degradation, swelling, and affinity-based mechanisms.[1] Most common routes of administration include the preferred non-invasive peroral (through the mouth), topical (skin), transmucosal (nasal, buccal/sublingual, vaginal, ocular and rectal) and inhalation routes.[2] There are many eye ailments which affected to eye and one can loss the eye sight also. Therefore many ophthalmic drug delivery systems are available. These are classified as conventional and non-conventional (newer) drug delivery systems.[3] Various ophthalmic vehicles such as inserts, ointments, suspensions and aqueous gels have been developed in order to lengthen the residence time of instilled dose and enhance the ophthalmic bioavailability. These systems however have not been used extensively because of some drawbacks such as blurred vision from ointments or low patient compliance from inserts. [4]

Newer research in ophthalmic drug delivery systems is directed towards amalgamation of several drug delivery technologies, that includes to build up systems which not only extend the contact time of the vehicle at the ocular surface, but which at the same time slow down

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theremoval of the drug. [5] There are various new dosage forms like insitu gel, collagen shield, mini disc, ocular film, ocusert, nanosuspension, nanoparticulate system, liposomes, niosomes, dendrimers, ocular iontophoresis etc. [6] Three types of insitu gelling ophthalmic delivery system have been developed on the basis of method employed to cause sol-to-gel phase transition on the eye surface namely pH triggered system, temperature dependent systems and ion activated systems [7].

OCULAR ANATOMY AND PHYSIOLOGY

The human eye is challenging subject for topical administration of the drugs. The basis of this can be found in the anatomical arrangements of surface tissue and in permeability of the cornea. The protective operation of eyelids and lacrimal system are such that there is rapid removal of material instilled into eyes unless the materials are suitably small in volume and chemically and physiologically compatible with surface tissues [8].

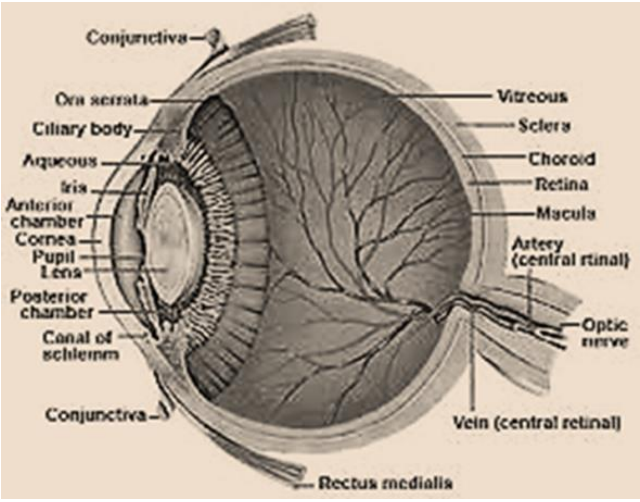

Figure 1: Human eye

The eye is referred to as a globe, is actually two spheres, one set in the other, as shown in fig. 2. The front sphere is the smaller of the two and is bordered anteriorly by the cornea, whereas the larger posterior sphere is an opaque fibrous shell encased by the sclera. The combined weight of both spheres has been given as 6.77-7.5 g. with a volume approximately 6.5 ml. The circumference of the eye is about 75 mm. Along with the rest of the orbital contents; the eye is located within the bony orbital cavity of the head. [9, 10]

Routes of Delivery 11

There are three main routes commonly used for administration of drugs to the eye: topical, intraocular and systemic. The topical route is the most common method to administer a medication to the eye. Introducing the drug directly to the conjunctival sac localizes drug effects, facilitates drug entry that is otherwise hard to achieve with systemic delivery and avoids first pass metabolism. The intraocular route is more difficult to achieve practically. Now research is concentrating on the development of intravitreal injections and use of intraocular implants to improve delivery to eye. In systemic route, several studies have shown that some drugs can distribute into ocular tissues following systemic administration. Oral administration of carbonic anhydrase inhibitors including acetazolamide, methazolamide demonstrates the capacity of a systemic drug to distribute into the cilliary process of eye.

DRUG ABSORPTION

The drug solution instilled as eye drops into the ocular cavity may disappear from the precorneal area of the eye by any or a composite of the following routes.12

- Nasolacrimal drainage
- Tear turnover
- Productive corneal absorption
- Non-productive conjunctiva uptake

Mechanism of drug absorption

Drug administered by instillation must penetrate the eye and do so primarily through the cornea followed by the non-corneal routes. These non-corneal routes involve drug diffusion across the conjunctiva and sclera.

Many ophthalmic drugs are weak bases and are applied to the eye as aqueous solution of their salts. The free base and the salts will be in equilibrium that will depend on the pH and the individual characteristics of the drug molecule. To aid in maintaining storage, stability and solubility, the medication may be acidic at the moment of instillation but usually, the neutralising action of the lacrimal fluid will convert it rapidly to the physiological pH range (PH 7.4) at which there will be enough free base present to begin penetration of the corneal epithelium. Once inside the epithelium (lipid rich) the dissociated free base dissociates immediately to a degree that the dissociated moiety then will tend to penetrate the stroma because it is water-soluble. At the junction of the stroma (lipid poor) and

endothelium (lipid rich), the same process that took place at the outer surface of the epithelium for the aqueous humour. Here it can readily diffuse to the iris and the ciliary body, the site of its pharmacological action.

Various pathways of drug absorption

The main route for intraocular absorption is across the cornea. Two features, which render the cornea an effective barrier to drug absorption, are its small surface area and its relative impermeability. Most effective penetration is obtained with drugs having both lipophilic and hydrophobic properties.[11]

Transcorneal permeation from the lacrimal fluid into the anterior chamber.

- Non-corneal drug permeation across the conjunctiva and sclera into the anterior uvea.
- Drug distribution from the blood stream via blood-aqueous barrier into the anterior chamber.
- Elimination of drug from the anterior chamber by the aqueous humor turnover to the trabecular meshwork and sclemm's canal.
- Drug elimination from the aqueous humor into the systemic circulation across the blood-aqueous barrier.
- Drug distribution from the blood into the posterior eye across the blood-retina barrier.
- Intravitreal drug administration.
- Drug elimination from the vitreous via posterior route across the blood-retina barrier.
- Drug elimination from the vitreous via anterior route to the posterior chamber.[13,14]

Barriers to ocular drug delivery [15]

- Drug loss from the ocular surface
- Lacrimal fluid-eye barriers
- Blood-ocular barriers

Advantages of ocular drug delivery systems [16-18]

- Increased accurate dosing. To overcome the side effects of pulsed dosing produced by conventional delivery.
- To provide sustained and controlled delivery.
- To increase the ocular bioavailability of drug by increasing the corneal contact time.
- To circumvent the protective barriers like drainage, lacrimation and conjuctival absorption.
- To provide targeting within the ocular globe so as to prevent the loss to other ocular tissues.
- To provide comfort, better compliance to the patient and to improve therapeutic performance of drug.

MATERIALS AND METHODS

Chemicals used

Ganciclovir, Gelatin, Hpmc K10, Hpmc K100, Citric Acid, Disodium Hydrogen Phosphate, Sodium Hydroxide, Tween 80, Benzalkonium Chloride, Distilled Water.

Equipment used

UV Spectrophotometer, Hot Air Oven, Melting point apparatus, Laminar air flow, Magnetic stirrer, Auto clave, France diffusion cell.

METHODOLOGY

PREFORMULATION STUDY

a) Identification of pure drug

The spectra was recorded for pure drug, the samples were prepared by the potassium bromide (KBr) disc method. The KBr discs were prepared by compressing the powder. The FT-IR spectra acquired were taken from dried samples. A FT-IR (S h i m dzu-840-os. Japan) was used for the analysis in frequency range between 4000-400 cm-1, with 4cm-1 resolution

b) Drug-polymers compatibility study

Drug polymer compatibility is one of the important parameter to be considered during pre-formulation studies, which can alter the physiochemical properties and bioavailability of the drugs. To develop effective, safe and stable formulation drug excipient compatibility is an important process and it helps in the selection of right excipient. Infrared spectroscopy is a techinique based on the vibrations of the atoms determining what fraction of the incident radiation is absorbed at a particular energy. The energy at which any peak in an absorption spectrum appears corresponds to the frequency of a vibration of a sample molecule.

C) Determination of drug melting point

Determining the melting point of a compound is one way to test if the substance is pure. A pure substance generally has a melting range (the difference between the temperature where the sample starts to melt and the temperature where melting is complete) of one or two degrees. Impurities tend to depress and broaden the melting range so the purified sample should have a higher and smaller melting range than the original, impure sample. Melting point of Ganciclovir was determined by capillary tube method described as follows, Filled a capillary tube with drug crystals about 3mm high. Put the capillary tube (open end down) into the crystals and tapped it on the bottom of the crystallization dish to get the crystals into the tube. Force the crystals to slide to bottom of the tube (open end up) on MEL-TEMP at a high enough level to make a rapid determination of melting point. Observe the melting process through the magnifying lens.

d) Solubility study

An excess amount of Ganciclovir was added to 10ml of aqueous solution of water soluble carriers like gelatine in the various ratios such as 1:1, 1:2 and 1:3 in the

screw capped bottles. Samples were shaken in an orbital shaker for the 24hrs at room temperature. Subsequently, the suspensions were filtered through a Waatmaan filter paper no 1.Filtered solutions were diluted properly with methanol. The diluted solutions were analysed for the Ganciclovir in UV at 293nm

Optimised method for ophthalmic in situ hydrogel preparation

The detailed procedure for preparing the Ganciclovir in situ gel forming system as a pH triggered system is outlined below. Formulation ingredients with their quantities were as given in above table. The buffer salts were dissolved in 75ml of purified water; hydroxypropyl methylcellulose was added and allowed to hydrate overnight. The solution was stirred with in a small quantity of water and benzalkonium chloride (BKC) was added to this solution, the drug solution was added to the polymer solution, the drug solution was added to the polymer solution. Purified water was then added to make up the volume to 100ml, and then the solution was filtered through 0-2mm filter paper.(when the drug solution and polymer solution were mixed immediate precipitation of Gelatin occurred due to the decrease in pH brought about by Gelatin. therefore the drug was incorporated in the sufficient quantity of 0.1M NaOH and then added to the polymer solution to get a clear solution of drug and polymer)

Evaluation of ophthalmic in situ gels

Visual clarity and appearance

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

Determination of pH

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

Determination of drug content

The drug content was determined by diluting 1mL of formulation to 50mL with freshly prepared simulated tear fluid having pH 7.4. An aliquot of 5mL was withdrawn and further diluted to 50mL with simulated tear fluid.Ganciclovir concentration was then determined at 272 nm using a UV-Visible spectrophotometer.

Physical evaluation methods

Shear stress measurement

Various concentrations of mucoadhesive material solutions such as 1%, 2% and 3% w/v are prepared. A drop of the solution is placed in between two plates and a one hundred gram weight was placed on the upper plate to spread the solution in between the plates. After a period of 15 minutes the weight kept on the upper plate is removed. The weights on the pan are gradually increased until the upper plate is detached from the lower plate. The experiment is again performed by prolonging the contact time of the solution between the plates for different intervals. The weight so placed is noted. Similarly, the test is repeated by using the following solutions.

Falling sphere method

A clean burette was taken and filled with 10% mucus solution and fixed in a stainless steel stand. Many mustard grains of equal weights were selected and are grouped. Each group of mustards is coated with the required concentration of the mucoadhesive material. A coated mustard grain is dropped into the burette and the time taken by the mustard to travel from zero to fifth mark is recorded. The same procedure is repeated by using mustards coated with different concentrations of the same mucoadhesive material. The whole set of experiment was performed for all the mucoadhesive materials.

Viscosity and rheological measurements

The rheological properties of solutions and gels were measured using a Brookfield DVIII programmable viscometer. The developed formulation was poured into the small adaptor of Brookfield DVIII programmable viscometer using spindle no.62 and and the angular velocity was increased gradually from 10 and 100rpm. The hierarchy of the angular velocity was reversed. The average of two readings was used to calculate the viscosity. The formulation was them poured into an ointment jar and the pH was raised to 7.4 by adding simulated lachrymal fluid.

In-Vitro dissolution studies

Dissolution testing is an in vitro method that characterizes how an API is extracted out of a solid dosage form. It can indicate the efficiency of in vivo dissolution but does not provide any information on drug substance absorption. Pharmacokinetic data supplements and provides additional information regarding API absorption rate.

Selection of the appropriate in vitro conditions (media and hydrodynamics) that simulate the in vivo conditions can lead to the generation of successful IVIVC or at the very least, in vitro-in vivo relations (IVIVR). Conditions that are optimal for QC purposes may not be applicable for establishing IVIVC so it may be necessary to use two dissolution tests to meet different objectives such as development needs or regulatory demands. 900 ml of phosphate buffer pH (7.4) was used as dissolution media.

The bath temperature as well as bowl temperature was maintained about 37±0.50C and paddle allowed rotating 50 rpm. 5ml of sample was withdrawn at time intervals 5, 10, 15, 20, 25, 30 min and dilution was made to 10ml. 5ml of fresh medium was replaced to dissolution jar. The diluted samples are analyzed spectrophotometrically at 241nm for Ketorolac and their % drug release was calculated.

Kinetics of In-vitro Drug Release

To study the release kinetics of in-vitro drug release, data was applied to kinetic models such as zero order ,first order, Higuchi and Korsmeyer-Peppas models.

RESULTS AND DISCUSSION

Preformulation Studies

Table 1: Solubility study

S.no	Solvent	Solubility
1	chloroform	Poorly soluble
2	Methanol	Slightly soluble
3	Water	Lightly soluble
4	acetone	Less soluble

Melting point:

Melting point of both the drugs complies with the standards, thus indicating the purity of the drug sample that is 2560C

Standard Calibration Curve of Ganciclovir:

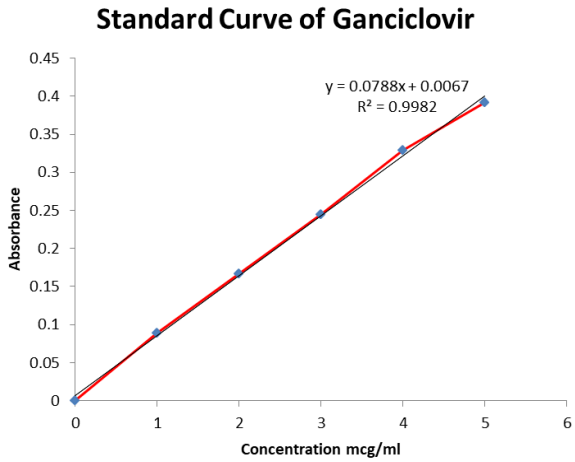

Figure 2: Standard curve of Ganciclovir simulated tear fluid pH 7.4

It was found that the estimation of Ganciclovir by UV Spectrophotometric method at λmax294.0 nm STF pH 7.4 had good reproducibility and this method was used in the study. The correlation coefficient for the standard curve was found to be closer to 1 that is 0.9982, at the concentration range, 1-5 µg/ml. The regression equation generated was y = 0.0806x.

FTIR compatibility Studies

The IR spectrum of Ganciclovir shown in Figure, reveals characteristic peaks in the Ganciclovir IR spectrum that occur at 695.84 cm-1 for the Aromatic -C-H Bending,1720.41 cm-1 for the C=O stretching, 1299.37 cm-1for the C-N stretching, 2779.22 cm-1 for the O-H stretching respectively. The peaks obtained in physical mixture spectrum matches with the peaks obtained in the spectrum of Ganciclovir, spectrum of Ganciclovir, as shown in Table 6.2. Therefore it can be concluded that there is no interaction of drugs with excipients. No considerable changes has been observed in vials containing drug excipients blend when kept for one month and two months study at 40oC.

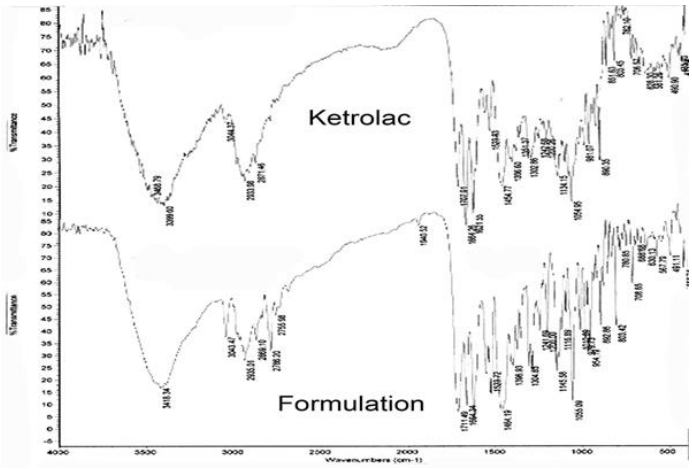

Figure 3: FTIR Spectra of Drug and Formulation

Table 2: pH, Gelling capacity and other visual parameters of the formulations

Formulation code	pH	Gelling capacity at 25^0C	Gelling capacity at 37^0C	Appearance	Clarity
KF1	6.25	-	++	Light yellow	Clear
KF2	6.09	-	+	Light yellow	Clear
KF3	6.19	-	+++	Light yellow	Clear
KF4	6.18	-	+++	Light yellow	Clear
KF5	6.24	-	++	Light yellow	Clear
KF6	6.37	-	+	Light yellow	Clear

-: No gelation; +: gels after few min and dissolves rapidly; ++: gelation immediate, remains for few hrs; +++: gelation immediate, remains for extend period

Table 3: Viscosity and % drug content of the formulations

Formulation code	Viscosity at different RPM (cps)			Drug content %
	10	25	50	
KF1	109	94	74	98.92
KF2	139	113	96	98.37
KF3	159	133	119	98.98
KF4	140	119	101	99.19
KF5	152	150	117	98.49
KF6	186	160	135	98.89

Viscosity was determined for all formulations at different rpm and results were shown in Table 3.The gels are typically pseudoplastic, exhibiting non-newtonian flow (shear thinning) characterized by decreasing viscosity with increasing the shear rate.

In-vitro Drug Release Studies from in-situ gels:

The Gelatin resins are hydrophilic substances that are not soluble in water. Rather, these polymers swell when dispersed in water forming a colloidal, mucilage-like dispersion. Gelatin readily absorbs water, get hydrated and swell. In addition to its hydrophilic nature, its cross-linked structure and although they are described as soluble gelatin do not dissolve but merely swell to a remarkable extent this makes Gelatin a potential candidate for use in controlled release drug delivery system.

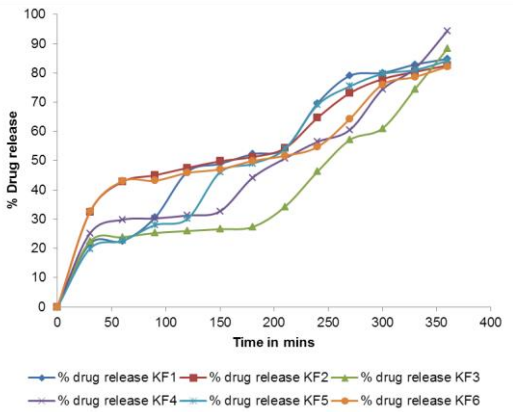

Figure 4: Invitro drug release profiles of prepared gel formulations

In vitro diffusion study for all formulations was carried out using fabricated dissolution testing apparatus. The percentage cumulative release of Ganciclovir from formulations KF1-KF6 was found to be in between 84 to 94%. The results were shown in table. The comparison of all formulation KF4 gives higher release, because of decreased the polymer concentration to increase the release the rate

Table 5: Data of various parameters of model fitting of optimized formulation

Drug	Zero Order R^2	Korsmeyer- Peppas		Higuchi R^2	First order R^2	Hixson- Crowell R^2
		n	R^2			
Ganciclovir KF4	**0.9882**	**0.8320**		**0.9990**	**0.9831**	**0.8879**

CONCLUSION

In the present work, an attempt has been made to develop ophthalmic in-situ gel of Ganciclovir. The IR spectra revealed that, there was no interaction between drugs and polymers. All polymers used were compatible with both the drugs. Simple mixing method that is cooling technique was employed to formulate the opthalamic in-situ gels. The evaluation parameters of the in-situ gel like gel pH, gel capacity, clarity, drug content, viscosity and in-vitro drug release studies were carried out. All the parameters were found to be within the limits.

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