Preparation And Evaluation Of Valacyclovir Hydrochloride Ocular Inserts By Hot Melt Extrusion Technique

Gauri Shadambikar

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PREPARATION AND EVALUATION OF VALACYCLOVIR HYDROCHLORIDE OCULAR INSERTS BY HOT MELT EXTRUSION TECHNIQUE

A thesis

Presented in partial fulfilment of requirements

For the degree of Master of Pharmaceutical Science

In the department of Pharmaceutics and Drug Delivery

The University of Mississippi

By

Gauri Shadambikar

May 2018
ABSTRACT

The objective of the current study was to investigate the processability of Hot Melt Extrusion to formulate Ocular Inserts of Valacyclovir Hydrochloride and Evaluate its drug release \textit{in vitro} and \textit{in vivo}. Valacyclovir is the prodrug of Acyclovir, which is used in treatment of ocular keratitis and HSV infections. The bioavailability of the prodrug is better than the acyclovir and it is proved to permeate extensively through the corneal barrier. The polymer of choice was Klucel\textsuperscript{®} Pharm MF and JF grades for the study. To optimize the formulation, different physical mixtures of the polymers and plasticizers (PEG 400 and Propylene glycol) were prepared. The physical mixture was extruded through co – rotating twin-screw Haake MiniLab ThermoFisher \textsuperscript{®} Scientific extruder. The obtained ocular inserts were cut into dimensions of 4mm×2mm×1mm to have suitable product instillation in the eye. Ocular inserts were evaluated for drug content, weight variation, uniformity of thickness and \textit{in vitro} and \textit{in vivo} drug release. The thermal characterization of ocular inserts was observed using Differential Scanning Calorimetry (DSC). Morphology of the ocular inserts was observed using Scanning Electron Microscopy (SEM). The polymer interactions or incompatibilities was assessed using FTIR. Klucel \textsuperscript{®} Pharm MF exhibited desired results than Klucel \textsuperscript{®} Pharm JF and was easy to extrude to prepare ocular inserts. The ocular inserts were able to provide sustained drug release compared to eyedrop solution and then dissolve completely in 9 hours.
DEDICATION

I dedicate my thesis to my wonderful family. I am especially grateful to my loving parents, Deepak and Anjali Shadambikar, who provided encouragement and pushed me to do my best. They have been a source of strength throughout this program. I am also grateful to my brother, Vishwesh; to all my friends Supriya, Sushrut, Pranav, Rohit, Siddhi and Abhishek. My love for all of you can never be quantified.
ACKNOWLEDGEMENT

I wish to thank my committee members, who were more than generous with their expertise and precious time. Special thanks to Dr. Michael Repka my advisor, for his encouragement throughout the entire process. Also, thanks to Dr. Soumyajit Majumdar for his countless hours of explaining and helping me with animal studies. Dr. Eman Ashour for guiding me throughout the project. Also, thankful for agreeing to serve on my committee.

I would like to acknowledge and thank my postdoc Dr. Bandari for helping in conducting my research. Special thanks to Miss. King for her continued support.

I thank my lab mates Vo and Sandeep for helping me with all the process equipment. My friends for discussing my project results, advising me on my experiments and for all the fun we had in last two years.

Last but not least, I would like to thank my parents for nurturing and supporting me throughout my life.
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CHAPTER I

INTRODUCTION
The application of therapeutic agents directly to the site of action ensures that the therapeutic agent is available in high concentration than achieved by oral route of administration. Eye drops usually provide low bioavailability due to effective eye protective mechanism occurring from reflex blinking\(^1\). This reduces pre-corneal residence time and thus reduces absorption to as low as 1% of the administered drug\(^2\). To maintain the bioavailability, eye drops need to be instilled often giving low patient compliance. Eye ointments and gels give sensation of being sticky and often cause blurring of the eye\(^3\).

Ocular inserts are small, flat, solid or semi-solid polymeric devices meant to be placed in the lower fornix to deliver drugs to the corneal surface\(^4\). The potential advantages provided by the ocular inserts is slow release of drug, increase in ocular residence time of the drug, reduced repeated administration of the medication leading to better patient compliance.

Ocular Inserts are classified based upon their solubility into three types: Soluble, Insoluble and Bioerodible\(^5\).

Soluble ocular inserts are the oldest type and have the potential advantage of being completely soluble and thus limiting intervention to administration only\(^6\). Soft contact lenses is a type of insoluble ocular inserts\(^7\). In bio-erodible ocular inserts the drug is dispersed in the hydrophobic matrix of bio-erodible polymers. The drug from this system is released when the insert is in contact with the tear fluid\(^8\).

Advantages of using ocular inserts over the conventional delivery systems are increased ocular residence, accurate dosing of the drug, sustained release of drugs, better
patient compliance and on-site action of the drug. The main advantage of using ocular inserts is increased contact between the conjunctival tissue and drug$^9$.

Hot Melt Extrusion (HME) offers many advantages to other pharmaceutical processing techniques. In extrusion process the polymers act as drug depots and/or drug release retardants upon solidification. Solvents and water are not necessary thereby reducing the number of processing steps and eliminating time-consuming drying steps. The intense mixing and agitation imposed by the rotating screw cause de-aggregation of suspended particles in the molten polymer resulting in a more uniform dispersion and the process is continuous and efficient$^{10}$. It is a promising technology to produce new drug delivery systems and for improving products already on the market. HME is a continuous pharmaceutical process that involves pumping polymeric materials with a rotating screw at temperatures above their glass transition temperature ($T_g$) and sometimes above the melting temperature ($T_m$) to achieve molecular level mixing of the active compounds and thermoplastic binders, polymers, or both$^{11}$.

Hydroxypropyl Cellulose (HPC) is a derivative of cellulose and has solubility in organic solvents and water. Often used as an excipient in pharmaceutical products. HPC is used in ophthalmic formulations as a lubricant$^{12}$. Marketed preparation Lacrisert is formulated using HPC which helps in conditions of dry eyes, corneal erosion and corneal sensitivity and stimulates artificial tears$^{13}$.

Klucel ® HPC grades (JF Pharm and MF Pharm) are used as controlled release matrix.
They have low glass transition and thus they are easy to extrude. The extrudates obtained from Klucel® are less porous and hence help in control release of drug14.

Valacyclovir hydrochloride, a prodrug of Acyclovir is used in the treatment of ocular herpes15. Herpes simplex virus can affect almost any part of the eye. If the infection, occurs only on the top of the cornea it is referred to as epithelial keratitis. It usually transmits through contact of the infected person16. Approach of prodrug is commonly used to transport drugs through the biological membranes.

![Structure of Valacyclovir Hydrochloride](image)

**Figure 1: Structure of Valacyclovir Hydrochloride**

Valacyclovir hydrolyses to form Acyclovir and L-valine. The viral thymidine kinase converts acyclovir to acyclovir monophosphate, which then by the host cell kinases is converted into acyclovir triphosphate. It is acyclovir triphosphate that inactivates and inhibits HSV specific DNA polymerases which prevents the viral DNA synthesis further without affecting the regular cellular processes17.

It is observed that the ocular penetration of the prodrug Valacyclovir hydrochloride is superior than the Acyclovir in the rabbit eye by carrier mediated transport system18. It is also known that Valacyclovir has affinity for peptide transporters which could be the reason for enhanced penetration than Acyclovir.
The main purpose of preparing ocular inserts of Valacyclovir Hydrochloride was to treat corneal keratitis with site specific drug delivery system.

Corneal Keratitis is condition were the cornea is inflamed. This condition has many causes, including chemical and physical injury, dry eyes and it can be caused by herpes simplex virus infection which is named as dendritic ulcer\textsuperscript{19}. To treat this condition, site specific drug release i.e. release of drug at the cornea is necessary. If the keratitis involves only the epithelial surface of the cornea it is called as superficial keratitis\textsuperscript{20}. This can be achieved by using ocular inserts as they would be directly in contact with the cornea after the administration.

Thus, it was attempted to prepare long acting ocular inserts of Valacyclovir hydrochloride using Hot Melt Extrusion Technique.
CHAPTER II

METHODOLOGY
**Materials**

Acyclovir was purchased from Ria International, New Jersey. Klucel® MF and JF Pharm, Methocel® were obtained as gift samples from Ashland and Colorcon Inc., respectively. All other reagents used were of Analytical Grade.

**Animal and Animal Tissue**

Complete eyes of male albino New Zealand rabbits were acquired from PelFreez Biologicals (Rogers, AR, USA). Male albino New Zealand rabbits weighing 2 to 3 kg were procured from Envigo (Indianapolis, IN, USA). All the in vivo studies were carried out in accordance with the University of Mississippi Institutional Animal Care and Use Committee approved protocols (Protocol #17-018).

**Pre-formulation**

Polymer for trials, Klucel® MF Pharm, Klucel® JF Pharm (Hydroxypropyl cellulose), Methocel® K4M, Methocel® K100M (Hydroxypropyl methylcellulose) were observed in Differential Scanning Calorimetric for their thermal analysis. The polymers were heated from 25°C to 200°C in nitrogen environment at heating rate of 10°C/min in aluminum sealed pans.

These plain polymers were extruded on the twin screw counter rotating hot melt extruder (Haake Minilab II, ThermoFisher Scientific). Plasticizers that are non-irritant to the eye such as Propylene Glycol, PEG 400 were tried during the trials (Table 1). During Extrusion a die of dimension 2mm×4mm× 1mm was used to have suitable size for the formulation instillation in the
eye. HPMC K100M was not easy to extrude and HPMC K4M extrudates obtained were brownish in color which could possibly be due to degradation of the polymer. The polymer of choice for ocular inserts was Klucel® MF Pharm. Different processing temperatures had varying effect on the appearance of the inserts.

Each insert contained 1 mg of Valacyclovir Hydrochloride. The dose of the ocular insert was calculated according to the marketed acyclovir formulation which is available as 3% w/w eye ointment. The acyclovir eye ointment regime was followed during the experiment.

**Method of Preparation of Ocular Inserts**

Valacyclovir Hydrochloride and polymer (Table 1) were sieved through mesh #60 and mixed with the plasticizer. The physical mixture was mixed together on mortar and pestle for 6-7 minutes and then blended again in the MaxiblendGlobe Pharma at 25 rpm for 10 minutes. The blend was fed through the hopper on the rotating screws with constant feeding rate of 1 gm per minute; with the screw speed of 50 rpm. The mixture takes around 7 minutes to form molten mass inside the barrel and form extrudates. The extrudates were cut into desired size (4mm × 2mm × 1mm), sterilized by UV radiation and packed into aluminum foils.
<table>
<thead>
<tr>
<th>Formulation</th>
<th>Polymer (g)</th>
<th>API (g)</th>
<th>Plasticizer (ml)</th>
<th>Temperature (⁰C) / (Screw speed of 50 rpm for all)</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Klucel® MF</td>
<td>Klucel® JF</td>
<td>PEG 400</td>
<td>PG</td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>8.5</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>F2</td>
<td>8.5</td>
<td>-</td>
<td>1</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>F3</td>
<td>7.5</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>F4</td>
<td>-</td>
<td>8.5</td>
<td>1</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>F5</td>
<td>-</td>
<td>7.5</td>
<td>2</td>
<td>0.5</td>
<td>-</td>
</tr>
</tbody>
</table>
CHAPTER III

CHARACTERIZATION OF OCCULAR INSERTS
Evaluation of Ocular Inserts

The inserts were evaluated for appearance, weight variation, uniformity of thickness, swelling index, drug content, surface pH, Thermal analysis (DSC), sterility, in vitro release, trans-corneal permeation and in vivo release. The surface of the inserts was observed using Scanning Electron Microscope (SEM). The drug polymer interactions were assessed using Fourier-Transform Infrared Spectroscopy (FTIR).

Physical Characterization

The surface of the ocular inserts was observed for color, surface smoothness, bubbles on the surface. Nine individual ocular inserts were weighed, and deviation was calculated. Surface uniformity of nine ocular inserts was calculated using digital Caliper. The uniformity was checked by placing the caliper on two different points of the ocular insert.

Insert was immersed in 5 ml of water for 10 minutes and then pH was checked using calibrated pH meter (Mettler Toledo, USA)\textsuperscript{21}.

Drug Content

Six inserts were weighed and dissolved in 50 ml of Methanol, dissolved by sonication and the absorbance of this solution was checked in UV Spectrophotometer at 254 nm. The results were extrapolated from the standard curve to determine the drug content.
Swelling Index

To determine the swelling index of the inserts, each insert was weighed initially and then placed into freshly prepared Isotonic Phosphate Buffer Saline (pH 7.4) at 37°C. The insert was removed at time intervals of 5, 10, 15, 20, 25, 30, 35 minutes, excess media was wiped using filter paper and the insert was reweighed. The swelling index was calculated as:

\[
SI(\%) = \frac{\text{Weight of Swollen Insert} - \text{Initial Weight of the Ocular Insert}}{\text{Initial Weight of the Ocular Insert}} \times 100
\]

The swelling index was carried out on nine ocular inserts from the batch.

Differential Scanning Calorimetry

DSC was carried out to check the thermal behavior of the API, Polymer and inserts under different conditions. The instrument used for analysis was TA Instruments Discovery Differential Scanning Calorimetric, Delaware. Sample was heated from 25°C to 200°C at a heating rate of 10°C/min under nitrogen environment (50 ml/min). Each time around 4-5 mg of sample was weighed in the aluminum pans and hermetically sealed. Empty pan was used as a reference and each DSC analysis was measured for three times.

Sterilization of the Inserts

The inserts were sterilized under UV Radiation for 30 minutes and the sterility test was conducted in aseptic conditions. Sodium Thioglycolate medium was used to check the sterility
of the inserts. Both positive control (growth promotion) and negative control (sterile) test were also carried out. The inserts after sterilization were placed into the sterile medium and then incubated for 48 hours\textsuperscript{24}. The unsterilized insert was also checked for sterility by placing it into the medium. The sterilized inserts were also checked for change in appearance, drug content and thermal analysis.

Preparation of Sodium Thioglycolate medium- 7.54 g of media was suspended into 200 ml of sterile distilled water and boiled till the media was completely dissolved. The media was distributed into 20 test tubes and sterilized by autoclaving at 121\textdegree C for 15 minutes\textsuperscript{25}.

\textbf{In vitro Release Study}

There is no specified official \textit{in vitro} test for the drug release from ocular formulations. Thus, a modified reported method was utilized in this study to check the release pattern for the inserts. Each weighed insert was placed in scintillation glass vial containing 10 ml freshly prepared Isotonic Phosphate Buffer Saline. The vials were placed in water bath shaker at 37 ± 2\textdegree C maintained at speed of 50 strokes / minute\textsuperscript{26}. Sample of 1 ml was withdrawn at each hour for up to 9 hours. With each withdrawn sample, the vial was replaced with same quantity of fresh medium. The samples were analyzed with UV spectroscopy at 254 nm against the blank to determine the amount of drug released from the inserts. The release study was carried out in triplicate.
Release Kinetics

The *in vitro* drug release kinetics were characterized by fitting the release study data obtained from different formulations in drug release kinetics equation (zero order), Higuchi, Korsmeyer- Peppas model \((M_t/M_\infty < 0.5)^2\). Goodness of fit for *in vitro* drug release was evaluated by regression coefficient \((R^2)\) value.

Ex Vivo Transcorneal Permeation Study

The eyes of freshly slaughtered young rabbits were obtained from PelFreez, Arkansas USA. The permeation study was performed using Valia-chein ® (Permi Gear) cells. Freshly excised rabbit cornea was placed between the donor and receptor compartments clamped together in such a way that the epithelial surface faced the donor compartment. The receptor compartment was filled with 5 ml of freshly prepared Isotonic Phosphate Buffer Saline and the donor compartment had the insert. The opening of the donor compartment and the receptor compartment was sealed using paraffin wax paper. The apparatus was maintained at 34 ± 2⁰ C with continuous stirring using magnetic stirrers. Due to difference in formulation concentration on the both sides of cell, a pressure difference is created across the corneal membrane. Because of this pressure difference the cornea bulges out towards the cell with less amount of formulation and thus mimicking the natural curvature of the cornea. The total corneal area available for the diffusion was 0.64 cm². The test was carried out for 4 hours. Sample was withdrawn at each hour from the receptor compartment and replaced using same quantity of the medium. The samples were analyzed using HPLC- UV at 254 nm using a
Phenomenex Luna® 5µm C18 100 Å 250 × 4.60 mm column with a flow rate of 0.75 ml/min. The mobile phase used was Methanol: Water: Glacial acetic acid (1:9:0.5 v/v).

**In vivo Bioavailability Study**

Selected formulation of ocular inserts was sterilized by UV radiation and used for *in vivo* drug release study. Eye drop formulation of Valacyclovir Hydrochloride was used as control. The protocol on the use of animals was approved by The Institutional Animal Care and Use Committee (IACUC). The *in vivo* bioavailability of Acyclovir from the prodrug Valacyclovir Hydrochloride was determined in Male New Zealand White Albino Rabbits weighing around 2-2.5 kg. The ocular inserts were placed in the lower fornix of the right eye in the rabbits. At the end, of two and six hours the rabbits were anesthetized using a combination of ketamine (35 mg/kg) and xylazine (3.5 mg/kg) injected intramuscularly. The anesthetized rabbits were euthanized by an overdose of pentobarbital injected through the marginal ear vein. The eye was enucleated and washed with DPBS. The tissues were separated for further analysis. All formulations were tested in triplicate.

**Bioanalytical Method for In Vivo Samples**

**Standard solution preparation**

To 190 µl of Aqueous Humor (AH) or to the weighed quantity of cornea, 10 µl of the standard solution was added and vortexed. To precipitate the proteins 600 µl of ice cold
methanol was added. Samples were centrifuged at 13,000 rpm for 30 minutes. The supernatant was analysed using HPLC-UV. Final concentrations of the standard solutions were in the range of 10-400 ng/ml.

The standard curve generated had $R^2$ greater than 0.94.

**Analytical Method for Acyclovir**

Acyclovir was analyzed using Waters HPLC system with 600 E pump controller, 717 plus auto sampler and 2486 UV detector. The mobile phase consisted of Acetonitrile: Water (1:9 v/v) with Phenomenex Luna® 5μm C18 100 Å 250 × 4.60 mm column with a flow rate of 0.75 ml/min. The detection wavelength was set at 254 nm. 20 μl of each sample was spiked into the column.

The assay was linear ($r^2= 0.99$) with the lowest detection limit at 10ng/ml.

**Analytical Method for Valacyclovir Hydrochloride**

VH was analyzed using Waters HPLC system with 600 E pump controller, 717 plus auto sampler and 2486 UV detector. The mobile phase consisted of Methanol: Water: Glacial Acetic Acid (1:9:0.5 v/v) with Phenomenex Luna® 5μm C18 100 Å 250 × 4.60 mm column with a flow rate of 0.75 ml/min. The detection wavelength was set at 254 nm. 20 μl of each sample was spiked into the column. The assay was linear ($r^2 = 0.98$) with the lowest detection limit at 10 ng/ml.
**Stastical Analysis**

The \textit{in vivo} bioavailability results were presented in their mean values ± standard deviation (SD). Stastical analysis for the \textit{in vivo} drug bioavailability study in ocular tissue was done by unpaired t-test (Graph Pad 5 Prism®, software USA). The confidence interval set for the statistical analysis was 95% and hence the p-value for the significant difference should be less than 0.05.

**Drug – Polymer Interactions**

It is an essential factor to determine the compatibility between the drug and polymer in a polymeric delivery system. The FTIR spectra was recorded using Agilent Technologies Cary 660 (Santa Clara, CA.). The measurements were performed in the scanning range of 4000 to 600 at ambient temperature.

**Scanning Electron Microscope**

The inserts were mounted on the aluminum stubs using glued carbon tabs and then sputter coated for 120s with gold using Hummer 6.2 sputter coated (Anatech USA, Union City, CA). During the process, the gas pressure was about 100 mTorr and the current was 15 mA. The morphology of the prepared inserts was observed using JSM-5600 Scanning Electron Microscope (JEOL USA Inc.) at an accelerating voltage of 5kV.
Stability study

A short-term stability study of 30 days was carried out at 25°C ± 2°C and 60 % RH. The stability samples were checked for appearance, drug content and thermal analysis. The thermal analysis parameter was observed in DSC. The sample was heated from 25°C to 200°C at a heating rate of 10°C/min in hermetically sealed aluminum pans in nitrogen environment at a flow rate of 50 ml/min.
CHAPTER IV

RESULTS AND DISCUSSION
Physical Characterization

Table 2: Physicochemical characteristics of Valacyclovir Hydrochloride ocular inserts

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Weight (mg) ± SD</th>
<th>Thickness (mm) ±SD</th>
<th>pH ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>5.29 ± 0.72</td>
<td>1.00 ± 0.038</td>
<td>7.02 ± 0.04</td>
</tr>
<tr>
<td>F2</td>
<td>5.69 ± 1.02</td>
<td>1.08 ± 0.12</td>
<td>7.04 ± 0.03</td>
</tr>
<tr>
<td>F3</td>
<td>4.98 ± 0.91</td>
<td>0.99 ± 0.056</td>
<td>7.01 ± 0.09</td>
</tr>
<tr>
<td>F4</td>
<td>5.19 ± 0.69</td>
<td>1.04 ± 0.084</td>
<td>6.78 ± 0.1</td>
</tr>
<tr>
<td>F5</td>
<td>4.97 ± 0.86</td>
<td>0.98 ± 0.041</td>
<td>6.99 ± 0.01</td>
</tr>
</tbody>
</table>

The above five formulations were evaluated for weight variation, uniformity of thickness and pH. The weight of the ocular inserts observed in all formulations was between 4.97 mg to 5.69 mg. The expected thickness for the ocular inserts was 1 mm, which was observed in F1 with minimum standard deviation. The pH of ocular inserts needs to be neutral to have less irritation and redness. The optimum pH was seen in F1 and F3 with minimum standard deviation.
Drug Content

Table 3: Drug Content of Ocular Inserts

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Drug Content (mg) ± SD</th>
<th>Drug Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>5.97 ± 0.053</td>
<td>99.5</td>
</tr>
<tr>
<td>F2</td>
<td>5.57 ± 0.128</td>
<td>92.95</td>
</tr>
<tr>
<td>F3</td>
<td>6.32 ± 0.195</td>
<td>105.38</td>
</tr>
<tr>
<td>F4</td>
<td>5.39 ± 0.13</td>
<td>89.90</td>
</tr>
<tr>
<td>F5</td>
<td>6.83 ± 0.14</td>
<td>113.91</td>
</tr>
</tbody>
</table>

The drug content of F3 and F5 was observed to be more than 100 %. Formulation F1 and F2 had the optimum amount of drug content. The ocular inserts drug content was carried out for n=3.

Differential Scanning Calorimetry

The melting point of Valacyclovir Hydrochloride was obtained as 129 °C which is similar to the melting point from literature. The glass transition of the pure polymer Klucel ® MF and JF grade started from around 90-95 °C and 100-110 °C respectively. The DSC studies confirm there is no degradation of the drug up to 140 °C in the physical mixture which is the maximum extrusion temperature for all the formulations.
Figure 2: DSC overlay of API, Pure Polymer (Klucel MF and JF)

Swelling Index

Water absorption and swelling increases with time due to polymer hydrophilicity. The integrity of matrix and swelling process is greatly influenced by the viscosity of the polymer. The inserts do not break after the process of swelling and get hydrated quickly, reaching around 40% of hydration in 5 minutes. The polymer swelling is necessary to initiate the bio adhesive character to the inserts. For the F1, the swelling of the insert reaches around 40% of hydration in 5 minutes. The...
F2 show similar trend of swelling as of F1. The formulations F4 and F5, lose their matrix integrity after a period of 15 minutes. The swelling index of these formulations was difficult to evaluate as the ocular inserts broke into parts after reaching maximum hydration at around 15 minutes. This could be due to the faster polymer hydration of Klucel Pharm JF. It was observed that F3 had similar trend as of F1 and F2, but it reached its maximum hydration at 15 minutes which could be because of less polymer concentration in the ocular inserts.

Figure 3: Swelling Index of Ocular Inserts
**In Vitro Release study**

It was observed that formulations, F4 and F5 did not show good swelling index as they could not withstand their polymer matrix. Formulation F3 had high drug content, hence it was not evaluated further. The *in vitro* release study was carried out on F1 and F2.

It was observed that the ocular inserts from formulations F1 and F2, released around 98% of drug in 8 hours. The inserts dissolved completely in 9 hours.

![In vitro Drug release from Ocular Insert](image)

**Figure 4**: In vitro Drug release from Ocular Insert
Release Kinetics

Table 4: Release Kinetics from F1 and F2 formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>ZeroOrderPlot $R^2$</th>
<th>Higuchi Plot $R^2$</th>
<th>Peppa’s Plot $R^2$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.949</td>
<td>0.9892</td>
<td>0.9732</td>
<td>0.50</td>
</tr>
<tr>
<td>F2</td>
<td>0.8916</td>
<td>0.9876</td>
<td>0.9619</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Formulation F1 was best fitted in zero order equation. Higuchi Plot was observed for both the formulations. This proved that the drug release from the ocular inserts is by diffusion mechanism. The ‘n’ value for Peppas plot was less than 0.5, hence it was Non-Fickian.

Ex Vivo Transcorneal Permeation

From the above formulations, only F1 and F2 were tried for transcorneal permeation.

The plot of cumulative amount of drug permeated through the cornea versus the time was used to obtain the rate of transport through the rabbit cornea. The steady state flux was calculated from the rate of transport of drug per unit surface area (equation 1). The permeability was calculated by dividing the steady state flux with initial drug concentration in the donor compartment (equation 2).
Flux = \frac{(dM/dt)}{A} \quad \text{Equation 1}

Where \( dM \) is the cumulative amount of drug that transported across the cornea, \( A \) is the total corneal surface area \((0.64 \text{ cm}^2)\).

Permeability = \frac{\text{Flux}}{C_i} \quad \text{Equation 2}

\( C_i \) is the initial drug concentration in the donor cell.

All the permeation studies were carried out in triplicates and the data is expressed as mean value ± SD.

The transcorneal flux for the ocular inserts F1 and F2 was found to be 0.314 ± 0.019 \( \mu g/\text{min/cm}^2 \) and 0.292 ± 0.016 \( \mu g/\text{min/cm}^2 \) respectively. For the control eyedrop formulation it was 0.662 ± 0.088 \( \mu g/\text{min/cm}^2 \). The flux for the control formulation was observed to be two folds more than the ocular inserts.

The permeability of the control formulation, F1 and F2 was found to be 11.033 ± 1.459, 5.229 ± 0.316 and 4.89 ± 0.521 \( \times 10^{-3} \text{cm/s} \) respectively.

We can conclude that the flux and permeability of control formulation i.e. Valacyclovir Hydrochloride in sterile water is more than the ocular inserts as it provides immediate release for short period of time. It can also lead to toxicity of the eye tissue as the drug permeation is more.
Whereas, ocular inserts on other hand provide sustained release and reduce the chances of toxicity which could be possible because of more drug permeation.

Figure 5: Transcorneal Flux across the rabbit cornea for Ocular Inserts and Control Formulation

Figure 6: Transcorneal Permeability through Rabbit cornea for Control and Ocular
Sterilization Study

Figure 7 : A- Before Incubation   B- After Incubation of 24 hours

The microbial growth in positive control was compared with the test tubes containing sterilized and unsterilized ocular inserts. No microbial growth was seen in the sterilized and unsterilized ocular inserts test tubes. Hence, we can say that the ocular inserts are sterile.

In Vivo Bioavailability Study

Based on the results of physicochemical characterization, \textit{in vitro} drug release and transcorneal permeation, F1 was the best optimized formulation for the ocular inserts. To evaluate the ocular bioavailability, F1 and control were administered in conscious rabbits.

Approximately 0.2 ml of AH was collected from each test eye into individual centrifugal vials. Cornea was collected and weighed. Small pieces of cornea were cut and placed into the individual centrifugal vials. Protein precipitation of the collected samples was carried out similar to the standard solution samples of bioanalytical method. The supernatant of the samples was analyzed using HPLC-UV.
It was expected that the Valacyclovir Hydrochloride gets converted into the active drug Acyclovir by esterification of the prodrug post administration in the eye. After 2 hours of administration of the formulations, detectable quantities of Acyclovir were observed in Aqueous Humor and Cornea. The amount of Acyclovir was more in Control than the ocular insert. It was 123.19 ± 12.01 ng/ml for ocular insert and 279.39 ± 29.799 ng/ml for ocular. The permeation of control formulation through the cornea as compared to the ocular insert was observed to be more even during the transcorneal studies. The corneal concentration in 2 hours was assessed to be 0.95 ± 0.14 ng/ml for ocular inserts. The control formulation was observed to be 1.5202 ± 0.4 ng/ml in concentration for 2 hours.

Similarly, the rabbits were euthanized after 6 hours of administration. The corneal and Aqueous Humor samples were collected for analysis. The concentrations of control formulation for cornea and aqueous humor were 0.830 ± 0.02 ng/mg and 223.159 ± 14.39 ng/ml respectively. It was observed that ocular inserts provided much sustained permeation and release of the drug than the control. The corneal and aqueous humor concentrations were observed to be increased at 6 hours, time point. The concentration in cornea and aqueous humor was 1.247 ± 0.067 ng/mg and 295.64 ± 14.60 ng/ml respectively.

The control and ocular inserts delivered the drug significantly to the cornea and aqueous humor. The ocular inserts provided sustained release which increased with time. The control on other hand had burst release in first stage and then reduced with time.
Also, during the trials in the rabbits, it was difficult to administer (50 μl) eyedrop formulation. Most of the formulation was lost due to rapid blinking by the rabbits. The ocular inserts administration was in the lower fornix of the rabbit eye, which due to swelling and bioadhesive properties retained its place in the lower fornix. No irritation was observed in the eye after ocular inserts insertion by rabbit movements.

**Figure 8 : In Vivo Drug Release of Acyclovir from Ocular Inserts and Control in Aqueous Humor.** (p-value for 120 min = 0.0011, p-value for 360 min = 0.0036)

**Figure 9 : In Vivo Drug Release of Acyclovir from Ocular Inserts and Control in Cornea**

(p-value for 120 min = 0.0829, p-value for 360 min = 0.0005)
Figure 10: Rabbit eye while placing the ocular insert in the lower fornix

Figure 11: Rabbit eye after 6 hours to check redness due to ocular insert
**Scanning Electron Microscopy**

The surface morphology of the F1 formulation was observed by Scanning Electron Microscopy. From the images it can be possibly assessed that the surface of the ocular inserts is irregular but no drug crystals can be identified on the surface. This suggests that the drug was completely miscible in the polymer matrix.

**Figure 12**: Representative SEM images of ocular inserts. A – surface of ocular inserts B-lateral view of ocular inserts.
Figure 13: FTIR Spectra of Ocular Insert, Propylene Glycol, Physical Mixture, API and Pure polymer

A qualitative FTIR spectral analysis was conducted to observe any interactions or incompatibilities among the polymer, plasticizer and API.

The FTIR spectra does not show any interaction between the physical mixture and API or pure polymer.

The characteristic peak of Valacyclovir Hydrochloride at 1727 cm\(^{-1}\) (C=O stretching) was also detectable in the ocular inserts.
Stability

The F1 formulation ocular inserts were kept for stability assessment for 30 days. The drug content was observed to be within the expected limits and the appearance was similar to the fresh inserts. The 30 days ocular inserts sample was observed to be stable.

Figure 14: Drug Content (%) for stability samples

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Drug Content (mg) ± SD</th>
<th>Drug Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>5.97 ± 0.053</td>
<td>99.5</td>
</tr>
<tr>
<td>30</td>
<td>5.92 ± 0.09</td>
<td>99.5</td>
</tr>
</tbody>
</table>
The DSC thermogram of 30 days ocular inserts was similar to the fresh ocular inserts. Thus, it was observed to stable over the period of 30 days.
CHAPTER V

CONCLUSION
It was observed that the ocular inserts dissolve completely in 9 hours proving the biodegradable nature of the ocular inserts. The drug was released over a period of 8 hours and thus improving the patient compliance in comparison to eyedrops and eye ointment.

The ocular inserts were soft in texture and turn into gel like consistency in the eye. The polymer and plasticizer used in the formulation were non-irritant and non-greasy and hence improve the therapy and acceptability of the formulation.
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VITA

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