



# Formulation and evaluation acyclovir gel containing ashwagandha

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## ABSTRACT :

Herpes Simplex Virus (HSV) infection , caused by either HSV1 or HSV 2 are prevalent worldwide disease. Acyclovir a potent antiviral agent , has been widely used for treatment of HSV infection. Topical application offer several advantage including targeted delivery system. In this study we aimed to formulate an acyclovir gel with physicochemical properties for topical application. The carbapol and triethylenamine gel forming agent were selected considering compatibility with acyclovir. The selected concentration of acyclovir was then incorporated into gel base using trituration .Furthermore the rheological properties of acyclovir were evaluated to ensure proper spreadability , viscosity , PH .

**Keywords :** Herpes , preformulation , ointment , virus , gel , acyclovir

## 1. INTRODUCTION

### 1.1 viruses

The word "virus" is defined from Latin word "poison" or "fatal material." A virus is a metabolically inactive, 'acellular,' ultra-small infectious particle that can only replicate inside one type of live host cell. Viruses are the most abundant biological entity on earth and have the ability to infect practically all living things, including humans, plants, animals, and microbes (Archea, Bacteria)<sup>1</sup>.

### 1.2 HERPES<sup>[2,3]</sup>

The herpesvirus family includes the Herpes Simplex Virus (HSV). The virus, which has been known about since the time of the ancient Greeks, commonly infects people and causes a wide range of illnesses, from simple mucocutaneous infections to those that can be fatal. Significant breakthroughs in our understanding of the molecular biology of HSV over the past 50 years have provided new insights into the aetiology and treatment of illness. Herpes is caused by the

Herpes Simplex Virus infection (HSV). Along with other symptoms, it results in sores or blisters developing in or near the mouth or genitalia.

### 1.2.1 TYPES OF HSV [2,3]

HSV-1 causes oral herpes, which usually affects the mouth and surrounding skin. HSV-2 causes genital herpes, which is usually sexually transmitted.

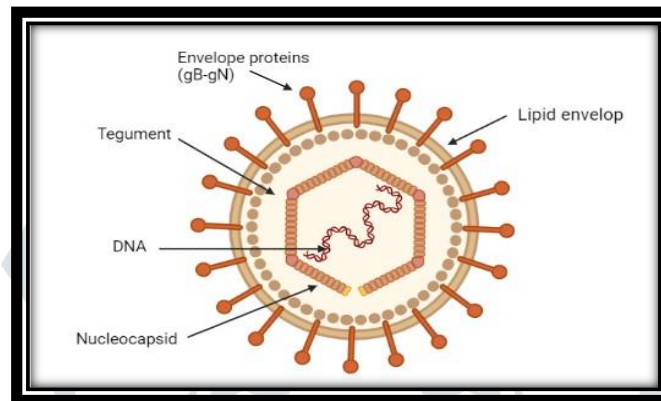


Figure No: 1 Structure of HSV

### 1.3 HERPES SIMPLEX: A DNA VIRUS [4]

Herpes viruses are a leading cause of human viral disease, second to the infections caused by airborne influenza viruses. A distinct characteristic of herpes viruses, compared to other viruses, is their ability to cause disease while they remain silent and reactivated time to time, e.g., shingles. 'Herpes' is a Latin term derived from the Greek word 'herpein' means 'to creep', due to the creeping or spreading nature of the skin lesions caused by herpes viruses. There are at least more than 20 viruses in the family of Herpes viridae, which is classified into 3 subfamilies: alpha ( $\alpha$ ), beta ( $\beta$ ) and gamma ( $\gamma$ ); and there are eight herpes virus types that frequently infect human beings (Table 1). Herpes viruses are larger viruses and their genome encodes at least 80 proteins. Many of those are not directly involved in the viral replication or structural modification but can contribute to the interaction with the host cell or host immune response.

### 1.4 TRANSDERMAL DRUG DELIVERY SYSTEM [5]

The biggest organ of the body, the skin, plays a significant role in medication distribution via the stratum corneum. The epidermis, dermis, and hypodermis are the three primary layers of skin. The transdermal drug delivery system has progressed in its ability to transport drugs through both systemic and topical routes to achieve therapeutic effects. Ethosomes, Niosomes, Transferosomal, Aquasomes, and other sophisticated transdermal delivery methods allow for excellent medication penetration across the skin barrier (stratum corneum). Several dosage forms, including as gels, lotions, creams, and ointments, are used as a substrate for the transport mechanism.

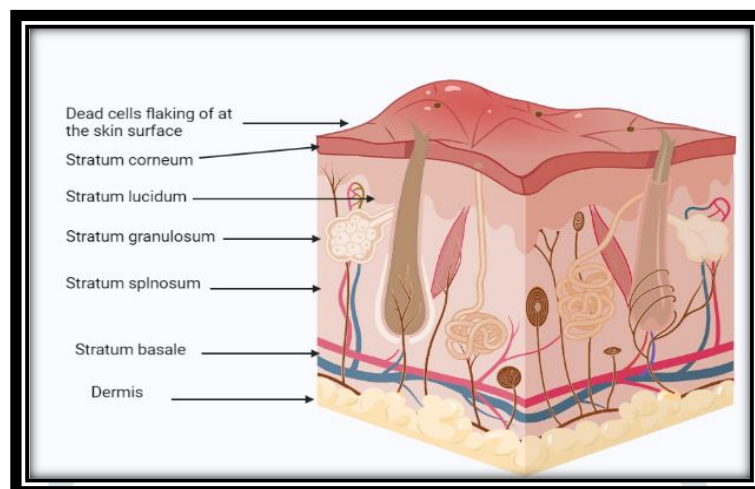


Figure no 2. Anatomy of skin

Gels are semisolid, significantly dilute cross-linked systems that do not flow in a steady state. Topical gels are intended to provide a therapeutically effective quantity of medication to the skin. Topical gels have a systemic action through the skin and are lighter than creams, making them easier to absorb. When compared to creams and ointments, they deliver quicker medication release, lower dose, and eliminate GI absorption.

### 1.5 ACYCLOVIR <sup>[6]</sup>

The most used antiviral for treating HSV infections is acyclovir. These prodrugs prevented HSV multiplication by converting to its active triphosphate form. Intravenous acyclovir is used to treat encephalitis, new-born disorders, severe infections in immunocompromised patients, and sporadic cases of severe orolabial or genital disease, oral medication is beneficial for non-life-threatening HSV infections (e.g., primary orolabial, genital). Additionally, it helps to suppress recurrent genital HSV infections by lowering viral shedding and clinical recurrence rates. Acyclovir (ACV) is a guanosine analog used to treat herpes simplex, varicella zoster, herpes zoster. Herpes labialis, Varicella zoster, herpes labialis, and acute herpetic keratitis are all conditions that can be treated with acyclovir, a nucleotide analogue anti-viral. When treating these viruses, acyclovir is typically used as the first line medication, Immunocompromised patients 12 years of age and older with recurrent herpes labialis are advised to use an acyclovir topical cream. Herpes zoster, genital herpes, and chickenpox are all approved for treatment with acyclovir oral pills, capsules, and liquids. Initial genital herpes and limited, non-life-threatening mucocutaneous herpes simplex in immunocompromised patients are indicated for treatment with an acyclovir topical ointment. It is recommended to use an eye ointment of acyclovir to treat acute herpetic keratitis.

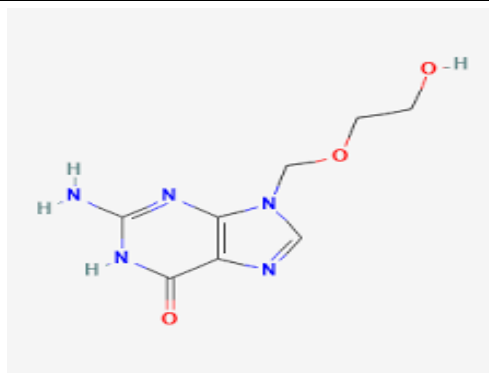


Figure no. 3 Structure of Acyclovir

### 3. Objective of study-

The objective of the present study is to formulate Acyclovir gel containing ashwagandha.

3.1 To carry out preformulation studies of the drug and excipients.

3.2 To study drug excipient compatibility.

3.3 To optimize and formulate Acyclovir & ashwagandha gel.

3.4 Characterization of prepared Acyclovir gel.

### 4. Materials and Methodology :

Acyclovir – (Drug Profile)

Sr. no	Property	Explanation
1.	Category	Antiviral Agent (synthetic nucleoside analogues)
2.	IUPAC	2-amino-9-[(2-hydroxyethoxy) methyl]-6,9-dihydro-3H-purin-6-one
3.	Chemical Formula	C <sub>8</sub> H <sub>11</sub> N <sub>5</sub> O <sub>3</sub>
4.	Molecular Weight	225.20g/mol
5.	Appearance	Almost white amorphous powder.
6.	Solubility	Ethanol, Water, Ether.
7.	pKa value	2.27 & 9.25
8.	BCS class	BCS Class III
9.	Half Life	2-4 hours
10.	Melting Point	256.5°C
11.	λ <sub>max</sub>	253 nm

12.	Absorption	The oral bioavailability of acyclovir is 10-20% but decreases with increasing doses. Acyclovir ointment is <0.02-9.4% absorbed.
13.	Distribution	9 -33%
14.	Metabolism	Metabolized by CYP2C9 pathway to t The oral bioavailability of the metabolite 4-hydroxyacyclovir.
15.	Elimination	Kidney (62 – 90% as unchanged drug)

Table No. 2 Drug Profile Acyclovir

#### 4.1 ABSORPTION-

The oral bioavailability of acyclovir is 10-20%. Acyclovir ointment is <0.02-9.4% absorbed.

#### 4.2 MECHANISM OF ACTION <sup>[12]</sup>

Acyclovir is converted to acyclovir monophosphate due to the action of viral thymidine kinase. Acyclovir monophosphate is converted to the diphosphate form by guanylate kinase. Acyclovir diphosphate is converted to acyclovir triphosphate by nucleoside diphosphate kinase, pyruvate kinase, creatine kinase, phosphoglycerate kinase, succinyl-CoA synthetase, and phosphoenolpyruvate carboxykinase and adenylosuccinate synthetase.

#### 4.3 ROUTE OF ELIMINATION <sup>[12]</sup>

The majority of acyclovir is excreted in the urine as unchanged drug. 90-92% of the drug can be excreted unchanged through glomerular filtration and tubular secretion.

#### 4.4 METABOLISM <sup>[12]</sup>

Acyclovir is <15% oxidized to 9-carboxymethoxymethylguanine by alcohol dehydrogenase and aldehyde dehydrogenase and 1% 8-hydroxylated to 8-hydroxy acyclovir by aldehyde oxidase. Acyclovir is converted to acyclovir monophosphate due to the action of viral thymidine kinase. Acyclovir monophosphate is converted to the diphosphate form by guanylate kinase.

#### 4.5 HALF LIFE <sup>[12]</sup>

The clearance of acyclovir varies from 2-4 hours depending on the creatinine clearance of the patient.

#### 4.6 CLEARANCE <sup>[12]</sup>

The renal clearance of acyclovir is 248mL/min/ 1.73m<sup>2</sup>. The total clearance in neonates is 105-122mL/min/1.73m<sup>2</sup>.

SR .No	Materials	Use
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1	Acyclovir	Antiviral agent
2	Carbopol	Gelling agent
3	Water	Solvent
4	Ashwagandha	Herbal base
5	Polyethylene glycol	Preservative
6	Triethalonamine	Gelling agent

#### 4.7 Batch Formula :

Ingredients	Batch 1	Batch 2	Batch 3	Use
Acyclovir	2 mg	3mg	5 mg	Antiviral agent
Carbopol	2 mg	3 mg	5 mg	Gelling agent
Ashwagandha	1mg	1.5 mg	2.5 mg	Herbal base
Water	10 ml	15 ml	25 ml	solvent
Polythelyne glycol	1drop	1 drop	1 drop	preservative

Table No.4 Formulation table.

## 5.Methodology-

### 5.1 Formulation of Acyclovir Gel-

Weigh accurately 10gm of carbopol add sufficient amount of water in it and continues stirring using mortle and pestle.

Well stirring properly untill carbopol uniformly mix in water and forming gel.

Cover the mortle with aluminium foil kept it for 2hrs. After 2hrs add few drops of triethylonamine for better gelling consistency .

Take another mortle add 5gm ashwagandha in it and make it semisolid with sufficient amount of water .In that same mortle add 5gm of acyclovir drug and stirring well.

Add Carbopol gel and propylene glycol in above mixture .

Then , Final gel was prepared.

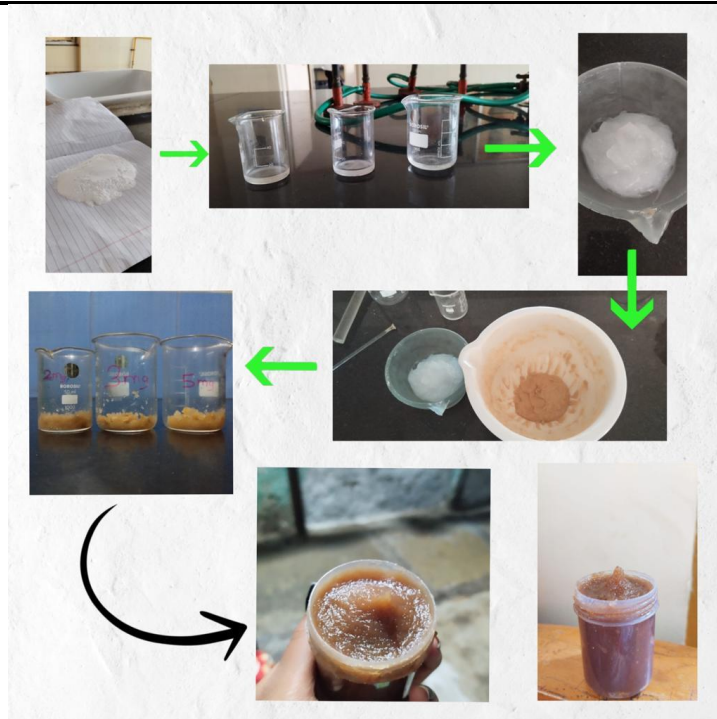


Figure no. Formulation process of gel

## 5.2 PREFORMULATION STUDIES:

During preformulation, drug compounds' physical and chemical properties are examined. Preformulation research's main objectives are to identify a medicinal ingredient's appropriate shape, assess its physiochemical properties, and get a better understanding of material stability, all of which will help create the ideal delivery method.

## 5.3 IDENTIFICATION TEST :

### 5.3.1 Physical Characteristics :

The drug's physical appearance, odour, colour, and texture were examined.

### 5.3.2 Estimation of Melting Point :

A material's melting point is used to identify it and gauge its purity. It was put to the test using Thiele's tube contraption. A glass capillary with flame at one end was filled with the medication. There was a melting point. Figured out by dipping the capillary containing the medication in paraffin liquid and keeping track of the temperature at which the drug sample melted.

### 5.3.3 Determination Of Absorption Maxima ( $\lambda$ max) of drug:

The Maximum peak of the spectrum was  $\lambda$  max of drug. The Drug's stock sample was produced with water . in beam UV spectrophotometer, this solution was scanned from 400-200nm. Max of drug was used to describe the spectrum's highest point.

#### 5.3.4 Development of Standard Calibration Curve for Acyclovir in water:

Acyclovir Concentrations in Beer range from 2 to 20  $\mu\text{g/ml}$ . To make the stock solution of Acyclovir, 10mg of Acyclovir was put to 100ml volumetric flask and was brought up to the mark with ethanol to archive a concentration 100 $\mu\text{g/ml}$ . Aliquots were taken (2,4,6,8,10 ml)and the amount was adjusted to 10ml to get the concentration in 2 to 20 $\mu\text{g/ml}$ , and analysed at a wavelength of 252nm.

#### 5.3.5 PH :

The pH of the formulated Acyclovir gel was measured by using a pH meter. 1g of the gel was weighed and dispersed in 10 ml of distil water. The pH meter was calibrated prior by a phosphate buffer of pH 4 and pH 9 prior to use.

#### 5.3.6 Viscosity :

The viscosity of formulated Acyclovir was determined by Brookfield viscometer at normally 37° C using disc spindle with a finest speed at 100rpm. The viscosity of gels were recorded.

#### 5.3.7 Spreadability :

The Spreadability of ACV gel was recorded by the parallel plate method. Weighed 0.5g of the gel was placed on a glass plate in 1cm diameter; a second glass plate was placed over it with 500 mg weight on allowed to rest for 5 minutes. A rise in diameter was recorded. Very minimum time taken for the Spreadability means good Spreadability.

$$S = W \times L / T$$

W= weight on the upper slide (2 slides)

L = length of the slide

T = time taken to separate both the slides.

#### 5.3.8 IR Spectroscopy :

The acquired drug sample's Fourier Transform Infrared (FT-IR) spectrum was compared to a typical FT-IR spectrum of pure drug as a reference. IR spectroscopy was also conducted to identify the compatibility of the drug. Acyclovir and physical mixture such as Acyclovir, Carbopol 940. All of the spectra were scanned at a resolution of 5 $\text{cm}^{-1}$  between 500 and 5000 $\text{cm}^{-1}$ .

#### 5.3.9 Differential Scanning Calorimetry :

Aluminum crucibles with a pierced aluminum lid were used to seal the weighed quantity of physical mixtures of drug and excipients. Shimadzu model DSC-60 thermo analytical system was used to obtain DSC thermograms. Analyses has been performed in the presence of nitrogen (nitrogen flow rate 50ml/min) at a standard heating rate of 10<sup>0</sup>C/minute over a temperature range of 30<sup>0</sup>C-350<sup>0</sup>C.

## 6.RESULTS

Evaluation test

### 6.1 preformulation studies :

Physical Characteristics of pure drug . Acyclovir occurs as a white crystalline powder having no odour n taste as well .PH - 6

### 6.2 Determination of melting point of drug :

The melting point of Acyclovir was found to be  $255.6 \pm 0.22$  (254.8<sup>0</sup>c to 256<sup>0</sup>c) and it complies with standard thus it indicates the drug purity of the sample.

Trial Number	Initial Melting Point	Final Melting Point
1	255 <sup>0</sup> C	254 .8 <sup>0</sup> C
2	254 <sup>0</sup> C	256 <sup>0</sup> C
3	255 <sup>0</sup> C	2556 <sup>0</sup> C
Mean ± SD	254.66±0.22 <sup>0</sup> C	255.6±0.22 <sup>0</sup> C

Table .5 Melting Point of drug

### 6.3 Determination of absorption maxima ( $\lambda$ max) of the drug :

Absorption maxima of Acyclovir was carried out using wavelength sweep from 400-200nm. At 252nm, the highest absorption was recorded. The maximum was employed in the study.

Sr. No	P/V	Wavelength (nm)	Absorbance (Au)
1	↑	252.00	0.687
2	↑	203.20	0.932
3	↑	197.00	0.388

4	↑	193.00	0.436
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#### 6.4 Standard Calibration Curve Of Acyclovir:

The Acyclovir standard calibration curve was estimated by plotting concentration Vs Absorption. In beer's range (2-20 $\mu$ g/ml), the standard calibration curve was determined to be linear at max 252nm. The obtained correlation coefficient ( $R^2$ ) was 0.9994 and the equation was  $y= 0.0739x + 0.0046$ .

Concentration ( $\mu$ g /ml )	Absorbance (nm)
0	0
2	0.165
4	0.295
6	0.444
8	0.594
10	0.748

#### 6.5 IR Spectroscopy :

Functional Group	Reported frequencies (cm <sup>-1</sup> )	Observed frequencies (cm <sup>-1</sup> )
N - H stretching ( Piperazine )	3500 - 3220	3288.99
C = O Stretching ( Ketone )	1740 - 1680	1716.65
C- O stretching (Carboxylic acid )	1320 -1210	1288.45
O – H stretching ( Carboxylic acid)	1440 -1395	1408.04

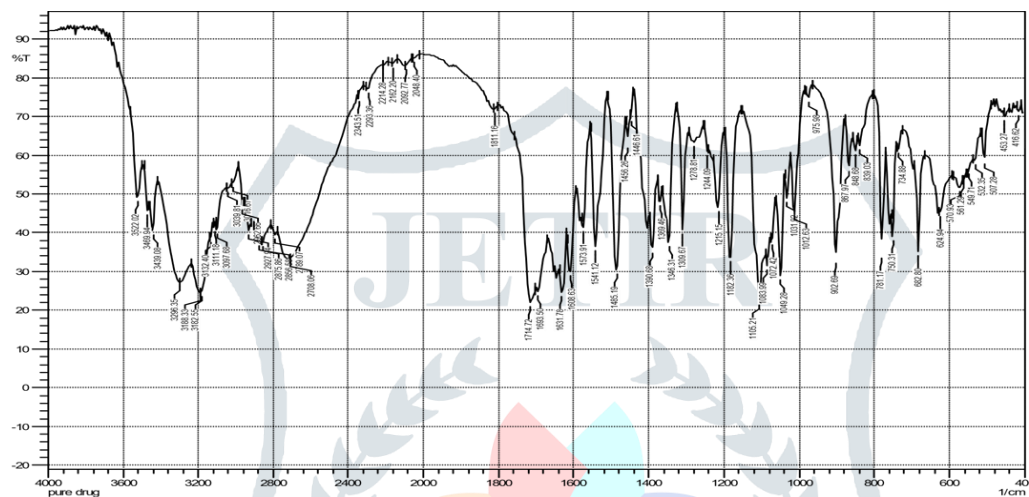


Figure 4: FTIR Spectrum of Pure Drug Acyclovir

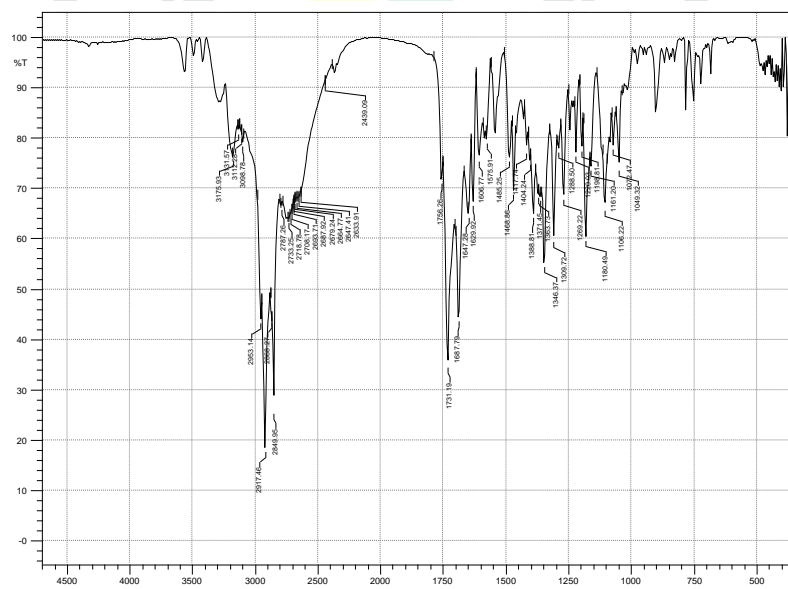
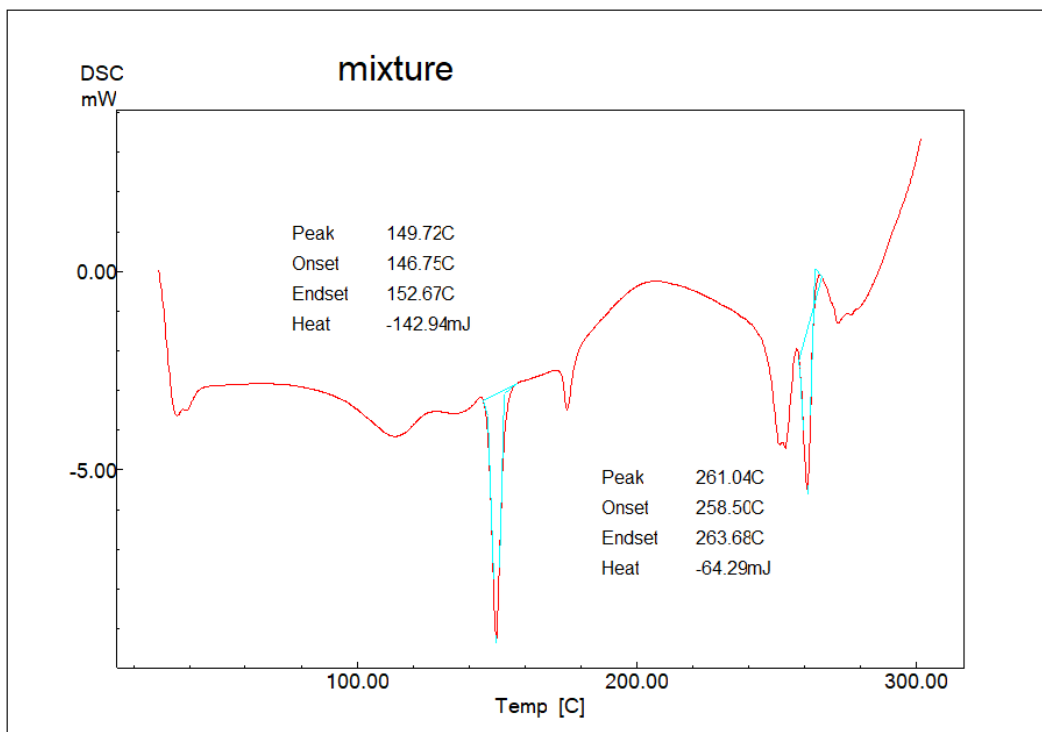
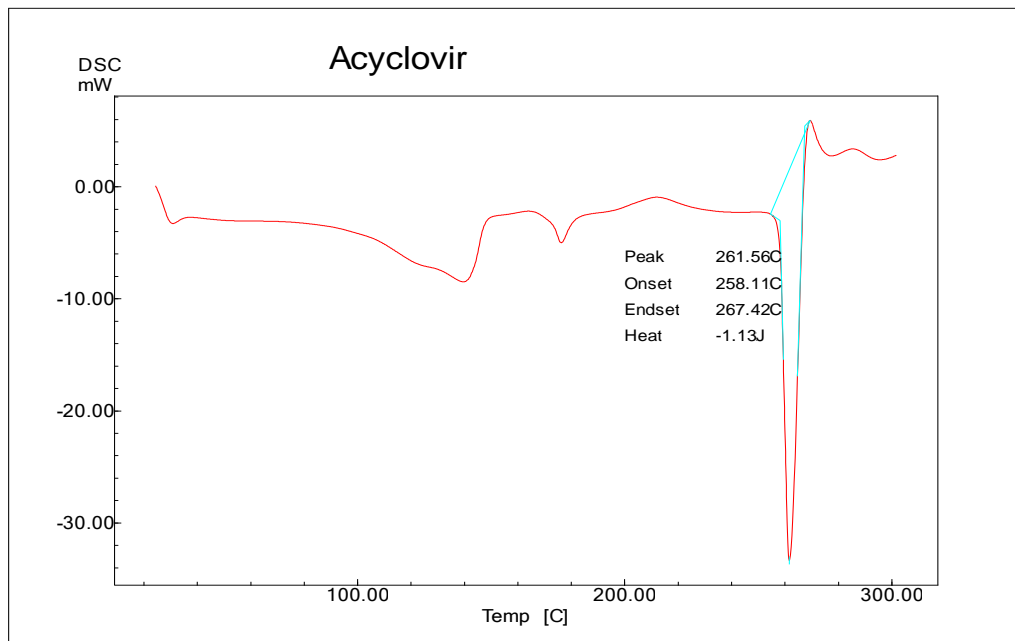


Figure 5: FTIR Spectrum of Drug and Mixture

## 6.6 DIFFERENTIAL SCANNING CALORIMETER :



## 7.CONCLUSION :

The present study has been satisfactorily performed and an attempt was made to formulate Acyclovir gel for transdermal delivery by enhancing permeability of the drug. From the experimental results it can be concluded that:

The formulation of an acyclovir gel offers a promising approach for topical treatment of HSV infection. This study provides a comprehensive methodology for the development of an acyclovir gel considering factors such as gel forming agent, acyclovir concentration, rheological properties. Formulated acyclovir gel effective for treatment of HSV infection.

## 8. REFERENCE :

- 1) Chattopadhyay D, Chawla-Sarkar M, Chatterjee T, Dey RS, Bag P, Chakraborti S, Khan MT. Recent advancements for the evaluation of anti-viral activities of natural products. *New Biotechnology*. 2009 Jun 1;25(5):347-68.
- 2) Chayavichitsilp P, Buckwalter JV, Krakowski AC, Friedlander SF. Herpes simplex. *Pediatrics in review*. 2009 Apr 1;30(4):119.
- 3) Whitley RJ, Roizman B. Herpes simplex virus infections. *The Lancet*. 2001 May 12;357(9267):1513-8.
- 4) Taylor TJ, Brockman MA, McNamee EE, Knipe DM. Herpes simplex virus. *Frontiers in Bioscience-Landmark*. 2002 Mar 1;7(4):752-64.
- 5) Forrester A, Farrell H, Wilkinson G, Kaye J, Davis-Poynter N, Minson T. Construction and properties of a mutant herpes simplex virus type 1 with glycoprotein gH coding sequences deleted. *J Virol* 1992; 66: 341-48.  
Prausnitz MR, Langer R. Transdermal drug delivery. *Nature biotechnology*. Nov;26(11):1261-8.
- 6) O'Brien JJ, Campoli-Richards DM. Acyclovir. *Drugs*. 1989 Mar;37(3):233-309
- 7) Mehta P. Acyclovir. *Pediatric Infectious Disease*. 2013 Oct 1; 5(4):178-8
- 8) Nilsen A, Aasen T, Halsos A, Kinge BR, Tjøtta EL, Wikström K, Fiddian AP. Efficacy of oral acyclovir in the treatment of initial and recurrent genital herpes. *The Lancet*. 2019 Sep 11; 320(8298):571-3.
- 9) Gopinath D, Ravi D, Rao BR, Apte SS, Renuka D, Rambhau D. Ascorbyl palmitate vesicles (Aspasomes): formation, characterization and applications. *International journal of pharmaceutics*. 2018 Mar 1; 271(1-2):95-113.
- 10) Kar M, Saquib M, Jain DK. Formulation Development and Evaluation of Aspasomes Containing Skin Whitening Agent. *Manipal Journal of Pharmaceutical Sciences*. 2020;6(1):8.
- 11) PC H, Chandrakala V. *International Journal of Modern Pharmaceutical Research*
- 12) Serrano M, Valverde JM, Guillén F, Castillo S, Martínez-Romero D, Valero D. Use of Aloe vera gel coating preserves the functional properties of table grapes. *Journal of agricultural and food chemistry*. 2006 May 31;54(11):3882-6.