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FORMULATION AND DEVELOPMENT OF ACYCLOVIR OINTMENT; A COMPREHENSIVE REVIEW

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ABSTRACT

The practice school is a preliminary step for the project work and was conducted in the domain of Pharmaceutics with the objective of promoting intellectual exchange and collaboration between academia and industry. The present review focuses on the formulation and development of acyclovir ointment, a topical antiviral preparation used in the treatment of herpes simplex and varicella-zoster viral infections. Acyclovir acts by inhibiting viral DNA polymerase and preventing viral replication within infected cells. Due to the poor aqueous solubility of acyclovir, suitable formulation strategies are necessary to enhance its topical delivery and therapeutic effectiveness. The study includes detailed information regarding viral diseases, antiviral drugs, mechanism of action of acyclovir, preformulation studies, compatibility studies, formulation techniques, evaluation parameters, and stability studies. The ointment formulation mainly contains polyethylene glycol bases which improve consistency, spreadability, and drug release. Various evaluation tests such as pH determination, viscosity, spreadability, diffusion studies, and skin irritation studies are important in ensuring the quality, safety, and efficacy of the formulation. Proper packaging, labeling, and storage conditions also play a significant role in maintaining product stability. Overall, acyclovir ointment is an effective topical antiviral formulation that provides improved therapeutic action and patient compliance in the management of viral infection.

KEY WORDS: Acyclovir, Antiviral Drug, Ointment, Pharmaceutics, Herpes Virus, Topical Drug Delivery, Preformulation Studies, Stability Studies, Polyethylene Glycol, Viral Infections.

INTRODUCTION

- Diseases are illnesses caused by viruses, which are microscopic infectious agents that can replicate only inside living host cells.
- Unlike bacteria, viruses lack the cellular machinery necessary for independent metabolism and reproduction.
- They consist primarily of genetic material—either DNA or RNA—enclosed in a protein coat, and in some cases, a lipid envelope.
- Once a virus enters a susceptible host cell, it hijacks the host's cellular mechanisms to produce new viral particles, often resulting in cell damage or death.
- Viral diseases affect humans, animals, and plants, and they range from mild, self-limiting infections such as the common cold to severe and life-threatening conditions like acquired immunodeficiency syndrome (AIDS), Ebola virus disease, and COVID-19.
- Transmission of viral diseases can occur through various routes, including respiratory droplets, direct contact, blood and body fluids, contaminated food or water, and vectors such as mosquitoes.

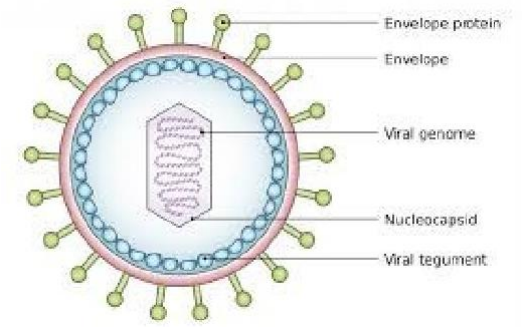


Figure no.1; virus.

TYPES OF VIRUSES

Viruses can be classified based on genetic material, structure, mode of replication, and host range.

1. Classification based on Genetic material:

a) DNA Viruses

These viruses contain DNA as their genetic material. Examples:

- Adenovirus
- Herpes simplex virus
- Variola virus (smallpox)

b) RNA Viruses

These viruses contain RNA as their genetic material. Examples:

- Influenza virus
- HIV
- Coronavirus (SARS-CoV-2)

2. Classification Based on Capsid Symmetry:

a) Icosahedral Viruses

Capsid has 20 triangular faces. Examples:

- Poliovirus
- Adenovirus

b) Helical Viruses

Genetic material is arranged in a spiral form. Examples**:**

- Tobacco mosaic virus
- Influenza virus

c) Complex Viruses

Have complex shapes that are neither purely helical nor icosahedral. Example**:**

- Bacteriophages

3. Classification Based on Presence of Envelope:

a) Enveloped Viruses Surrounded by a lipid envelope. Examples:

- HIV
- Influenza virus
- Herpes virus

b) Non-Enveloped (Naked) Viruses Lack an envelope.

Examples:

- Poliovirus
- Adenovirus

4. Classification Based on Host Range:

a) Animal Viruses

Infect humans and animals Examples:

- Rabies virus
- Influenza virus

b) Plant Viruses

Infect plants Example:

- Tobacco mosaic virus

c) Bacterial Viruses (Bacteriophages)

Infect bacteria Example:

- T4 phage

5. Classification Based on Mode of Replication (Baltimore Classification)

Table 1; classification based on mode of replication.

Group	GenomeType	Example
I	dsDNA	Herpesvirus
II	ssDNA	Parvovirus
III	dsRNA	Rotavirus
IV	+ssRNA	Poliovirus
V	−ssRNA	Influenza

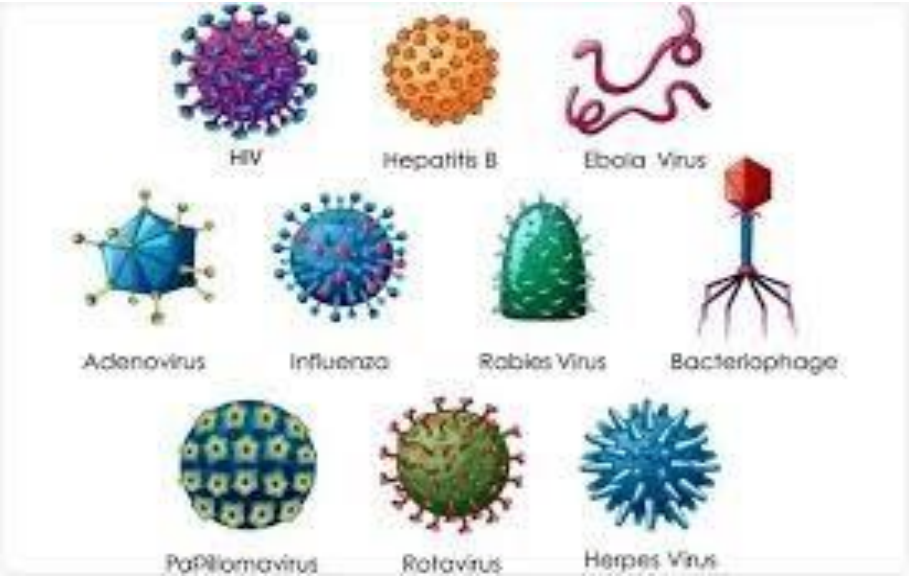


Figure no.2 Types of virus.

Major Modes of Viral Transmission

1. Direct Transmission

Virus passes directly from an infected person/animal to another susceptible host.

Types:

- Direct contact – physical contact such as touching, kissing, sexual contact.
- Droplet spread – respiratory droplets (from coughing, sneezing, talking) carrying virus to another person nearby.

2. Indirect Transmission

Virus is spread via intermediaries — not by direct physical contact.

Routes include:

- Airborne Transmission

Virus particles (aerosols) remain suspended in air and can infect people who inhale them even at a distance.

Example: Measles and varicella viruses.

- Vehicle-borne Transmission

Virus is transmitted by inanimate objects ("vehicles" or fomites) such as:

- Contaminated water
- Contaminated food
- Surfaces (doorknobs, utensils, toys)

Example: Norovirus spread via contaminated food/water.

- Vector-borne Transmission

A living organism, usually an arthropod (e.g., mosquito, tick), passes virus from one host to another.

Example: Dengue virus, Zika virus transmitted by mosquitoes.

3. Other Transmission Categories

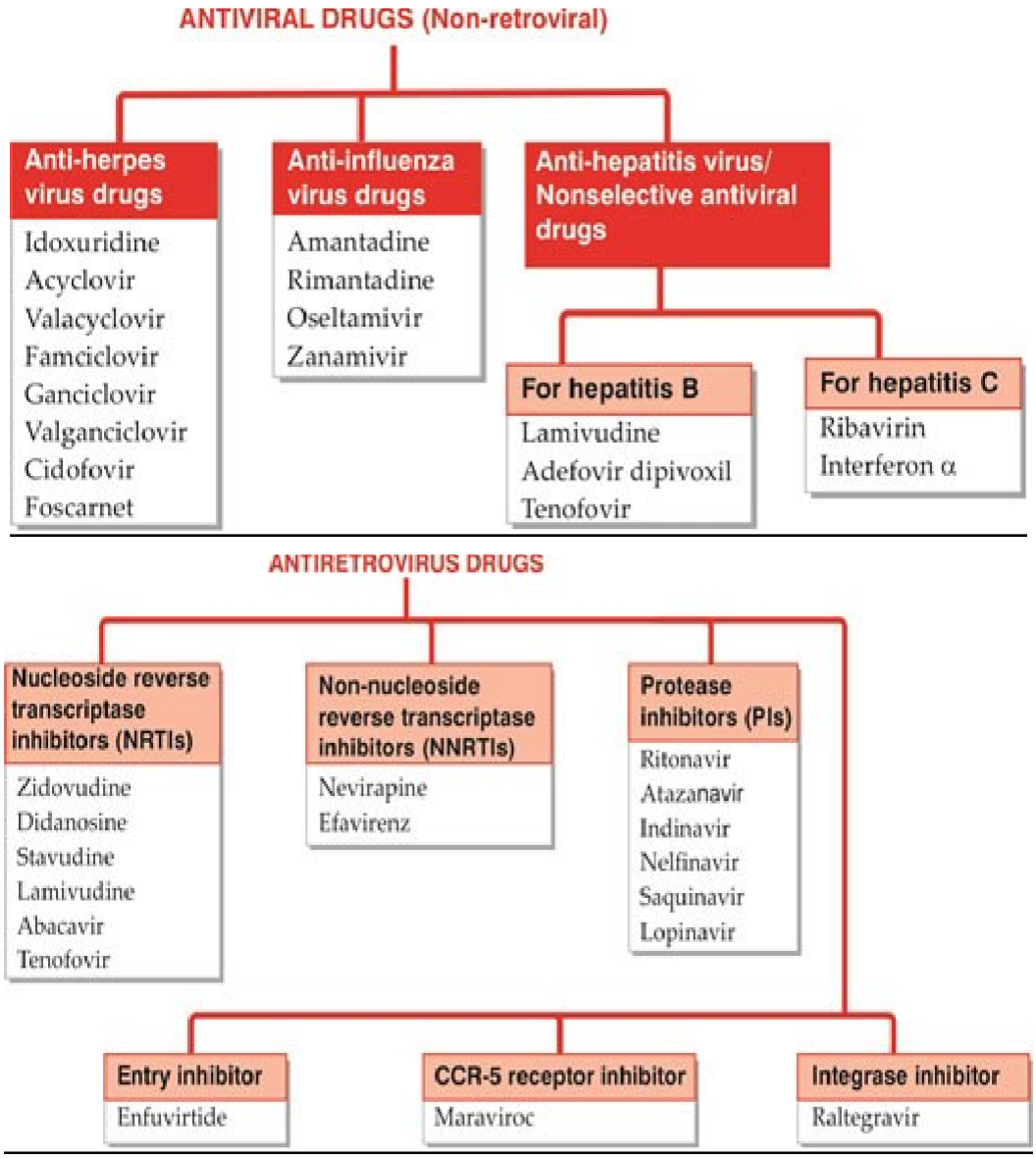
Some texts also list:

- Common vehicle transmission – a single source (like contaminated water) exposing many people.
- Iatrogenic / nosocomial transmission – spread in healthcare settings via medical procedures or surfaces. Top of Form
- Vertical transmission– parent to child (e.g., transplacental, perinatal).

ANTI-VIRAL DRUGS

Antiviral agents are drugs or substances that prevent viruses from multiplying in the body. They work by inhibiting specific stages of the viral life cycle—such as viral entry into cells, replication of viral genetic material, assembly, or release of new virus particles—thereby reducing the severity and duration of viral infections rather than directly killing the virus.

CLASSIFICATION



Acyclovir

Acyclovir is an antiviral medication used to treat infections caused by herpes viruses, including cold sores, shingles, genital herpes, and chicken pox**.** Acyclovir acts as a specific inhibitor of herpes virus DNA polymerase. It shows good in vitro activity against herpes simplex and varicella-zoster viruses.

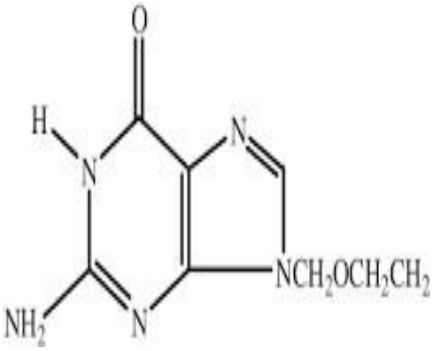


Figure no.3; chemical structure of acyclovir

Chemical name: 9-[(2-Hydroxyethoxy) methyl] guanine

Molecular formula: $C_8H_{11}N_5O_3$

Molecular weight: 225.20 g/mol

This deoxiguanosine analogue antiviral drug requires a virus specific enzyme for conversion to the active metabolite that inhibits DNA synthesis and viral replication.

MECHANISM OF ACTION

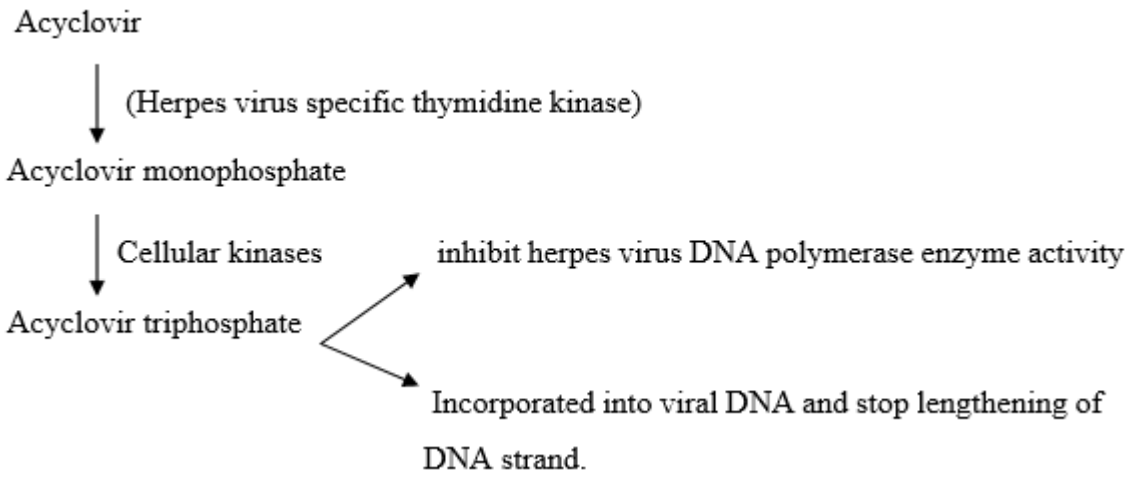


Table 2; formulation chart.

Formulation	Strength available
Tablet	200mg,400mg,800mg
Syrup	200mg/5mL
Injection	250mg,500mg,1g
Ointment	5%

PATENT RELATED TO THE DRUG

1. US6514980B1: Nucleoside analogs in combination therapy of herpes simplex infections. Filed: 26/7/2000

Assignee: Novartis pharmaceuticals corp

Overview: This patent relates to therapeutic methods for treating infections caused by herpes simplex virus (HSV) using nucleoside analog compounds in combination therapy.

2. US7223387B2: antiviral formulation comprising propylene glycol and an isopropyl alkanoic acid ester.

   Filed: 30/12/2002

   Assignees: meda pharma SARL, medivir AB

   Overview: This patent describes **topical antiviral pharmaceutical compositions** specifically formulated to treat or prevent infections caused by herpes viruses (such as HSV-1 and HSV-2, which cause cold sores and genital herpes).

3. US8592434B2: Mucoadhesive buccal tablets for treatment of orofacial herpes.

   Filed: 9/12/2009

   Assignee: ligand pharmaceuticals Inc.

   Overview**:** the tablet formulation is intended to adhere to the inside of the mouth (buccal mucosa) and to slowly release the antiviral drug over time**, improving drug exposure and therapeutic effect compared with standard oral tablets or topical creams.**

INNOVATORS AND GENERIC FORMS

1. Zovirax

   Manufacturer: glaxosmithKline.

   Formulation: oral tablet, capsule suspension, topical cream/ointment, IV injection.

   Indications: herpes zoster, genital herpes, chickenpox, cold sores.

2. Sitavig Manufacturer: cipher pharmaceuticals Inc. Formulation: buccal muco-adhesive tablets.

   Indication: for treatment of recurrent herpes liabialis in immunocompetent adults.

GENERIC ACYCLOVIR PRODUCTS

1. Blenheim pharmacal, Inc.

   Formulation: Capsule (200mg)

   Packaging: Available in bottle of 25 or 40 capsule for oral use

2. Hikma pharmaceuticals.

   Formulation: Injection (500mg and 1g vials) Approval: FDA.

3. Physician total care, Inc.

   Formulation: Suspension (200mg/5ml) Approval: FDA ANDA.

4. BTA Pharmaceuticals, Inc.
   Formulation: Ointment (50mg/1g) Packaging: Available in 5g and 15g tubes.

PREFORMULATION STUDIES OF ACYCLOVIR

Preformulation is initial phase of drug development characterizing the physical and chemical properties of active pharmaceutical dosage form and its excipients to develop a stable, safe and effective dosage form**.**

OBJECTIVES

- To formulate stable and effective dosage form
- To increase drug stability
- To improve drug bioavailability
- To reduce drug excipient incompatibly
- To determine its kinetics and stability Various preformulation parameters evaluated are:

1. PHYSICOCHEICAL PROPERTIES

a) Organoleptic properties
b) Solubility
c) Melting point
d) Partition coefficient (log P)
e) pka
f) pH
g) hygroscopicity

2. MICROMERITIC PROPERTIES

a) Particle size
b) Flow property

3. COMPATIBILITY STUDIES

a) FTIR
b) DSC

4. STABILITY STUDIES

a) Thermal stability

1. PHYSICOCHEMICAL PROPERTIES

a. organoleptic properties

Table 3; organoleptic properties.

Parameters	observation
Appearance	White to off white crystalline powder
Odour	odourless
Taste	Slightly bitter taste

b. solubility

Acyclovir is practically insoluble in chloroform and ether. But slightly soluble in water and methanol. It is determined using shake flask method**.**

Procedure:

- Add excess acyclovir to a known volume of solvent(water)
- Shake for 24-72 hours at constant temperature (usually 25°C or 37°C)
- Filer the solution using membrane filter
- Analyze the supernatant using UV-spectroscopy Solubility of acyclovir can be enhanced by:
- Use of co solvent like propylene glycol, ethanol.
- Use of surfactant like sodium lauryl sulphate.
- Conversion into salt form.
- Complexation with solubilising agents like cyclodextrin.

c. melting point

The melting point of acyclovir is approximately256–257°C (with decomposition**)**.it is determined using capillary melting point apparatus. Powdered sample of acyclovir is packed into a thin capillary tube. The tube is placed in a heated block. Thermometer placed in it. The temperature at which substance begins and finishes melting is noted.

d. Partition coefficient

The partition coefficient of acyclovir is 1.5-1.7. Its lipid solubility is enhanced by using base like propylene glycol.

- Prepare saturated solution of n- octanol in water.
- Add excess amount of acyclovir to a known volume of the two-phase system.
- Shake vigorously for 20-30 min (may take several hours) at controlled

temperature (usually 25°C).

- Allow phases to separate (by centrifugation or setting).
- Sample both phases and determine the compound concentration using UV spectrophotometer. Calculate the log p value.

e. ionization constant(pKa)

The ionization constant (pKa) of acyclovir is defined in literature because acyclovir is un-ionized or neutral molecule under physiological pH condition.

f. pH

Acyclovir's stability is affected by pH. The drug is known to be chemically stable in mildly acidic to neutral environment and degrades faster in alkaline pH. pH stability studies are conducted by dissolving the drug in buffer solutions of varying pH (pH 3 to 10),storing at room temperature or elevated temperatures, and analyzing degradation over time using HPLC.

g. Hygroscopicity

Acyclovir is slightly hygroscopic, absorb small amount of moisture from atmosphere, but still require protection from heat for optimal stability.

2. micromeritic properties

a. particle size

Micronized acyclovir used in topical formulation has a particle size in the range of 5-20 micrometer. Particle size of acyclovir is determined using laser diffraction method.

b. flow property

Table 4; flow property.

Parameter	significance
Angle of repose (θ)	35° – 42°
Bulk density	0.40 – 0.55 g/cm³
Tapped density	0.55 – 0.70 g/cm³
Carr's index	20 – 30 %
Hausner's ratio	1.25– 1.40

Acyclovir exhibits poor flow properties. This is due to low bulk density, fine particle size, High cohesiveness. It can be overcome by combining micronized acyclovir with flow enhancing excipients such as magnesium stearate, colloidal silicon dioxide and talc.

3. COMPATIBILITY STUDIES

a. FTIR

FTIR Spectroscopy is commonly used to identify the characteristic functional groups of acyclovir and to Asses drug-excipient compatibility by detecting any changes in the IR spectrum.

- No peak or disappearance of characteristic peaks→ indicates compatibility.
- Peak shifts, broadening, or new peaks→ suggest potential interaction or degradation.

Acyclovir is compatible with,

- Propylene glycol
- White soft paraffin

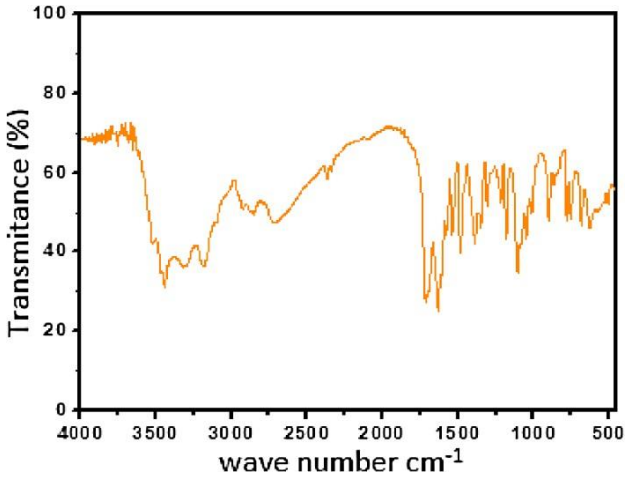


Figure no.4; FTIR sample of acyclovir

b. HPLC

High performance liquid chromatography (HPLC) is a gold standard analytical technique for detecting chemical incompatibilities.

4. STABILITY STUDIES

a. thermal stability

Acyclovir begins to degrade at temperature of 400 °C.

Thermal behavior of acyclovir in solid state, studied by some thermal analysis technique such as,

- Differential scanning calorimetry(DSC)
- Thermogravimetric analysis(TGA)

Avoid high temperature processes like excessive drying or heating during manufacture .store below 25°C, preferably refrigerated to minimize thermal degradation.

FORMULATON OF ACYCLOVIR OINTMENT

Pharmaceutical formulation is the process of combining a drug (active pharmaceutical ingredient, API) with other substance (excipients)to produce a final medicine product in a specific dosage form (e.g., tablet, cream, ointment, injection) that delivers the drug effectively, safely, and in a form.

METHODS OF FORMULATION

A. COMPOSITION

Table 5: composition of acyclovir ointment.

Ingredient	Quantity (per 100 g)	Function
Acyclovir	5 g	Active antiviral agent; inhibits viral DNA replication (used in herpes infections)
Polyethylene Glycol (PEG) 400	40 g	Solvent; improves drug solubility and spreadability
Polyethylene Glycol (PEG) 3350 / 4000	55 g	Ointment base; provides consistency and enhances drug release

B. EQUIPMENT REQUIRED

Figure no.5; colloid mill.

Figure no.6; Cream filling machine.

Figure no.7; High viscosity filling machine.

Figure no.8; Homogenizer stirrer.

Figure no.9; Ointment manufacturing plant.

Figure no.10; paste kettle.

C. MANUFACTURING STEPS

1. Preparation of Ointment Base

- Transfer oil-phase components (e.g., paraffin base) into a **stainless-steel jacketed**

vessel.

- Heat to about **60–70°C** until completely melted.
- Maintain temperature and ensure uniform mixing using a **mechanical stirrer**.

2. Drug Incorporation

- Acyclovir is poorly soluble, so it is usually **dispersed**, not dissolved.
- Levigate acyclovir with a small quantity of **propylene glycol or liquid paraffin** to form a **smooth paste**.
- This step ensures:

✓ Uniform distribution

✓ Prevention of grittiness

2. Mixing and Homogenization

- Slowly add the drug paste into the molten base under **continuous stirring**.

Use a **high-shear mixer or homogenizer** to achieve:

✓ Uniform dispersion

✓ Smooth texture

- Maintain temperature to keep base semi-fluid during mixing.

4. Cooling Process

- Gradually cool the mixture while stirring continuously.
- Controlled cooling prevents:

✓ Drug sedimentation

✓ Phase separation

- Cooling is typically done to around **30–40°C**.

5. Final Homogenization

- Perform final homogenization to ensure:

✓ Consistency

✓ Uniform drug distribution

✓ Desired viscosity

EVALUATION OF OINTMENTS

All the prepared ointments were characterized for the parameters such as appearance, odor, color, homogeneity, pH, spreadability, hardness, water number, and viscosity measurements.

Organoleptic characteristics

All blank formulations (i.e., formulations without active ingredient) and drug-loaded formulation were tested for physical appearance, color, texture, phase separation, and homogeneity. These characteristics were evaluated by visual observation. Homogeneity and texture were tested by pressing a small quantity of the formulated cream and gels between the thumb and index finger. The consistency of the formulations and the presence of coarse particles were used to evaluate the texture and homogeneity of the formulations. Immediate skin feel (including stiffness, grittiness, and greasiness) was also evaluated.

PH

About 2.5 g of all formulations were taken in dry beaker and 50 ml of water was added. Beaker containing ointments was heated on water bath at 60–70°C. The pH of ointments determined using a pH meter (pH Tutor, Eutech Instruments). The determinations were carried out in triplicate and the averages of three readings were noted.

Spreadability

Spreadability of the formulation was determined by an apparatus suggested by Multimer with some modifications. It consists of a wooden block having a pulley at one end with fixed glass slide on block. An excess of ointment (3 g) placed on ground plate. The ointment was sandwiched between this plate and another glass plate having the dimension of fixed ground plate and provided with the hook. A 1 kg weight was placed on the top of the two plates for 5 min to expel air and to provide a uniform film of the ointment between the plates. Excess of ointment was scrapped off from the edges. The top plate was then subjected to pull of 240 g. With the help of spring attached to the hook and time required by the top plate to cover a distance of 10 cm was noted. A shorter interval indicates better spreadability. Spreadability was calculated using the following formula:

$S = M \times L/T$

Where, S = Spreadability

M = Weight in the pan (tied to the upper slide) L = Length moved by the glass slide and

T = Time (in seconds) taken to separate the slide completely each other.

Viscosity

Brookfield Synchro-Lectric Viscometer (Model RVT) with Helipath Stand was used for

rheological studies. The sample (50 g) was placed in a beaker and was allowed to equilibrate for 5 min before measuring the dial reading using a T-D spindle at 10, 20, 30, 50, 60, and 100 rpm. At each speed, the corresponding dial reading on the viscometer was noted. The spindle speed was successively lowered and the corresponding dial reading was noted. The measurements were carried in triplicate at ambient temperature. Direct multiplication of the dial readings with factors given in the Brookfield Viscometer catalog gave the viscosity in centipoises (CPS).

Water number

Water number is the maximum amount of water that can be added to100 g of base at a given temperature. It was determined by continuously stirring the base with the addition of distilled water. When no more water was absorbed into the base evidenced by droplets of water, remaining in the container was taken as end point.

Hardness/strength

Hardness of formulations was determined using Texture Pro CT V1.3 (Build 15 Brookfield Engineering Labs, Inc.) texture analyzer. It is based on the speed of the displacement of probe into sample (ointment) at a given distance. The probe was moving down at a speed of 2 mm/s till a 7 g surface trigger was attained. At this point, the probe was in full contact with the sample surface. Then, the probe continued to penetrate to a depth of 4 mm at a speed of 2 mm/s. At this point, the probe returned to its starting position. The penetration depth of a standard4 mm needle (P/2N) at a constant 10 kg load force was measured to represent the hardness of the formulation. The peak load (maximum force) was registered and is considered a measure of firmness of the product – the bigger the force the thicker/harder is the sample. All tests were conducted 2 times at room temperature (25±2°C) and values of peak force were expressed in g.

In vitro diffusion study

Franz diffusion cell was used for the drug release studies. Ointment was evenly applied onto the surface of cellulose membrane. The cellulose membrane was clamped between the donor and the receptor chamber of diffusion cell. The receptor compartment was filled with phosphate buffer pH 7.4, and the assembly was maintained at 37°C±0.5under constant magnetic stirring. With reference to Scale-up and Post-approval Changes guidelines laid by FDA, 300 mg of ointment was applied to the membrane on the donor compartment and then covered with aluminum foil to prevent drying out. Aliquots were withdrawn at predetermined time intervals

over a period of 1 h and amount of salicylic acid released was analyzed at 275 nm using UV spectrophotometer.

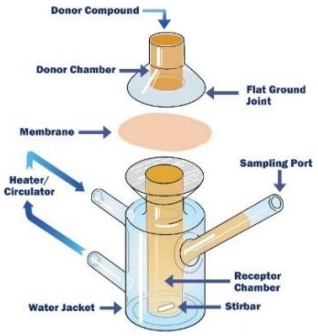


Figure no.11; Franz diffusion cell.

Ex vivo permeation study

The rat skin was used by cleaning with a mild skin cleanser, removing any hair and subdermal fat and fascia were used. The prepared rat skin was mounted on the Franz diffusion cell (with effective diffusion area3.14 cm2 and 7 ml cell volume) with stratum corneum facing upward .The receptor compartment was filled with phosphate buffer pH 7.4,and the assembly was maintained at 37°C±0.5 under constant magnetic stirring. The amount of drug permeated through rat skin was carried out as per method described in diffusion Study

Skin irritation study

Healthy albino rats of 25–30 g body weight were used for skin irritancy and sensitization model. The animals were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 22±2°C and 35–60% humidity). Standard palletized feed and tap water were provided ad libitum. The study was approved by the Institutional Animal Ethical Committee of R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, India, registered under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India (registration No. 951/09/C/CPCSEA).This test was performed on albino rats weighing between 150 and200 g. Animals are divided into four groups, each batch containing five animals. Dorsal hairs at the back of the rats were removed 1 day before the commencement of the study and kept individually in cages to avoid contact with the other rats. Two groups of each were used for control and standard irritant. Other two groups were used as test. The 50 mg of each formulation were applied over one square centimeter area of whole and abraded skin of different animals. Aqueous solution of 0.8% formalin was used as standard irritant. At the end of the study, animal skin was evaluated for the skin irritancy and sensitization effect. The animals were observed for 7 days for any signs

of edema and erythema.

Stability testing

The developed ointment formulations were subjected to stability study as per the International Conference on Harmonization (ICH) guidelines. The formulated ointment was filled in the collapsible tubes and stored at different temperatures and humidity conditions, namely, 25°C±2°C /60%±5% RH, 30°C±2°C /65%±5% RH, and0°C±2°C /75%±5% RH for a period of 3 months and studied for appearance, pH, viscosity, and spredability.

PACKAGING, LABELLING, AND STORAGE

- Acyclovir ointment is **packed in**
- Primary package- aluminium tubes
- Secondary package-carton box
- **Label** must include;
- Name of preparation
- Quantity
- Route of administration
- Composition
- Direction of use
- Storage condition
- Warning and precautions
- Manufacturer details
- Batch number, manufacturing date, expiry date

Package/Label Display Panel;

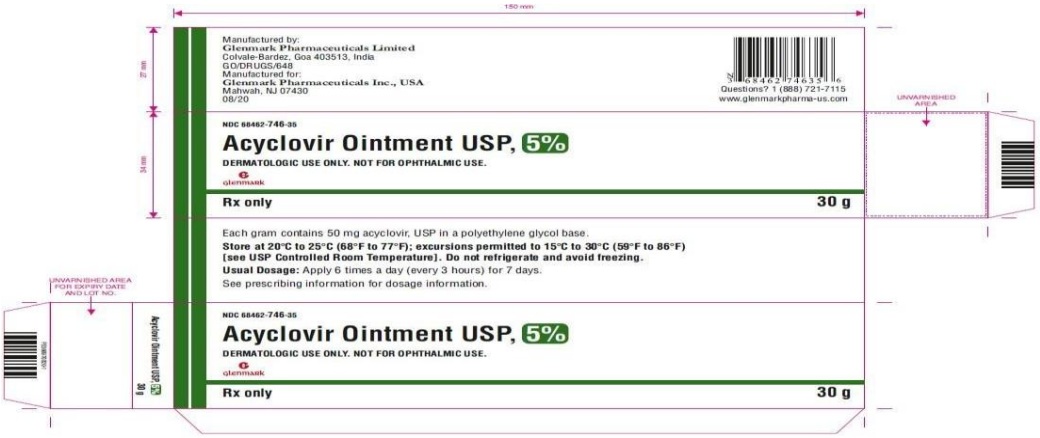


Figure no.12; Label display panel of acyclovir ointment.

➢ **Storage;**

- Stored at controlled room temperature **15-25°C**.
- Do not freeze.

CONCLUSION

In conclusion, the formulation and evaluation of Acyclovir ointment play an important role in the development of an effective topical antiviral therapy for the treatment of herpes simplex infections and other viral skin diseases. The formulation process involves the careful selection of suitable bases, stabilizers, preservatives, and penetration enhancers to ensure proper drug release, stability, patient acceptability, and therapeutic efficacy. A well-designed ointment formulation improves the bioavailability of acyclovir at the site of infection and enhances patient compliance due to ease of application and reduced systemic side effects.

The evaluation studies carried out on acyclovir ointment are essential to confirm the quality, safety, and effectiveness of the final product. Various physicochemical parameters such as appearance, pH, spreadability, viscosity, homogeneity, drug content uniformity, extrudability, and in-vitro drug diffusion are evaluated to ensure the formulation meets pharmaceutical standards. Stability studies also help determine the shelf life and storage conditions of the ointment. Microbial testing and skin irritation studies further confirm the safety and suitability of the preparation for topical use.

Overall, the review highlights that acyclovir ointment is a promising and widely used antiviral dosage form with significant therapeutic benefits. Continuous research and advancement in formulation techniques may further improve drug penetration, stability, and patient outcomes. Therefore, proper formulation and systematic evaluation are crucial for producing a safe, stable, and effective acyclovir ointment for clinical use.

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