FORMULATION AND EVALUATION STUDIES OF

ACYCLOVIR TOPICAL GELS FOR ANTI-VIRAL ACTIVITY

Dissertation submitted to

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(An ISO 9001-2000: Certified Institution)

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CERTIFICATE

This is to Certify that this dissertation entitled "**FORMULATION AND EVALUATION STUDIES OF ACYCLOVIR TOPICAL GELS FOR ANTI-VIRAL ACTIVITY"** by **Mr. A. Sivakumar** for the award of "**Master Of Pharmacy"** degree, comprises of the bonafide work done by him in the Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences for Girls, Tiruchirapalli, under my supervision and guidance and to my full satisfaction.

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THE PRINCIPAL
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We are pleased to inform you that the following two students of your college have been awarded scholarship for the year 2007-2008.

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This is to certify that this dissertation entitled "**FORMULATION AND EVALUATION STUDIES OF ACYCLOVIR TOPICAL GELS FOR ANTI-VIRAL ACTIVITY"** by **Mr.A. Sivakumar** for the award of "**Master of Pharmacy"** degree, comprises of the bonofide work done by him in the Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences for Girls, Tiruchirapalli, his work was supervised by **Mrs.K.Reeta Vijaya Rani, M.Pharm., (Ph.D)**., Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences for Girls, Tiruchirapalli.

I recommend this research work for acceptance as project for the partial fulfillment of the degree of "**Master of Pharmacy"** of the Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences for Girls, Tiruchirapalli, for the year March 2008.

Place : Tiruchirapalli
Date:

(Dr.R.Senthamarai)

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A.SIVAKUMAR

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INTRODUCTION

Continuous intravenous infusion is recognised[1] as a superior mode of drug administration not only to by pass hepatic "first pass" metabolism, but also to maintain constant drug level in the body. This provides direct entry of drug into the systemic circulation but entails certain risks.

Recently, the benefits of I.V. drug infusion can be duplicated without its hazards by using skin as the port of drug administration to provide continuous transversal drug infusion into the systemic circulation.

Topical administration is employed to deliver a drug immediately at the point of application, so enough drugs is absorbed into the systemic circulation to cause therapeutic effects. To provide continuous drug infusion through an intact skin, several topical formulations are used one of this is "Gels".

Gels mainly used for the purpose of topical dosage form especially which is to deliver drug across a localized area of the skin.

The demanding expectations of topical include:

1. Formulation of gel should have both physical and chemical stability
2. Formulation that have one (or) more components are non-sensitizing, non-irritating.
3. Formulation should have acceptability of the patient.
4. Formulation must have ability to release therapeutic levels of drugs and various

factors influence the absorption through the skin.

SKIN CHARACTERISTICS[2, 3]

The purpose of topical dosage form is to conveniently deliver drugs across a localized area of the skin. Medications are applied to the skin in the form of ointments, creams, gels etc. The absorption of substances from outside the skin,

including entrances into the blood stream is referred to as percutaneous absorption. It is necessary to understand the skin characteristics to develop an ideal topical dosage form.

SKIN

The skin is an organ because it consists of tissues structurally joined together to perform specific activities. It is one of the larger organs of the body in terms of surface area. For the average adult, the skin occupies a surface area of approximately 2 sq.m (3000 sq.inches)

It is a common site of administration for dermatological drugs to achieve a localized pharmacological action. Here, the drug molecules diffuse to a target in the skin to produce its action before it is distributed to the blood circulation for elimination.

The skin also serves as a port of administration for a number of systemically active drugs whereby those drugs applied topically are first absorbed into the blood circulation and then transported to the target for elicitation of its therapeutic effect.

STRUCTURE

Structurally, the skin consists of two principal parts. The outer, thinner portion, which is composed of epithelium, is called the epidermis. The epidermis is cemented to the inner, thicker, connective tissue part called the dermis. Beneath the dermis is a subcutaneous layer. This layer, also called the superficial fasica or hypodermis, consists of aerolar and adipose tissues.

Fibers from the dermis extend down into the subcutaneous layer and anchor the skin to it. The subcutaneous layer, in turn, is attached to underlying tissues and organs. The pH of the skin is normally between 5.0-6.0.

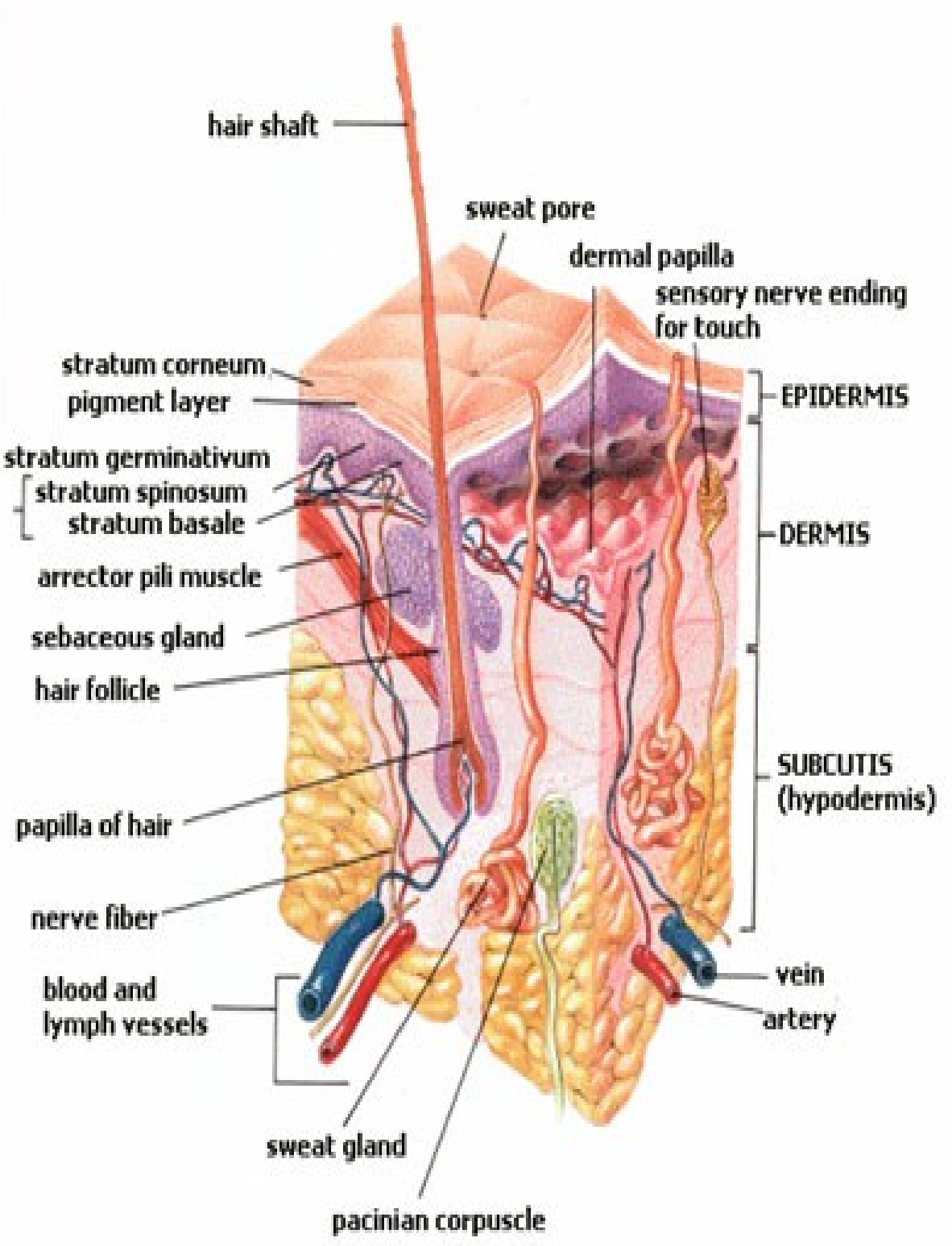


Figure 1: Structure of skin underlying subcutaneous tissue

Epidermis

The epidermis is composed of stratified squamous epithelium and contains four distinct types of cells. They are 1) Keratinocytes 2) Melanocytes Non-pigmented granular dedtrocytes formerly known as 3) Langerhans cells and 4) Granstein cells. The keratinocytes of the epidermis are organized into the following cell layers, from the deepest to the most superficial region.

1. Stratum basale
2. Stratum sponosum
3. Stratum granulosum
4. Stratumlucidum
5. Stratum corneum.

Dermis

The second principle part of the skin, is composed of connective tissue containing collagenous and elastic fibres. Numerous blood vessels, nerves, glands and hair follicles are embedded in the dermis.

The upper region of the dermis, about one fifth of the thickness of the total layer is named the papillary region or layer. It consists of loose connective tissue containing fine elastic fibres. Its surface area is greatly increased by small, finger like projection called dermal papillae (pa-PIL-e).

These structures project into the epidermis and many contain loops of capillaries. Some dermal papillae also contain corpuscles of touch, also called Meissner's corpuscles, never endings that are sensitive to touch.

The remaining portion of the dermis is called the reticular region or layer. It consists of dense, irregularly arranged connective tissue containing interlacing

bundles of collagenous and coarse elastic fibres. A small quantity of adipose tissue, hair follicles, nerves, oil glands and the ducts of sweat glands occupy spaces between the fibres. The combination of collagenous and elastic fibres in the reticular region provides the skin with strength, extensibility and elasticity.

The reticular region is attached to underlying organs, such as bone and muscle, by the subcutaneous layer. The subcutaneous layer also contains nerve endings called lamellate or paining corpuscles that are sensitive to pressure.

The permeability barrier of the skin consists of

1. The stratum corneum (10-50 micro meter thick)
2. The viable epidermis (100 micro meter thick)
3. The papillary layer of dermis (100-200 micro meter thick)

The composite structure of skin is pierced at various places by hair follicles and sweat glands, which occupy about 0.1-1% of total skin surface area.

Factors that affect the dissolution of drugs from topical preparations

Drugs incorporated in topical preparation may exert effect in two different ways.

- First is related to the pharmacological properties of the drug substance.
- In second exerted effect is non-specific, ointment, creams and gels constitute by far the vast majority.

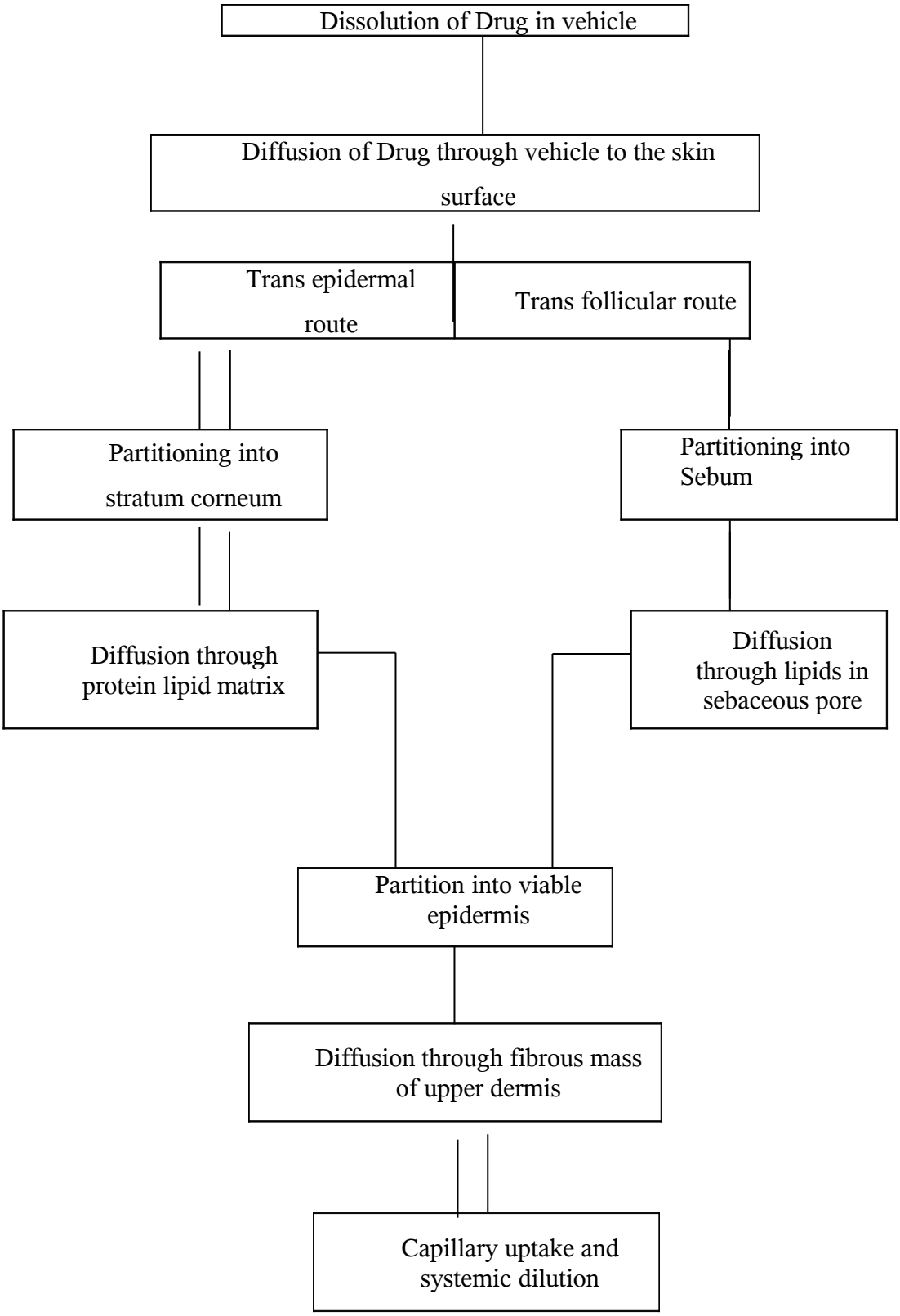

Dissolution of Drug in vehicle
Diffusion of Drug through vehicle to the skin surface
Trans epidermal route
Trans follicular route
Partitioning into stratum corneum
Partitioning into Sebum
Diffusion through protein lipid matrix
Diffusion through lipids in sebaceous pore
Partition into viable epidermis
Diffusion through fibrous mass of upper dermis
Capillary uptake and systemic dilution

Figure 2: Scheme of Events for Percutaneous Absorption[4]

FACTORS AFFECTING TRANSDEMAL PERMEABILITY

The principle of transport mechanism across mammalian skin is by passive diffusion[5] through primarily transepidermal route at steady state or through transappendegel route at non-steady state. The factors influencing and causing in transdermal permeability can be classified into three major categories:

1. Physico chemical properties of penetrants
2. Physico chemical properties of drug delivery system
3. Pathological & Physiological conditions of the skin.

Various physico-chemical propreties of the drug like partition co-efficient, concentration in the vehicle, conditions, molecular size and molecular weight play a vital role in deciding the percutaneous absorption.

The affinity of the vehicle for the drug[6] molecules solubility of the drug in the vehicle, pH of the vehicle can influence the release rate of the drug. The composition of the formulation has a great influence on percutaneous absorption of the drug .It may not only affect the drug release but also the permeability of the stratum corneum by means of hydration or absorption promoting effect[7]**.**

MECHANISTIC ANALYSIS OF DRUG PERMEATION THROUGH THE SKIN[2]

Before a topically applied drug can either locally or systemically, which must penetrate the stratum corneum as a skin barrier. Drug molecules may diffuse by three different routes. The intact skin, the hair follicle region and the sweat gland ducts. But there is no total agreement on the mechanism responsible for permeation through intact skin. The major pathway is diffusion through the intact, cornified cells of the stratum corneum. From this the dominant pathway to stratum corneum is by diffusion.

MECHANISM OF DIFFUSION THROUGH THE STRATUM CORNEUM[2]

The stratum corneum is a multicellular membrane and the intercellular regions are filled with lipid rich amorphous material. In the dry membrane, the intercellular volume may reach 5% of the total volume. Although molecules diffuse through intercellular regions, the available evidence indicates that for water-soluble, non-electrolytes diffusion is not primarily intercellular.

The transcellular permeation is explained on the basis of relatively smaller diffusion coefficient. Thus, molecules diffuse through intercellular route and also penetrate by transcellular mechanism.

The stratum corneum has a finite thickness and so there is a period of transient diffusion (lag time) after applying the drug to the skin, during which the rate of transfer through the skin rises to reach a steady state. The lag time (t), is related thickness of the membrane (h), and the diffusion coefficient (D) of the drug, by the relationship, $t=h^2/6D$.

The lag time has a direct bearing on the rate of the onset of skin penetration by the drug. Generally it ranges from minutes to days for the transepidermal route and from seconds to minutes for the trans follicular route. Thus an early pharmacological response would be dependant on some drug penetration by way of the transfollicular route. However, once the steady state has been established, the contribution of the shunt to the overall diffusion becomes negligible and bulk diffusion then occurs largely through the transepidermal route.

Damage or destruction of the stratum corneum barrier by keratolytic agents, cracking of the skin and by physical damage by increased absorption. Thus, the stratum corneum functions physiologically as the principle diffusion barrier. Once molecules pass the horny layer, they permeate rapidly through the living tissues of the epidermis and the dermis into the systemic circulation.

MECHANISM OF PERCUTANEOUS ABSORPTION[4]

The mechanism of percutaneous absorption for topical medication, which is important to asses the entire drug delivery system than the drug alone. It is important to consider.

1. Process of drug dissolution in the vehicle
2. Diffusion of the drug

These factors can be better understood by considering Fick's law, which describes drug transport across the skin, according to this,

$$J=P.C$$

Where,

J = the flux (is the amount of material passing through the barrier per unit area unit time)

C = the difference in concentration on both sides of the membrane

P = permeability constant

$$P=Km.Dm/h$$

Where,

Km = Partition coefficient of the drug molecule between the membrane and the vehicles in which the drug is dissolved.

Dm = diffusion constant of the drug in the skin

h = thickness of the membrane

The concentration of the drug in the vehicle, the thickness of the membrane barrier, the mobility of the drug molecule in the barrier and the relative solubility of the drug in the skin and the vehicle affect the percutaneous absorption of a drug.

Drugs applied to the skin surface reach the orifices of the sweat glands and the hair follicles directly. Once a substance passes through the stratum corneum, there is apparently no further hindrance to penetration of the remaining epidermal layers.

Diffusion through the horny layer is a passive process, which is affected only by the substance being absorbed, the medium in which the substance is dispersed and by ambient conditions. On the other hand percutaneous absorption is a complicated process, of which epidermal diffusion is the first phase and clearance from the dermis, the second. The latter depends on effective blood flow, interstitial fluid movements, lymphatic and combination with dermal constituents.

GELS AS TOPICAL APPLICATION

Gels are “Semisolid system in which liquid phase constrained within three dimensional polymeric matrix in which a high degree of physical or chemical cross liking has been introduced”. This network limits fluid flow by entrapment and immobilization of solvent molecules.

This network structure is also responsible for a gel resistance to deformation and clear as water in appearance and visually aesthetically pleasing as in gelatin deserts, their clarity ranges from clear to whitish translucent.

Preservatives may be incorporated into the gels especially for those prepared from natural sources. Appropriate preservatives depending upon the use and the gelling agent include the parabens (0.2%), benzoic acid (0.2%) and chlorcresol (0.1%).

The gels, are being used more frequently in therapeutic and cosmetic because of several properties such as

1. Semisolid state
2. High degree of clarity
3. Ease of application
4. Ease of removal and use.

The gels provide a faster release of drug substances, independent of water solubility of the drug.

GEL CHARACTERISTICS

Ideally, gelling agents should be inert, safe and non-reactive to other formulation components. The gelling agent should provide a reasonable solid like nature during storage that can be broken easily when subjected to the shear forces generated by squeezing a tube or during topical application. The gel should exhibit little viscosity change under the temperature variations of normal use and storage. The gel characteristics should match the intended use. A topical gel should not be tacky.

FORMULATION CONSIDERATIONS

In the formulation of gel, the efficiency is often dependent on the composition of the vehicle. The ability of a drug in gel formulation to penetrate the skin and exert its effect depends on two consecutive physical events. The drug must first diffuse out of the vehicle to the skin surface and then, it must penetrate the natural barrier to enter into the site of action. Many so called 'Vehicle effects' reported in the literature are the consequences of these two diffusional processes. These two processes are intimately related and are dependent upon physico - chemical properties of the drug, vehicle and the barrier.

FORMATION OF GEL

All polymer solutions are prone to settling to gels because the solute consists of long flexible chains of molecular thickness that find to become entangled, attract each other by secondary valence force. Cross linking of dissolved polymer molecule also causes their solutions to gel.

Gel often contract on standing and some of the interstitial liquid is squeezed out. This phenomenon called syneresis is due to crystallization or the formation of additional contact points between polymer segments on aging.

Pharmaceutical gels are random coil networks and hence further discussion or random coiled will be worthwhile. Random coil relation mechanisms are rooted in the polymer-polymer and polymer-solvent interactions with a given polymer the gel network increases in strength with increase in polymer concentration this result in a reduction of interparticle distances which subsequently leads to chain entanglement and further development of cross links. Continual addition of polymer strengthens the gel network and results in increased resistance and viscoelasticity.

Although the gel network is basically formed through polymeric interactions, the nature of the polymer-solvent affinity, actually determines the integrity of the gel. Classical theory is distinguished between three categories of solvents.

1. Free solvents that are very mobile
2. Solvent bound as a salvation layer usually through hydrogen bonding
3. Solvent entrapped within the network structure.

The ratio of the three solvent types in a given gel, are dependent on the polymer concentration and the solvent affinity for the polymer. Solvent affinity governs the extension of this random coil. The greater the solvent affinities the more coil expands and entangles with adjacent coils to form cross-links.

In a good solvent, solvent molecules interpenetrate the polymer chains and the salvation layer is enhanced, which facilitates random expansion and network formation. In a poor solvent, the polymer chain contract to minimize solvent contact reducing the effective number of cross-links and weakens the gel network structure.

Gelation theory can be readily applied when formulating gel products and some of the desirable attributes of gel formulations are in the following order. For optimum consumer appeal the gel should have good optical clarity and sparkling appearance. To preserve product integrity, the gel should maintain its viscosity at all temperatures.

STABILITY TESTING OF GEL FORMULATION

Stability is one of the important factor apart from the formulation. So evaluation of stability in gels is perhaps one of the important criteria. Chemical integrity of the dispersed active ingredients and the physical characteristics the gel system needs to be studied together. Most gels exhibit non-newtonion rheological behavior and they can be evaluated by a Rotational Viscometer like Brookfield Viscometer and Cone Plate Viscometer which operate at different shear rates. Similarly gels can be evaluated for spreadability.

A useful empirical test is the measurement force required to extrude the material from a deformable tube. While not strictly a test of product characteristics due to inclusion of the force necessary to deform the container, the method applies shear rate exceeding the yield value and exhibiting plug flow. Such flow can be measured by the use of pentrometer.

Yield value within the range of 100-1000 dyne/cm^2 is classified as spreadable. Below this range the material is too soft and flowing, above this range, it is hard and can not be spread. Apart from the above test, other tests which are used to examine semisolid products in general are also used for gels.

Freeze-thaw cycling test can be used to see whether separation or synthesis occurs. Storage of samples at various temperatures gives good information about the storage requirements for that gel. The elevated temperature should not be more than 45^0-50^0C.

Gels and jellies now days have gained more importance and extensive studies on release characteristics have revealed that the active ingredients are better percutaneously absorbed from the gel based formulation, than from the creams and ointment base.

The ointment base has limitation in miscibility with the alcohol and the emulsion based creams lead to crack if the alcohol content is more. It will be no wonder if the gel based formulation greatly replaces ointments and creams in future.

VIRAL DISEASE [8]

The Herpes virus family contains over a hundred species of enveloped DNA viruses that affect humans and animals. They are characterized by their ability to establish latent infections enabling the virus to persist indefinitely within infected hosts and to undergo periodic reactivation.

Herpes simplex virus belongs to a family of viruses called *Herpesviridae*. They are composed of a central DNA core and a protein capscid with 162 hollow cylindrical apsomers. This nucleocapsid is surrounded by an envelope forming a virus particle with an overall diameter of 130-180nm.

Herpes simplex virus usually affects tissues of ectodermal origin, such as skin, mucous membrane and the nervous system. After the attachment to specific receptors on the surface of the human cells the virion loses its envelope to the cell membrane and enters the cells by pinocytosis.

The DNA released into the cells travels to the nucleus. In namely thymidine kinase and DNA polymerase. Viral proteins synthesized in the cytoplasm are transferred to the nucleus where the nuleocapscid is assembled. The nucleocapscids packed with such particles before it ultimately undergoes cell lysis and releases the infectious particles.

Groups	Species (Official name)	Species (Common name)	Site of latent infection
Alpha-herpes virus	Human herpes virus type 1	Herpes simplex virus type1(HSV-1)	Neurons
	Human herpes virus type 2	Herpes simplex virus type2(HSV-2)	Neurons
	Human herpes virus type 3	Varicella zoster virus (VZV)	Neurons
Beta-herpes virus	Human herpes virus type 4	Cytomegalovirus	Secretory glands, kidneys, other organs and tissues
	Human herpes virus type 5	Human B cell lymphtropic virus	Lymphoid tissues
	Human herpes virus type 6	R K virus	Lymphoid tissues
Gamma-herpes virus	Human herpes virus type 7	Epstein-Barr virus	Lymphoid tissues
	Human herpes virus type 8	--------	-------

Virus types

The family of Herpes viruses is very large, and its members infect most animal species. There are eight recognized herpes viruses that affect humans. These viruses are divided into three groups are listed in Table 1.

Table 1: Virus types

Worldwide, more than 90 percent of humans have been exposed to HSV-1 and are seropositive for this virus by the fourth decade of life. HSV type 1 is usually isolated from lesions in and around the mouth and is transmitted by direct contact or droplet spread from the cases or carriers. HSV type 2 is responsible for the majority of genital herpes infections and is commonly transmitted venerially.

HERPES SIMPLEX VIRUS INFECTION

Herpes Simplex Virus (HSV) is one of the most common agents infecting humans of all ages. The virus occurs and produces a variety of illness, including mucocutaneous infections, infections of the CNS, and occasionally infections of the visceral organs. Infections in children can include neonatal disease, mucocutaneous infections during childhood and adolescence, and serious disease in individuals who are immunocompromised.

Genital HSV infection in older adolescents and adults is a major public health problem. This increased prevalence of genital HSV infections posses major threats to newborns because most infections in neonates are acquired perinatally. Neonatal HSV infection is a disease with high morbidity and mortality rates.

CLINICAL FEATURES

The clinical manifestations depend on the site of action, age and immune status of the host, and the antigenic type of the virus.

Cutaneous infections

The most common site is the face on the cheeks, chin, around the mouth and on the forehead. Lesions may also appear on the buttocks in infants as napkin rash. “Eczema herpeticum” is a generalized eruption caused by herpes infection in children suffering from eczema.

Mucosal infections

The buccal mucosa is the site most commonly affected. Gingivostomatitis and pharyngitis are the most frequent conditions in primary infection and *Herpes labialis* in recurrent infection. The vesicles may ulcerate and become secondarily infected.

Ophthalmic infections

The most common cause is corneal blindness and acute keratoconjuctivitis may occur by itself or by extension from facial herpes. Follicular conjunctivitis with vesicle formation on the lids is another manifestation.

Nervous system infections

HSV can cause sacral autonomic dysfunction also rarely transverse myelitis or the Guillian Barre syndrome. HSV has been implicated in the etiology of Bell’s palsy. HSV encephalitis has an acute onset with fever and focal neurological symptoms. Brai biopsy was employed in diagnosis for instituting early specific therapy.

Genital infections

In men, the lesions occur mainly on the penis or in the urethra causing urethritis. In women the cervix, vagina, vulva and perineum are affected. The primary infection is usually more serious, accompanied by systemic features like

fever and malaise. Both type of HSV may cause genital lesions, though HSV 2 is responsible more frequently and causes many more recurrences.

DIAGNOSIS

The diagnosis of herpes virus infection may be made by microscopy, antigen or DNA detection, virus isolation or serology.

Microscopy: Smears are prepared from the lesions, preferably from the base of vesicles and stained with 1% aqueous solution of toluidine blue for 15 seconds. The virus particle may also be demonstrated under the electron microscope.

Virus isolation: Human embryonic kidney, human amnion and many other cells are susceptible for this. Vesicle fluid, Spinal fluid, Saliva and Swabs may be used. Typical cenotaphic changes may appear as early as in 24-48hrs but cultures should be observed for two weeks.

Serology: Serological methods are useful in the diagnosis of primary infections. Antibodies develop within a few days of infection and rise in the titre of antibodies may be demonstrated by ELISA, neutralization or complement fixation tests.

TREATMENT

Idoxyuridine is used topically in eye and skin infections were one of the first clinically successful antiviral agents. The introduction of **Acyclovir** and **Vidarabine** enabled the effective management of deep and systemic infections. Early treatment with intravenous acyclovir has improved the outcome of encephalitis. Oral and **topical use** may help in less serious conditions. **Valacyclovir** and **Famciclovir** are more effective oral agents.

VARICELLA-ZOSTER VIRUS INFECTIONS [9]

Varicella Zoster Virus (VZV) is one of the most common viral infections of the peripheral nervous system. Latent infection of neurons in the sensory ganglia of the spinal cord and brain stem follows chicken pox, and reactivation leads to a

painful, vesicular skin eruption in the distribution of sensory dermatomes (Shingles), most frequently thoracic or trigeminal.

The virus may be transported along the sensory nerves to the skin , where it establishes on active infection of epidermal cells. In small portions of patients, weakness is also apparent in the same distribution. Although the factors that give rise to reactivation are not fully understood, decreased cell-mediated immunity is of major importance in some cases.

Affected ganglia show neuronal destruction and loss, usually accompanied by abundant mononuclear inflammatory infiltrates, regional necrosis with hemorrhage may also be found. Pheripheral nerve shows axonal degeneration after the depth of the sensory neurons. Focal destruction of the large motor neurons of the anterior horns or cranial nerve motor nuclei may be seen at the cones ponding levels. Intranuclear inclusions generally are not found in the pheripheral nervous system.

Pathophysiology

The host immunologic mechanisms suppress replication of the virus. Reactivation can occur if host immune mechanisms are compromised. This may be caused by medications, illness, malnutrition, or by the natural decline in immune function with aging. Upon reactivation, the virus migrates along sensory nerves and produces sensory loss, pain and other neurologic complications. If motor nerve roots are also involved, weakness can develop in addition to sensory changes.

Mortality/Morbidity

- Severe pain and insomnia are most bothersome to patients. About 95% of patients with zoster experience severe pain during the illness.
- Other presentations of zoster, including ocular (keratitis) and spinal cord (myelitis) presentations may result in additional morbidity.

- Bacterial super infection (impetgenzation) of vesicular skin lesions can occur. The vesicular eruption of VZV infection may be more difficult to diagnose in patients with darker skin.

Causes

Sex: Varicella Zoster Virus infection occurs with equal frequency in males and females.

Age:

- After primary infection, zoster can occur at any age. However, the risk of zoster increase with age.
- The risk of postherapeutic neuralgia also increases with advancing age.
- **Herpes zoster (Shingles)**
    - The most common presentation is the shingles vesicular rash, which most commonly affects a thoracic dermatome.
    - After a prodermal illness of pain and paresthesias, erythematous muscles papules develop and progress to vesicles within 24 hours. The vesicles eventually crust and resolve.
    - Pain and sensory loss are the usual symptoms, but motor weakness also occurs and is frequently missed on examination. Motor weakness results when the viral activity extends beyond the sensory root to involve the motor root.

Diagnosis

Diagnosis is usually clinical. Smears are prepared by scraping the base of the early vesicles and stained with toluidine blue. Electron microscopy of the vesicle fluid may demonstrate the virus with typical herpes morphology,

Prophylaxis and Treatment

A live varicella vaccine was developed by serial passage in tissue culture. Given subcutaneously, it induced good antibody response but it was liable and had to be stored frozen. A modified lyophilized form of the vaccine is now available which can be stored between 2^0C-8^0C. Specific treatment is indicated mainly in immunodeficient and elderly subjects and those with complications such as varicella pneumonia, encephalitis and disseminated zoster. **Acyclovir** and **Famciclovir** are effective. Corticosteroids are contraindicated in varicella as they enhance the risk of pneumonia and disseminated disease.

Antiviral agents[10]

Table 2: Antiviral agents

Drug Name	Acyclovir	Valacyclovir	Famciclovir (Famvir)	Penciclovir (Denavir)
Description	Inhibits activity of HSV-1, HSV-2, available as oral suspension, tablet, capsule, injection and topical.	Prodrug rapidly converted to active drug acyclovir, use in adolescent HSV infection.	Transformed to active nucleoside analogue penciclovir, inhibits HSV DNA synthesis	1% cream approved for orolabial HSV infection and inhibit viral DNA synthesis
Adult Dose	200mg or 400mg 5times for 7-10 days, 800mg 3times for 2 days.	1000mg for 10days and 500mg for 3 days	250mg for 7-10 days	Apply and cover lesion for 4 days
Pediatric Dose	15mg/kg 5times for 7days, 5mg/kg IV for 7-10 days	Not established	Not established	Not FDA approved for children
Contraindications	Hypersensitivity	Hypersensitivity	Hypersensitivity	Hypersensitivity
Interactions	Zidovudine or probenecid prolongs half-life and can increase CNS toxicity	Zidovudine,probencid and cimetidine prolongs half-life and can increase CNS toxicity	Probenecid or cimetidine may increase toxicity.	Not reported
Precautions	Renal dysfunction can occur during high dose IV administration ; can minimize effect by using slow infusion.	Caution in renal failure and with co administration of nephrotoxic drugs.	Caution in renal failure and with co administration of nephrotoxic drugs.	Mild erythema possible, do not apply to mucosal surfaces.

LITERATURE REVIEW

Jocelyne Piret et al[11]., have carried out the topical efficacies of foscarnet and acyclovir incorporated into a polyoxypropylene-polyoxyethylene polymer were evaluated and compared to that of 5% acyclovir ointment by use of a murine model of cutaneous herpes simplex virus type 1 infection in mice, whereas the foscarnet formulation has less of an antiviral effect but a single application given 24 hrs postinfection resulted in a significantly higher efficacy of the formulation of acyclovir than of the acyclovir ointment. Acyclovir incorporated within the polymer was also significantly more effective than the acyclovir ointment when treatment was initiated on day 5 postinfection. The higher efficacy of the acyclovir formulation than of the acyclovir ointment is attributed to the semi viscous character of the polymer, which allows better penetration of the drug into the skin.

Min Jiang et al[12]., have studied In vitro evaluation of percutaneous absorption of Acyclovir product using intact and Tape-stripped human skin, Acyclovir ointment (~10 mg) spiked with H-labeled acyclovir was applied onto the stratum corneum/epidermis side. The skin sections were continually perfused on the dermis side with sterilized culture medium. After 24 hours, the percentages of acyclovir-derived radioactivity in different components were obtained with the intact skin sections through human skin layers and therefore can potentially be used for dermal formulation characterization and development.

L. Trottet et al[13]., were studied all acyclovir cream formulations were bioequivalent, Topical acyclovir cream containing 40% propylene glycol (PG), the optimum found for skin penetration in vitro skin permeation study compared the innovator cream with two generics containing about 15% PG and 10 generics containing 0–15% PG were tested and ACV analyzed by LC-MS-MS the studies suggest that not all marketed ACV creams are bioequivalent to the clinically proven innovator.

Sintov et al[14]., The present invention relates to an antiviral topical pharmaceutical composition for treating viral diseases of the skin or mucosa comprises a poorly soluble antiviral nucleoside derivative in gel formulation, it enhances the absorption of acyclovir and the formulation containing a gelling agent carboxylic acid salt or dicarboxylic acid salt, to compare several acyclovir gels and one w/o cream that were formulated. The in vitro percutaneous absorption/penetration of acyclovir from the gel or cream vehicles was carried out using Franz diffusion cell system and the efficacy of a drug-containing gel and ZOVIRAX commercial cream was evaluated as compared to a non-treatment control. A guinea pig model was selected for the cutaneous HSV-1, because the model mimics the human herpes simplex labialis infection.

S. S. Dubhashi et al[15]., studied skin permeation of acyclovir by HPTLC method, in this study Separation of guinea pig skin proteins and acyclovir was achieved by employing a mobile phase consisting of chloroform–methanol–ammonia (15:9:4, v/ v/v) on precoated silica gel 60F254 aluminum plates. Densitometnic analysis was carried out at 255 nm. The average recoveries of 101.8 and 100.1% were recorded for two marketed preparations studied. The method was employed to optirmize topical liposomal gel formulation of acyclovir on basis of maximum skin permeation.

Chun Yang et al[16]., have worked Chemical stability, Enzymatic hydrolysis and Nasal uptake of amino acid ester prodrugs of Acyclovir and the work was to improve nasal absorption of impermeable small drug molecules by amino acid prodrug approach, Acyclovir was selected as a model drug.

Castela N et al[17]., Ganciclovir ophthalmic gel in herpes simplex virus rabbit keratitis: intraocular penetration and efficacy A chronic administration of three ganciclovir gels (0.2%, 0.05%, 0.0125%) was compared with a placebo gel and a 3% acyclovir ophthalmic ointment in the treatment of HSV-1 rabbit keratitis. All the ganciclovir gels showed a clinical efficacy: the efficacy was slower than using

acyclovir ointment, no significant difference could be shown between the 0.2% and 0.05% ganciclovir gels or the 0.05% ganciclovir gel and the acyclovir treatment on viral isolation, The distribution of ganciclovir and acyclovir into the rabbit eyes were similar and this study suggests a comparable activity on HSV-1 superficial keratitis between 0.05%, 0.2% ganciclovir gels and 3% acyclovir ointment.

Hoh HB, Hurley C et al[18]., Randomized trial of ganciclovir and acyclovir in the treatment of herpes simplex dentric keratitis, this study was designed to assess the relative efficacy of topical ganciclovir 0.15% gel and acyclovir 3% ointment in the treatment of herpes simplex dendritic keratitis, both treatment were administered on a five times daily basis to patients suffering from herpes simplex keratitis There was no statistically significant difference detected in the rate of healing between the two treatment groups over the course of the trial the relative efficacy of topical ganciclovir and acyclovir in the treatment of herpes simplex dendritic keratitis showed that both treatment were equally effective in the viral induced corneal ulceration**.**

Eric M. Morrel et al[19]**.,** Transport delivers unprecedented levels of acyclovir with novel five percent gel formulation, the topical treatment of dermatological conditions were announced preliminary results from a recently completed Phase I safety and pharmacokinetic study with the SoloVir electro kinetic transdermal system and five-percent acyclovir gel formulation. Treatment of a herpetic episode with the SoloVir will consist of a single ten minute treatment, each subject received four treatments, a single ten minute application delivered approximately 40 micrograms of acyclovir to the circulation after passing through stratum corneum and locally depositing in the epidermis.

C. Martnez-Sancho et al[20]., Poly (D, L-lactide-co-glycolide) micro spheres for long-term intravitreal delivery of acyclovir : influence of fatty and non-fatty additives. In this work, acyclovir containing Poly (D,L-lactide-co-glycolide) micro spheres were prepared by the solvent evaporation method. Seven additives were incorporated in the micro spheres to modulate the in vitro release rate of the drug and these are evaluated by scanning electro microscopy, Granulometric analysis showed that particle size distribution. The release of acyclovir from these micro spheres was adjusted to a zero order kinetics.

Massimo Fresta et al[21]., Studied ocular tolerability and in vivo bioavailability of PEG-coated and polyethyl-2-cyanoacrylate nanosphere-encapsulated Acyclovir and were prepared by an emulsion polymerization process in the micellar phase and characterized, presence of PEG also resulted in a changing the zeta potential but zeta potential was not influenced in vitro drug release was determined in both 7.4 phosphate buffer and plasma. The ocular tolerability was evaluated by the in vivo Draize test and the aqueous humour acyclovir was determined by HPLC, from this the two formulations showed significant increase of drug levels with the free drug.

Elaine D. Mackowiak et al[22]**., studied Prevention and Treatment of Cold sores, the** antiviral drugs approved by FDA for the treatment of herpes labialis are Penciclovir 1% cream, acyclovir 5% ointment, and valcyclovir , Acyclovir was the first oral antiviral drug approved by FDA for use in HSV-2 and VZV infections in immunocompetent patients and for HSV-1 infections in immunocompromised patients, the addition of sodium lauryl sulfate 5% to a foscarnet 3% gel formulation increased its protective effect against HSV-1, oral famciclovir (Famvir) in doses of 250 mg or 500 mg twice daily started 24 hours before laser skin resurfacing reduced reactivation of cold sores by 90% in a series. Another drug, valacyclovir was recently approved by FDA for the prevention of cold sores in people infected with HIV. Valacyclovir is the prodrug of acyclovir.

Loice Kikwai et al[23]., have studied in vitro and in vivo evaluation of Topical formulations of Spantide II . The lotion and gel was formulated with and without n-methyl-2-pyrollidine as a penetration enhacer. The release of Spantide II from gel was studied using microporous polyethylene and polypropylene membranes in Franz Diffussion cell, in vitro percutaneous absorption from gel and cream was evaluated using hairless rat skin in above setup and the in vivo anti-inflammatory was evaluated in mouse model. Among different gels studied, PF127 gel showed highest (70fold) release compared with HPMC and HPC gels. Lotion and gels with or without n-methyl-2-pyrollidine showed no detectable levels of Spantide II in the receiver compartment of the Franz diffusion cell.

Aly, A. M et al[24]**.,** have studied Formulation and *in vitro* evaluation of fluconazole topical gel, Seven gel formulations were prepared using sodium carboxymethylcellulose, sodium alginate, hydroxypropyl methylcellulose (HPMC), plantago, agar, pectin, and tragacanth containing 2% w/w fluconazole. The *in vitro* release was conducted using an artificial cellophane membrane. The permeation of fluconazole through hairless mouse skin was also studied. The receptor solution was saline phosphate buffer (pH 7.4). It was found that, plantago and sodium carboxy methyl cellulose are the best gel bases for this purpose. Fluconazole permeated through the hairless mouse skin exceeded the MIC from all the tested gel formulation within 30 minutes, except in the case of pectin where the MIC was reached between 30 and 90 minutes. It is concluded that fluconazole can be used topically, as gel formulations.

Jocelyne Piret et al[25]., carried out Sodium Lauryl Sulfate increases the efficacy of a topical formulation of Foscarnet against Herpes Simplex Virus Type 1 Cutaneous lesions in Mice, the addition of 5% SLS to this gel formulation markedly reduced the mean lesion score. The improved efficacy of the foscarnet formulation containing SLS could be attributed to an increased penetration of the antiviral agent into the epidermis also inhibited the HSV-1 strain. Foscarnet in phosphate-buffered saline decreased in a dose-dependent manner the viability of cultured human skin fibroblasts. This toxic effect was markedly decreased when foscarnet was incorporated into the polymer matrix. The use of gel formulations containing foscarnet and SLS could represent an attractive approach to the treatment of herpetic mucocutaneous lesions, especially those caused by acyclovir-resistant strains.

Reddy M Sreenivasa et al[26]**.,** have studied Preparation and evaluation of minoxidil gels for topical application in alopecia, using carbopol, hydroxypropyl cellulose, hydroxypropyl methylcellulose and combination of hydroxypropyl cellulose, hydroxypropyl methylcellulose. The gels were evaluated for drug content, viscosity determination, in vitro permeation (across dialysis membrane and mouse skin), skin irritation and stability at 4, 25 and 37° tests. The drug content of the gels was found to range from 96.40±0.57 to 98.10±0.32%. The viscosity of the gels ranged between 13,780±100 and 24,950±150 cps. The drug permeation across dialysis membrane from all the formulations at the end of 24 h was almost same and ranged between 92.05±1.52 and 93.52±1.95%, the percentage release of drug was found to increase in the following order of the polymer composition: HPC>Carbopol>HPMC>HPMC+HPC. All the gel formulations released almost similar amounts of drug (90.05±1.92 to 91.56±1.65%) across the mouse skin. The prepared gels did not produce any dermatological reactions and the gels were found to be physically stable at all temperature conditions for 3 months.

Joseph M Beaurline et al[27]., have carried out Gel formulations for topical drug delivery, relates to improved pharmaceutical gel formulations for the topical delivery of drugs, gel formulations including a drug 4-amino-2-ethoxymethyl-α,α-dimethyl-1H-imidazo[4,5-c]quinoline -1-ethanol, gelling agents colloidal silicon dioxide, triacetin and propylene glycol. The drug is preferably, which has been found to be sufficiently soluble and chemically stable in gel formulations, inclusion of propylene glycol thickens the gel formulations that may act as a solvent for the colloidal silicon dioxide and as a solubilizer for the drug and then heating with mixing to a temperature of about 50-55° C when the drug appears to be completely dissolved, the resulting mixture is sheared on a high speed propeller mixer until a homogeneous gel is formed. Drug release test was carried by using a modified Franz diffusion cell 10 of the type, a section of synthetic membrane 11 (microporous polyethylene film) was used and 0.1M sodium acetate buffer, pH 4.0) used as a medium. A modified Franz diffusion cell 20 of the type is used in In Vitro Skin Penetration Test Method using two types of skin are, hairless mouse skin and human cadaver skin.

Purushothamam rao K et al[28]., have studied development and evaluation of controlled release ciprofloxacin hydrochloride medicated dental paste, Formulations using various mucoadhesive polymers such as MC, HPMC, HEC, and NaCMC as release retardants. The formulations were subjected for various physicochemical tests like pH, spreadability, extrudability, drug content, in-vitro release and stability studies, the release studies were carried over a period of 6hrs and release was dependant on the type of polymer used. Stability studies showed no significant variations in pH, spreadability, viscosity, extrudability and drug content.

Devalina Law et al[29]**.**, have studied Ritonavir-PEG 8000 at ritonavir is a substrate of P-glycoprotein and the oral absorption of ritonavir could be limited by both dissolution and permeability. The effect of enhanced dissolution rate on oral absorption was explored; specifically PEG-amorphous ritonavir solid dispersions were prepared with different drug loadings. In vitro evaluation was conducted in 0.1N HCL with a USP apparatus I, the dissolution measurements of the two solid

phases indicated a 10-fold improvement in this comparison study. In vivo study results indicate that amorphous solid dispersions containing 10-30% drug exhibited significant increases in AUC and Cmax over crystalline drug. However, both in vitro dissolution and bioavailability decreased with increasing drug load.

Antiviral activity of thiazole derivatives in chick embryo was studied by **Alka Tripathi et al[30].,** The antiviral activity was evaluated against SRV in vitro and in vivo method. The compounds were also tested against Ranikhet disease virus (RDV) in a stationary culture of chorioallantoic membrane of chick embryo, in vitro exhibited a mild inhibitory effect and showed significant inhibition in vivo ranging from 65%-75%.

Alvara Jimenez-Kairuz et al[31] has studied mechanism of Lidocaine release from carbomer-lidocaine hydro gels, delivery rates of free base lidocaine were measured in a Franz-type biocompartmental device using water and Nacl 0.9% solution as receptor media. Carbomer-lidocaine hydro gels behave as a reservoir and pH suggests that, under the main conditions assayed, dissociation of ($R{=}COO^{-}\ LH^{+}$) is that controls the releasing rate. Accordingly, release rate was increased upon addition of a second counter ion (i.e. Na^{+}), into the matrix of the gel.

Bryan J. Campbell et al[32]., have studied Systemic absorption of topical Lidocaine in normal volunteers, patients with post-herpetic Neuralgia and patients with acute Herpes Zoster, the study was to characterize the absorption profile of lidocaine from patch and gel formulations in normal volunteers, patients with post-herpetic neuralgia and patients with acute herpes zoster., the primary active metabolite of lidocaine after application gel or patches was minimal in all types of patients. Considering the systemic absorption and toxicity of lidocaine seems not to be a significant risk.

Lalezari J et al[33]., A randomize double blind, placebo-controlled trial of cidofovir gel for the treatment of acyclovir-unresponsive mucocutaneous herpes simplex virus infection in patients with AIDS, The safety and efficacy of cidofovir gel for treatment of acyclovir-unresponsive herpes simplex virus infections in AIDS patients was evaluated in a randomized, double-blind, multicenter trial. Cidofovir (0.3% or 1%) or placebo gel was applied once daily for 5 days Application site reactions occurred in 25% of cidofovir-treated and 20% of placebo-treated patients; none was dose-limiting. Cidofovir therapy provided significant benefits in lesion healing, virologic effect, and pain reduction.

Ying-Yue Wang et al[34]., have studied In vitro and in vivo evaluations of topically applied capsaicin and nonivamide from hydro gels and compare with commercialized creams. Both skin stripping technique and Hexameter were applied to evaluate the level of capsaicin and nonivamide retained in stratum corneum and skin erythema in vivo, the in vitro capsaicin permeation showed higher levels in cationic chitosan and anionic CMC hydro gels than creams and the cream reduced in vivo skin erythema however, the dose-dependence was not observed in hydro gels, so this study indicates correlation between in vitro skin permeation and in vivo erythema response.

SCOPE AND PLAN OF WORK

Acyclovir is a broad-spectrum anti-viral agent against Herpes Simplex Virus (HSV) and Varicella Zoster Virus (VZV). Two conditions like Chicken pox and Shingles caused by VZV, which infects mucous membrane, skin and neurons.

Acyclovir is poorly water soluble and poor oral bioavailability, hence intravenous administration is necessary if high concentrations with fewer side-effects.

Therapy for this disease is based on the application of anti-viral agents to inhibit virus growth. Topical semi-solid preparations are designed to produce local activity. Creams, gels, ointments and pastes are some of the topical semi-solids in use for many decades.

These are evolved with little understanding or absorption mechanism. But now gels have gained more and more importance because the gel formulations have better percutaneous absorption than Creams and Ointments.

Gels are gaining more popular due to ease of application, undetectable to eye and neither tacky nor greasy.

Hence a study on formulation and evaluation of Acyclovir gel was as a principal objective for anti-viral activity.

Acyclovir appears to be more active anti-viral agent and is usually well tolerated. It works by stopping the spread of the HSV in the body. Non-availability of Acyclovir gels in the market is one of the reasons for the study. So the aim of project work is to develop gel formulation containing Acyclovir

The following objectives are the main considerations in the research work:

- Compatibility study of Drug with different polymers like Carbopol, Hydroxypropyl methyl cellulose and Sodium Carboxy methyl cellulose.

- Designing of trial formula for different concentration of each polymer

- Optimization of the formula to attain all gel characteristics

- Selection of suitable formula from each polymer and preparation of the gel formulations

- Evaluation of the prepared gels for
    - Drug content
    - pH
    - Viscosity
    - Spreadability
    - Extrudability

- In-vitro drug release for each gel formulation and compare the results and also study the effect of permeation enhancer for each formulation. From this the best formulation is selected for further studies

- Stability study of the selected formulation to perform for three months in different storage conditions

- In-vivo evaluation of the selected formulation to perform by using Albino Rabbits and comparison with marketed Acyclovir topical formulation.

PROFILE OF DRUG AND CHEMICALS

Acyclovir – Drug profile[35]

Chemistry

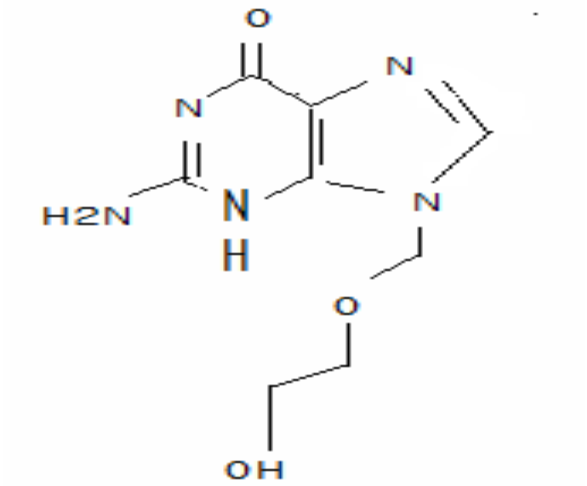


Figure 3: 2-amino-9-((2-hydroxyethoxy)methyl)-3,9-dihydro-6H-purin-6-one

Descripton[36]

Colour : White crystalline powder

Odour : Characteristic

Taste : Bitter to alkaline

Solubility: Slightly soluble in water, very slightly soluble in alcohol, freely soluble in Di methyl sulfoxide and dilute solutions of mineral acids and alkali hydroxides.

CLINICAL PHARMACOLOGY [10]

Acyclovir contains a partial nucleoside structure, the sugar ring is replaced by an open-chain structure. It is selectively converted into aciclo-guanosine monophosphate (aciclo-GMP) by viral thymidine kinase, which is far more effective (3000 times) in phosphorylation than cellular thymidine kinase.

Subsequently, the monophosphate form is further phosphorylated into active triphosphate form, aciclo-guanosine triphosphate (aciclo-GTP), by cellular kinase. Aciclo-GTP is a very potent inhibitor of viral DNA polymerase, it has approximately 100 times greater affinity for viral than cellular polymerase.

As a substrate, aciclo-GMP is incorporated into viral DNA, resulting in chain termination.Viral enzymes cannot remove aciclo-GMP from the chain, which results in inhibition of further activity of DNA polymerase. Aciclo-GTP is fairly rapidly metabolized within the cell, possibly by cellular phosphatases.

Acyclovir is specific to viral-infected cells with low toxicity and which is less toxic than earlier generation of antiviral agents and as such represents a major therapeutic advance.

Acyclovir can be considered a prodrug, it is administered in an inactive or less active and is metabolized into a more active species after administration.

Acyclovir is active against most species in the herpes virus family, in descending order of activity:

- Herpes simplex virus type I (HSV-1)
- Herpes simplex virus type II (HSV-2)
- Varicella zoster virus (VZV)
- Cyto megalo virus (CMV)
- Activity is predominantly against HSV and VZV, it is only limited efficiency against EBV and CMV.

Pharmacokinetics

Acyclovir is poorly water soluble and has poor oral bioavailability, hence intravenous administration is necessary if high concentrations are required. When orally administered,

- Bioavailability - 10-20%
- Protein binding - 30%
- Metabolism - Viral thymidine kinase
- Elimination half life - 2 -3 hours
- t_{max} - 1-2 hours
- Acyclovir has a high distribution rate
    - Excretion - Renally excreted, partly by glomerullar filtration and Partly by tubular secretion.

Adverse Effects

- Common adverse drug reactions associated with systemic Acyclovir therapy include : nausea, vomiting and headache.
- Infrequent adverse effects include: confusion, dizziness, edema, sore throat, constipation, abdominal pain and weakness**.**
- Rare adverse effects include: coma, seizures, neutropenia, leucopenia, crystalluria, hepatitis and anaphylaxis.

Therapeutic Use

Anti-viral activity against Herpes Simplex Virus and Varicella Zoster Virus infections in immunocompromised patients.

Toxicity

Its use should be avoided during pregnancy**.**

Drug interaction

Zidovudine or probenecid prolongs half-life and can increase CNS toxicity

Dosage forms

Intravenous Infusion; Capsules; Tablets; Suspension; Topical Cream; Topical Ointment

Dose[37]

Oral

200mg 5 times daily every four hours for 5-10 days,

400mg 5 times daily for 5 days in severly immunocompromised patients

800mg 4-5 times daily for 5-7 days.

Topical

5% ointment/cream 5-6 times daily every 3-4 hours for 5-10 days

Contraindications

Hypersensitivity

Trade Name	Preparation	Dose	Manufacturer
ACIV	Tablets	200 and 800mg	Zee lab
ACIVIR	Tablets, Injection, Cream 5%, Ointment 3%	200,400 and 800mg 10ml 5gm 5gm	Cipla
ACIVIRALL	DT-Tablets	200 and 400mg	Finecure
ALOVIR	Tablets	200,400 and 800mg	Adley
AXOVIR	Tablets, Injection	200,400 and 800mg 250 and 800mg vials	Samarth
CLOVIRAX	DT-Tablets, Cream 5%	200,400 and 800mg 5 gm	Puerhealth
CYCLOVIR	Tablets, Cream 5%	200mg 5 gm	Zydus Cadilla
HERPAX	Tablets, Cream 5%, Ointment 5%	200,400 and 800mg 5gm 5gm	Microvision
HERPEX	DT-Tablets, Cream 5%	200 and 800mg 5 gm	Torrent
LOVIR	Tablets	400 and 800mg	Eli lilly
OCUVIR OCUVIR EYE OINTMENT OCUVIR SKIN	DT-Tablets Eye ointment 3% Cream 5%	200,400 and 800mg 5 gm 5 gm	FDC
ZOVIRAX ZOVIRAX OPHTHALMIC OINTMENT	Tablets, Suspension Ointment 3%	200,400 and 800mg 400 mg/5ml 5 gm	GSK
VIRODERM	Ointment 5%	5 gm	Emcure

Table 3: Acyclovir products in market[37]

Carbomer[38]

Structural formula

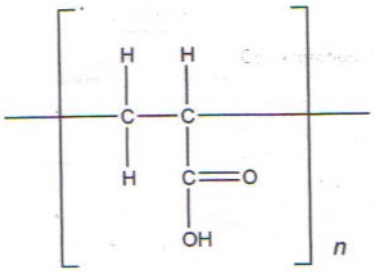


Figure 4: Acrylic acid monomer unit in carbomer resin

Carbomer polymers are formed from repeating units of acrylic acid. The monomer unit is shown above. The polymer chains are crosslinked with allyl sucrose or allylpentaerythritol. They contain between 56%and 68% of carboxylic acid (COOH) groups calculated on the dry basis.

Synonyms: Acritamer ; acrylic acid polymer ; Carbopol ; carboxy poly methylene, polyacrylic acid ; Pemulen ; Ultrez

Molecular Weight: $7x10^5$ to $4x10^9$

Description: Carbomers are white-colored, 'fluffy', acidic, hygroscopic powders with a slight characteristic odour.

Solubility: soluble in water and, after neutralization, in ethanol (95%) and glycerin.

Viscosity: Carbomers dispersed in water to form acidic colloidal dispersions of low viscosity that, when neutralized, produce highly viscous gels. Carbomer powders should first be dispersed into vigorously stirred water, taking care to avoid the formation of indispersible lumps, then neutralized by the addition of a base.

During preparation of gel, the solution should be agitated slowly with a broad , paddle like stirrer to avoid air bubbles. Neutralised aqeous gels are more viscous at pH 6-11. The viscosity is considerably reduced at pH values less than 3 or greater than 12 or presence of strong electrolytes. Gels rapidly lose viscosity. On exposure to ultraviolet light, but this can be minimized by the addition of a suitable antioxidant.

Stability and Storage conditions: Carbomers are stable, hygroscopic materials that may be heated at temperatures below 104^0C for up to 2 hours without affecting their thickening efficiency. However, exposure to excessive temperatures can result in discoloration and reduced stability. Complete decomposition occurs with heating for 30 minutes at 260c. Dry powders form of carbomer does not support the growth of molds and fungi. But microorganisms grow well in unpreserved aqueous dispersions.

At room temperature, carbomer dispersions maintain their viscosity during storage for prolonged periods. Similarly, dispersion viscosity is maintained, or only slightly reduced, at elevated storage temperatures if an antioxidant is included in the formulation or if the dispersion is protected from the light.

The UV stability of carbomer gels may also be improved by using triethanolamine as the neutralizing base. Carbomer powder should be stored in airtight, corrosion-resistant container in a cool, dry place.

Incompatibilities: Carbomers are discolored by resorcinol and are incompatible with phenol, cationic polymers, strong acids and high levels of electrolytes. Trace levels of iron and other transition metals can catalytically degrade carbomer dispersions. Carbomers also form pH dependant complexes with certain polymeric exipients.

Applications in pharmaceutical formulation: Carbomers are mainly used in liquid or semisolid pharmaceutical formulation as suspending or viscosity-increasing agent.

Use	Concentration (%)
Emulsifying agent	0.1-0.5
Gelling agent	0.5-2.0
Suspending agent	0.5-1.0
Tablet binder	0.5-10.0

Table 4: Uses of carbomers

Regulatory Acceptance: Included in the FDA Inactive Ingredients Guide (oral suspensions and tablets; ophthalmic, rectal and topical preparations). Included in nonparenteral medicines licensed in Europe.

Grade of Carbopol	Acrylic acid cross linked with	Viscosity
Carbopol 931	Penta erythritol	3000-7000cps of a 1% Aqueous dispersion
Carbopol 934	Sucrose	30,500-39,400cps of a 0.5% Aqueous dispersion
Carbopol 940	Penta erythritol	40,000-60,000cps of a 0.5% Aqueous dispersion
Carbopol 941	Penta erythritol	4000-11,000cps of a 0.5% Aqueous dispersion
Carbopol 1342	Co-polymer of acrylic acid & long chain allyl methacrylate cross linked with allyl ether of Penta erythritol	9,500-26,000cps of a 1% Aqueous dispersion

Table 5: Specification of Carbopols

Hydroxy propyl methyl cellulose[38]

Structural formula

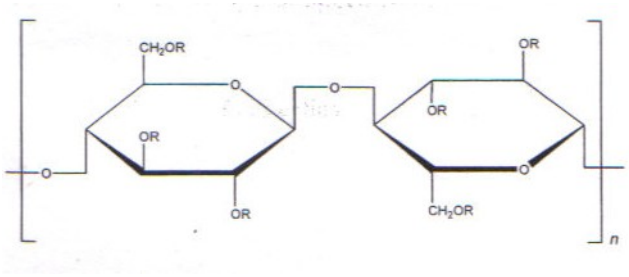


Figure 5: **Structure of Hydroxy propyl methylcellulose**

Hydroxypropyl methyl cellulose is water soluble non ionic polysaccharide polymers. It is available in several grades that vary in viscosity and extent of substitution. HPMC defined in the USP 25 specifies the substitution type by appending a four digit number to the non proprietary name: e.g., HPMC 1828. The first two digits refer to the approximate percentage content of the methoxy group (OCH_3). The second two digits refer to the approximate percentage content of the hydroxypropoxy group ($OCH_2CH(OH)CH_3$).

Chemical Name: Cellulose, 2-hydroxypropyl methyl ether

Synonyms: Benecel MHPC;cellulose, hydroxypropyl methyl ether; HPMC; Methocel; methylcellulose propylene glycol ether; methyl hydroxypropylcellulose.

Molecular weight: 10,000-1,500,000

Description: HPMC is an odorless and tasteless, white or creamy-white fibrous or granular powder.

Solubility : soluble in cold water and insoluble in hot water; practically insoluble in chloroform, ethanol(95%) and ether, but soluble in mixtures of ethanol and dichloramethane, mixtures of methanol and dichloramethane, and mixtures of water and ethanol.

Viscosity: Wide ranges of viscosity types are commercially available. To prepare an aqueous solution HPMC is dispersed and thoroughly hydrated in about 20-30% of the required amount of water. The water should be vigorously stirred and heated to 80-90^{0}c, then the remaining HPMC is added. Cold water should then be added to produce the required volume.

Stability and Storage conditions: HPMC powder is a stable material, although it is hygroscopic after drying. Solutions are stable at pH 3-11. Increasing temperature reduces the viscosity of solutions. The gel point is 50-90^{0}C, depending upon the grade and concentration of material.

Aqueous solutions are providing good viscosity, stability during long term storage. However, aqueous solutions are liable to microbial spoilage and should be preserved with an antimicrobial preservative. HPMC powder should be stored in a well-closed container, in a cool, dry place.

Incompatibilities: HPMC is incompatible with some oxidizing agents. Since it is nonionic, HPMC will not complex with metallic salts or ionic organics to form insoluble precipitates.

Applications in pharmaceutical formulation: HPMC is widely used in oral and topical pharmaceutical formulations.

Use	Concentration (%)
Film forming agent	2.0-20.0
Gelling agent(ophthalmic)	0.45-1.0
Tablet binder	2.0-5.0

Table 7: Uses of Hydroxypropyl methyl cellulose

HPMC is also used as an emulsifier, suspending agent and stabilizing agent in topical gels and ointments. It is also used in the manufacture of capsules, plastic bandages, and as a wetting agent for hard contact lenses.

Regulatory status: Accepted as a food additive in Europe. Included in the FDA Inactive Ingredients Guide (ophthalmic preparations; oral capsules, suspensions, syrups and tablets; topical and vaginal preparations). Included in nonparenteral medicines licensed in the UK.

Carboxy methyl cellulose-Sodium[38]

Structural formula

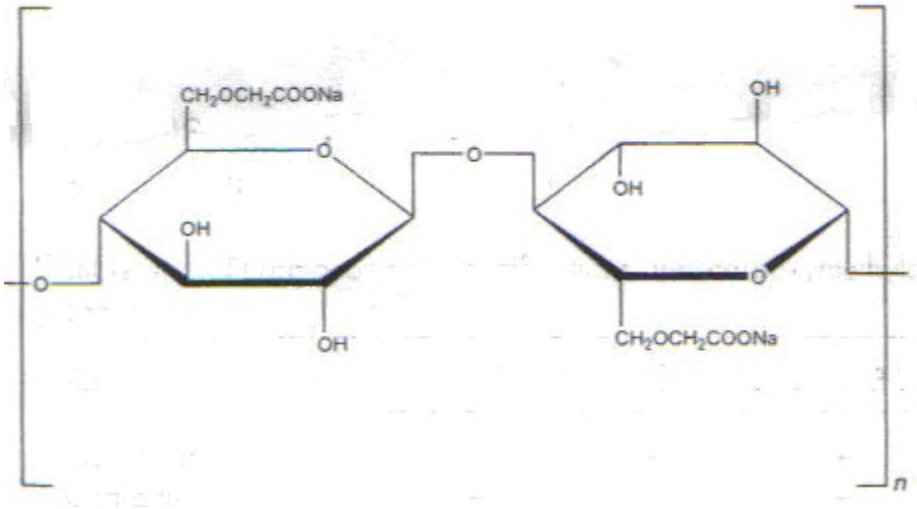


Figure 6: Structure of Carboxy methyl cellulose-Sodium

Chemical Name: Cellulose, carboxymethyl ether, sodium salt

Synonyms: Akucell; Aquasorb; Blanose; cellulose gum; CMC sodium; E466; Finnfix; SCMC; sodium carboxymethyl cellulose; sodium cellulose glycolate; sodium CMC; Tylose CB.

Molecular weight: 90,000-700,000

Description: Carboxymethylcellulose sodium occurs as a white to almost white, odorless, granular powder.

Solubility: Practically insoluble in acetone, ethanol, ether and toluene. Easily dispersed in water at all temperatures, forming clear, colloidal solutions.

Viscosity: It is available in low, medium and high viscosity grades. Prolonged heating at high temperatures will depolymerize the gum and permanently decrease the viscosity. The viscosity of sodium carboxymethylcellulose solutions is fairly stable over a pH range of 4-10. The optimum pH range is neutral.

Stability and Storage conditions: Carboxymethylcellulose sodium is a stable, though hygroscopic material. Under high humidity conditions, it can absorb a large quantity (>50%) of water. Solutions exhibit maximum viscosity and stability at pH 7-9.

Aqueous solutions stored for prolonged periods should contain antimicrobial preservative. The bulk material should be stored in a well-closed container in a cool, dry place.

Incompatibilities: Carboxymethylcellulose sodium is incompatible with strongly acidic solutions and with the soluble salts of iron and some other metals, such as aluminium, mercury and zinc. Carboxymethylcellulose sodium forms complex with gelatin and pectin also with collagen.

Applications in pharmaceutical formulation: Carboxymethylcellulose sodium is widely used in oral and topical formulations, primarily for its viscosity-increasing properties.

Use	Concentration (%)
Emulsifying agent	0.25-1.0
Gelling agent	3.0-6.0
Oral solutions	0.1-1.0
Tablet binder	1.0-6.0
Injections	0.05-0.75

Table 6: Uses of carboxymethylcellulose sodium

Regulatory status: Accepted as a food additive in Europe. Included in the FDA Inactive Ingredients Guide (dental preparations, inhalations, intra-articular, intradermal, intra-lesional, IM and subcutaneous injections; oral capsules, drops, solutions, suspensions, syrups and tablets; topical and vaginal preparations). Included in nonparenteral medicines licenced in the UK.

Triethanolamone[38]

Structural formula

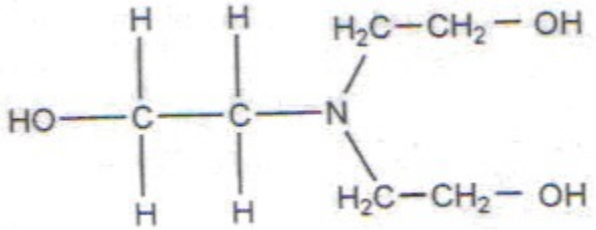


Figure 7: Structure of Triethanolamine

Synonyms : Daltogen; TEA; Tealan; triethanolamine; trihydroxytriethylamine; tris(hydroxyl)amine

Chemical Name : 2, 2', 2''-Nitrilotriethanol
Molecular weight: 149.19

Description: clear, colorless to pale yellow- colored viscous liquid having a slight ammonical odour.

Solubility: Soluble in chloroform and miscible with acetone, ethanol, methanol and water.

Stability and Storage conditions: Triethanolamine may turn brown on exposure to air and light. It should be stored in airtight container protected from light, in a cool, dry place.

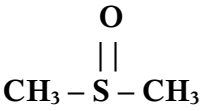
Incompatibilities: It will react with mineral acids to form crystalline salts and esters and also react with copper to form complex salts, discoloration and precipitation can take place in the presence of heavy metal salts.

Category: Neutralizing agent, Emulsifying agent.

Regulatory status: Included in the FDA Inactive Ingredients Guide (rectal, topical and vaginal preparations). Included in nonparenteral medicines licensed in the UK.

Dimethyl sulfoxide[38]

Structural formula:



Synonyms: Deltan ; dimexide ; dimethyl sulfoxide ; DMSO ; kemsol ; methyl sulfoxide; Rimso-50 ; sulphinylbismethane.

Chemical Name: Sulfinylbismethane

Molecular weigh: 78.13

Description: colorless, viscous liquid; miscible with water, alcohol and ether; slightly bitter taste; odourless; extremely hygroscopic; absorbing up to 70% of its own weight in water with evaluation of heat.

Stability and Storage conditions: DMSO is reasonably stable to heat, temperature between 40-60^{0}C it has been partially break down, which is indicated by changes physical properties. When heated to decomposition and toxic fumes are emitted. It should be stored in airtight, light resistance containers. Contact with plastics should be avoided.

Applications in Pharmaceutical formulation: Penetration Enhancer and Solvent, which enhances the topical penetration of drugs from the stratum corneum and the use of DMSO to improve transdermal deliver has been reported.

Incompatibilities: DMSO can react with oxidizing materials.

Compatibility Study

The drug Acyclovir and the polymers namely Carbopol, Hydroxypropyl methyl cellulose and Sodium Carboxy methyl cellulose were analyzed by Fourier Transform Spectrophotometer (FTIR).

The IR spectrums were interpreted and the compatibility of the drug with the polymers were confirmed.

S.No	Sample	Assignments(cm-1)						
		(O-H)st (alcoholic)	(C-O-C)st	(C=O)St	(O-H)st (carboxylic)	(C-H)st	(N-H)st	(C=N)st
1	Acyclovir(ACV)	3307, (1049,1306)*	1104	1712	------	2856	3442	1634
2	Carbopol	------	------	1716	3443	2941	------	------
3	HPMC	3442, (1059,1317)*	1117	------	------	2926	------	------
4	Sodium CMC	------	1064	1638	3439	2926	------	------
5	ACV+ Carbopol	3300, (1050,1304)*	1104	1713	3185	2927, 2862	3442	1634
6	ACV+HPMC	3305,1307*	1104	1712	------	2866	3442	1633
7	ACV+ Sodium CMC	3301,1306*	1104	1713	3185	2860	3442	1633

st – stretching, *Primary alcohol

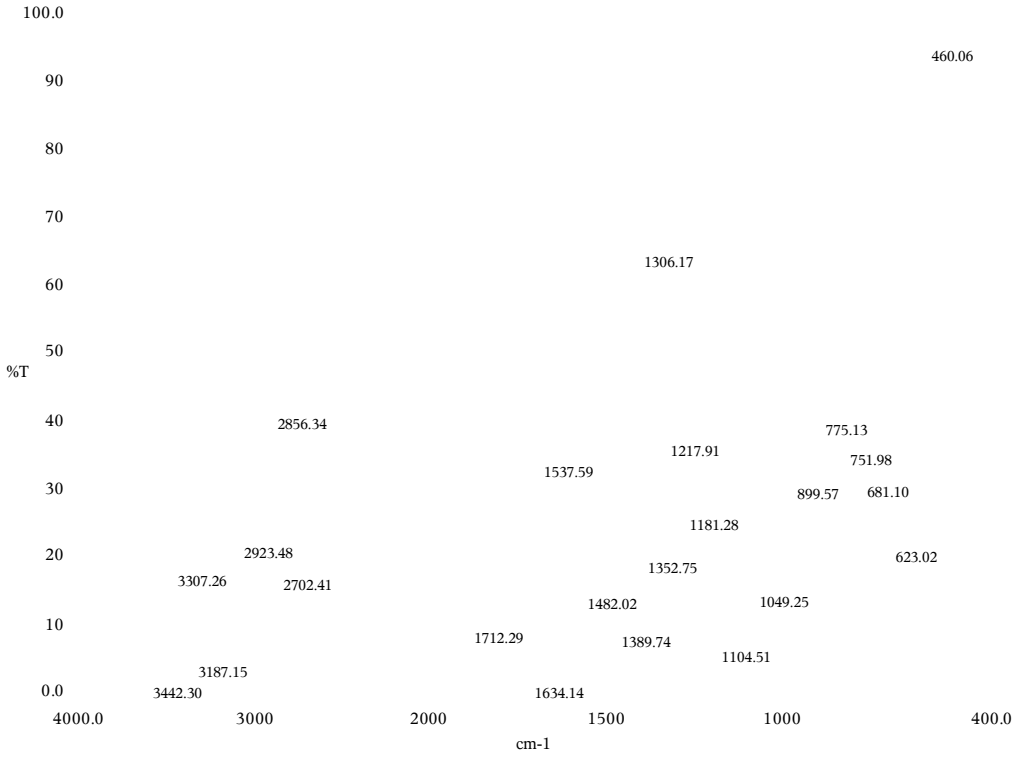
Table 8: Interpretation of IR Spectrums[39]

FIGUIRE: 8 IR SPECTRUM OF ACYCLOVIR

ACIC
St.Joseph's College (Autonomous)
Trichy-2

FTIR SPECTRUM

Spectrum Name: Sam=Acyclovir.sp



Sam=Acyclovir.pk

SAM_AC~2.SP 3601 4000.00 400.00 3.52 99.48 4.00 %T 5 1.00

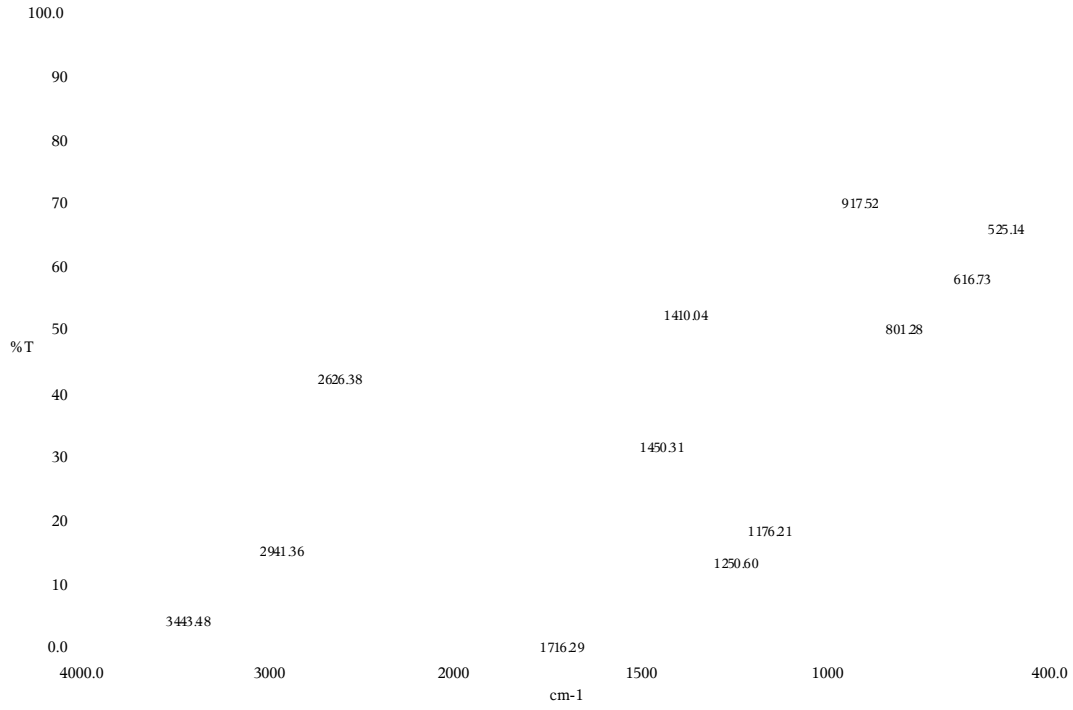
REF 4000 98.80 2000 95.59 600
3442.30 5.89 3307.26 7.01 3187.15 7.73 2923.48 27.28 2856.34 25.59
2702.41 21.48 1712.29 5.98 1634.14 3.52 1537.59 21.36 1482.02 18.45
1389.74 16.53 1352.75 25.12 1306.17 39.24 1217.91 44.25 1181.28 28.83
1104.51 11.46 1049.25 21.93 899.57 36.74 775.13 43.11 751.98 40.38
681.10 36.73 623.02 29.59 460.06 87.28

FIGUIRE: 9 IR SPECTRUM OF CARBOPOL

ACIC
St.Joseph's College (Autonomous)
Trichy-2

FT IR SPECTRUM

Spectrum Name: Sample =Carbopol.sp



Sample =Carbopol.pk

SAMPLE~1.SP 3601 4000.00 400.00 3.22 100.00 4.00 %T 5 1.00

REF 4000 99.56 2000 87.05 600
3443.48 10.48 2941.36 20.11 2626.38 49.11 1716.29 3.22 1450.31 36.98
1410.04 40.05 1250.60 19.44 1176.21 24.53 917.52 77.19 801.28 57.06
616.73 65.44 525.14 72.61

FIGURE: 10 IR SPECTRUM OF HYDROXY PROPYL METHYLCELLULOSE

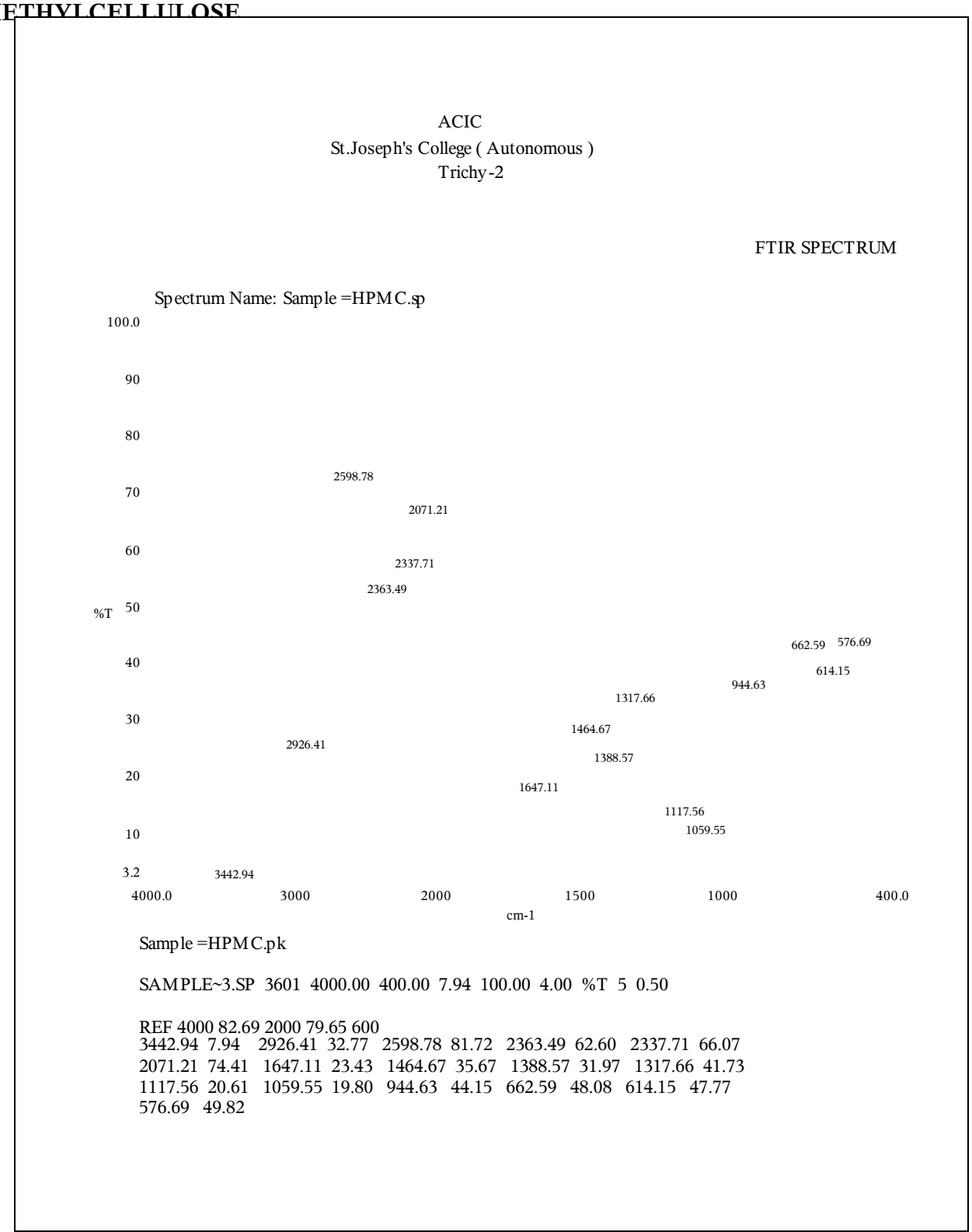


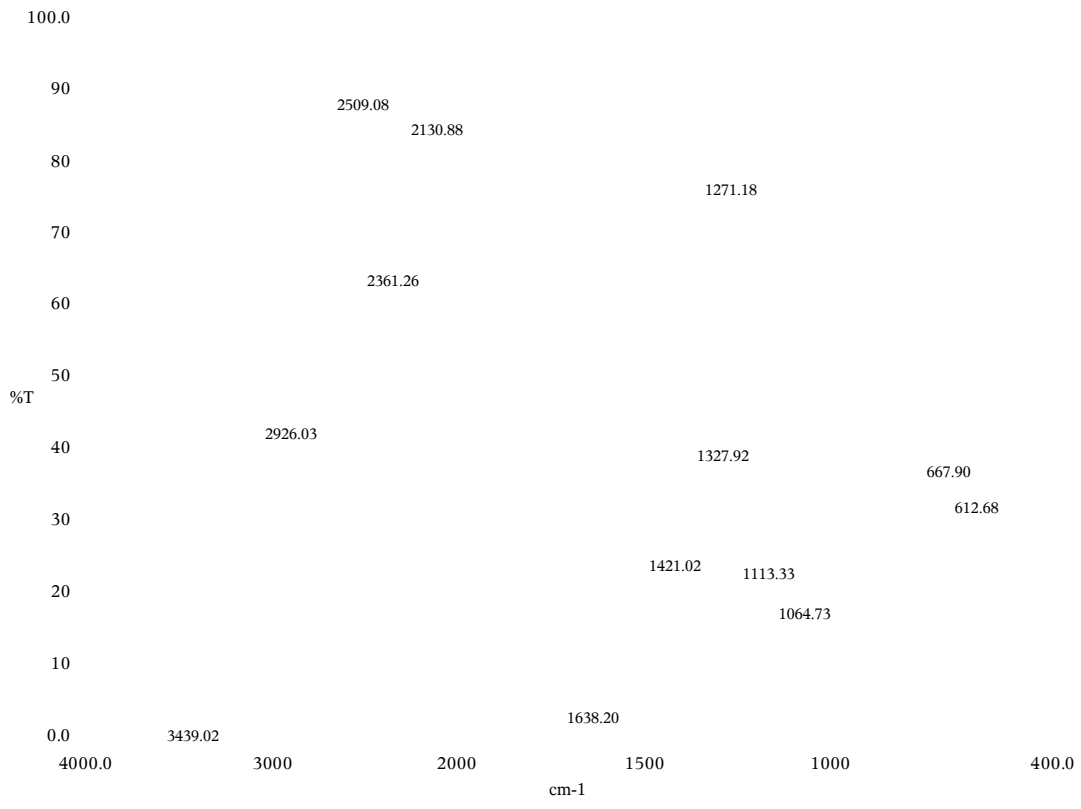
FIGURE: 11 IR SPECTRUM OF SODIUM CARBOXY

ME

ACIC
St.Joseph's College (Autonomous)
Trichy-2

FTIR SPECTRUM

Spectrum Name: Sample =CMC-Sodium.sp



Sample =CMC-Sodium.pk

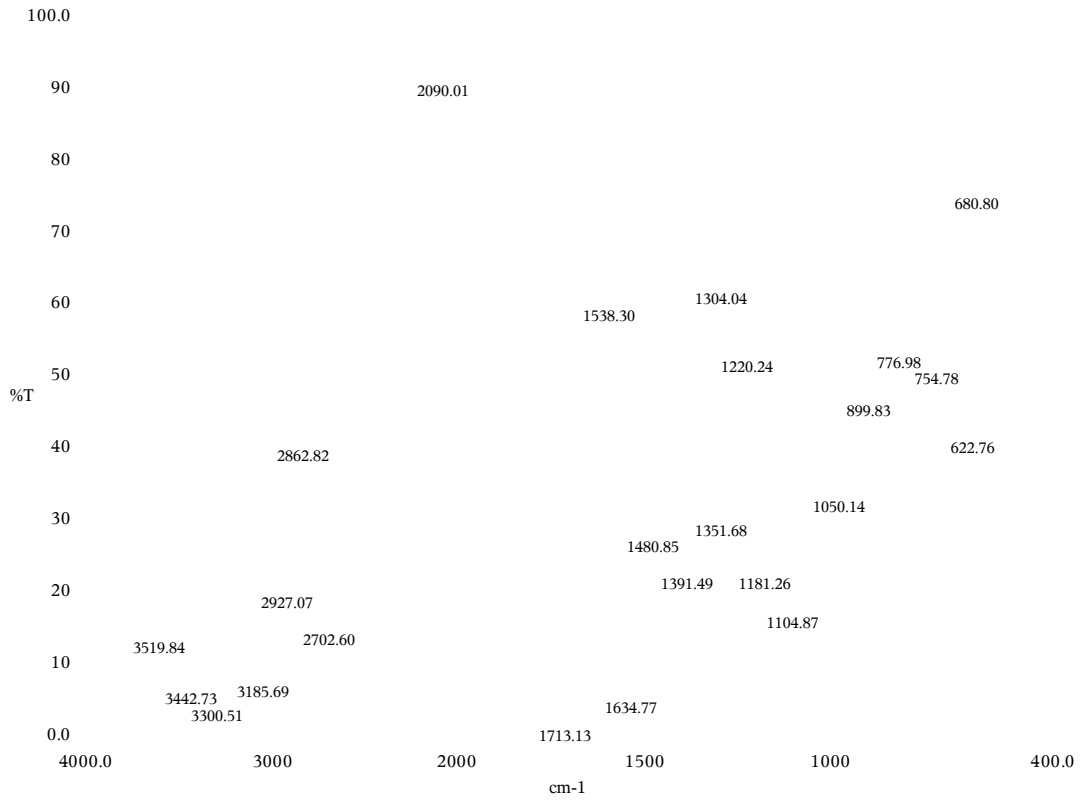
SAMPLE~2.SP 3601 4000.00 400.00 3.17 98.47 4.00 %T 5 0.50

REF 4000 98.30 2000 96.72 600
3439.02 3.17 2926.03 49.71 2509.08 97.31 2361.26 69.22 2130.88 89.65
1638.20 10.06 1421.02 28.95 1327.92 48.04 1271.18 66.67 1113.33 27.63
1064.73 25.24 667.90 43.02 612.68 42.68

ACIC
St.Joseph's College (Autonomous)
Trichy-2

FTIR SPECTRUM

Spectrum Name: Sam=Acyclovir+Carbopol.sp



Sam=Acyclovir+Carbopol.pk

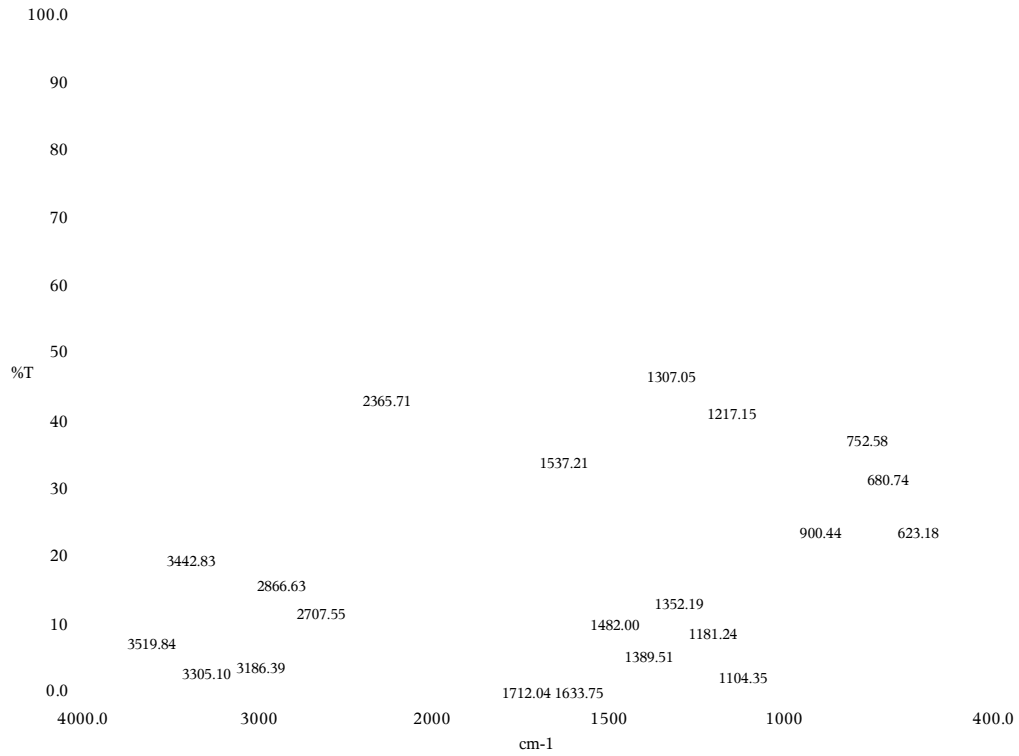
SAM_AC~3.SP 3601 4000.00 400.00 3.43 99.81 4.00 %T 5 1.00

REF 4000 99.67 2000 99.55 600
3519.84 18.13 3442.73 10.27 3300.51 10.56 3185.69 9.34 2927.07 23.65
2862.82 25.35 2702.60 25.40 2090.01 95.90 1713.13 3.43 1634.77 6.33
1538.30 42.65 1480.85 33.58 1391.49 26.18 1351.68 33.95 1304.04 39.29
1220.24 34.30 1181.26 26.14 1104.87 21.07 1050.14 38.22 899.83 51.27
776.98 55.13 754.78 56.61 680.80 57.52 622.76 44.89

ACIC
St.Joseph's College (Autonomous)
Trichy-2

FTIR SPECTRUM

Spectrum Name: Sample =HPMC +Acyclovir.sp



Sample =HPMC +Acyclovir.pk

SA2AFD~1.SP 3601 4000.00 400.00 3.64 99.57 4.00 %T 5 1.00

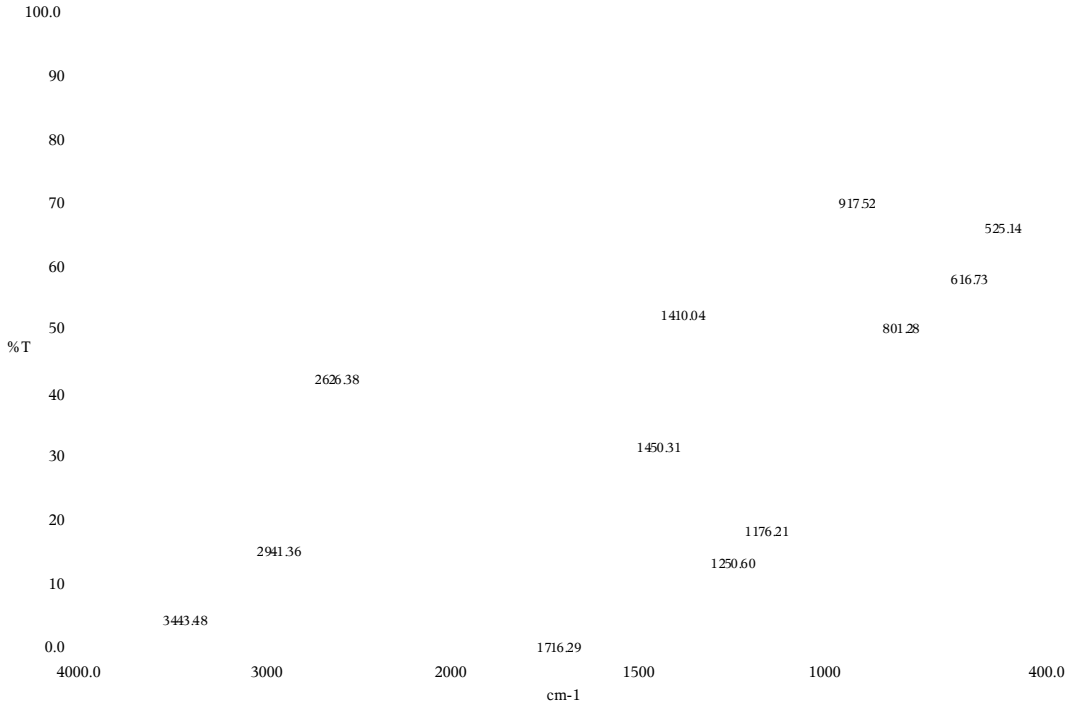
REF 4000 99.27 2000 96.12 600
3519.84 14.97 3442.83 7.08 3305.10 7.99 3186.39 7.97 2866.63 22.80
2707.55 22.08 2365.71 52.98 1712.04 4.60 1633.75 3.64 1537.21 21.54
1482.00 15.17 1389.51 13.45 1352.19 19.86 1307.05 30.42 1217.15 27.93
1181.24 17.82 1104.35 6.38 900.44 29.67 752.58 42.82 680.74 37.28
623.18 28.90

FIGUIRE: 9 IR SPECTRUM OF CARBOPOL

ACIC
St.Joseph's College (Autonomous)
Trichy-2

FT IR SPECTRUM

Spectrum Name: Sample =Carbopol.sp



Sample =Carbopol.pk

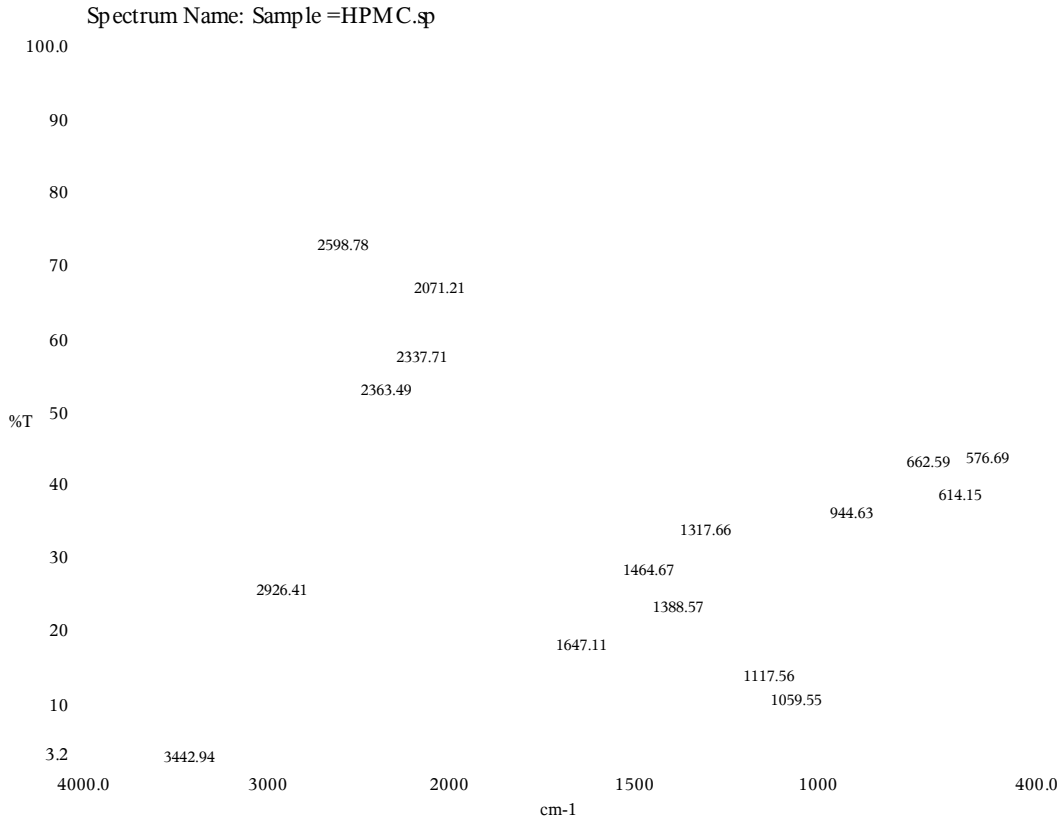
SAMPLE~1.SP 3601 4000.00 400.00 3.22 100.00 4.00 %T 5 1.00

REF 4000 99.56 2000 87.05 600
3443.48 10.48 2941.36 20.11 2626.38 49.11 1716.29 3.22 1450.31 36.98
1410.04 40.05 1250.60 19.44 1176.21 24.53 917.52 77.19 801.28 57.06
616.73 65.44 525.14 72.61

FIGURE: 10 IR SPECTRUM OF HYDROXY PROPYL METHYLCELLULOSE

ACIC
St.Joseph's College (Autonomous)
Trichy-2

FTIR SPECTRUM



Sample =HPMC.pk

SAMPLE~3.SP 3601 4000.00 400.00 7.94 100.00 4.00 %T 5 0.50

REF 4000 82.69 2000 79.65 600
3442.94 7.94 2926.41 32.77 2598.78 81.72 2363.49 62.60 2337.71 66.07
2071.21 74.41 1647.11 23.43 1464.67 35.67 1388.57 31.97 1317.66 41.73
1117.56 20.61 1059.55 19.80 944.63 44.15 662.59 48.08 614.15 47.77
576.69 49.82

FIGURE: 11 IR SPECTRUM OF SODIUM CARBOXY METHYLCELLULOSE

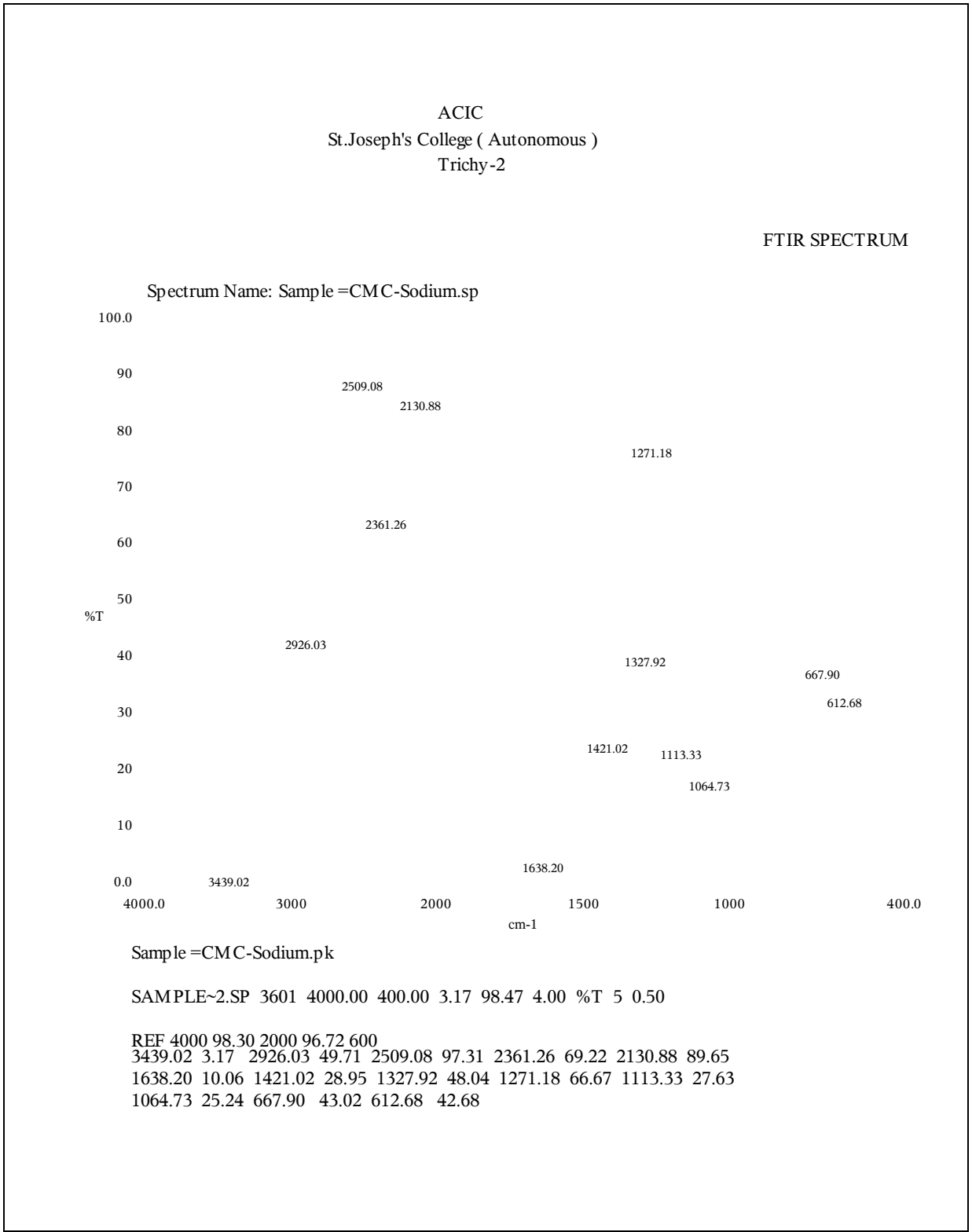
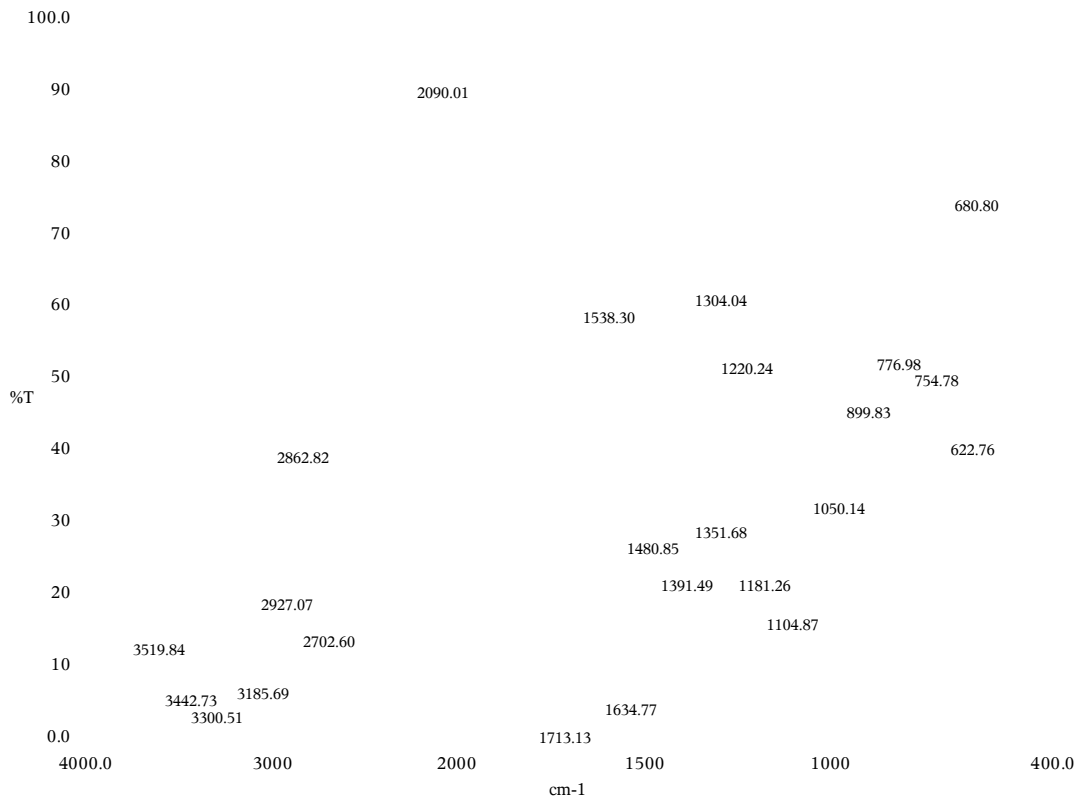


FIGURE: 12 IR SPECTRUM OF CARBOPOL + ACYCLOVIR

ACIC
St.Joseph's College (Autonomous)
Trichy-2

FTIR SPECTRUM

Spectrum Name: Sam=Acyclovir+Carbopol.sp



Sam=Acyclovir+Carbopol.pk

SAM_AC~3.SP 3601 4000.00 400.00 3.43 99.81 4.00 %T 5 1.00

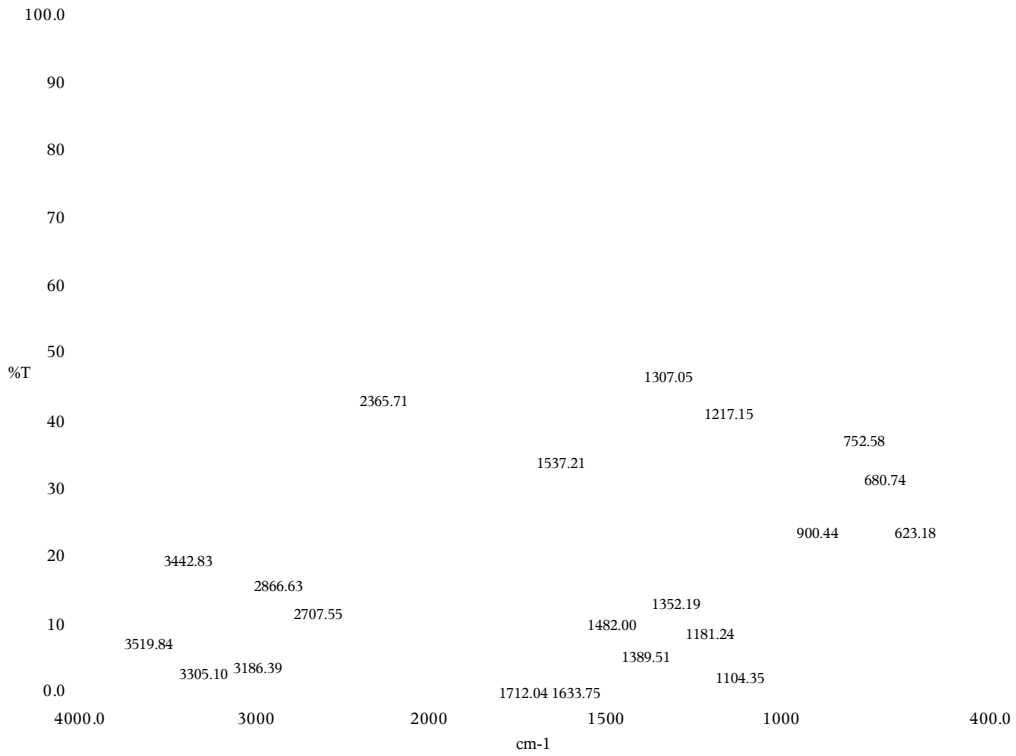
REF 4000 99.67 2000 99.55 600
3519.84 18.13 3442.73 10.27 3300.51 10.56 3185.69 9.34 2927.07 23.65
2862.82 25.35 2702.60 25.40 2090.01 95.90 1713.13 3.43 1634.77 6.33
1538.30 42.65 1480.85 33.58 1391.49 26.18 1351.68 33.95 1304.04 39.29
1220.24 34.30 1181.26 26.14 1104.87 21.07 1050.14 38.22 899.83 51.27
776.98 55.13 754.78 56.61 680.80 57.52 622.76 44.89

FIGURE: 13 IR SPECTRUM OF HPMC + ACYCLOVIR

ACIC
St.Joseph's College (Autonomous)
Trichy-2

FTIR SPECTRUM

Spectrum Name: Sample =HPMC +Acyclovir.sp



Sample =HPMC +Acyclovir.pk

SA2AFD~1.SP 3601 4000.00 400.00 3.64 99.57 4.00 %T 5 1.00

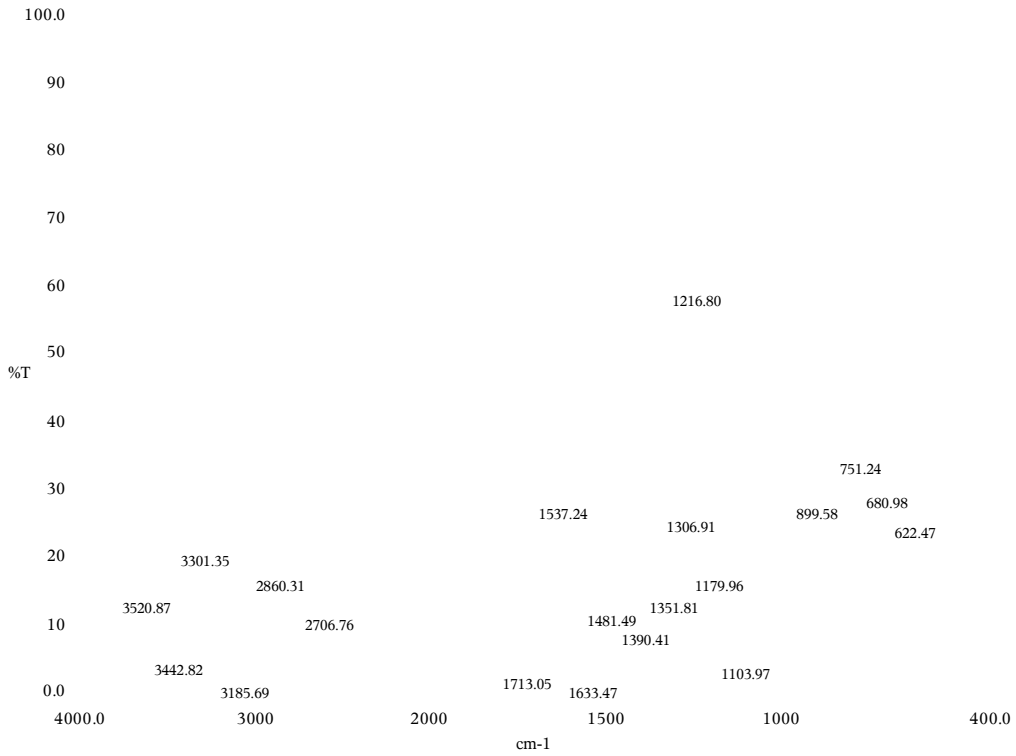
REF 4000 99.27 2000 96.12 600
3519.84 14.97 3442.83 7.08 3305.10 7.99 3186.39 7.97 2866.63 22.80
2707.55 22.08 2365.71 52.98 1712.04 4.60 1633.75 3.64 1537.21 21.54
1482.00 15.17 1389.51 13.45 1352.19 19.86 1307.05 30.42 1217.15 27.93
1181.24 17.82 1104.35 6.38 900.44 29.67 752.58 42.82 680.74 37.28
623.18 28.90

FIGURE: 14 IR SPECTRUM OF SODIUM CMC + ACYCLOVIR

ACIC
St.Joseph's College (Autonomous)
Trichy-2

FTIR SPECTRUM

Spectrum Name: Sample =CMC+Acyclovir.sp



Sample =CMC+Acyclovir.pk

SAFB60~1.SP 3601 4000.00 400.00 3.54 99.61 4.00 %T 5 1.00

REF 4000 99.26 2000 96.55 600
3520.87 18.21 3442.82 9.49 3301.35 8.74 3185.69 7.62 2860.31 21.99
2706.76 18.51 1713.05 5.35 1633.47 3.54 1537.24 21.58 1481.49 16.81
1390.41 15.18 1351.81 21.15 1306.91 33.25 1216.80 38.09 1179.96 22.58
1103.97 8.93 899.58 33.26 751.24 38.32 680.98 35.42 622.47 28.82

EXPERIMENTAL WORK

S.NO	Materials	Source
1	Acyclovir	Microlabs, Hosur
2	Carbopol-934	Kemphasol, Mumbai
3	Carbopol-940	Kemphasol, Mumbai
4	Hydroxy propyl methyl cellulose	HIMEDIA LBS, Mumbai
5	Carboxy methyl cellulose-sodium	Kemphasol, Mumbai
6	Propyl paraben	NATIONAL CHEMICALS
7	Methyl paraben	NATIONAL CHEMICALS
8	Dimethyl sulfoxide	SUVIDHINATH LABS, BARODA
9	Triethanolamine	REACHEM LABS, CHENNAI
10	0.1M Hydrochloric acid	--------
11	Cellophane membrane	--------

Table 9: Materials used in Research work

Preparation of 0.1M Hydrochloric acid[40]

Solution of 0.1M Hydrochloric acid was prepared by diluting 8.5 ml of Hydrochloric acid to 1000ml with water.

S.NO	Instruments and Equipments	Make
1	Remi stirrer	REMI Equipments, Mumbai
2	UV-Spectrophotometer	SHIMADZU UV-1700, Japan
3	pH meter	ELICO, LI120
4	Digital Balance	Sartorius, Mumbai
5	Heating plate	Tempo Equipments, Mumbai
6	Viscometer	Brookfield
7	Oven	Tempo Equipments, Mumbai
8	Refrigerator	ALLWYN
9	Diffusion cell	-------
10	Magnetic stirrer	REMI Equipments, Mumbai
11	Remi Centrifuge	REMI Equipments, Mumbai
12	Environment Test Chamber	HECO

Table 10: Instruments and Equipments used in Research work

FORMULATION OF GELS

Acyclovir gels were formulated using different polymers like Carbopol 934, Carbopol 940, Hydroxy propyl methyl cellulose and Sodium Carboxy methyl cellulose. Different concentrations of polymer were used in the formulation of gels. The concentrations chose varied with the polymer used. After initial trials, the concentrations that gave products of good consistency were selected for the formulation. The concentration of drug taken in all the formulation remained constant.

Table 11 to 14 gives the different concentrations of polymers used in the formulation.

Preparation of Carbopol- 934 gels

Ingredients	Formula for 100gms		
	A_1(gms)	A_2(gms)	A_3(gms)
Acyclovir	1.0	1.0	1.0
Carbopol-934	0.5	1.0	1.5
Triethanolamine	0.5	0.5	0.5
Purified water	98	97.5	97
Methyl paraben	0.002	0.002	0.002

Table 11: Formulations with varying Carbopol-934 concentrations

Procedure

1. Accurately weighed quantity of Acyclovir was dispersed in purified water with constant stirring and the drug solution was heated to 50^0C.

2. Methyl paraben was added as a preservative.

3. The carbopol-934 was added to the solution under stirring while temperature Was maintained at 50^0 C.

4. The dispersion of gelling agent was neutralized by addition of triethanolamine solution to attain the neutral pH . Stirred slowly till a clear gel was obtained.

Preparation of Carbopol- 940 gels

Ingredients	Formula for 100gms		
	B_1(gms)	B_2(gms)	B_3(gms)
Acyclovir	1.0	1.0	1.0
Carbopol-940	0.5	1.0	1.5
Triethanolamine	0.5	0.5	0.5
Purified water	98	97.5	97
Methyl paraben	0.002	0.002	0.002

Table 12: Formulations with varying Carbopol-940 concentrations

Procedure

1. Accurately weighed quantity of Acyclovir was dispersed in purified water with constant stirring and the drug solution was heated to 50^0 C.

2. Methyl paraben was added as a preservative.

3. The carbopol-940 was added to the solution under stirring while temperature was maintained at 50^0 C.

4. The dispersion of gelling agent was neutralized by addition of triethanolamine solution to attain the neutral pH. Stirred slowly till a clear gel was obtained.

Preparation of Hydroxy propyl methyl cellulose gels

Ingredients	**Formula for 100gms**			
	C_1(gms)	**C_2(gms)**	**C_3(gms)**	**C_4(gms)**
Acyclovir	1.0	1.0	1.0	1.0
Hydroxy propyl methyl cellulose	1.0	1.5	3.0	4.0
Purified water	98	97.5	96	95
Methyl paraben	0.002	0.002	0.002	0.002

Table 13: Formulations with varying Hydroxy propyl methyl cellulose concentrations

Procedure

1. Accurately weighed quantity of Acyclovir was dispersed in purified water with constant stirring and the drug solution was heated to 50^0 C.

2. The solution was maintained at 50^0 C, HPMC was gradually added to the Solution under stirring until a thick viscous gel was formed.

3. Methyl paraben was added finally to the preparation as a preservative.

4. Formulation was allowed to settle down to room temperature

Preparation of Sodium Carboxy methyl cellulose gels

Ingredients	Formula for 100gms		
	D_1(gms)	D_2(gms)	D_3(gms)
Acyclovir	1.0	1.0	1.0
Sodium Carboxy methyl cellulose	2.0	3.0	4.0
Purified water	97	96	95
Methyl paraben	0.002	0.002	0.002

Table No 14: Formulations with varying Sodium carboxy methyl cellulose concentrations

Procedure

1. Accurately weighed quantity of Acyclovir was dispersed in purified water with Constant stirring.

2. Sodium Carboxy methyl cellulose was added under stirring to the above solution.

3. Methyl paraben was added to the dispersion under stirring as a preservative.

4. The dispersion was allowed to stand for complete hydration of Sodium CMC. Finally the weight was adjusted to 100gm by adding purified water.

EVALUATION OF GELS

The prepared gels were proposed to be evaluated for Drug content, pH, Viscosity, Extrudability, Spreadability, In vitro release characteristic and the selected gel formulation subjected for Stability and In-vivo study by using Albino Rabbits.

Standard curve of Acyclovir

100 mg of accurately weighed Acyclovir was dissolved in little amount of 0.1M hydrochloric acid and made up to required volume 100 ml with 0.1M hydrochloric acid[41]. So that each ml of stock solution required concentration of 5, 10, 15, 20, 25, 30, 35 and 40 µg/ml was made up with 0.1M hydrochloric acid. The absorbance of the dilute sample was measured spectrophotometrically at 255nm using 0.1M hydrochloric acid in UV- spectrophotometer[42]. The standard plot was made with concentration (µg /ml) on X axis and Absorbance on Y axis.

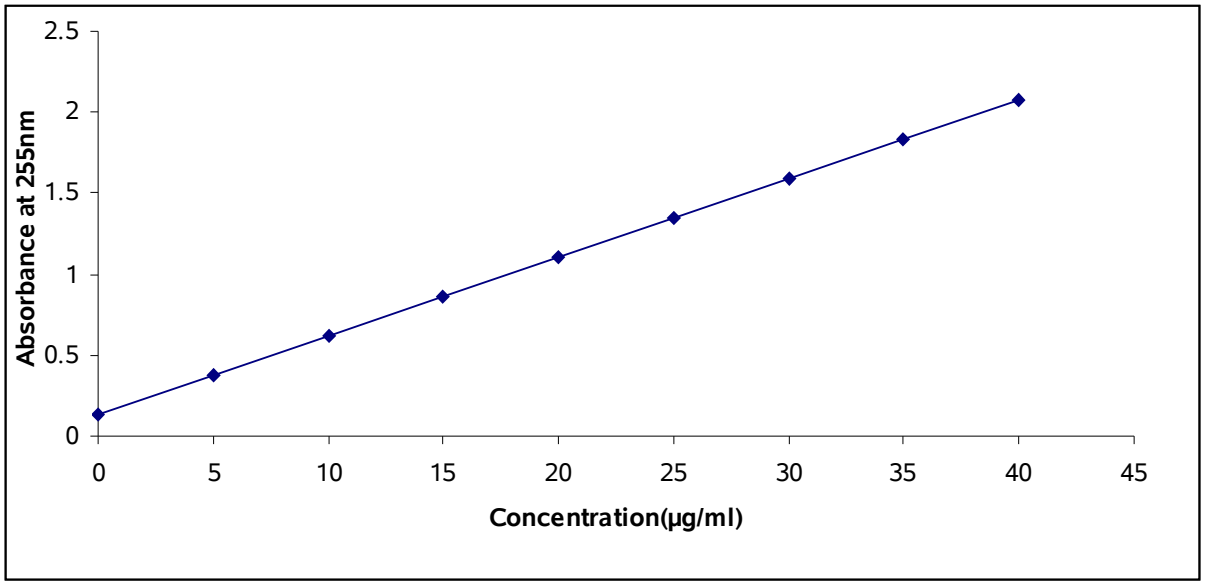
Table 15: Standard curve of Acyclovir

S.No	Concentration (µg/ml)	Absorbance at 255nm
1	5	0.399
2	10	0.647
3	15	0.896
4	20	1.119
5	25	1.347
6	30	1.513
7	35	1.726
8	40	1.947

Average of three readings

r=0.9932 a=0.1366 b=0.0486

Figure 15: Standard curve of Acyclovir



Estimation of Drug content

1gm of Acyclovir gel was dissolved in sufficient quantity of 0.1M hydrochloric acid to get the clear solution, volume was made up to 100ml with 0.1M hydrochloric acid. 1ml of the solution was diluted to 10ml with 0.1M hydrochloric acid solution. Absorbance was measured at 255nm using UV spectrophotometer.

The amount of Acyclovir was determined from the standard calibration curve and the percentage drug content in different formulations was calculated. Results were tabulated as follows

Formulation	Drug content (mg)	Drug content (%)
A_2	10.172	101.72
B_2	9.81	98.1
C_3	9.78	97.8
D_2	9.69	96.9

Average of three readings

Table 16: Drug content in the gel formulations

pH Measurements

pH measurements of the gel were carried out using a digital pH meter by dipping the glass electrode completely into the gel system so as to cover the electrode. The results were tabulated as follows

Formulation	pH
A_2	6.9
B_2	7.2
C_3	7.1
D_2	6.8

Average of three readings

Table 17: pH of gel formulations

Determination of viscosity

Viscosities of the gels were determined by using Brookfield Viscometer (model-RVTP).Spindle type, RV-7 at 20 rpm. 100gm of the gel was taken in a beaker and the spindle was dipped in it and rotated for about 5 minutes and then reading was taken. The results were shown in Table 18

Formulation	Viscosity in cps
A_2	43,000
B_2	41,000
C_3	36,000
D_2	51,000

Average of three readings

Table 18: Viscosity of gel formulations

Extrudability

It is useful empirical test to measure the force required to extrude the material from the tube. The formulations were filled in a collapsible metal tubes with a nasal tip of 5mm opening tube extrudability was then determined by measuring the amount of gel, extruded the tip when a pressure was applied on tube gel. The extrudability of the formulation was checked and the results were tabulated.

Formulation	Extrudability
A_2	+++
B_2	+++
C_3	+
D_2	++

+++Excellent, ++Good, +Not satisfactory

Table 19: Extrudability of gel formulations

Determination of spreadability

One of the criteria for a gel meet ideal quality is that it should possess good spreadability. About 1 gm of gel formulation was weighed and kept at the center of the glass plate of standard dimensions (10x10cm) and another glass plate placed over it carefully, that the gel was sandwiched between the two slides. 2 kg weight was placed at the center of the plate (avoid sliding of the plate). The diameter of the gel in cms, after 30 minutes was measured and the results were tabulated in Table 20

Formulation	Time taken (minutes)	Spreadability (cm)
A_2	30	8.0
B_2	30	7.8
C_3	30	7.4
D_2	30	7.7

Average of three readings

Table 20: Spreadability of gel formulations

In vitro Drug release pattern of Acyclovir gels

The In vitro release of Acyclovir from the gel formulation was studied by open ended cylinder method. This diffusion cell apparatus consists of a glass tube with an inner diameter of 2.5 cm, open at the both ends. One end of the tube tied with Cellophane membrane, which serves as a donar compartment.

1 gm of Acyclovir gel was taken in this compartment and placed in a beaker containing 200ml of 0.1M Hydrochloric acid stirring at moderate speed, maintaing the temperature at 37 ± 1^0C. Periodically 5ml of samples were withdrawn and after each withdrawal using 0.1M Hydrochloric acid was replaced into the diffusion medium to maintain the sink condition through out the experimentation. Then the samples were assayed by spectrophotometrically at 255nm in UV-Spectrophotometer using 0.1M Hydrochloric acid as blank.

Table 21: Drug release profile of Formulation A_2 (1% Carbopol-934)

Time (minutes)	Absorbance at 255nm	Concentration (µg/ml)	Amount of drug release(mg)	Percentage drug release*
30	0.205	1.407	0.281	2.81
60	0.461	6.674	1.334	13.34
90	0.592	9.370	1.874	18.74
120	0.705	11.695	2.339	23.39
150	0.747	12.559	2.511	25.11
180	0.763	12.880	2.577	25.77
210	0.884	15.378	3.075	30.75
240	1.074	19.286	3.857	38.57
270	1.143	20.707	4.141	41.41
300	1.237	22.641	4.528	45.28
360	1.516	28.382	5.672	56.72
420	1.610	30.316	6.063	60.63
480	1.714	32.456	6.491	64.91

*Average of three readings

Table 22: Drug release profile of Formulation B_2 (1% Carbopol-940)

Time (minutes)	Absorbance at 255nm	Concentration (µg/ml)	Amount of drug release (mg)	Percentage drug release*
30	0.186	1.016	0.203	2.03
60	0.365	4.699	0.934	9.34
90	0.481	7.086	1.417	14.17
120	0.520	7.888	1.577	15.77
150	0.617	9.884	1.976	19.76
180	0.676	11.098	2.219	22.19
210	0.747	12.559	2.511	25.11
240	0.843	14.534	2.906	29.06
270	0.906	15.831	3.166	31.66
300	0.963	17.004	3.400	34.00
360	1.184	21.551	4.310	43.10
420	1.243	22.765	4.553	45.53
480	1.394	25.872	5.147	51.47

*Average of three readings

Table 23: Drug release profile of Formulation C_3 (3% Hydroxypropyl methyl cellulose)

Time (minutes)	Absorbance at 255nm	Concentration (µg/ml)	Amount of drug release (mg)	Percentage drug release*
30	0.162	0.522	0.104	1.04
60	0.312	3.609	0.721	7.21
90	0.396	5.337	1.067	10.67
120	0.443	6.304	1.260	12.60
150	0.503	7.539	1.507	15.07
180	0.532	8.135	1.627	16.27
210	0.598	9.493	1.898	18.98
240	0.687	11.325	2.265	22.65
270	0.763	12.883	2.577	25.77
300	0/846	14.596	2.919	29.19
360	0.904	15.790	3.158	31.58
420	1.116	20.152	4.030	40.30
480	1.207	22.024	4.404	44.04

*Average of three Readings

Table 24: Drug release profile of Formulation D_2 (3% Sodium Carboxy methyl cellulose)

Time (minutes)	Absorbance at 255nm	Concentration (µg/ml)	Amount of drug release (mg)	Percentage drug release*
30	0.136	------	------	------
60	0.269	2.724	0.544	5.44
90	0.313	3.629	0.725	7.25
120	0.361	4.617	0.923	9.23
150	0.414	5.707	1.141	11.41
180	0.474	6.942	1.388	13.88
210	0.543	8.362	1.672	16.72
240	0.613	9.802	1.960	19.60
270	0.686	11.304	2.260	22.60
300	0.734	12.292	2.458	24.58
360	0.816	13.979	2.795	27.95
420	0.903	15.769	3.153	31.53
480	0.976	17.271	3.454	34.54

*Average of three Readings

In vitro drug release of gel formulations with Dimethyl sulfoxide (DMSO) as a permeation enhancer.

Table 25: Drug release profile of Formulation A_2 with Dimethyl sulfoxide

Time (minutes)	Absorbance At 255nm	Concentration (µg/ml)	Amount of drug release (mg)	Percentage drug release*
30	0.243	2.251	0.450	4.50
60	0.514	7.765	1.553	15.53
90	0.646	10.481	2.096	20.96
120	0.761	12.847	2.569	25.69
150	0.804	13.732	2.746	27.46
180	0.823	14.123	2.824	28.24
210	0.946	16.654	3.330	33.34
240	1.141	20.666	4.133	41.33
270	1.276	23.444	4.688	46.88
300	1.407	26.139	5.222	52.22
360	1.651	31.160	6.232	62.32
420	1.798	34.185	6.837	68.37
480	1.907	36.427	7.285	72.85

*

*Average of three Readings

Table 26: Drug release profile of Formulation B_2 with Dimethyl sulfoxide

Time (minutes)	Absorbance at 255nm	Concentration (µg/ml)	Amount of drug release (mg)	Percentage drug release*
30	0.214	1.592	0.318	3.18
60	0.398	5.378	1.075	10.75
90	0.541	8.320	1.664	16.64
120	0.634	10.234	2.046	20.46
150	0.703	11.654	2.330	23.30
180	0.721	12.024	2.404	24.04
210	0.911	15.934	3.186	31.86
240	0.984	17.436	3.487	34.87
270	1.073	19.267	3.853	38.53
300	1.124	20.316	4.063	40.63
360	1.343	24.823	4.964	49.64
420	1.461	27.251	5.450	54.50
480	1.605	30.213	6.042	60.42

*Average of three Readings

Table 27: Drug release profile of Formulation C_3 with Dimethyl sulfoxide

Time (minutes)	Absorbance At 255nm	Concentration (µg/ml)	Amount of drug release (mg)	Percentage drug release*
30	0.201	1.325	0.265	2.65
60	0.396	5.337	1.067	10.67
90	0.474	6.942	1.388	13.88
120	0.523	7.950	1.590	15.90
150	0.614	9.823	1.964	19.64
180	0.692	11.427	2.285	22.85
210	0.746	12.534	2.507	25.07
240	0.816	13.979	2.795	27.95
270	0.932	16.366	3.273	32.73
300	1.003	17.827	3.565	35.65
360	1.104	19.905	3.981	39.81
420	1.271	23.341	4.668	46.68
480	1.394	25.872	5.174	51.74

*Average of three Readings

Table 28: Drug release profile of Formulation D_2 with Dimethyl sulfoxide

Time (minutes)	Absorbance at 255nm	Concentration (µg/ml)	Amount of drug release (mg)	Percentage drug release*
30	0.194	1.181	0.236	2.36
60	0.304	3.444	0.688	6.88
90	0.396	5.337	1.067	10.67
120	0.464	6.736	1.347	13.47
150	0.497	7.415	1.483	14.83
180	0.583	9.185	1.837	18.37
210	0.612	9.781	1.956	19.56
240	0.698	11.551	2.310	23.10
270	0.761	12.847	2.569	25.69
300	0.864	14.967	2.993	29.93
360	0.938	16.489	3.297	32.97
420	1.042	18.629	3.725	37.25
480	1.163	21.119	4.223	42.23

*Average of three Readings

Table 29

Compare the percentage release of Acyclovir from gel formulations with and without Dimethyl sulfoxide (DMSO).

Time (Mins)	Gel Formulations				Gel Formulations with DMSO			
	A_2	B_2	C_3	D_2	A_2	B_2	C_3	D_2
30	2.81	2.03	1.04	-----	4.50	3.18	2.65	2.36
60	13.34	9.34	7.21	5.44	15.53	10.75	10.67	6.88
90	18.74	14.17	10.67	7.25	20.96	16.64	13.88	10.67
120	23.39	15.77	12.60	9.23	25.69	20.46	15.90	13.47
150	25.11	19.76	15.07	11.41	27.46	23.30	19.64	14.83
180	25.77	22.19	16.27	13.88	28.24	24.04	22.85	18.37
210	30.75	25.11	18.98	16.72	33.34	31.86	25.07	19.56
240	38.57	29.06	22.65	19.60	41.33	34.87	27.95	23.10
270	41.41	31.66	25.77	22.60	46.88	38.53	32.73	25.69
300	45.28	34.00	29.19	24.58	52.22	40.63	35.65	29.93
360	56.72	43.10	31.58	27.95	62.32	49.64	39.81	32.97
420	60.63	45.53	40.30	31.53	68.37	54.50	46.68	37.25
480	64.91	51.47	44.04	34.54	72.85	60.42	51.74	42.23

*Average of three Readings

Figure 16: In vitro release profile of Acyclovir from 1% Carbopol – 934 gel

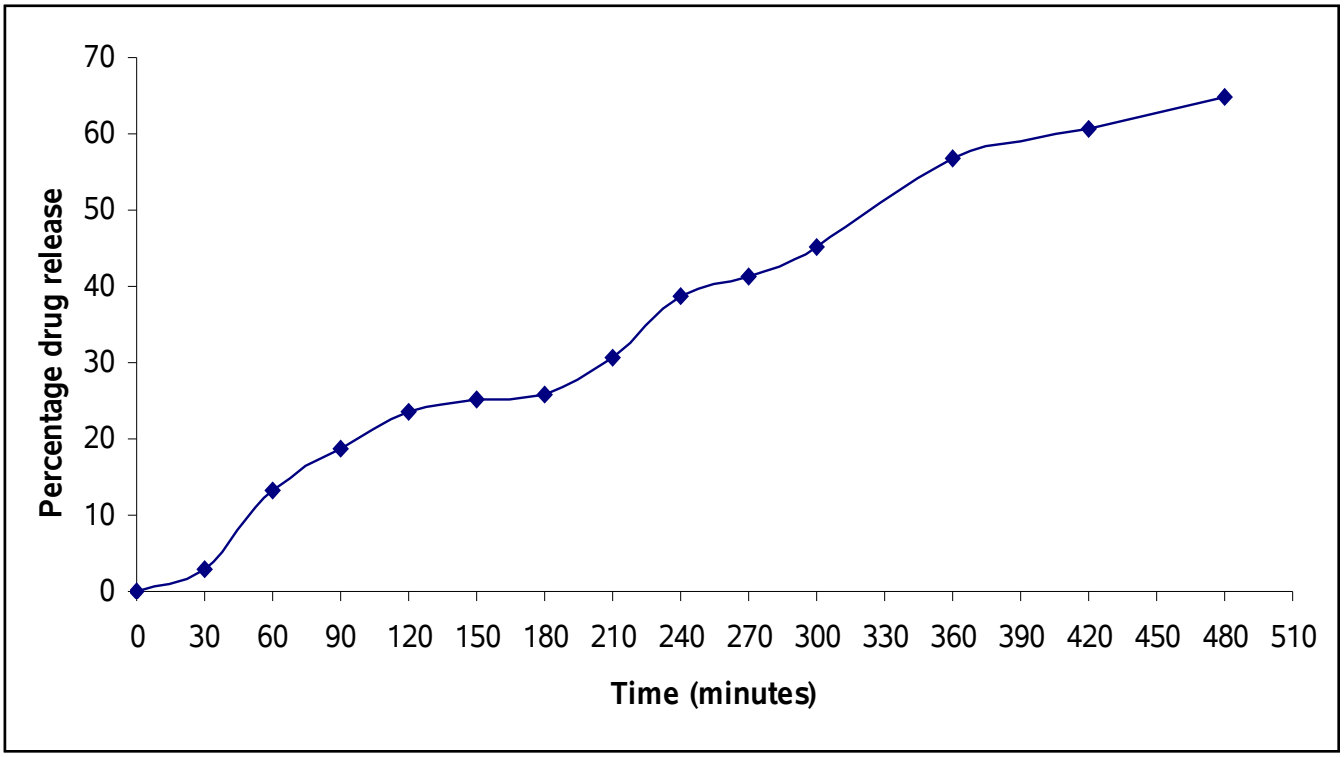


Figure 17: In vitro release profile of Acyclovir from 1% Carbopol – 940 gel

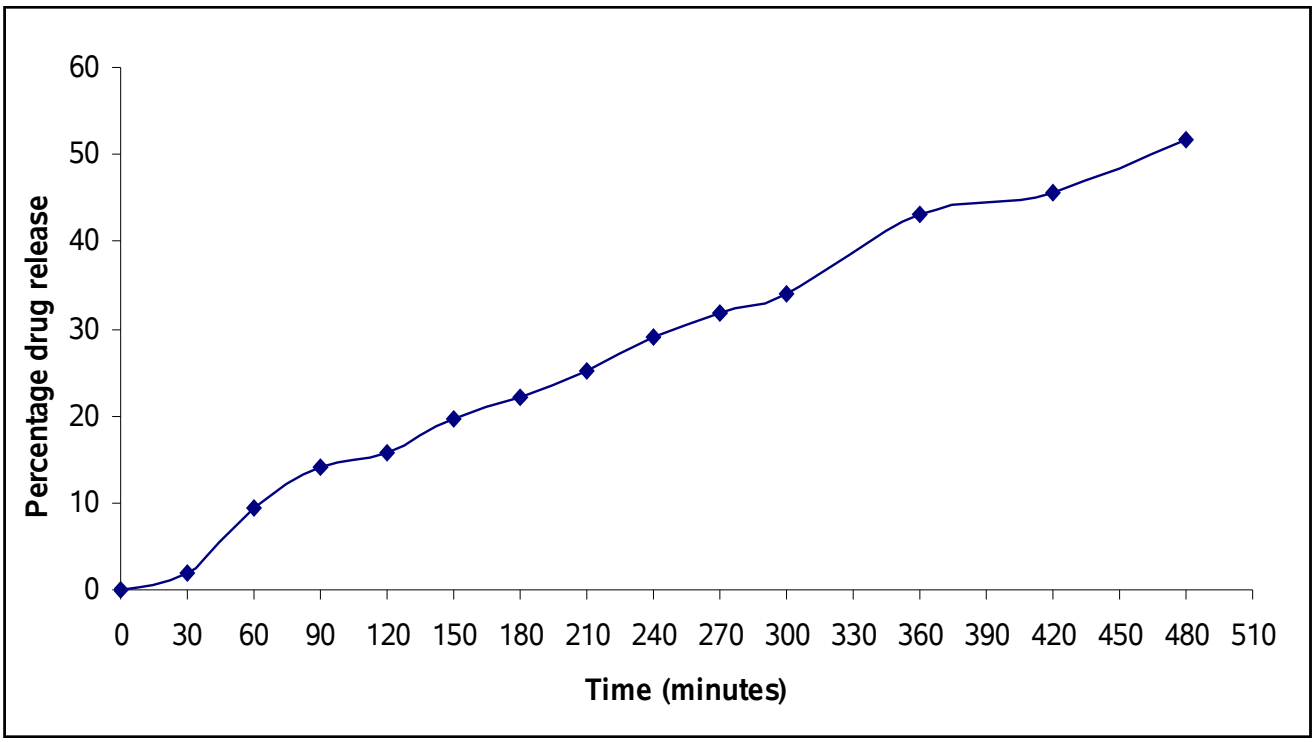


Figure 18: In vitro release profile of Acyclovir from 3% Hydroxy propyl methyl cellulose gel

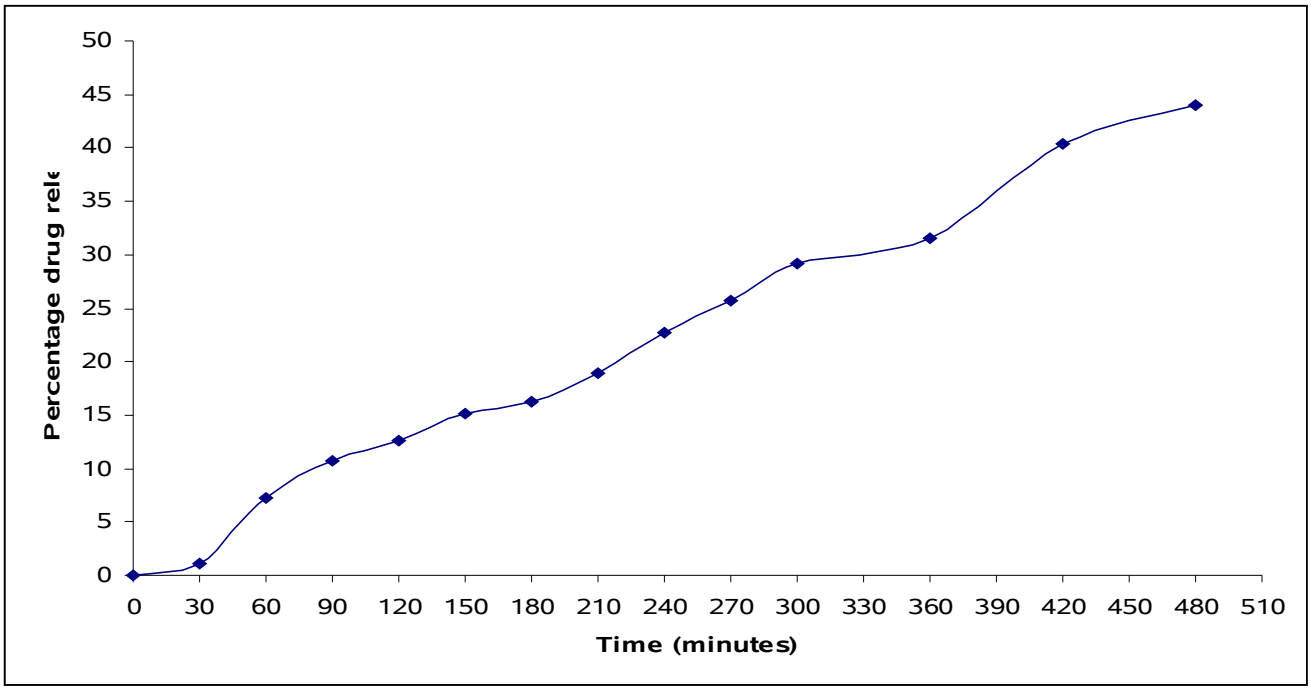


Figure 19: In vitro release profile of Acyclovir from 3% Sodium carboxy methyl cellulose gel

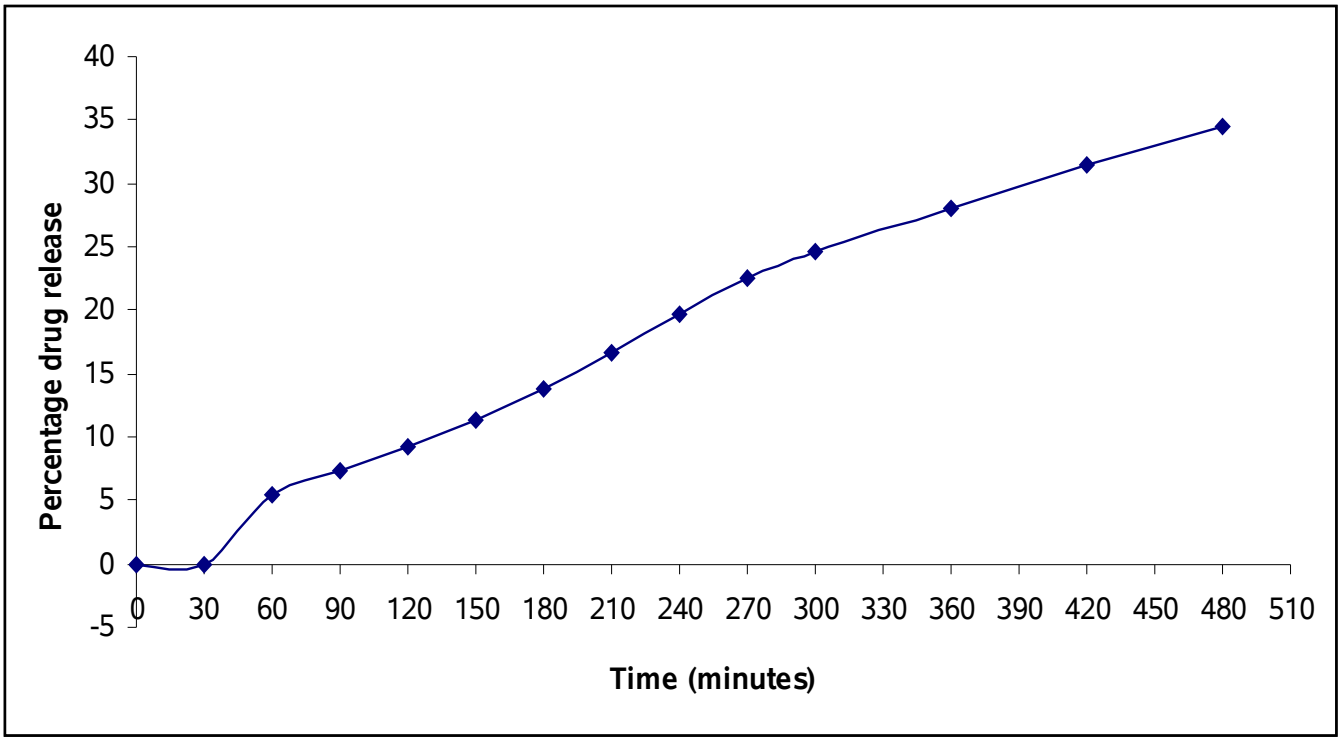


Figure 20: Comparative in vitro release profile of Acyclovir from 1% Carbopol -934 gel formulation with permeation enhancer

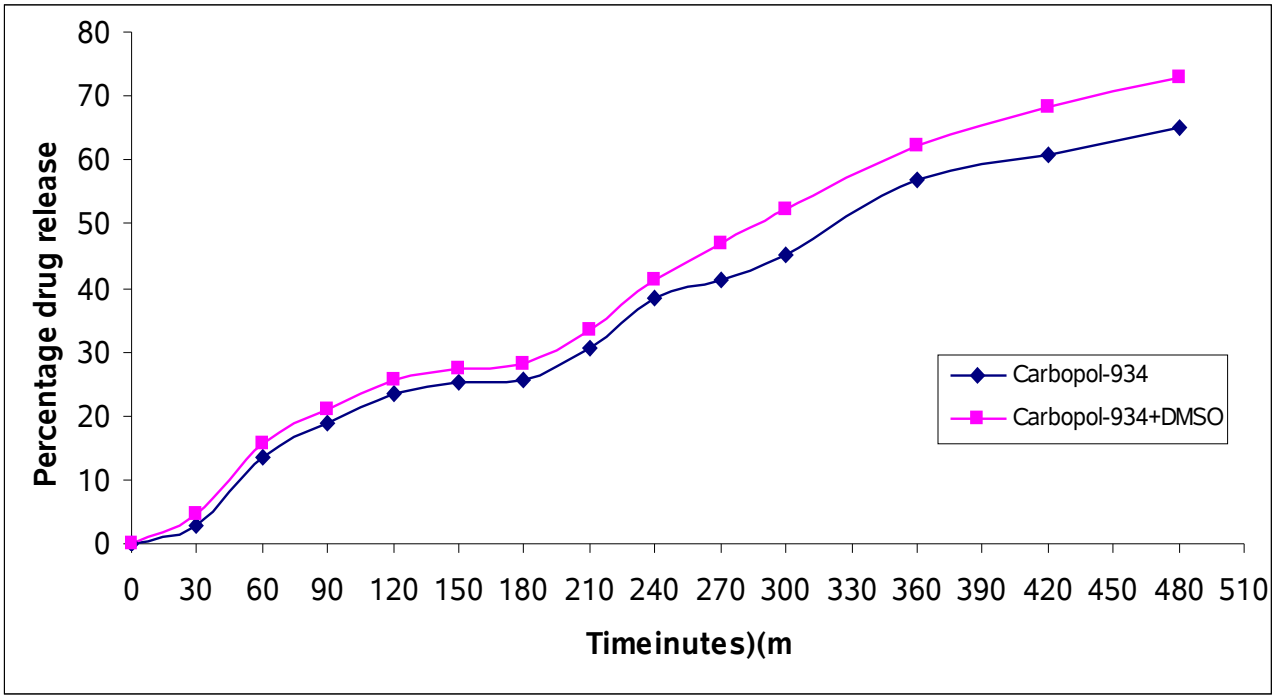


Figure 21: Comparative in vitro release profile of Acyclovir from 1% Carbopol -940 gel formulation with permeation enhancer

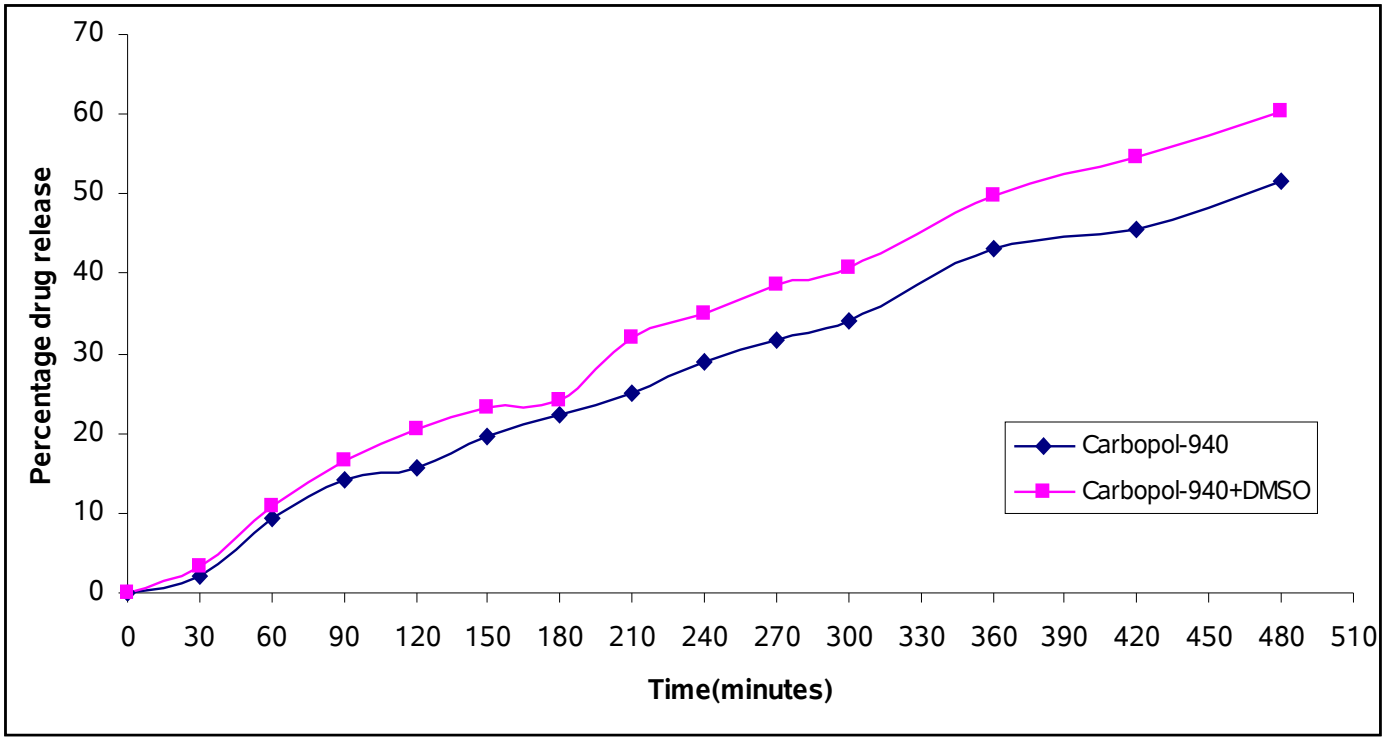


Figure 22: Comparative in vitro release profile of Acyclovir from 3% Hydroxy propyl methyl cellulose gel formulation with permeation enhancer

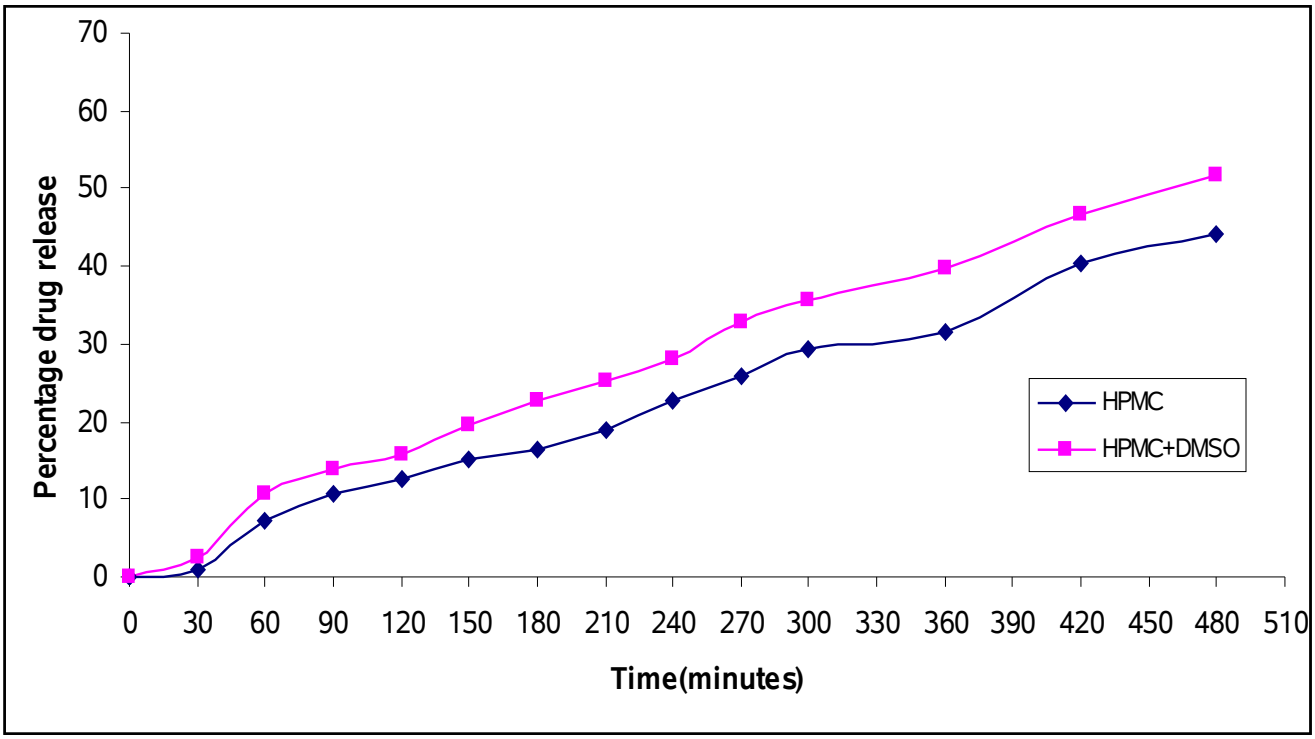


Figure 23: Comparative in vitro release profile of Acyclovir from 3% sodium carboxy methyl cellulose formulation with permeation enhancer

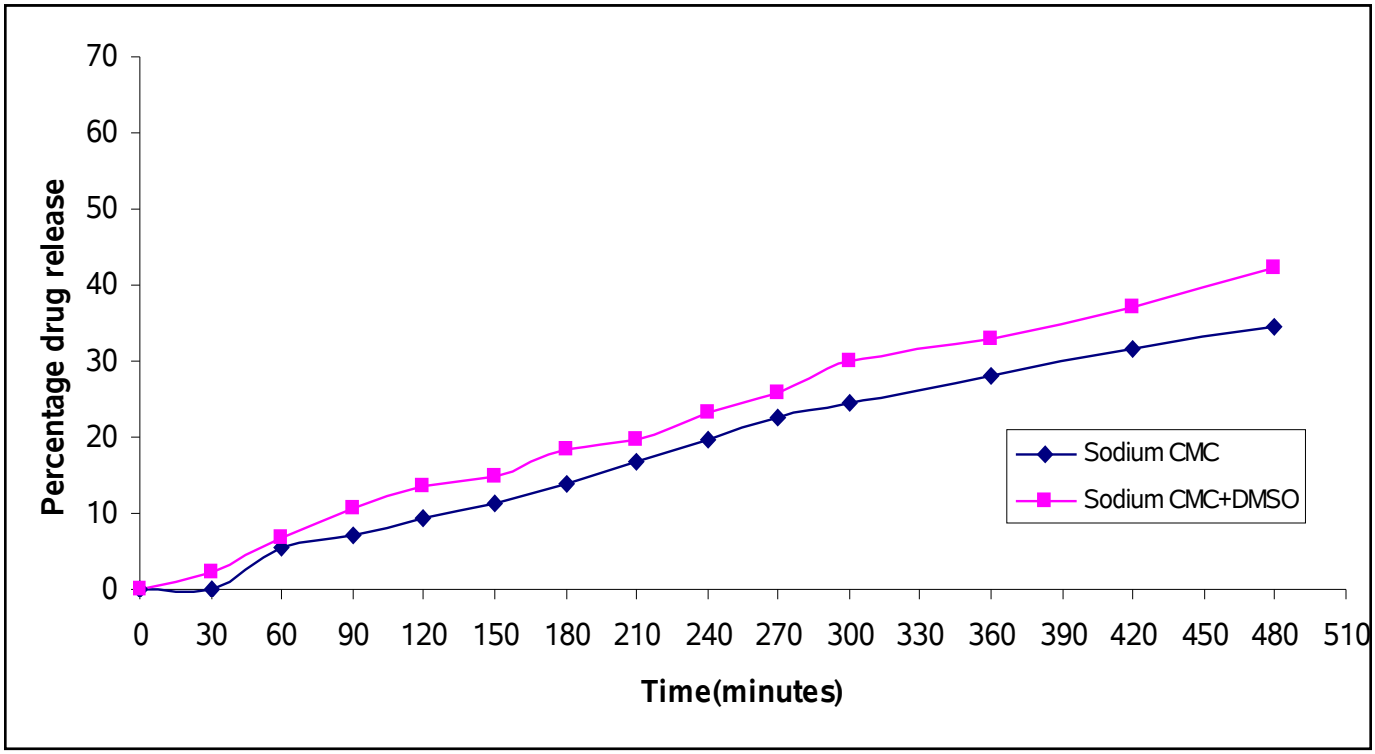


Figure 24: Comparative in vitro release profile of Acyclovir from different gel formulations

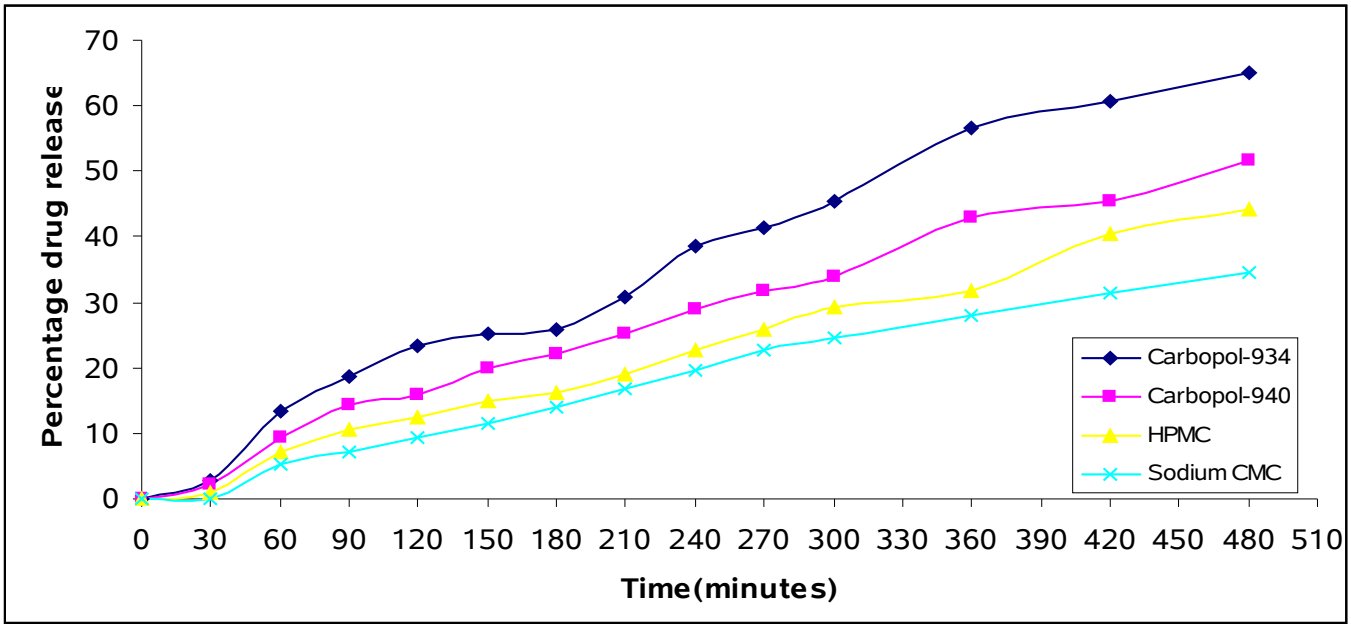
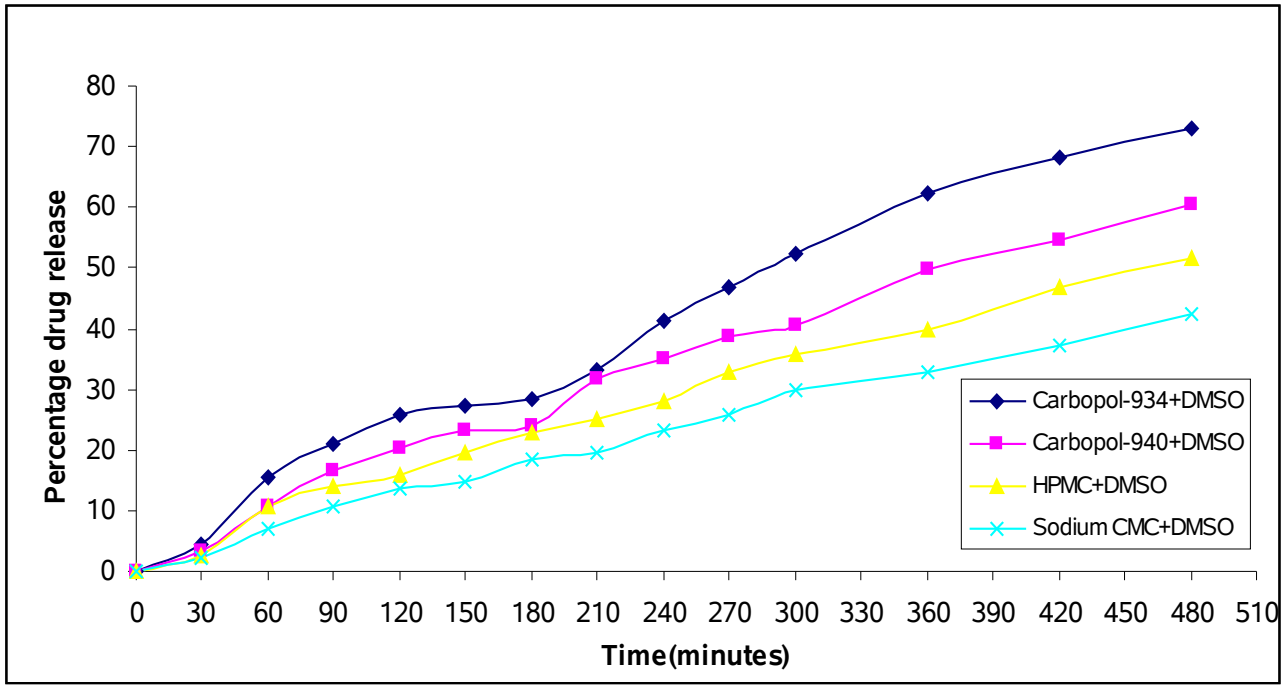


Figure 25: Comparative in vitro release profile of Acyclovir from different gel formulations ith permeation enhancer



Stability studies of the selected gel formulation [43]

The assessment procedure for the stability of a pharmaceutical product lies in the capability of a formulation to retain its physical, chemical and therapeutic specifications. A general methodology for predicting the stability is accelerated stability analysis in which the materials are subjected to elevated temperatures. This does not hold good for gels, as they melt at higher temperature conditions.

Thus the most commonly applied temperatures are refrigeration (4-5^{0}C), room temperature (25-30^{0}C) and 37±5^{0}C. Then the samples were checked at the regular intervals of 1, 2 and 3 months. Different parameters considered for analysis are shown below.

Physical parameters

1. Visual Appearance
2. pH
3. Viscosity
4. Extrudability
5. Phase separation
6. Leakage
7. Nature

Chemical parameters

- Drug content analysis of active ingredients

Method

The selected formulation was filled into aluminium collapsible tubes and stored at

a. Room temperature

b. 37±5^{0}C

c. 4-5^{0}C

The gel formulation was stored for a period of three months. Samples were withdrawn at monthly intervals for a period of three months and assessed for the drug content. At the end of third month they were evaluated for physical parameter and integrity of the product.

1. Physical evaluation of gels

The physical parameters considered for the evaluation were Visual appearance, Nature of the product, pH, Viscosity, Leak, Phase separation and Extrudability.

2. Chemical evaluation

The drug content of the formulation was estimated by withdrawing samples from different corners of the tube. The samples were mixed together and 1gm was taken for the assay. The estimation of drug content was carried out as per the procedure.

Table 30: Physical evaluation of formulation A_2 (1% Carbopol-934)

Parameters	Room Temperature	37±5^{0}C	4-5^{0}C
Visual appearance			
Initial	Transparent	Transparent	Transparent
Final	Transparent	Transparent	Transparent
pH			
Initial	6.9	6.9	6.9
Final	7.1	7.0	6.9
Viscosity (cps)			
Initial	43,000	43,000	43,000
Final	43,000	43,500	43,000
Extrudability			
Initial	+++	+++	+++
Final	+++	+++	+++
Phase separation	Not found	Not found	Not found
Leakage	Not found	Not found	Not found
Nature			
Initial	Smooth	Smooth	Smooth
Final	Smooth	Smooth	Smooth

+++Excellent.

Chemical evaluation

The drug content of the formulation was estimated over a period of 3 months. The results were tabulated as follows.

Table 31: Drug content of formulation A_2 (1% Carbopol-934)

Storage condition	Withdrawal period (monthly)			
	0	1	2	3
4-5^{0}C	101.72	101.54	100.04	99.36
Room Temperature	101.72	100.86	99.48	98.93
37±5^{0}C	101.72	100.55	99.08	98.24

Each reading represents the average of three determinations

Skin irritation test[44]

The primary skin irritation test was performed on healthy albino rabbits, weighing between 2.0-3.5 kg. The gel formulation film was prepared and used as test patches, while adhesive tape (USP) was used as control. The test was conducted on unbraided skin of the rabbits. The control and test patches were placed on the left and right dorsal surfaces of the rabbits respectively. The patches were removed after 24 hours with the help of alcohol swab and the skin was examined for erythema and edema[45], and its shown in figure No.26 & 27.

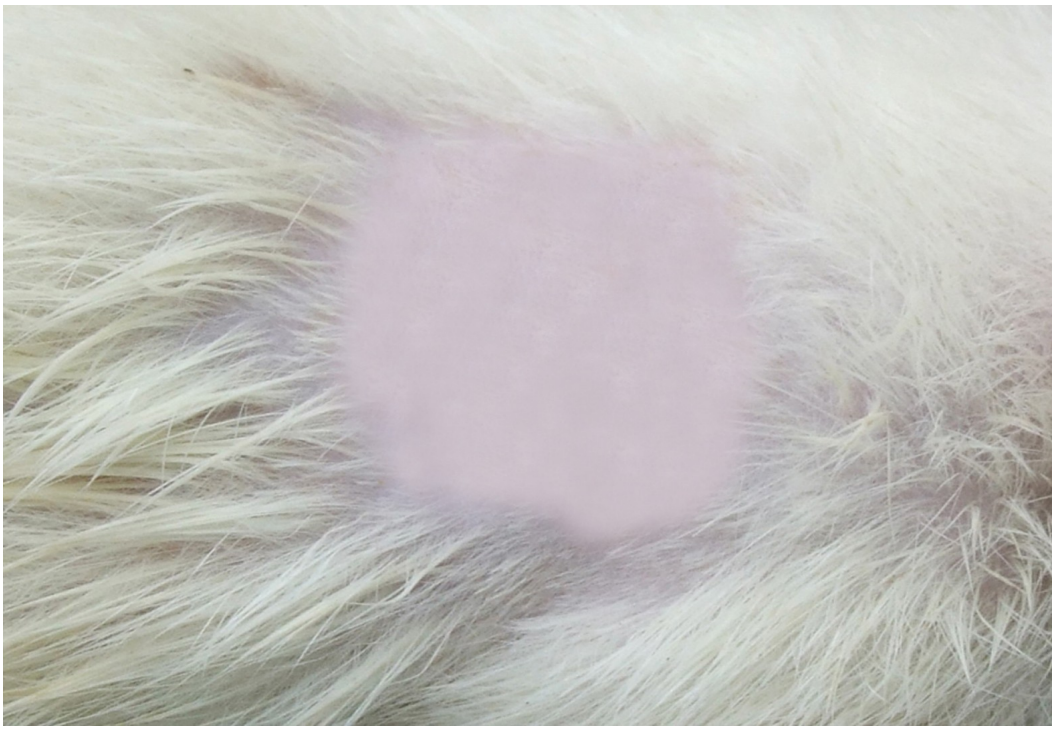


Figure 26: **Initial skin appearance for irritation test**

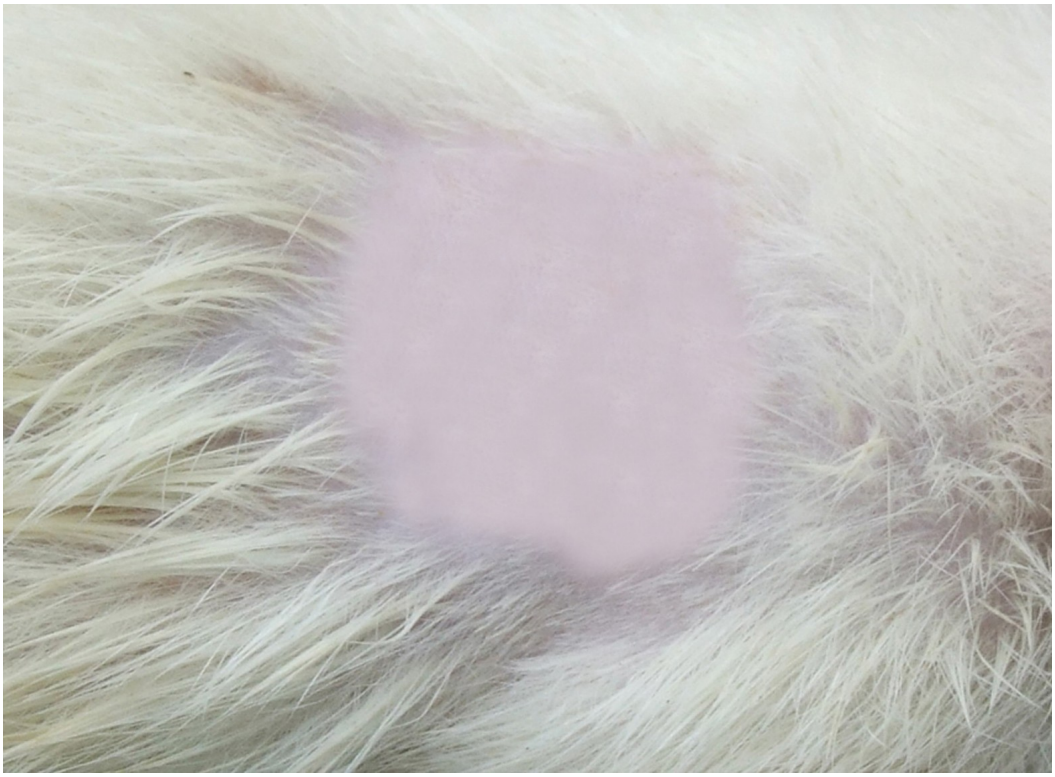


Figure 27: **Skin appearance after 24 hrs for irritation test**

In vivo studies of the selected gel formulation

Selection of Animal model

Rabbits were chosen as an animal model for the In vivo evaluation because rabbits had the advantage of ease of safety handling and experimentation.

Experiment

In vivo studies were performed by using Albino Rabbits, weighing from 1.5 to 2.0 kg selected for this study and they were fed with vegetables and water. The animals were divided into two groups, each group containing six animals.

The groups under treatment were designed as follows,

Group - I: Marketed ACIVIR Cream as Standard
Group - II: Selected gel formulation as Test.

The standard and test sample were administered at the unbraided area of the skin. After administration, exactly 1 ml of blood samples were collected from the marginal ear veins of rabbits using sterile butterfly needles and syringes at one hour intervals from zero hour to a period of two biological half lives of the drug and then transferred into sterile vials containing 1ml of sterile anticoagulant solution (10 mg of sodium citrate in 100 ml of sterile water). Then the plasma was separated from the blood by centrifugation at 3000rpm in Remi centrifuge for 10 minutes and then supernatant plasma was collected. The plasma was transferred to screw capped vials and kept in the refrigerator till the samples were analyzed.

Figure 28

Blood sampling for In vivo studies of Acyclovir topical gel

Determination of plasma drug concentration

The plasma levels of drug were determined by Shimadzu UV-Spectrophotometer. The each sample was diluted with equal quantity of distilled water. Then the prepared solutions were analyzed by UV-Spectrophotometer at 255nm by using distilled water as blank. The absorbances of drug were enlighted in Table 32 & 33. From the absorbance the drug concentration in blood samples at different time intervals both standard and test group animals were calculated from the standard graph and the results are given in the Table 34.

Pharmacokinetic parameters[46]

From all the above data obtained, Area Under Curve (AUC) was plotted in graph taking Time (in hours) on X-axis and plasma drug concentration (µg/ml) in Y-axis. The AUC was determined by using Trapezoidal rule.

AUC =(1/2)(C1+C2)(t2-t1)+(1/2) (C2+C3)(t3-t2)+……+(1/2) (Cn-1+Cn)(tn- tn-1)

Where, C = Drug concentration

t = Time

Elimination rate constant (Ke)

It is calculated by the following equation $Ke = 0.693/t_{/2}$

Elimination half life ($t_{/2}$)

The **($t_{/2}$)** values were obtained by the extrapolation of Time Vs plasma concentration curve'

Peak plasma concentration (C_{max})

C_{max} was obtained from the Time Vs Plasma concentration curve.

t_{max}

t_{max} was obtained from the Time Vs Plasma concentration curve.

Relative Bioavailability

The relative bioavailability of drug was determined by using the following formula

$$\text{Relative Bioavailability} = \frac{\text{Area Under Curve for Test}}{\text{Area Under Curve for standard}}$$

Absorption rate constant (Ka)

The **(Ka)** values were obtained by the extrapolation of Time Vs log concentration in semilogrthmic curve by Residual (or) Feathering method.

The above all pharmacokinetic parameters were shown in Table 34

Table 32

Plasma drug concentration at different time intervals for Standard (ACIVIR cream)

Time (hours)	Absorbance at 255nm	Concentration* (µg/ml)	AUC (µg hr/ml)
0	0	0	2.154
1	0.346	4.308	10.666
2	0.964	17.024	19.493
3	1.204	21.962	17.970
4	0.816	13.979	10.851
5	0.512	7.724	7.322
6	0.473	6.921	-----

*Average of three readings

Table 33

Plasma drug concentration at different time intervals for Test-A_2 (1%Carbopol gel formulation)

Time (hours)	Absorbance at 255nm	Concentration (µg/ml)	AUC (µg hr/ml)
0	0	0	
1	0.494	7.353	3.676
2	1.074	19.288	13.320
3	1.319	24.329	21.778
4	1.102	19.864	22.096
5	0.974	17.230	18.547
6	0.721	12.024	14.627

*Average of three readings

Table 34

Pharmacokinetic Parameters for Standard (ACIVIR Cream) and Test (Acyclovir gel)

S.No	Pharmacokinetic Parameters	Units	Standard	Test
1	AUC	(µg hr/ml)	98.032	146.322
2	Relative Bioavailability	----------	---------	1.492
3	C_{max}	(µg/ml)	21.962	24.329
4	t_{max}	Hours	3	3
5	Ke (Elimination rate constant)	Hour-1	0.234	0.230
6	$t/_2$ (Elimination half life)	Hours	2.96	3.01
7	Ka (Absorption rate constant)	Hour-1	0.621	0.575

Figure 29: Area Under curve (AUC) of Acyclovir for Standard (ACIVIR cream)

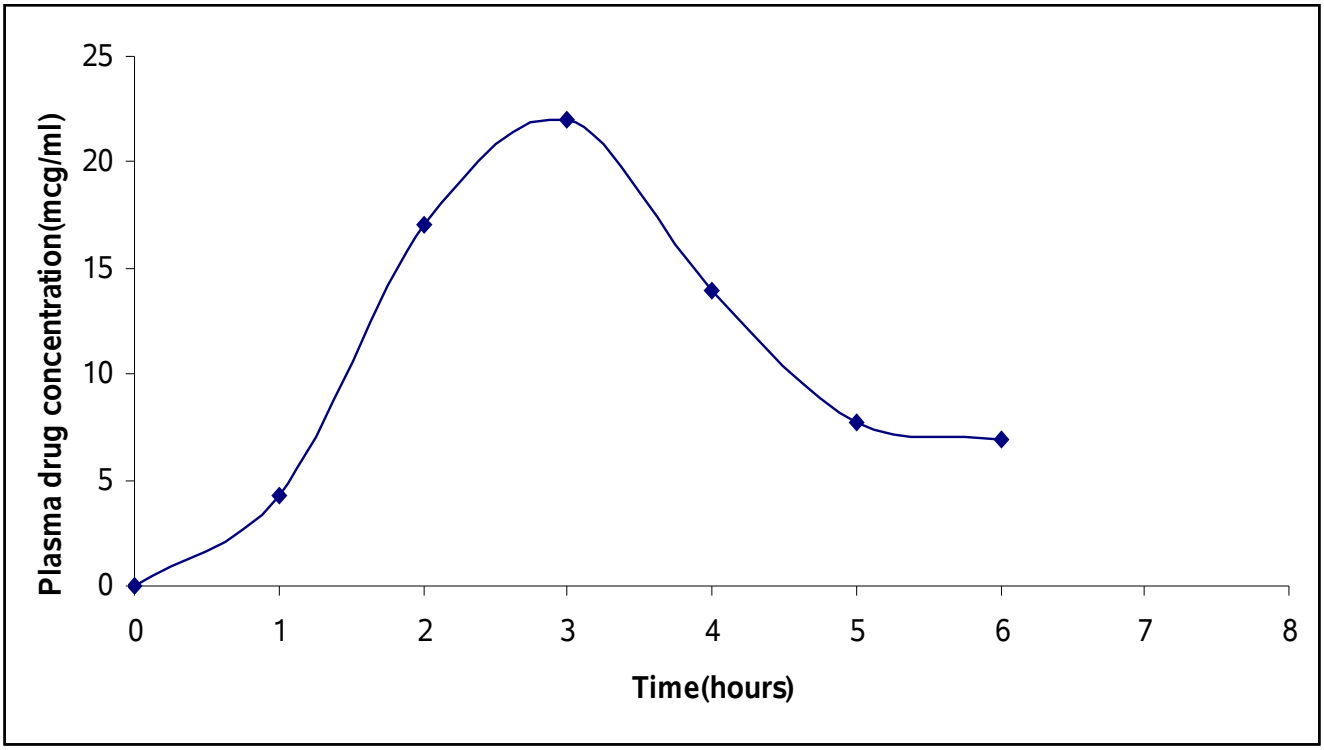


Figure 30: Area Under curve (AUC) of Acyclovir for Test (Acyclovir gel)

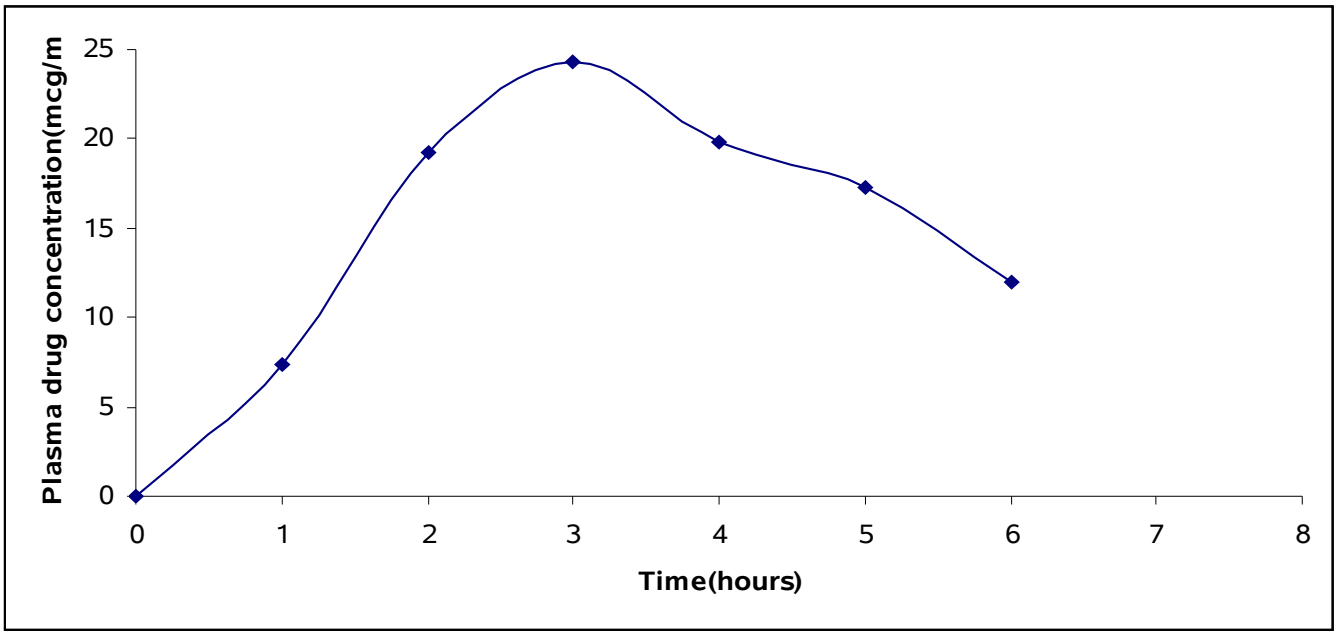
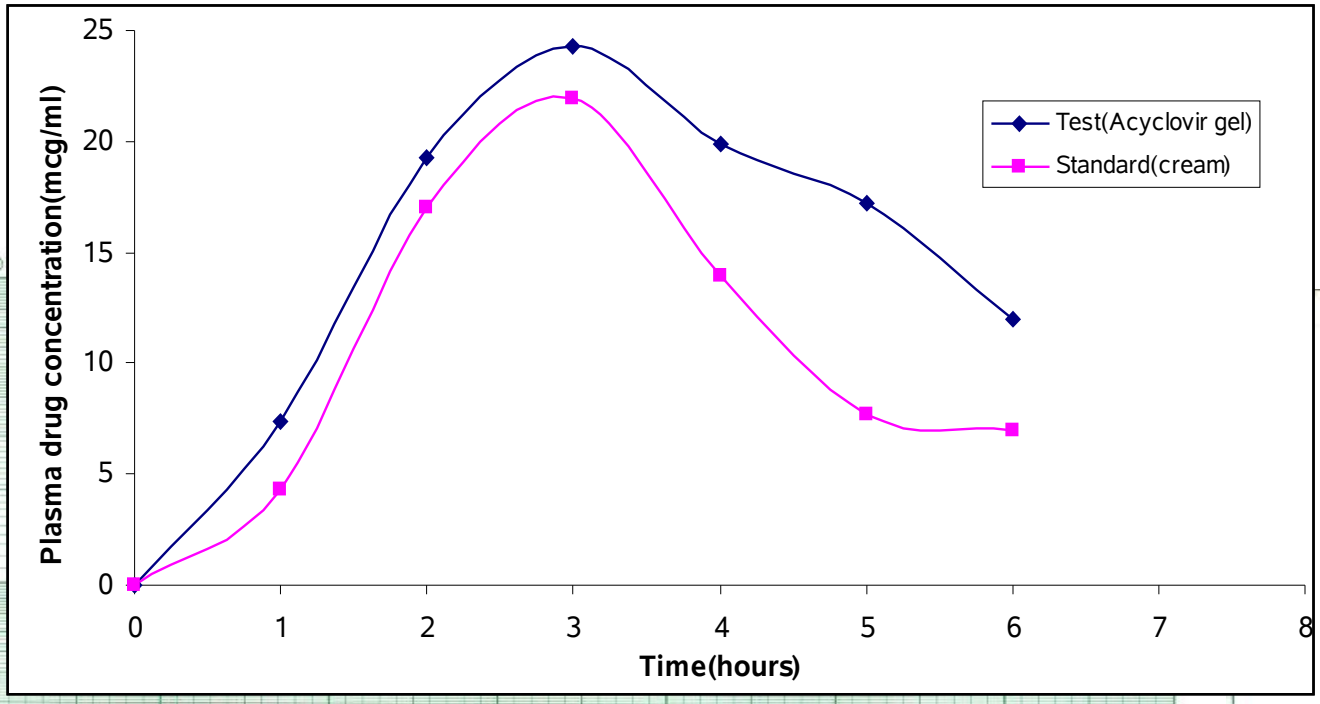


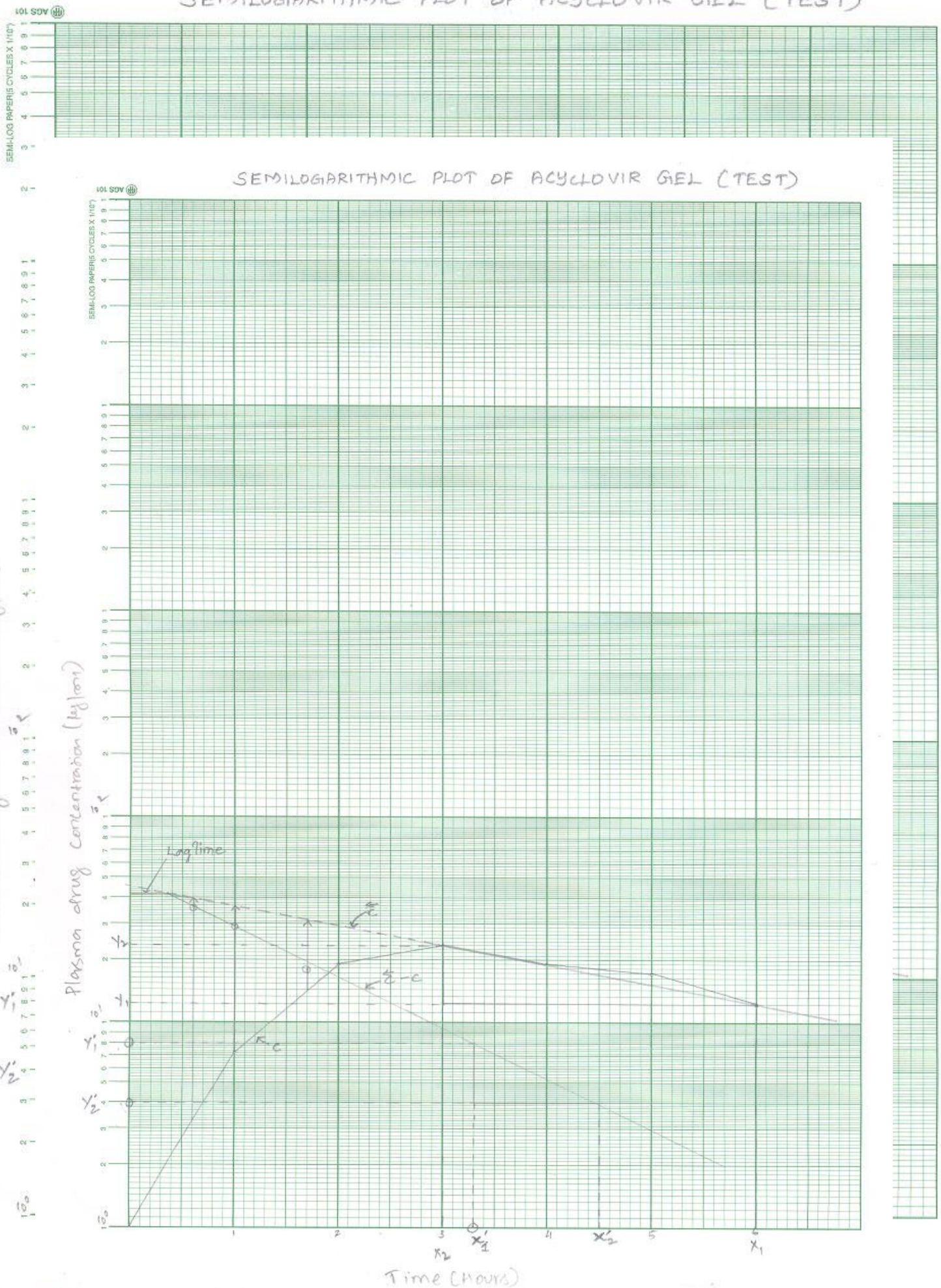
Figure 31: Comparative Area Under curve (AUC) of Acyclovir for Test and Standard


Plasma drug concentration(mcg/ml)
25
20
15
10
5
0
0
1
2
3
4
5
6
7
8
Time(hours)
Test(Acyclovir gel)
Standard(cream)

Plasma drug Concentration (log|ml)
Log time
Time (Hours)

Figure 32

SEMILOGARITHMIC PLOT OF ACYCLOVIR GEL (TEST)



RESULTS AND DISCUSSION

Compatibility Study

With reference to the IR-spectrum, the drug Acyclovir was compatible with all the polymers namely Carbopol, Hydroxypropyl methyl cellulose and Sodium carboxy methyl cellulose were used in the gel formulation.

Formulation of Acyclovir topical gels using various gelling agents

Gel formulations of acyclovir were prepared using different polymers namely Carbopol-934, Carbopol-940, Hydroxypropyl methyl cellulose and Sodium carboxy methyl cellulose as per the procedure.

Carbopol-934 as a gelling agent

Formulations with formula A_1 (0.5%Carbopol-934), A_2 (1.0%Carbopol-934) and A_3 (1.5%Carbopol-934) were prepared. A_1 showed low consistency and A_3 showed very high viscosity. The gel formulation A_2 (1.0% carbopol-934) exhibited desired consistency.

Carbopol-940 as a gelling agent

Formulations with formula B_1 (0.5%Carbopol-940), B_2 (1.0%Carbopol-940) and B_3 (1.5%Carbopol-940) were prepared. B_1 showed low consistency and B_3 showed very high viscosity. The gel formulation B_2 (1.0% carbopol-940) exhibited desired consistency.

Hydroxypropyl methyl cellulose as a gelling agent

Formulations with formula C_1 (1.0%HPMC), C_2 (1.5%HPMC), C_3 (3.0%HPMC) and C_4 (4.0%HPMC) were prepared. C_1 and C_2 showed low consistency and C_4 was highly viscous. The formulation C_3 (3.0%HPMC) exhibited desired consistency.

Sodium Carboxy methyl cellulose as a gelling agent

Formulations with formula D_1 (2.0%SodiumCMC), D_2 (3.0%SodiumCMC) and D_3 (4.0% SodiumCMC) were prepared. D_1 showed low consistency and D_3 showed very high viscosity. The gel formulation D_2 (3.0%SodiumCMC) exhibited desired consistency.

Evaluation of Acyclovir gels

All the optimized gel formulations were subjected to evaluation studies

Estimation of drug content

The amount and percentage of drug present in gel formulation using different polymers were estimated as per the procedure. The prepared gel using 1% carbopol-934(A_2) showed maximum drug content (101.72%) compared to other formulations, The results were shown in the Table 16.

pH Measurements

The pH measurements of all the gel formulations were carried out by using digital pH meter. The pH of the formulations were ranged from 6.8 to 7.2 and the results were shown in Table 17

Determination of viscosity

The viscosity of the gels were determined using Brookfield Viscometer. The viscosity of the formulations were ranged from 36,000 to 51,000cps and the results were shown in Table 18

Extrudability

The extrudability of the gel formulations were checked as per the procedure. Extrudability of carbopol and HPMC gels were excellent than sodiumCMC gel and the results were shown in Table 19.

Determination of Spreadability

The spreadability of gels was determined as per the procedure. From spreadability data is observed that the formulation with 1.0% carbopol-934 showed maximum (8cm), where as the formulations with 1%carbopol-940, 3%, HPMC and SodiumCMC 3% were showed significant spreadability. The results were tabulated in Table 20

In vitro drug release of gel formulations

In vitro drug release of gel formulations were carried out as per the procedure. The percentage release of drug from different gel formulations at the end of 8hrs was determined.

1.0 % carbopol-934 shows maximum release (64.91%). The addition of DMSO as permeation enhancer improves the drug release from gel formulation.

1.0% carbopol-940 also showed a similar release pattern, but the release was lesser (51.47%). The addition of DMSO as permeation enhancer improves the drug release from gel formulation.

In case of HPMC and SodiumCMC gels the release was much lesser than carbopol gels. The addition of DMSO as permeation enhancer drug release was improved.

Based on the drug release A_2 (1.0 % carbopol-934) was the best formulation and the percentage release was found to be 64.91% . So, stability and In vivo studies were carried out for A_2 formulation.

The percentage release of drug from different gel formulations was shown in Table 21 - 29 and Figure 16 -25.

Stability studies for the formulation A_2 (1.0 % carbopol-934)

Stability study for the best formulation was done as per the procedure. The gel was both physically and chemically stable at 4-5^{0}c, Room temperature and 37±5^{0}c. The results were tabulated in Table 30 & 31.

Skin irritation test

Skin irritation test was carried out as per the procedure, there was no erythema and edema and any kind of reaction. Thus the gel was found to be safer for topical use. The observations were shown in the figure 26 & 27.

In vivo studies for the selected gel formulation

In vivo studies were carried out as per procedure. The blood samples were drawn at different time intervals for Standard and Test group of animals were analyzed for the absorbance at 255nm in UV-Spectrophotometer. The absorbance values were interpreted with standard curve the plasma drug concentration and the pharmacokinetic parameters were determined.

The bioavailability of Acyclovir in Test and Standard were estimated by the measurement of Area Under Curve (AUC) and the relative bioavailability was estimated. The bioavailability of the drug in Test was more than the Standard.

The t_{max} was 3hours for both Test and Standard and (peak plasma concentration) C_{max} was found to be 24.329 and 21.962 respectively. The elimination rate constant (Ke) for Standard and Test was found to be 0.230 and 0.234 $hour^{-1}$.

The elimination half life ($t/_2$) for Standard and Test was found to be 3.01 and 2.96hours. The absorption rate constant (Ka) was determined by Residual method was found to be 0.575 and 0.621$hour^{-1}$ for Test and Standard respectively. The results were shown in Table 32 – 34.

SUMMARY AND CONCLUSION

The present work describes a study on “Formulation and Evaluation studies of Acyclovir topical gels for Antiviral activity”

Acyclovir is a broad spectrum antiviral agent against Herpes Simplex Virus and Varicella Zoster Virus, which is specific to viral-infected cells with low toxicity and which is less toxic than earlier generation of antiviral agents and as such represents a major therapeutic advance. This drug was selected for the study because it has good percutaneous absorption and appears to be more active as antiviral activity and is well tolerated. The polymers namely Carbopol-934, Carbopol-940, Hydroxypropyl methyl cellulose and Sodium carboxy methyl cellulose were used for formulation of gels and studied for their drug release from the gel formulations.

It is evidence from the IR spectrum that all the polymers used in the gel formulations were compatible with the drug Acyclovir.

Different formulations of Acyclovir were prepared by using Carbopol-934, Carbopol-940, Hydroxypropyl methyl cellulose and Sodium carboxy methyl cellulose in varying proportions. Carbopol gels were transparent, non-greasy and smooth on application. SodiumCMC and HPMC gels were opaque, non-greasy and sticking on application.

The gel was prepared using 1%Carbopol-934 has maximum drug content (101.72%) than the others.

The pH of the formulations ranged from 6.8 to 7.2 and viscosity is from 36,000 to 51,000cps.

Extrudability of carbopol and HPMC gels were excellent than the SodiumCMC gel.

The spreadability data shown that the formulation with 1%Carbopol- 934 has the highest value (8cm), where as the others have significant values.

In vitro release studies of the formulations were carried out across the cellophane membrane using a diffusion cell. The release was highest for the formulation A_2 (1%Carbopol-934) and on the addition of DMSO as a permeation enhancer the drug release was improved.

The formulation B_2, C_3 and D_2 also have significant percentage release and on addition of DMSO as a permeation enhancer the drug release from gel formulation was improved. Hence based on the above results, out of 13 formulations A_2 was chosen as the best formulation.

Stability studies were carried out by placing the gels in collapsible tube at 4-5^0c, Room temperature and 37 ± 5^0C for 3 months and also analyzed for various physical and chemical parameters. The result indicates that the prepared gel was both stable physically and chemically at all storage conditions.

From the skin irritation test it was observed that the formulation A_2 was found to be safer for topical use.

In vivo studies were carried out by collecting blood samples from albino rabbits at regular intervals. The plasma drug concentration and pharmacokinetic parameters were determined. From the above data it was observed that the bioavailability of the drug in Test was higher than Standard.

From this investigation, it was concluded that formulation A_2 with 1% Carbopol-934 may be the best formulation having good in vitro release profile, stability and bioavailability. Based on the results from the study further utility of the dosage form may depend on pharmacokinetic data. Forthcoming research work of antiviral activity may contribute in the challenging area.

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