CHAPTER-III

Experimental Investigation
Profiles of acyclovir and polymers are discussed in this chapter.

3.1 DRUG PROFILE:

3.1.1 Acyclovir:

Category: Antiviral

Systematic (IUPAC) name:

2–Amino–9–(2–hydroxyethoxymethyl)–3H–purin–6–one.

Structure:

![Structure of Acyclovir](image)

3.1.2 Clinical use:

Acyclovir is indicated for the treatment of herpes simplex virus and varicella zoster virus infections, including genital herpes simplex (treatment and prophylaxis), labial herpes simplex (cold sores), herpes zoster (shingles), acute chickenpox in immunocompromised patients, herpes simplex encephalitis, acute mucocutaneous
HSV infection in immunocompromised patients and herpes simplex keratitis.
(Sean.c.Sweetman et al., 2002)

3.1.3 Mechanism of action:

Acyclovir differs from nucleoside analogues as it contains only a partial nucleoside structure. The sugar ring is replaced by an open-chain structure. It is selectively converted into a monophosphate form 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 the active triphosphate form, aciclov - GTP by cellular kinases. Aciclov - GTP is a very potent inhibitor of viral DNA polymerase; it has approximately 100 times higher affinity to viral than cellular polymerase. Its monophosphate form also incorporates into the viral DNA, resulting in chain termination. It has showed that the viral enzymes cannot remove acyclic- GTP from the chain – which results in inhibition of further activity of DNA polymerase. acyclic - GTP is fairly rapidly metabolized within the cell, possibly by cellular phosphatases. (Joel et al., 2001, Sean C. Sweetman et. al., 2002).

Therefore the acyclovir can be considered as a prodrug. It is administered in an inactive form and metabolism converting it into a more active species.

3.1.4 Pharmacokinetics:

Acyclovir is poorly water soluble and has poor oral bioavailability (10-20%) hence intravenous administration is necessary if high concentrations are required. When orally administered, peak plasma concentration occurs after 1-2 hours. Acyclovir has a high distribution rate; only 30% is protein – bound in plasma. The
elimination half life of acyclovir is approximately 3 hours. It is renally excreted partly by glomerular filtration and partly by tubular secretion. (Joel et al., 2001, Sean C. Sweetman et al., 2002).

3.1.5 Toxicity:

Since acyclovir can also be incorporated into the cellular DNA, it is a chromosome mutagen; therefore, its use should be avoided during pregnancy. However it has not been shown to cause any teratogenic or carcinogenic effects. The acute toxicity (LD50) of acyclovir when given orally is greater than 1 mg / kg due to the low oral bioavailability.

3.1.6 Adverse drug reactions:

Common adverse drug reactions associated with systemic acyclovir therapy (oral or IV) include nausea, vomiting, diarrhea, headache, agitation, vertigo, confusion, dizziness, oedema, sore throat, constipation, abdominal pain, rash and weakness. (Joel et al., 2001, Sean C. Sweetman et al., 2002).

3.1.7 Rare adverse effects include:

Coma, leucopenia, fatigue, seizures, crystalluria, hepatitis, neutropenia, anorexia, toxic epidermal necrolysis, anaphylaxis, stevens johnson syndrome, additional common adverse effects, when acyclovir is administered IV, include encephalopathy and injection site reactions.

The injection formulation is alkaline (pH 11) and extravasations may cause local tissue pain and irritation. Renal impairment has been reported when acyclovir is
given in large, fast doses intravenously, due to the crystallization of acyclovir in the kidneys.

3.1.8 Other medical problems:

Its use was restricted in dehydration and kidney diseases. They may increase blood levels of acyclovir increasing the chance of side effects.

Nervous system problems Acyclovir may make these problems worse.

3.1.9 Drugs interacting with acyclovir:

Phenytoin, Probenecid and Zidovudine, AZT.

3.1.10 Dosage forms:

Acyclovir is commonly marketed as tablets (200 mg, 400 mg and 800 mg), topical cream (5% w/w), intravenous injection (25 mg/ml) and ophthalmic ointment (3% w/v)

Cream preparations are used primarily for labial herpes simplex. The intravenous injection is used when high concentration of acyclovir is required. The ophthalmic ointment preparation is only used for herpes simplex keratitis. (Sean. C. Sweetman et al., 2002).

3.1.11 Dosing:

The dose of acyclovir will be different for different patients.

- For oral dosage forms (Capsules, oral suspension tablets.)
- For treatment of genital herpes
Adults and children 12 years of age and older – 200 mg five times a day for ten days. (Joel et al., 2001, Sean C. Sweetman et al., 2002).

- **For prevention of recurrent outbreaks of genital herpes infections:**
  
  Adult and children 12 years of age and older 200–400 mg two to five times a day for five days or up to twelve months, depending upon the outbreaks of infection.

- **For treatment of chickenpox:**
  
  Adults and children who weigh 40 kilogram, 800 mg four times a day for five days. Children 2 years of age and older weighing 40 kg or less, dose is calculated based on body weight. The usual dose is 20 mg per kilogram of body weight up to 800 mg four times a day for five days.

- **For treatment of shingles:**
  
  Adults and children 12 years of age and older – 800 mg five times a day for seven to ten days.

- **For injection dosage form in treatment of herpes of the brain, genitals, mucous membranes, or for the treatment of shingles:**
  
  Adults and children 12 years of age and older, dose is based on body weight. The usual dose in 5 to 10 mg per kg of body weight, injected slowly into a vein over at least a one – hour period and repeated every eight hours for five to ten days. Children up to 12 years of age – dose is based on body weight. The usual dose is 10 mg to 20 mg of acyclovir per kg of body weight, injected
slowly into a vein over at least a one–hour period and repeated every eight hours for seven to ten days.

- **For treatment of widespread herpes virus infection in newborns:**
  Infants from birth to 3 months of age usual dose is 10 mg of acyclovir per kg of body weight, injected slowly into a vein over at least a one–hour period and repeated every eight hours for ten days.

### 3.2. POLYMER PROFILE:

#### 3.2.1 Yeast Profile:

**Table: 3 Yeast Profile**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>Ascomycota</td>
</tr>
<tr>
<td>Subphylum</td>
<td>Saccharomycotina</td>
</tr>
<tr>
<td>Class</td>
<td>Saccharomycetes</td>
</tr>
<tr>
<td>Order</td>
<td>Saccharomycetales</td>
</tr>
<tr>
<td>Family</td>
<td>Saccharomycetaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Saccharomyces</td>
</tr>
<tr>
<td>Species</td>
<td><em>Saccharomyces bayanus, Saccharomyces boullardii, Saccharomyces Cerevisiae, Saccharomyces uvarum</em></td>
</tr>
</tbody>
</table>
The science or study of fungi is called mycology. The yeast cell is surrounded by a true cell wall. Yeast are larger than most bacteria; yeast vary considerably in size, ranging from 1 to 5 μm in width and from 5 to 30 μm or more in length. They are commonly egg–shaped, but some are elongated and some spherical. Each species has a characteristic shape but even in pure culture there is considerable variation in size and shape of individual cells, depending on age and environment. Yeast has no flagella or other organelles of locomotion. (Darling et. al., 1972).

Yeast and molds can grow in a substrate or medium, containing sugars that inhibit most bacteria. Yeast and molds can tolerate more acidic conditions than most other microbes. Some yeast is facultative that is, they grow under both aerobic and anaerobic conditions. Yeast is usually aerobic.

3.2.2 Classification of yeast (Gilland, 1971).

*Saccharomyces* is a genus in the kingdom of fungi that includes many species of yeast, the name Saccharomyces meaning, 'Sugar Fungi'. The inability to utilize nitrate and ability to ferment various carbohydrates are typical characteristics of *Saccharomyces*. Colonies can grow and mature in 3 days and exhibit a yellow-tan colour.

The best known is *Saccharomyces cerevisiae*, strains of which are used in the fermentation of beer and wine and in baking. It is found in nature on ripe fruits. Thus *Saccharomyces cerevisiae* is yeast of great economic importance. Its cells are elliptical 6-8 by 5μm. They multiply asexually by budding, where has formed on a cell, a raised scar remains. During budding, the nucleus divides by constriction and a portion of it enters the bud along with other organelles (Brown, 1970).
The cytoplasmic connection is closed by the bud along with other organelles. The cytoplasmic connection is closed by the laying down of cell wall material. Under appropriate conditions, *S. cerevisiae* form asci. The cytoplasm of the cell differentiates into four thick walled spherical spores, although the number of spores can be fewer. The cells from which asci develop are diploid and the nuclear divisions which precede spore formation are meiotic. It may be noted that many strains of the yeasts are heterothallic, and the ascospores are of mating types. Mating type is specifically controlled by a single gene which exists in two allelic states a and α, and segregation at reduction division preceding ascospore formation gives rise to two a and two α ascospores. (Cytoplasmic DNA hypothesis Science, 1969, Gilland, 1971, Darling et al., 1972)

Fusion normally occurs only between cells of differing mating types; a process termed legitimate copulation such fusions results in diploid cells which form asci containing viable ascospores. Many studies have been made of yeast genetic (Brown, 1970). For instance, by means of hybridization.

Many members of this genus are considered important in food production. One example is *Saccharomyces cerevisiae*, which is used in making wine, bread and beer. Other members of this genus like *Saccharomyces bayanus*, used in making wine, *Saccharomyces boullardii* used in medicine, *Saccharomyces uvarum* is also used in making beer.

The presence of yeast in beer was first suggested in 1680. Although the genus was not named *Saccharomyces* until 1837. It was not until 1876, that Louis Pasteur demonstrated the involvement of living organisms in fermentation and in 1888,
Hansen isolated brewing yeast and propagated leading to the importance of yeast in brewing. The use of microscopes for the study of yeast morphology and purity was crucial to understanding their functionality.

3.2.3 Baker’s yeast:

In modern baking practice, pure cultures of selected strains *S. cerevisiae* is mixed with bread dough to bring about desired changes in texture and flavour. Desirable characteristics of *S. cerevisiae* strains selected for commercial production of baker’s yeast includes the ability to ferment, the sugar in the dough vigorously and to grow rapidly, there as well as other characteristics for which the strain was selected should be relatively stable. Carbon dioxide produced during fermentation is responsible for leavening, or rising of dough (Baker *et al.*, 1974).

The quality of product depends on the proper selection of yeast and incubation conditions as well as on choice of raw materials.

In manufacture of Baker’s yeast, stock strain is inoculated into a medium which frequently contains molasses and corn steep liquor. The medium is adjusted to an acidic pH 4-5 which helps to retard bacterial growth. The inoculated medium is aerated during the incubation period.

At the end of incubation the yeast cells are harvested by centrifugation and washed by suspending the cells in water and then centrifuging the cells out. The cells are finally recovered on a filter press, small amount of vegetable oil is added as a plasticizer, and then this mass of the cells is molded into blocks.
3.3 GUAR GUM

3.3.1 Nonproprietary Names

BP: Guar Galactomannan
Ph Eur: Guar Galactomannan
USP-NF: Guar Gum

3.3.2 Synonyms

E-412; Galactosol; guar flour; Guar Galactomannum; jaguar gum; Meyprogat;
Meyprodur; Meyprofin.

3.3.3 Chemical Name and CAS Registry Number

Galactomannan Polysaccharide (9000-30-0)

3.3.4 Empirical Formula and Molecular Weight

\[(\text{C}_6\text{H}_{12}\text{O}_6)_n \approx 220000\]

3.3.5 Structural Formula

Guar gum consists of linear chains of \((1\rightarrow4)\)-\(\beta\)-D mannopyranosyl units with \(\alpha\)-D- galactopyranosyl units attached by \((1\rightarrow6)\) linkages. The ratio of D-galactose to D-mannose units is between 1:1.4 and 1:2.

Functional Category

Suspending agent; tablet binder; tablet disintegrant; viscosity increasing agent.

3.3.6 Application in Pharmaceutical Formulation or Technology.

Guar gum is galactomannan, commonly used in cosmetics, food products, and pharmaceutical formulations. It has also been investigated in the preparation of
sustained release matrix tablets in the place of cellulose derivatives such as methyl cellulose (Gohel et. al., 2002).

In pharmaceuticals, guar gum is used in solid dosage forms as a binder and disintegrant (Efentakis et.al., 1997). It is used in oral and topical products as a suspending, thickening and stabilizing agent and also as a controlled release carrier, guar gum has also been examined for use in colonic drug delivery (Dharamsi et al., 2004, Mohini Chourasia et al., 2006, QuF et al., 2006, Sheng et al., 2006; Shirwaikar et al., 2008).

Guar gum based three layer matrix tablets have been used experimentally in oral controlled release formulations (Das et. al., 2007). Therapeutically guar gum has been used as part of the diet in patients with diabetes mellitus (Leung et.al., 1988).

**Table : 4 Use of Guar Gum**

<table>
<thead>
<tr>
<th>Use</th>
<th>Concentration 1% w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emulsion stabilizer</td>
<td>1</td>
</tr>
<tr>
<td>Tablet binder</td>
<td>up to 10</td>
</tr>
<tr>
<td>Thickener for lotions and creams</td>
<td>up to 2.5</td>
</tr>
</tbody>
</table>

**3.3.7 Description**

The USP-NF27 describes guar gum as gum obtained from the ground endosperms of the *Cyamoposis tetragonolobus*. It consists chiefly of a high molecular
polysaccharide, composed of galactan and mannan units combined through glycoside linkages, which may be described chemically as a galactomannan. (Baker et al., 1974)

The main components are polysaccharides composed of D-galactose and D-mannose in molecular ratios of 1:1.4 and 1:2. The molecule consists of linear chains of (1→4)-ß-D mannopyranosyl units with α-D- galactopyranosal units attached by (1→6) linkages. Guar gum occurs as an odorless or nearly odorless, white to yellowish-white powder with a bland taste.

**Typical properties:**

Acidity/Alkalinity pH = 5-7.0 (1% w/v aqueous dispersion)

Density: 1.492 gm/cm³

**Solubility:**

Practically insoluble in organic solvents. In cold or hot water guar gum disperses and almost swells immediately to form a highly viscous thixotropic sol. The optimum rate of hydration occurs at pH 7.9- 9.0. Finely milled powders swell more rapidly and are more difficult to disperse. Two to four hours in water at room temperature are required to develop maximum viscosity.

**Viscosity:**

Dynamic Viscosity of 1% w/v dispersion is 4.86 pas/4860 cps. It is dependent upon temperature, time, concentration, pH, and rate of agitation and the particle size of the guar gum powder. Synergistic rheological effects may occur with other suspending agents such as xanthan gums.
3.3.8 Stability and Storage conditions:

Aqueous guar gum dispersions have a buffering action and are stable at pH 4.0-10.5. However prolonged heating reduces the viscosity of dispersions.

The bacteriological stability of guar gum dispersions may be improved by the addition of mixture of 0.15% methyl paraben and 0.02% propyl paraben as a preservative. In food applications, benzoic acid, citric acid, sodium benzoate, or sorbic acid may be used.

Guar gum powder should be stored in a well closed container, in a cool, dry place.

3.3.9 Incompatibility:

Guar gum is compatible with most other plant hydro colloids such as tragacanth. It is incompatible with acetone, ethanol (95%), tannins, strong acids and alkalies. Borate ions if present in the dispersing water will prevent the hydration of guar gum. However, the addition of borate ions to hydrated guar gum produces cohesive structural gel and further hydration is then prevented. Gel formed can be liquefied by reducing the pH below 7, or by heating.

3.3.10 Safety:

Guar gum is widely used in foods, and oral and topical pharmaceutical formulations. Excessive consumption may cause gastro intestinal disturbance such as flatulence, diarrhea, nausea. Therapeutically daily oral dose up to 25g of guar gum have been administered for patient with diabetes mellitus.
Although it is generally regarded as non toxic and non irritant material, the safety of guar gum when used as the appetite suppressant has been questioned. When consumed, the gum swells in the stomach to promote the feeling of fullness. However it is claimed that premature swelling of guar gum tablets may occur and cause obstruction or damage to the oesophagus, consequently appetite suppressants containing guar gum in tablet form have been banned in UK (Leung et. al., 1988). However appetite suppressants containing micro granules of guar gum are claimed to be safe.

The use of guar gum for pharmaceutical purposes is unaffected by the ban.

3.3.11 Handling Precautions:

Observe normal precaution appropriate to the circumstances and the quantity of material handled. Guar gum may be irritating to the eyes. Eye protection, gloves, and dust mask or respirator are recommended.

3.3.12 Regulatory Status:

It is GRAS listed as it was accepted as food additive in Europe. Included in the FDA, Inactive Ingredients Database (oral suspensions, syrups and tablets; topical preparations; vaginal tablets). Also included in non-parenteral medicines licensed in the UK. Included in Canadian list of acceptable non medicinal ingredients.
3.4 Ethyl cellulose

3.4.1 Nonproprietary Names:

BP: Ethyl cellulose

USP-NF: Ethyl cellulose

3.4.2 Synonyms:

Aquacoat ECD; Aqualon; Ashacel; E462; Ethocel; ethylcellulosum.

3.4.3 Chemical Name and CAS Registry Number:

Cellulose ethyl ether [9004-53-7]

3.4.4 Empirical Formula and Molecular Weight:

Ethyl cellulose is partially ethoxylated. Ethyl cellulose with complete ethoxy substitution (DS=3) is \( C_{12}H_{23}O_6(C_{12}H_{22}O_5)nC_{12}H_{23}O_5 \) where ‘n’ can vary to provide a wide variety of molecular weights. Ethyl cellulose, an ethyl ether of cellulose, is a long chain polymer of \( \beta \) – anhydroglucose units joined together by acetal linkages

3.4.5 Structural Formula:
3.4.6 Functional Category:

Coating agent; flavouring agent; tablet binder; tablet filler; viscosity increasing agent.

3.4.7 Application in Pharmaceutical Formulations or Technology

Ethyl cellulose is widely used in oral and topical formulations. The main use of ethyl cellulose in oral formulations is as a hydrophobic coating agent for tablets and granules (Handa et al., 2000, Chowdary et al., 2004, Das et al., 2007, Morkhade et al., 2007).

Ethyl cellulose coatings are used to modify the release of a drug, (Handa et al., 2000) to mask an unpleasant taste, or to improve the stability of a formulation; for example, where granules are coated with ethyl cellulose to inhibit oxidation. Ethyl cellulose, dissolved in an organic solvent or a solvent mixture, can be used on its own to produce insoluble films. Higher viscosity ethyl cellulose grades tend to produce stronger and more durable films. Ethyl cellulose films may be modified to alter their solubility, (The U.S.P (26), The N.F (21), 2003), (Martin and Swarbrick, 1991) or a plasticizer. Drug release through ethyl cellulose coated dosage forms can be controlled by diffusion through the film coating. This can be a slow process unless a large surface area (e.g pellets or granules compared with tablets) is utilized. Ethyl cellulose coated beads and granules have also demonstrated the ability to absorb pressure and hence protect the coating from fracture during compression (Allen, 1975). High viscosity grades of ethyl cellulose are used in drug microencapsulation (The U.S.P (26), The N.F (21), Acyclovir Official Monograph, 2003, Herdan, 1953).
Table: 5 Uses of Ethyl Cellulose

<table>
<thead>
<tr>
<th>Use</th>
<th>Concentration (1% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microencapsulation</td>
<td>10.0 – 20.0</td>
</tr>
<tr>
<td>Sustained- release tablet coating</td>
<td>3.0 - 20.0</td>
</tr>
<tr>
<td>Tablet coating</td>
<td>1.0 – 3.0</td>
</tr>
<tr>
<td>Tablet granulation</td>
<td>1.0 – 3.0</td>
</tr>
</tbody>
</table>

3.4.8 Description:

Ethyl cellulose is a tasteless, free flowing, white to light tan colored powder.

3.4.9 Typical Properties:

Density (bulk): 0.4 gm/cm$^2$.

Glass transition temperature: 129 - 133° C. (Morkhade et al., 2007).

Moisture content:

Ethyl cellulose absorbs very little water from humid air or during immersion, and that small amount evaporates readily (Chowdary et al., 2004, Morkhade et al., 2007).

Solubility:

Ethyl cellulose is practically insoluble in glycerin, propylene glycol, and water. Ethyl cellulose that contains less than 46.5% of ethoxyl groups is freely soluble in chloroform, methyl acetate, and tetrahydrofuran, and in mixtures of aromatic hydrocarbons with ethanol (95%). Ethyl cellulose that contains not less than 46.5% of
ethoxyl groups is freely soluble in chloroform, ethanol (95%), ethyl acetate, methanol and toluene.

Specific gravity: 1.12 – 1.15 g/cm².

**Viscosity:**

The viscosity of Ethyl cellulose is measured typically at 25° C using 5%w/v ethyl cellulose dissolved in solvent blend of 80% toluene:20% ethanol(w/w). Grades of ethyl cellulose are commercially available. They may be used to produce 5%w/v solutions in organic solvent blends with viscosities nominally ranging from 7 to 100 mPas (7 – 100 Cp). Specific ethyl cellulose grades, or blends of different grades, may be used to obtain solutions of a desired viscosity. Solutions of higher viscosity tend to be composed of longer polymer chains and produce strong and durable films. The viscosity of ethyl cellulose solution increases with an increase in ethyl cellulose concentration.

**3.4.10 Stability and Storage conditions:**

Ethyl cellulose is a stable, slightly hygroscopic material. It is chemically resistant to alkalis, both dilute and concentrated, and to salt solutions, although it is more sensitive to acidic materials than are cellulose esters.

Ethyl cellulose is subject to oxidative degradation in the presence of sunlight or UV light at elevated temperatures. This may be prevented by the use of antioxidant and chemical additives that absorb light in the 230 – 340 nm range.
Ethyl cellulose should be stored at a temperature not exceeding 32° C (90° F) in a dry area away from all sources of heat. It should not be stored next to peroxides or other oxidizing agents.

3.4.11 Incompatibilities:

Incompatible with paraffin wax and microcrystalline wax.

3.4.12 Safety:

Ethyl cellulose is widely used in oral and topical pharmaceutical formulations. It is also used in food products. Ethyl cellulose is not metabolized following oral consumption and is therefore a noncalorific substance. Ethyl cellulose is generally regarded as a nontoxic nonallergenic and nonirritating material.

As ethyl cellulose is not considered to be a health hazard, the WHO has not specified an acceptable daily intake (Chowdary et al., 2004) The highest reported level used in an oral product is 308.8 mg in an oral sustained release tablet. (Chandran et al., 2008).

3.4.13 Handling Precautions:

It is important to prevent fine dust clouds of ethyl cellulose from reaching potentially explosive levels in the air. Ethyl cellulose is combustible and may be irritant to the eyes and eye protection should be worn.

3.4.14 Regulatory Status:

GRAS listed. Accepted for use as a food additive in Europe. Included in FDA inactive ingredients Database (oral capsules, suspensions and tablets; topical
emulsions and vaginal preparations). Included in nonparenteral medicines licensed in Europe. Included in Canadian List of Acceptable Non Medicinal Ingredients.

3.5 Egg Albumin

3.5.1 Nonproprietary Names:

Egg albumin, Oval albumin

3.5.2 Synonyms:

Ovalbumin, Ovoconalbumin

Albuconn, Albumisol

3.5.3 CAS Registry no: 9006-59-1 (Takafumi et al., 1981).

3.5.4 Molecular weight:

About 45,000 – 66,500

3.5.5 Functional category:

Stabilizing Agent, Therapeutic Agent, Coating Agent.

3.5.6 Pharmaceutical applications:

In pharmaceuticals, Egg albumin is used as an excipient in parenteral formulation. Albumin has also been used to prepare microspheres and microcapsules for experimental drug-delivery systems; Albumin has also been used as a co solvent for parenteral drugs, and prevent adsorption of other proteins to surfaces (Gupta et al., 1989).

3.5.7 Description:

Egg albumin is obtained from the hen’s eggs. In the solid state, albumin appears as yellow, brownish amorphous lumps, scales or powder. Denaturation can be
induced by heating to 56°C by vigorously shaking by electric around or by using various acids. (Takafumi et al., 1981).

Structure is a complex protein consisting of a single polypeptides chain of about 400 residues, a maximum of two phosphate residues per mole, and an oligosaccharide side chain of composed of mannose & glucosamine residues. (Gupta et al., 1989).

### 3.5.8 Typical Properties:

**Acidity /Alkalinity:**

\[ \text{pH} = 6.7 - 7.3 \ (1\% \text{w/v Solution in } 0.9\% \text{ w/v sodium chloride}) \]

Isoelectric point : 4.63

Osmolarity : 4-5% w/v aqueous solution.

**Solubility:**

Freely soluble in dilute salt solutions and water aqueous solution containing 40% w/v albumin can be readily prepared at pH 7.4. (Vural et al., 1990).

Moisture: Maximum 7.5%.

### 3.5.9 Storage:

Store at room temperature (15 to 25°C recommended). Keep well closed and protected from direct sunlight and moisture.

### 3.5.10 Precautions:

Keep away from heat. Keep away from sources of ignition.
3.6 MATERIALS:

The materials employed in the present investigation were given in table: 3.6

Table: 3.6 Materials employed in the present study.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Materials used in the study</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Acyclovir U.S.P</td>
<td>Obtained as gift sample from Matrix laboratory, Hyderabad.</td>
</tr>
<tr>
<td>2.</td>
<td>Bakers yeast food grade</td>
<td>Sigma Aldrich, Bangalore</td>
</tr>
<tr>
<td></td>
<td>(Saccharomyces Cerevisiae)</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Guar gum (3,500-4,000 cps)</td>
<td>E.Merck, Mumbai.</td>
</tr>
<tr>
<td>4.</td>
<td>Egg albumin</td>
<td>Hi media, Mumbai</td>
</tr>
<tr>
<td>5.</td>
<td>Ethyl cellulose 22 cps</td>
<td>E. Merck, Mumbai</td>
</tr>
<tr>
<td>6.</td>
<td>Acetonitrile HPLC grade</td>
<td>S. D Fine chemicals, Mumbai</td>
</tr>
<tr>
<td>7.</td>
<td>Dichloromethane I.P.</td>
<td>S. D Fine chemicals, Mumbai</td>
</tr>
<tr>
<td>8.</td>
<td>Span 80</td>
<td>S. D Fine chemicals, Mumbai</td>
</tr>
<tr>
<td>9.</td>
<td>Double Distilled water</td>
<td>E.Merck, Mumbai</td>
</tr>
<tr>
<td>10.</td>
<td>Deionised water</td>
<td>Core Parentrals, Mumbai</td>
</tr>
<tr>
<td>11.</td>
<td>Glutaraldehyde</td>
<td>S. D Fine chemicals, Mumbai</td>
</tr>
<tr>
<td>12.</td>
<td>DMSO (Dimethyl Sulphoxide)</td>
<td>S. D Fine chemicals, Mumbai</td>
</tr>
<tr>
<td>13.</td>
<td>Sulfrhodamine-B</td>
<td>Himedia, Mumbai</td>
</tr>
<tr>
<td>14.</td>
<td>Trichloro acetic acid</td>
<td>S. D Fine chemicals, Mumbai</td>
</tr>
<tr>
<td>15.</td>
<td>Tris base</td>
<td>Himedia, Mumbai</td>
</tr>
<tr>
<td>16.</td>
<td>Tri basic sodium phosphate I.P.</td>
<td>S. D Fine chemicals, Mumbai</td>
</tr>
<tr>
<td>17.</td>
<td>Hydrochloric acid I.P.</td>
<td>Qualigens chemicals, Mumbai</td>
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<tr>
<td>18.</td>
<td>Sodium Hydroxide I.P.</td>
<td>Qualigens chemicals, Mumbai</td>
</tr>
<tr>
<td>19.</td>
<td>Petroleum Ether</td>
<td>Qualigens chemicals, Mumbai</td>
</tr>
<tr>
<td>20.</td>
<td>Liquid Paraffin I.P.</td>
<td>S D Fine Chemicals, Mumbai</td>
</tr>
</tbody>
</table>
3.7 Equipment employed in the present study were given in Table: 3.7

Table: 3.7 List of Equipment

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Equipment</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Digital balance</td>
<td>Torrel, India.</td>
</tr>
<tr>
<td>2.</td>
<td>Electronic balance</td>
<td>Shimadzu, Japan.</td>
</tr>
<tr>
<td>3.</td>
<td>Probe sonicator</td>
<td>Vibronics, Mumbai.</td>
</tr>
<tr>
<td>5.</td>
<td>Autoclave</td>
<td>Remi equipments, Mumbai.</td>
</tr>
<tr>
<td>6.</td>
<td>High speed centrifuge</td>
<td>Remi equipments, Mumbai.</td>
</tr>
<tr>
<td>7.</td>
<td>FT – IR Spectro photometer</td>
<td>Shimadzu, Japan.</td>
</tr>
<tr>
<td>8.</td>
<td>High Performance Liquid Chromatography</td>
<td>Shimadzu, Japan.</td>
</tr>
<tr>
<td>10.</td>
<td>pH meter</td>
<td>LI 127, Elico, Hyderabad.</td>
</tr>
<tr>
<td>17.</td>
<td>Dissolution test apparatus</td>
<td>Electro Lab, Mumbai.</td>
</tr>
</tbody>
</table>
Brief details of the drug acyclovir and polymers (Yeast, Guar gum, Egg albumin and Ethyl cellulose) used in the formulation of microcapsules of acyclovir are given below.

3.8 Solubility study of acyclovir in various solvents: Solubility is important physicochemical properties of drug substances which determines its systemic absorption and in turn its therapeutic efficacy. The solubility of acyclovir was checked in general by the U.S.P method

**Table: 3.8 Solubility of acyclovir in various solvents**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Description term</th>
<th>Parts of solvent required for one part of solute</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Very soluble</td>
<td>Less than one part</td>
</tr>
<tr>
<td>2</td>
<td>Freely soluble</td>
<td>From 1 – 10</td>
</tr>
<tr>
<td>3</td>
<td>Soluble</td>
<td>From 10 – 30</td>
</tr>
<tr>
<td>4</td>
<td>Sparingly soluble</td>
<td>From 30 – 100</td>
</tr>
<tr>
<td>5</td>
<td>Slightly soluble</td>
<td>From 100 – 1000</td>
</tr>
<tr>
<td>6</td>
<td>Very slightly soluble</td>
<td>From 1000 – 10000</td>
</tr>
<tr>
<td>7</td>
<td>Practically insoluble or insoluble</td>
<td>Greater than or equal to 10000</td>
</tr>
</tbody>
</table>

3.9: Preparation of standard graph

**Preparation of standard graph for acyclovir using distilled water, 0.1N HCl pH 1.2 and Phosphate buffer pH 6.8.**

Accurately weighed 100 mg of acyclovir was dissolved in 100 ml of the respective solution (distilled water, 0.1N HCl pH 1.2 and Phosphate buffer pH 6.8.) to give a solution of 1mg/ml (1000µg/ml) concentration and these served as the standard stock solution
From the above solution 1, 2, 3, 4 and 5 ml were taken and transferred to 100 ml standard flask and made up the volume to 100 ml (1 ml = 10 µg/ml). The concentration of the final solution was 10, 20, 30, 40 and 50 µg/ml.

The absorbance of these solutions were measured against reagent blank at 225 nm using Elico model SL – 146 UV – visible spectrophotometer.

3.10 Methods: (Bishop et al., 1998).

Principle: A pre-treatment of yeast cells were carried out to assess the importance of cell viability. The yeast cells were pre-treated with sodium azide, a respiratory inhibitor to prevent the cells from performing any energy dependant process.

Parameters used: Different amounts of drug concentration, stirring speed and temperature.

3.10.1 Preparation of acyclovir microcapsules using baker’s yeast: (Bishop et al., 1998).

Pre-treatment of yeast cells: Pre-treatment of yeast cells was carried out prior to the encapsulation process to assess the importance of cell viability. A suspension of fresh yeast (100 ml of 50% solids) was treated overnight with sodium azide (2 g), a respiratory inhibitor used to prevent the cells from performing any energy dependant process. Sterilization by autoclaving is a thermally destructive process denaturing any carrier protein molecules likely to be involved in facilitated diffusion process. The pre-treated dead yeast cells with intact cell membrane were used for the encapsulation of acyclovir.
Preparation of acyclovir microcapsules using pre treated yeast cells: Acyclovir, pre treated yeast cells and distilled water was taken in the ratio of 1:2:4 respectively. This suspension was agitated in a magnetic stirrer for 4 hours and the cells were then centrifuged for 10 minutes at 2000 rpm. The supernatant solution was decanted and the cells were washed 5 times with distilled water and dried in a lyophillizer for 48 hours.

Table: 3.10.1 Formulation and process variables for different batches of acyclovir microcapsules using pre treated baker’s yeast

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Sample No</th>
<th>Temperature</th>
<th>Drug (mg) in 5ml buffer (pH5)</th>
<th>Yeast (g) in 10ml water</th>
<th>Water (ml)</th>
<th>Volume taken for stirring (ml)</th>
<th>RPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FY-1</td>
<td>25°C</td>
<td>200</td>
<td>0.4</td>
<td>20</td>
<td>35</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>FY-V</td>
<td></td>
<td>400</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FY-IX</td>
<td></td>
<td>800</td>
<td>1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>FY-II</td>
<td>30°C</td>
<td>200</td>
<td>0.4</td>
<td>20</td>
<td>35</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>FY-VI</td>
<td></td>
<td>400</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FY-X</td>
<td></td>
<td>800</td>
<td>1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>FY-III</td>
<td>35°C</td>
<td>200</td>
<td>0.4</td>
<td>20</td>
<td>35</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>FY-VII</td>
<td></td>
<td>400</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FY-XI</td>
<td></td>
<td>800</td>
<td>1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>FY-IV</td>
<td>40°C</td>
<td>200</td>
<td>0.4</td>
<td>20</td>
<td>35</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>FY-VIII</td>
<td></td>
<td>400</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FY-XII</td>
<td></td>
<td>800</td>
<td>1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
hours. The effect of temperature and the concentration of drug on the formulation of acyclovir microcapsules with pre-treated baker’s yeast were studied. Formulation variable (different amounts of drug concentration) and process variables (stirring speed and temperature) were evaluated to obtain the microcapsules of optimum properties. The compositions of variables were given in Table 3.10.1.

3.10.2 Preparation of acyclovir microcapsules using guar gum: (Das et al., 2007).

The microcapsules of acyclovir with guar gum were prepared by water-in-oil-oil (w/o/o) solvent diffusion method. A weighed amount of acyclovir (0.2 to 0.8 gm) and guar gum was dissolved in 30 ml of a mixture of acetonitrile and dichloromethane (1:1 v/v). Initially 50 ml of water-in-oil emulsion was formed by the addition of 20 ml of deionised water into the drug polymer solution with constant stirring at 500 rpm for 10 minutes. The w/o primary emulsion formed was then slowly added to 50 ml of light liquid paraffin containing span 80 (0.1 ml) as a surfactant with constant stirring for 2 hours. Crosslinking agent, glutaraldehyde (1 ml) was added and the stirring was further continued for 1 hour. The microcapsules obtained were separated by filtration, and washed with petroleum ether to remove oil. Finally they were air dried for a period of 24 hours in desiccator. Formulation and process variables such as amount of drug and stirring speed were evaluated to obtain the microcapsules of optimum properties. The compositions of different variables were given in Table 3.10.2.
Table: 3.10.2: Formulation and process variables for different batches of acyclovir microcapsules using guar gum

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>FG I</th>
<th>FG II</th>
<th>FG III</th>
<th>FG IV</th>
<th>FG V</th>
<th>FG VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyclovir (mg)</td>
<td>200</td>
<td>400</td>
<td>800</td>
<td>200</td>
<td>400</td>
<td>800</td>
</tr>
<tr>
<td>Water (ml)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Solvent (ml)</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Liquid paraffin (ml)</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Span 80 (ml)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Glutaraldehyde (ml)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Speed (rpm)</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1500</td>
<td>1500</td>
<td>1500</td>
</tr>
</tbody>
</table>

3.10.3 Preparation of acyclovir microcapsules using egg albumin: (Takafumi et al., 1981).

Microcapsules of acyclovir were prepared with egg albumin by the water-in-oil-in-oil (w/o/o) double emulsion (heat coagulation) method. A weighed amount of acyclovir and the egg albumin were dissolved in 30 ml water. The initial w/o emulsion was formed by addition of albumin solution drop wise to 50 ml liquid paraffin containing 0.1ml of span 80 and was stirred for 20 minutes. The formed w/o albumin emulsion was added drop wise to 50ml of hot liquid paraffin (80°C ) and stirred for one hour. The formed albumin microcapsules were collected by centrifugation process. The upper layer was discarded. The lower layer was washed four times with petroleum ether to remove traces of reactants and dried in an oven at 40°C. Formulation and process variables such as amount of drug and stirring speed were evaluated to obtain the microspheres of optimum properties. The compositions of different variables were given in Table 3.10.3.
### Table: 3.10.3: Formulation and process variables for different batches acyclovir microcapsules using egg albumin

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>FA I</th>
<th>FA II</th>
<th>FA III</th>
<th>FA IV</th>
<th>FA V</th>
<th>FA VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyclovir (mg)</td>
<td>200</td>
<td>400</td>
<td>800</td>
<td>200</td>
<td>400</td>
<td>800</td>
</tr>
<tr>
<td>Water (ml)</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Liquid paraffin (ml)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Span 80 (ml)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Speed (rpm)</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1500</td>
<td>1500</td>
<td>1500</td>
</tr>
</tbody>
</table>

### 3.10.4 Preparation of acyclovir microcapsules using ethyl cellulose: (Das et al., 2007)

Microcapsules of acyclovir were prepared with ethyl cellulose by water-in-oil-oil (W/O/O) emulsion solvent diffusion method. A weighed amount of acyclovir (0.2 to 0.8gm) and ethyl cellulose were dissolved in 30ml of a mixture of acetonitrile and dichloromethane (1:1 v/v). The initial 50ml water-in-oil emulsion was formed by the addition of 20 ml of deionised water to the drug polymer solution with constant stirring at 500 rpm for 10 minutes. The W/O primary emulsion was then slowly added to 50ml light liquid paraffin containing span 80 (0.1 ml) as a surfactant with constant stirring for 2 hours. The glutaraldehyde (1 ml) was added and stirring was continued for further 1 hour. The microcapsules resulted were separated by filtration and washed with petroleum ether to remove liquid paraffin. Finally they were air dried over a period of 24 hours in a desiccator. Formulation and process variables such as amount of drug and stirring speed were evaluated to obtain the microspheres of optimum properties. The compositions of different variables were given in table 3.10.4.
Table: 3.10.4: Formulation and process variables for different batches acyclovir microcapsules using ethyl cellulose

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>FE I</th>
<th>FE II</th>
<th>FE III</th>
<th>FE IV</th>
<th>FE V</th>
<th>FE VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyclovir (mg)</td>
<td>200</td>
<td>400</td>
<td>800</td>
<td>200</td>
<td>400</td>
<td>800</td>
</tr>
<tr>
<td>Water (ml)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Solvent (ml)</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Liquid paraffin (ml)</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Span 80 (ml)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Glutaraldehyde (ml)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Speed (rpm)</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1500</td>
<td>1500</td>
<td>1500</td>
</tr>
</tbody>
</table>

3.11 Characterization of acyclovir microcapsules:

3.11.1. Particle size determination of acyclovir microcapsules using optical microscopy

Particle size in the range of 0.2 – 100 µm can be measured by optical microscopy. In this method, the size is expressed as $d_p$ (projected diameter), which describes the diameter of a sphere having the same area as the asymmetric particle when observed under a microscope. The method directly gives number distribution, which can be further converted to weight distribution (Herdan, 1953).

The acyclovir formulations of present study were suspended in a suitable vehicle such as paraffin oil. The sample of suspension was mounted on a slide and placed on a mechanical stage. The size of the particle was estimated with the help of the eye piece micrometer. The diameter of 200 particles was determined by the number of divisions of eye piece micrometer. The particles were arranged on the basis of size ranges. The number of particles in each size range were then converted and
tabulated. Then the percent number of particles in each interval and percent undersize were calculated. A histogram and cumulative undersize curve were plotted (Allen, 1975).

The details of particle size determination of acyclovir microcapsules were given in table 3.2 to table 3.4 and fig. 3.1 to fig. 3.9 and table 4.1 to table 4.6 and fig. 4 to fig. 21 respectively.

3.11.2. Bifocal Microscopy of acyclovir microcapsules:

The popularity of bifocal microscopy arises from its ability to produce clear morphology of the specimens at various magnifications. The light passes through a small pinhole and expands to fill the entrance aperture of the microscope objective lens. The objective lens focuses the light to a small spot on the specimen at the local place of the objective lens. Light reflected back from the illuminated spot on the specimen was collected by the objective and was partially reflected by a beam splitter. The details of bifocal microscopy of acyclovir microcapsules were given in fig. 22 (a-k) respectively.

3.11.3 Scanning Electron Microscopy:

Surface morphology of microcapsules was studied using scanning electron microscopy (SEM) using Phillips 200 FEI, Japan. Microcapsules were sprinkled on to double side tape, sputter-coated with platinum and examined in the microscope at 15 KV. The results were given in fig. 23 (a-x).
3.11.4. Determination of entrapment efficiency of acyclovir microcapsules by UV – Spectrophotometer

Entrapment efficiency of acyclovir microcapsules prepared with different – Encapsulating agents was determined by the following procedure

Accurately weighed amount (100 mg) of the microcapsule were taken and 60 ml of 0.1 M sodium hydroxide was added and dispersed well for 15 minutes. Then sufficient quantity of 0.1 M sodium hydroxide was added to produce 100 ml. Mixed well and filtered. 15 ml of the filtrate was taken and 50 ml of water and 5.8 ml of 2 M Hydrochloric acid and sufficient water was added to produce 100 ml. To 5ml of this solution sufficient volume of 0.1 M hydrochloric acid was added to produce 50 ml and mixed well. The absorbance of the resulting solution was measured at 254nm, using 0.1 M hydrochloric acid in the reference cell. Acyclovir content of the microcapsules was determined from the calibration curve. The results were given in table 7 and table 8.

The entrapment efficiency (%) was calculated according to the following equation:

\[
\text{Entrapment efficiency } (\%) = \frac{\text{Actual acyclovir content in microcapsules}}{\text{Theoretical acyclovir content}} \times 100
\]

3.11.5 Analysis of acyclovir microcapsules using FT-IR spectroscopy:

Fourier transform - IR spectra were recorded on Shimadzu FT-IR-281-spectrophotometer. The FT-IR spectra of the acyclovir pure drug, acyclovir microcapsules prepared using various polymers and dead Saccharomyces Cerevisiae with intact cell wall were studied and recorded. Samples were prepared in KBr disks with hydrostatic press at a force of 5.2T cm\(^{-2}\) for 3 min. The scanning range was 450-4000 cm\(^{-1}\) and the resolution was 1 cm\(^{-1}\). The pure drug acyclovir functional group
were present in all formulations of FT–IR spectra. The pure drug functional group peaks correlated with the microcapsules formulation peaks. The details were given in fig. 5.0 to fig. 5.12 and fig. 6.0 to fig. 6.16 respectively.

3.11.6 Thermal Analysis of acyclovir microcapsules by Differential Scanning

Calorimetric Method:

Thermal Analysis is a group of technique in which a physical property of a substance or its reaction products were measured as a function of temperature while the substance was subjected to a controlled temperature program.

Thermograms of the pure drug acyclovir and all the formulations of acyclovir microcapsules made using various polymers were recorded on a Seiko DSC 220C model differential scanning calorimeter (Tokyo, Japan). About 10mg of formulations were sealed in aluminium pans and heated at a rate of 10°C/min from 30°C-200°C. The details were given in fig. 7.0 to fig. 7.10 respectively.

3.11.7 Estimation of drug content of acyclovir microcapsules by HPLC technique

The drug content of acyclovir microcapsules was estimated by the HPLC technique. The drug content of acyclovir pure drug, acyclovir microcapsules and the marketed product was determined. The data was given in table 8.0 to 8.9 and table 10.1.

3.11.7.1 Optimization of chromatographic conditions for the estimation

Proper selection of the chromatographic method depends upon the nature of the sample (ionic or neutral molecule), its molecular weight and solubility. The drugs
selected for the present study was polar in nature and hence reverse phase or ion exchange chromatography can be used.

3.11.7.2 Selection of detection wavelength for acyclovir

Sample of 10µg/ml of acyclovir was prepared individually and using solvent mixtures of acetonitrile and water (1:1). These solutions were scanned in the UV region of 200-400nm and the UV spectrum were recorded. From the spectra, the absorption maxima (λ max) 254 nm was selected for acyclovir.

3.11.7.3 Optimized Chromatographic conditions

The following chromatographic conditions were selected for the estimation of the selected drug acyclovir and formulations of acyclovir microcapsules (acyclovir with yeast, guar gum, egg albumin and ethyl cellulose).

3.11.7.4 Chromatographic conditions for acyclovir

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stationary phase</td>
<td>Princeton SPHER C18 (250× 4.6mm i.d., 5μ )</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>Acetonitrile + Disodium hydrogen ortho</td>
</tr>
<tr>
<td></td>
<td>phosphate buffer of pH 3.0</td>
</tr>
<tr>
<td>Mobile phase ratio</td>
<td>5.95 % v/v</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.7ml/min</td>
</tr>
<tr>
<td>Sample volume</td>
<td>20µl using Rheodyne 7725 injector</td>
</tr>
<tr>
<td>Detection</td>
<td>254nm using UV-VIS Detector</td>
</tr>
<tr>
<td>Run Time</td>
<td>15 mins</td>
</tr>
<tr>
<td>Channel Name</td>
<td>2487 Channel – 1</td>
</tr>
</tbody>
</table>
3.11.8 In vitro release studies of acyclovir microcapsules prepared by using pre-treated baker’s yeast (FY-I to FY-XII) (LP)

The in vitro release studies were performed in a modified diffusion cell. Dialysis membrane was tied to the one end of open ended glass tube, which was immersed into 250ml of receptor fluid kept in 500ml beaker.

The apparatus was operated at 100 rpm over a magnetic stirrer maintained at 37 ± 0.5°C. The acyclovir microcapsules were taken into the dialysis membrane.

The release study of acyclovir microcapsules was done for the first two hours in 0.1N hydrochloric acid (pH 1.2) and the dissolution for the next 6 hours was continued in phosphate buffer pH 6.8. Sample of 5 ml was withdrawn every hour and was replaced with 5 ml of the fresh dissolution fluid. The samples were analysed spectrophotometrically at 254 nm with suitable dilution. The details of in vitro dissolution data with respect to the amount released, cumulative drug released, % cumulative drug released and other relevant data were given in table 3.6 to table 3.17.

3.11.9 In vitro release studies of acyclovir microcapsules prepared by using various polymers (guar gum, egg albumin and ethyl cellulose) (USP)

The in vitro release studies were performed in a modified diffusion apparatus as per USP specification. Microcapsules equivalent to 100 mg of acyclovir was accurately weighed and the in vitro release studies were performed. A 500 ml beaker with 187.5 ml of dissolution fluid (0.1 N HCl, pH 1.2) was kept on a magnetic stirrer. The dissolution test was performed at 100 rpm and the temperature was set at 37°C ± 0.5°C. The dialysis membrane was tied to the bottom of two side open glass tube and it was immersed in the dissolution fluid so that it touches the surface of the
dissolution fluid. Samples were withdrawn every half an hour for the first 2 hours (pH 1.2). After 2 hours, 62.5 ml of 0.2 M tribasic sodium phosphate solution was added to change the pH from 1.2 to 6.8 and samples were withdrawn every 1 hour interval upto 6 hours. Sample of 5 ml was withdrawn and replaced with 5 ml of the fresh medium. The samples were diluted suitably and analysed spectrophotometerically at 254 nm.

*In vitro* release studies for eighteen acyclovir microspheres were performed (FG-I to FG-VI), (FA-I to FA-VI) and (FE-I to FE-VI) and the dissolution data were given in table 3.18 to table 3.35.

### 3.11.10 Mathematical modeling of acyclovir microcapsules

In order to investigate the mode of release from the microcapsules, the release data were analyzed with the following mathematical models.

\[ Q_t = K_0 t \quad \text{(Zero Order Kinetics)} \]

\[ \log \left( \frac{Q_t}{Q_0} \right) = -K_1 t / 2.303 \quad \text{(First order Kinetics)} \]

\[ Q_t = K_{KP} t^n \quad \text{(Korsmeyer and Peppas equation)} \]

\[ Q_t = K_H t^{1/2} \quad \text{(Higuchi’s equation)} \]

Where, \( Q_t \) is the percent of drug released at time ‘t’, \( K_0, K_1, K_{KP} \) and \( K_H \) are the coefficients of Zero order, First order, Korsmeyer- Peppas, and Higuchi’s equations. Korsmeyer-peppas model was widely used when the release mechanism was not well known or when more than one type of release phenomenon was involved. The ‘n’ values can be used to characterize diffusion release mechanism as: 0.5 = Fickian diffusion; 0.5<n<1= Non- fickian diffusion, 1 = Class II transport. The results were compiled in table 8 to table 11.
3.11.11 Stability studies as per the ICH guidelines

The prepared microcapsules of acyclovir with baker’s yeast, guar gum, egg albumin and ethyl cellulose were packed in High Density Poly Ethylene (HDPE) containers and were subjected to stability studies at the following different temperature and humidity conditions,

25 ± 2°C and 60 ± 5 % RH and 40± 2°C and 75 ±5 % RH as prescribed by the International Conference on Harmonization (ICH) (Q1A (R2))

Samples were stored in the above mentioned conditions for 180 days. The parameters were evaluated (physical appearance and acyclovir content) periodically on 15, 30, 60, 90, 120 and 180th day.

Acyclovir content was evaluated by UV analysis at $\lambda_{\text{max}}$ of 254 nm. The results were given in table 3.1 to table 3.8 and fig. 3.0 to fig. 3.8 for physical appearance and acyclovir content.

3.12 Preparation and evaluation of oral suspension of acyclovir microcapsule

Based on the analysis of data obtained from the prepared microcapsules of acyclovir (using baker’s yeast, guar gum, egg albumin and ethyl cellulose) it was observed that the microcapsules of acyclovir prepared with Baker’s yeast i.e., (FY IV, FY VIII and FY XII) showed a better result. Hence, these microcapsules were formulated into oral suspension and evaluated for the following quality control tests:

- **Physicochemical parameters**
- **Drug content estimation**
- **Wt/ml**
- **Particle size and size distribution**
- Sedimentation volume
- Rheology
- Zeta potential
- *In vitro* drug release study
- Accelerated stability study.

The results were compared with the marketed oral suspension of acyclovir (Zovirax).

### 3.12.1 Procedure for preparation of oral suspension of acyclovir using acyclovir microcapsules

Based upon entrapment efficiency data, three samples of acyclovir microcapsules prepared with baker’s yeast were selected.

**Table: 1.1 Selected Samples and entrapment efficiency**

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Sample code</th>
<th>Entrapment efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FY IV</td>
<td>96.66</td>
</tr>
<tr>
<td>2</td>
<td>FY VIII</td>
<td>97.61</td>
</tr>
<tr>
<td>3</td>
<td>FY XII</td>
<td>96.42</td>
</tr>
</tbody>
</table>

Microcapsules with better entrapment efficiency were formulated into suspension and compared with marketed sample (Zovirax). A suitable loading dose of the pure drug was added to the sample based on the entrapment efficiency.

The above product was triturated using a glass mortar and pestle. 0.1 gm of sodium carboxy methyl cellulose as the suspending agent and 0.002 gm of methyl paraben as the preservative was added to each of the formulation. The mixture was
made to a pourable form by slow addition and trituration of about 6 ml of distilled water. The suspension was transferred to a suitable container. Finally, the mortar was rinsed with 4 ml of distilled water and the volume was made up to 10 ml of suspension.

The above suspension was prepared thrice for each sample so that all the evaluations could be done accordingly.

Table: 1.2 Materials used for the preparation of acyclovir suspension

<table>
<thead>
<tr>
<th>S.No</th>
<th>Ingredients</th>
<th>FY IV</th>
<th>FY VIII</th>
<th>FY XII</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prepared microcapsule sample</td>
<td>800 mg</td>
<td>800 mg</td>
<td>800 mg</td>
</tr>
<tr>
<td>2</td>
<td>Pure drug (loading dose)</td>
<td>120.72 mg</td>
<td>130.32 mg</td>
<td>58.96 mg</td>
</tr>
<tr>
<td>3</td>
<td>Sodium CMC</td>
<td>0.1 gm</td>
<td>0.1 gm</td>
<td>0.1 gm</td>
</tr>
<tr>
<td>4</td>
<td>Methyl paraben</td>
<td>0.002 gm</td>
<td>0.002 gm</td>
<td>0.002 gm</td>
</tr>
<tr>
<td>5</td>
<td>Distilled water</td>
<td>10 ml</td>
<td>10 ml</td>
<td>10 ml</td>
</tr>
</tbody>
</table>

3.12.2 Evaluation of oral suspension of acyclovir microcapsules

3.12.2.1 Physicochemical parameters:

The physicochemical parameters that were evaluated include general appearance, colour, odour, taste and pH.

a) Appearance of Phases:

This test was done by visual examination of the dispersed phase and dispersion medium. For preparation of dispersion phase of suspension usually purified
water and syrup were used as dispersion medium. The uniformity in appearance of prepared samples and marketed product was monitored visually and results were compiled in table: 01.

b) Colour, odour and taste:

Colour, odour and taste are the organoleptic properties which play a vital role in the preformulation study. These properties can create impact on the patient. Hence, it is very much important to monitor them to ensure product quality. Colour, odour and taste was noted for all the 6 samples and results were compared with that of marketed formulation.

c) pH:

pH of the phases of suspension also contribute to stability and characteristics of formulations. So pH of the prepared suspensions and marketed sample was recorded using a pH meter to ensure optimum pH environment being maintained in the prepared suspension.

3.12.3 Drug content estimation:

For proper dosing of the dosage form it is necessary that the active ingredient is uniformly distributed throughout the dosage form. So samples are withdrawn from the dispersed phase after micronisation and after mixing with dispersion medium, assayed to find out % drug content. About 1ml of the sample was withdrawn, extracted and diluted to 100 ml with 0.1 N HCl. The absorbance of the resulting solution was measured at 254 nm against reagent blank. The practical yield was calculated for the prepared samples. Drug content was estimated using the formula:

\[
\% \text{ Drug content} = \left(\frac{\text{Practical yield}}{\text{Theoretical Yield}}\right) \times 100.
\]
The results were compared with that of the marketed sample. The results were tabulated in table 02 and fig. 01.

3.12.4 Wt/ml (Pycnometer method):

The mass density or density of a material is defined as its mass per unit volume. The symbol most often used for density is ρ (the Greek letter rho). Density is also defined as its weight per unit volume; although, this quantity is more properly called specific weight.

Density determination of liquids is done by pycnometer. Density determination by pycnometer is a very precise method. The pycnometer is a glass flask with a close-fitting ground glass stopper with a capillary hole through it. This fine hole releases a spare liquid after closing a top-filled pycnometer and allows for obtaining a given volume of measured and/or working liquid with a high accuracy. Pycnometer was first filled with distilled water and the weight of water was calculated (empty pycnometer weight subtracted). The procedure was repeated for the prepared samples and marketed suspension and its weight was calculated (measured weight minus weight of empty pycnometer). Wt/ml results were tabulated in the table 03 and fig. 02.

3.12.5 Particle size and size distribution:

Optimum Particle size of drug particle in the dispersed phase plays a vital role for the stability of final suspension. So this test was carried out to microscopically analyze and find out particle size range. Eye piece of the microscope was fitted with a micrometer. The eye piece micrometer was calibrated using a standard stage.
micrometer. The sample of suspension was mounted on a slide and placed on a mechanical stage. The size of the particle was estimated with the help of the eye piece micrometer. The diameter of 100 particles was determined by the number of divisions of eye piece micrometer. This is then converted to microns. The particles were arranged on the basis of size ranges. The number of particles in each size range were then converted and tabulated. Then percent number of particles in each interval and percent undersize were calculated. A histogram and cumulative undersize curve were plotted and the results were shown in table 05 to Table07 and fig 03 to fig.14.

3.12.6 Sedimentation volume:

The measurement of the sedimentation volume is one of the most common basic evaluative procedures. The sedimentation volume is the simple ratio of the height of sediment to initial height of the suspension. The larger the value better is the suspendability.

**Sedimentation Volume (F) or Height (H) for Flocculated Suspensions**

\[ F = \frac{V_u}{V_O} \quad (A) \]

Where, \( V_u \) = final or ultimate volume of sediment

\( V_O \) = original volume of suspension before settling.

The evaluation was conducted for a period of 10 days for the prepared samples and marketed formulation. About 10 ml of 3 samples and marketed suspension were taken in a measuring cylinder and the initial volume noted before settling of particles on the first day. Followed by that the volume of the sediment in the measuring cylinder was noted each day up to last day. Sedimentation volume is a ratio of the final or ultimate volume of sediment (\( V_u \)) to the original volume of sediment (\( V_O \)) before settling. The results were compiled in table 08 and fig. 15.
3.12.7 Rheological studies:

Rheological methods can help in determining the settling behavior of the suspension. Brookfield viscometer with variable shear stress control was used for evaluating viscosity of prepared samples and marketed suspension. It consist of T-bar spindle which is lowered into the suspension and the dial reading was noted which is a measure of resistance, the spindle meets at various levels in the suspension. The results were compiled in table 09.

3.12.8 In vitro release:

The in vitro release studies were carried out for 1 ml of prepared samples and marketed suspension (quantity equivalent to 80 mg) using paddle method. The dissolution vessels were filled with 900ml of 0.1N HCl. Paddle were rotated with 25 rpm and the set temperature was kept constant at 37 ± 0.5°C. The time of sampling was every 1 hr up to 8 hrs. Sample of 5ml was withdrawn and fresh dissolution medium was replaced to maintain sink conditions. After two hours of dissolution, the media was changed to pH 6.8 and the dissolution was continued for 6 hours. Samples were analyzed by using UV spectrophotometer at 254 nm after suitable dilution. Concentration of the drug was calculated from standard equation obtained from calibration curve. Cumulative percentage of drug release and percentage drug unreleased was calculated and respective graphs were plotted. The results were compiled in table 10 to table 19 and fig. 16 to fig. 33.
3.12.9 Stability testing:

It is not possible to conduct accelerated temperature studies as it can be done for solutions. The formulation exhibiting thixotropic properties a rise in temperature would change the properties. In this physical form, the preparation would exhibit parameters that could not be extrapolated to those that would exist in the normal system. The valid temperature data could be obtained that will be useful in the estimation of the physical stability of a product at normal storage conditions. The extended aging tests must be employed under various conditions to obtain the desired information. The results were compiled in table 20 and table 23 and fig. 34 to fig. 37.