"FORMULATION OPTIMIZATION AND CHARACTERIZATION OF NANOSUSPENSION OF ACYCLOVIR"

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BY

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UNDER THE GUIDANCE OF

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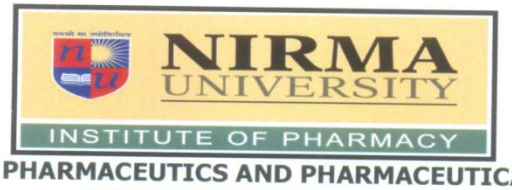

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CERTIFICATE

This is to certify that the dissertation work entitled "Formulation optimization and characterization of nanosuspension of Acyclovir" submitted by Ms. Dhruvi Mehta with Regn. No. (11MPH103) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutics" is a bonafide research work carried out by the candidate at the Department of Pharmaceutics and Pharmaceutical Technology, Institute of Pharmacy, Nirma University under our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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DECLARATION

I hereby declare that the dissertation entitled "Formulation optimization and characterization of nanosuspension of Acyclovir" is based on the original work carried out by me under the guidance of Dr. Jigar N. Shah, Assistant professor, Department of Pharmaceutics and Pharmaceutical Technology, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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C. Abbreviations

Short Name	Abbreviation
IP	India Pharmacopoeia
BP	British Pharmacopoeia
USP	United States Pharmacopeia
PhEur	European Pharmacopoeia
USPNF	United States Pharmacopoeia National Formulary
FTIR	Fourier Transfer Infra Red
UV	Ultra Violet
HPMC	Hydroxy Propyl Methyl Cellulose
HPC	Hydroxy Propyl Cellulose
PVP	Poly Vinyl Pyrrolidone
SLS	Sodium Lauryl Sulphate
KBr	Potassium Bromide
°C	Degree Centigrade
Conc.	Concentration
CP	Cumulative Percentage Release
µg	Microgram
RH	Relative Humidity
W/W	Weight By Weight
W/V	Weight By Volume

Formulation optimization and characterization of Nanosuspension of Acyclovir

Mehta D. U. , Shah J. N. , Mehta T. A.

Oral administration has been the most favorable route of drug delivery. Nevertheless, many hydrophilic drugs, administered orally exhibit low oral bioavailability mainly due to low permeability. For such BCS class III drugs, gastrointestinal permeation is the rate-controlling step in the absorption process. Many approaches have been tried for improving permeability like Particulate Drug delivery System, Vesicular Drug Delivery System, Polymeric Micelles, Micro Emulsion/Nano Emulsion, SMEDDS/ SNEDDS, Nanosuspension etc. Nanosuspensions are submicron colloidal dispersions of nano sized drug particles stabilized by surfactants. Acyclovir, a class III drug shows only 20% bioavailability. It is having very low permeability (Log P is -1.56) Also, it is having very high onset of action (within 24 hours). Acyclovir is highly hydrophilic compound, so its absorption through intestinal membrane carries out via active transport. The oral cavity is highly vascularized but the surface epithelium is avascular, thus potentially reducing the bioavailability of drug absorbed from the gut to these regions. An interesting property of the oral mucosa is their ability to phagocytose particles, e.g., by Langerhans cells and epithelial cells. Direct nanoparticle uptake is one of the promising methods of improving the bioavailability of active ingredients, especially for compounds that are soluble in water but that have low permeability. Transcytosis of the nanoparticle takes place with the help of M cells, which are located in Peyer's patches in the small intestine. But, the problem with this approach is that these M cells represent typically less than 1% of the total intestine area, which makes the selective delivery to these sites more difficult. So, surface modification technology was incorporated which includes the use of Eudragit coating. So, with the help of Emulsification Solvent evaporation method, the preparation of nanosuspension of Acyclovir was taking place, along with Eudragit EPO as Polymeric stabilizer and Tween 80 as Surfactant stabilizer. 3^2 full factorial design was applied for the optimization of the formulation parameters. Various evaluation parameters like Particle size and Zeta potential, % Entrapment efficiency, % Drug release, Ex vivo diffusion study, Differential scanning colorimetry (DSC), Transmission Electron Microscopy (TEM) study etc. were performed and the best batch was optimized.

CHAPTER 1
AIM AND OBJECTIVE OF PRESENT INVESTIGATION

AIM AND OBJECTIVE OF THE PRESENT INVESTIGATION

Oral administration has been the most common and favorable route for delivery of the drugs. Oral administration of medicines utilizes the highly absorptive capacity of the gut to deliver drugs to the systemic circulation. Thus, it is the most widely used and accepted route of drug delivery to the adult population.

The biopharmaceutical classification system (BCS) was proposed as an in vitro evaluation method to assess intestinal absorption of various types of compounds is basically depending upon two parameters- solubility and permeability. Drugs with high solubility and low permeability are classified as BCS class III drugs. Due to their inauspicious physicochemical and chemical properties which are difficult to change many drug molecules show poor permeability. Ordinarily, BCS class III drugs are not adapted to oral formulations.

Acyclovir, a prototype anti-viral agent is used orally for the treatment and prophylaxis of initial and recurrent episodes of genital and labial herpes and for the acute treatment of herpes zoster, for the treatment of varicella (chickenpox) in immunocompetent individuals. Acyclovir, a class III drug shows only 20% bioavailability, also it has very high onset of action (within 24 hours). Acyclovir is highly hydrophilic compound, so its absorption through intestinal membrane carries out via active transport. The oral cavity is highly vascularized but the surface epithelium is avascular, thus potentially reducing the bioavailability of drug absorbed from the gut to these regions. An interesting property of the oral mucosa is their ability to phagocytose particles, e.g., by Langerhans cells and epithelial cells.

Nanoparticles are key factor to increase the range of formulations for oral delivery in terms of improving drug solubility, drug permeability, drug stability and bioavailability. The fundamental rationale for application of these nano technologies for oral drug delivery is the inherent ability of the nano-carrier to modulate pharmacokinetics of the incorporated drug molecules. This is frequently achieved by polymeric protection of the pharmacophore from the destructive elements within the gastrointestinal tract (GT). Nanosuspension is one of the promising approaches to improve the permeability of BCS Class III drugs. Various methods

are used for the preparation of nanosuspension like, Media milling, high pressure homogenization, Ultrasonication method, Super critical fluid method, Emulsification solvent evaporation method etc. Here, Emulsification Solvent Evaporation method has been applied to prepare nanosuspension as it was the most convenient and useful method to prepare nanoparticles from polymers. Polymers used as stabilizers for the formulation of nanoparticles.

Along with acyclovir, Eudragit EPO was used as polymeric stabilizer as it is having property to stabilize the nanoparticles, by this way it prevented them from aggregation. Along with Eudragit EPO, Tween 80 was used, which is having property of permeation enhancement. 3^2 full factorial design was applied to optimize the nanosuspension. Effect of concentration of Eudragit EPO and effect of concentration of tween 80 was studied. Various evaluation parameters like Particle size and Particle size distribution, Zeta potential, % Entrapment efficiency, In vitro Diffusion study, Ex vivo diffusion study, Differential Scanning Colorimetry (DSC), Transmission Electron Microscopy (TEM) were carried out and from that the best batch was optimized.

Hence, the aim of current investigation was to prepare nanosuspension of acyclovir using Emulsification solvent evaporation method.

CHAPTER 2
INTRODUCTION

2. INTRODUCTION

Oral administration has been the most common and favorable route for delivery of the drugs. The oral absorption of a drug mainly depends on the water solubility of the drug and on the drug's membrane permeability. Oral administration of medicines utilizes the highly absorptive capacity of the gut to deliver drugs to the systemic circulation. This is the most widely used and accepted route of drug delivery to the adult population[1].

2.1 Oral Drug Delivery Systems

Oral drug delivery systems are gaining popularity and acceptance as technologies evolve. Together with easy administration, they also offer innovative solutions for some key challenges faced by the Pharma Industry. With conventional drug delivery systems (tablets, capsules, etc) the mouth is merely the entry point of the medication with the active being absorbed in the gastro intestinal (GI) tract. Oral technologies, however, are designed to deliver the active directly via the oral cavity with the mouth becoming the site of administration, application and absorption. Absorption into the blood capillaries occurs via diffusion of the drug through the non-keratinized buccal (lining the mucosa) and sublingual mucosa, thereby giving the drug direct access to the circulation system and correspondingly significantly reduces the so-called "first-pass effect" (the hepatic metabolism of orally administered drugs by gastrointestinal enzymes, resulting in a significantly reduced amount of unmetabolized drug reaching the systemic circulation).

2.1.1 Oral drug delivery systems possess some important characteristics and properties:

1) Broad range of applications (local or systemic, food, cosmetic and pharmaceutical products).

2) Non-invasive, ideal for pediatric/geriatric patients.

3) No water and no chewing are needed for administration resulting in a substantial improvement of patient compliance and convenience.

4) Significantly reduced "first-pass effect":

- Improved bioavailability in comparison to conventional solid dosage forms.
- Improved stability for pH sensitive drugs (mouth saliva pH ≈6.5).
- Accurate dosing and less waste of expensive drugs through metabolic/gastric degradation.
- Improved safety through lower dosage and milder GI side effects.
- Reduced level of metabolites and related side effects.

5) Versatile drug release (immediate/controlled).

6) Customized colour, shape, dimension, taste.

7) Adaptable to existing processing and packaging machinery.

8) Cost-effective.

9) Extension of market exclusivity for drug entities at the end of their patent life[2].

During the past few decades, noteworthy medical advances have been made in the field of drug delivery with the improvement of new dosage forms and techniques. For the drugs which are not absorbed by oral route, other routes of drug delivery such as injection, transdermal, pulmonary or any other are employed. However, oral route among different probable routes is most preferable because it offers significant advantages of therapeutic effectiveness and patient compliance[2].

The biopharmaceutical classification system (BCS) was proposed as an in vitro evaluation method to assess intestinal absorption of various types of compounds is basically depending upon two parameters- solubility and permeability. Drugs with high solubility and low permeability are classified as BCS class III drugs. Due to their inauspicious physicochemical

and chemical properties which are difficult to change many drug molecules show poor permeability. Ordinarily, BCS class III drugs are not adapted to oral formulations[3].

BCS High Permeability Criteria of a drug substance is considered to be "highly permeable" when the extent of absorption in humans is determined to be ≥ 90% of an administered dose based on a mass balance determination or in comparison to an i.v. reference dose[4].

2.1.2 Novel Drug Delivery Systems

Novel drug delivery system is a novel approach to drug delivery system that addresses the limitations of traditional drug delivery. Novel drug delivery system attempts to eliminate all the disadvantages associated with conventional drug delivery systems.

The benefits of new drug delivery system include:

(1) The required therapeutic conc. is well maintained with minimum fluctuations.

(2) Reproducible and predictable release rates for longer duration.

(3) Elimination of side effects.

(4) Decreased frequency of administration.

(5) Increased patient compliance.

The novel drug delivery systems include:-

Liposomes, Niosomes, Submicron Emulsion, Multiple Emulsion, Nanoparticles, and Microspheres[2].

2.1.3 Problems associated with Oral route:

Various drugs exhibit relatively low bioavailability due to;

- Poor Solubility.
- Poor Permeability.
- Degradation in the gastro-intestinal lumen or during absorption

- Extensive first-pass metabolism.
- Efflux by various efflux pump like p-gp, MDR(Multi Drug Resistance) proteins, & other ABC (ATP Binding Cassette) family proteins[5].

2.2 Potential Absorption Barriers

Various barriers for the intestinal permeability of drugs are the mucous layer, the apical and basal cell membrane and cell contents, the tight junctions and the wall of lymph and capillaries.

Mucous

A mucous layer consisting of water glycoproteins (mucins), electrolytes, proteins and nucleic acids covers the epithelial cells of the entire intestine. The layer is bound to the apical surface by the glycocalyx, a 500 nm thick glycoprotein structure which is covalently linked to lipids and proteins of the brush border membrane. The unstirred water layer is composed partially of the mucous layer, and it is supposed that the minimal thickness of the unstirred water layer, 100-50μm corresponds with the mucous layer. The mucous layer maintains the pH of the epithelial surface at 6 by acting as a buffer, thus creating an acidic microclimate.

Apical cell membrane

The shape of the apical cell membrane is like a 1μm thick brush border, and it consists of a 10nm thick double layer of polar lipid molecules containing a hydrophobic and a lipophilic part. The main constituents of lipid are phosphatidylcholine, phosphotidyl ethanolamine, sphingomyline (zwitterionic), phosphatidyl serine, phosphatidylinositol, phosphatidic acid (anionic), cholesterol and lipids. For the preservation of membrane structures divalent metal ions may be necessary, Ca^{2+} chalets with negatively charged phospholipids, thus decreasing membrane permeability and lipophilicity. Proteins are entrenched in the lipid bilayer by their hydrophobic segments. For the optimal activities of membrane bound enzymes, fluid state of the membrane is required; cell preserves the membrane transition temperature (Tin), the

temperature at which the transition from the stiff gel to the fluid liquid crystalline state occurs, below environmental temperature. A regulating action of cholesterol is employed on membrane structure, increasing fluidity of gel-state membrane and decreasing fluidity of liquid crystalline membrane. Sphingomyline has been proposed to enhance the assembling influence of cholesterol. The membrane order is also influenced by natural fatty acids, their cis-double bonds distracting phospholipid organisation. For this reasons fluidity of fluid state membranes may increase with decreasing cholesterol/phospholipid molar ratio or increasing total lipid/protein ratio and double bond index thus increasing permeability. For example, In rat colonocytes lipid fluidity decreases from proximal to distal, transition temperatures amounting to 23-24^{0}C and 26-27^{0}C respectively corresponding with a high enzyme activity in the proximal segment. The transport of molecules across the phospholipid bilayer is commonly correlated with lipid-water coefficient. Subsequently the absorption of strongly hydrophilic substances is restricted by the lipid bilayer e.g. certain antibiotics and peptides. For this reason the transcellular transport of water, ions and polar solutes (e.g. monosaccharides) require other mechanisms e.g. Diffusion through pores and carrier mediated transport.

Basal cell membrane

The basal cell membrane is composed of a 9 nm thick phospholipids bilayer which contains proteins. The lipid fluidity of the baso lateral membrane surpasses apical membrane fluidity probably because of lower content of glycosphingo lipids. Hence the barrier function of the basal membrane is possibly less prominent than that of apical membrane.

Tight Junctions

Tight junctions (zonula occludentes) are regions of close communication between apical ends of epithelial cells. They are constructed of a network of strands, the permeability of tight junction increases with the decreasing strand number, thus determining the ‘leakiness’ of epithelium. The small intestine contains leaky epithelium, and intestinal permeability decreases in the distal direct ion running parallel with apical cell membrane permeability; the proximal colon is temperately leaky, the distal colon moderately tight. The medium sized

solutes (e.g. disaccharides), ions and water thus establishing route for passive ion permeation. Tight junctions are cation selective & they have been suggested to be impermeable for cations with a molecular weight higher than 350 nm or a diameter exceeding 0.8 nm. Alternatively, it is conceivable that a distribution of pore sizes exists, with a large number of small pores and a few large ones. The structure of tight junction is destabilized by exposure to hypertonic solutions and by Ca^{2+} depletion. In hamster small intestine sodium coupled solute transports have been suggested to increase junctional permeability towards small peptides, sugars and amino acids.

Capillary wall

The location of capillary wall is 500 nm underneath the basal membrane. The endothelial cell membrane contains small perforations of 0.4-1 nm radius and the blood capillary wall is fenestrated, fenestrate radius amounting 20-30 nm. On the other hand lymphatic capillaries are provided with an intracellular junction of larger size, permitting passage of particles with a radius up to 300 nm. Particles with a radius smaller than 6 nm are not retained by basement membrane surrounding fenestrated capillaries. Due to the existence of large pores, the intestinal blood and lymph capillaries are not considered to execute an important barrier for drug absorption. However, it is conceivable that strongly hydrophilic drugs will be transported slowly across the capillary wall, compared with hydrophilic compounds, as their absorption site will be limited to the pore area[6].

2.3 Nanoparticles used for oral formulations

In pharmaceutics, ≈90% of all medicines, the active ingredients is in the form of solid particles. With the development of in nanotechnology, it is now possible to produce drug nanoparticles that can be utilized in a variety of innovative ways. New drug delivery pathways can now be used that can increase drug efficacy and reduce side effects. The enteric system has been specifically designed for the uptake of foreign substances to maintain homeostasis in the body. Despite the extensive research and success stories with other routes for drug delivery, the oral route is still the most preferred route because of its basic functionality and the advantages that ensue.

The oral cavity is highly vascularized but the surface epithelium is avascular, thus potentially reducing the bioavailability of drug absorbed from the gut to these regions. An interesting property of the oral mucosa is their ability to phagocytose particles, e.g., by Langerhans cells and epithelial cells.

Nanoparticles are key factor to increase the range of formulations for oral delivery in terms of improving drug solubility, drug permeability, drug stability and bioavailability. The fundamental rationale for application of these nano technologies for oral drug delivery is the inherent ability of the nano-carrier to modulate pharmacokinetics of the incorporated drug molecules. This is frequently achieved by polymeric protection of the pharmacophore from the destructive elements within the gastrointestinal tract (GT). These carriers are designed for site-specific drug release in the small intestine to achieve maximal bioavailability in the systemic circulation. This is achieved by variation in carrier chemical composition, size, interface forces, morphology, surface decoration, associated charge and hydrophile–lipophile balance[7].

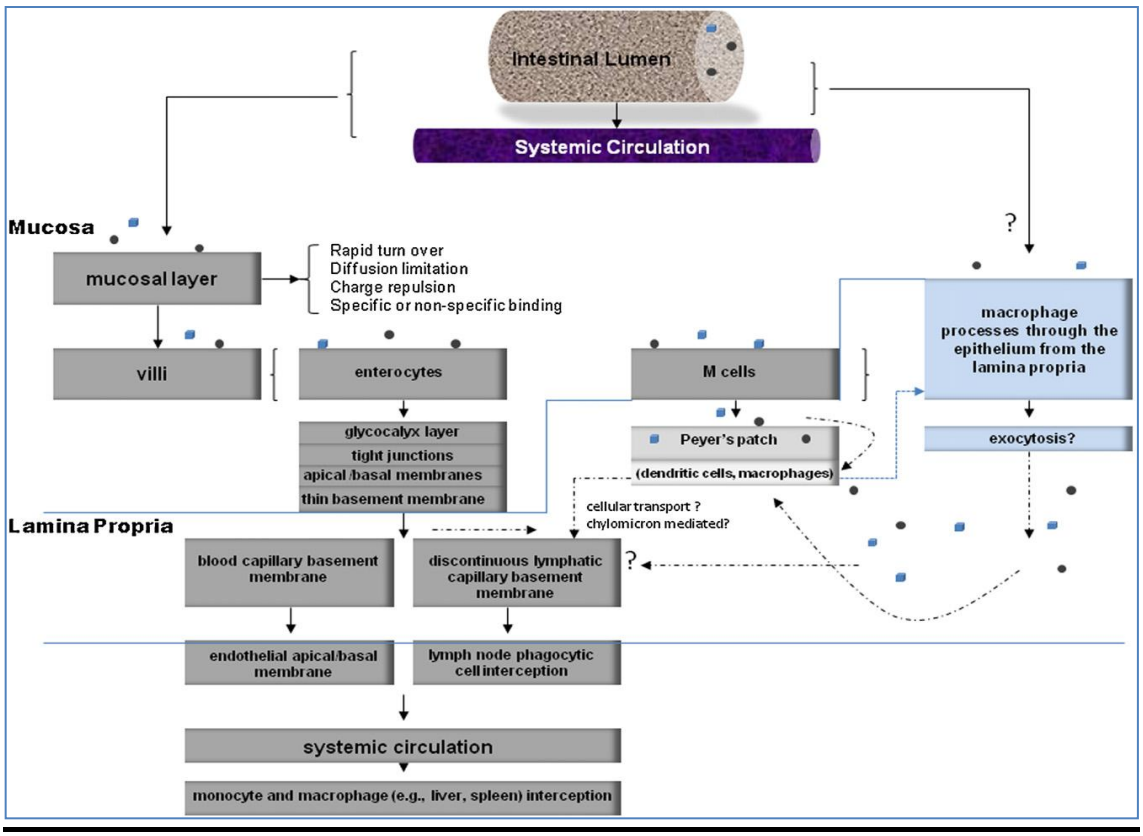


Figure 2.1: Absorption barriers located in the Intestine[7]

2.4 Absorption mechanism of nanoparticles

The absorption of Active Pharmaceutical Ingredients (API) through the small intestine occurs via two main mechanisms, active and passive transport. Active transport involves the uptake of the active ingredient through specific channels on the surface of the epithelial cells. The cells use their own energy to capture and absorb nutrients even when the concentration of the nutrient inside the cell is higher than the concentration outside the cell. Active transport is the main mechanism by which cell captures and absorb highly soluble minerals like calcium and iron. This active uptake is controlled by hormones that regulate the concentration of minerals and other nutrients in the body. It is having a control systems that regulate the absorption of nutrients and nutraceuticals and drugs. These control systems maintain a certain homeostatic level of substances in the blood, in a way that, if there is any excess of the active ingredient

in the blood, additional doses are not adsorbed (active adsorption mechanism), and that this excess is accumulated in tissue or secreted after the compound has entered the bloodstream.

Passive transport occurs by a simple diffusion across the epithelial tissue. In this case the rate and extent of uptake is a function of the difference in the activity of the mineral or nutrient across the epithelial tissue. The activity of a solute is calculated as the product of its concentration times its activity coefficient. The activity coefficient reflects the solubility of the solute in the solvent. Poorly soluble ingredients (e.g. hydrophobic compounds in water) have a large activity coefficient, thus inducing a large driving force for the nutrient (active ingredient) to permeate. Most hydrophobic compounds are highly permeable through the intestines and transport using passive and active diffusion, however highly hydrophilic substances tend to have low permeability and absorb via active transport[8].

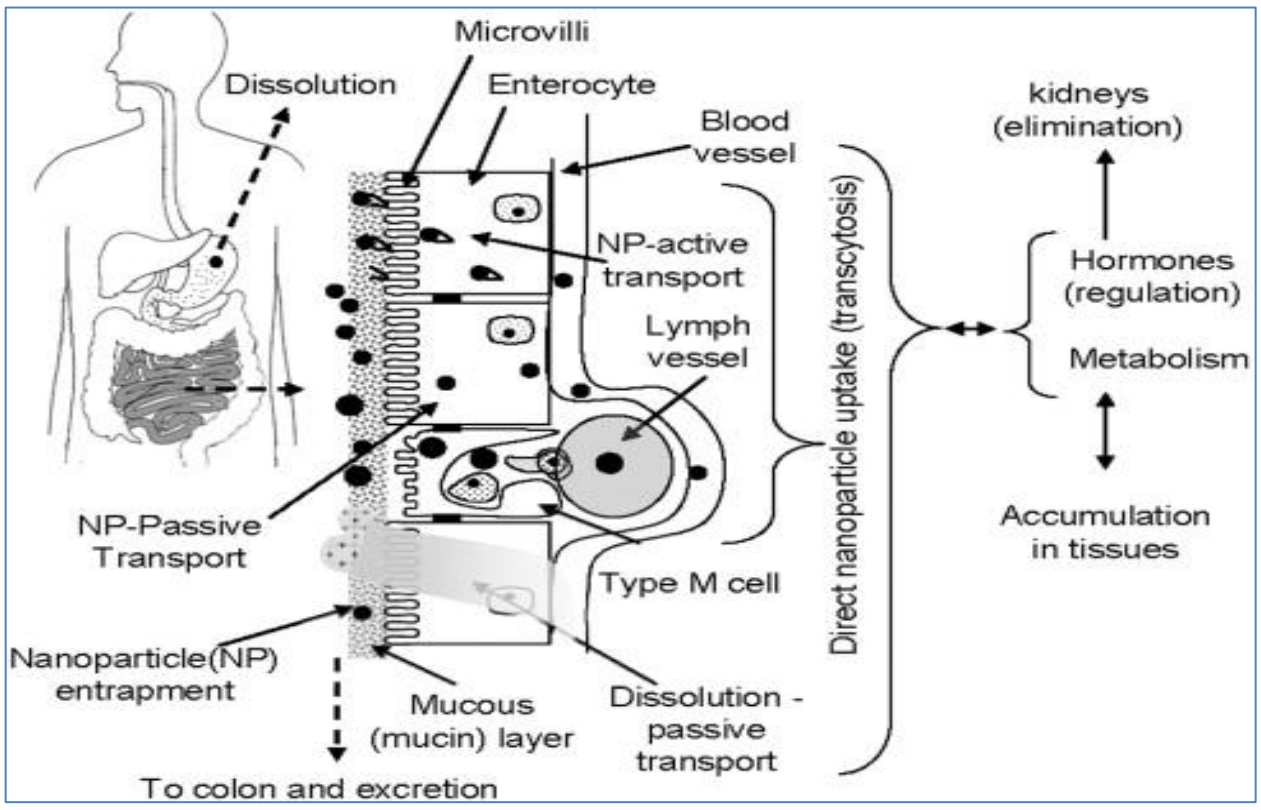


Figure 2.2: Mechanism of active ingredient uptake using nanoparticle systems[9]

Direct nanoparticle uptake is yet another method of improving the bioavailability of active ingredients, especially for compounds that are soluble in water but that have low permeability. There are three main routes of nanoparticle uptake: paracellular uptake, transcellular (transcytosis) uptake by enterocytes (which represent 90–95% of the epithelial cells of the intestine) and transcellular uptake by M (microfold) cells. The paracellular uptake (uptake through the interstitial space between epithelial cells) is considered to be the least effective of the three mechanisms because the space between cells ranges between 0.3 and 1 nm, which is too small for most nanoparticles to permeate. The transcellular (transcytosis) uptake of nanoparticles is illustrated in Figure 1.12. There are two methods of nanoparticle transcytosis, one is passive in which case the nanoparticle diffuses through the epithelial cell and the second method of involves the presence of receptors on the surface of the cells (represented as small "N" marks in Figure 1.12) that capture nanoparticles with specific surface chemistry. The same basic mechanisms of transport apply to enterocytes and M cells. However the M cells are more permeable than enterocytes, and therefore M cells have been used as the target for numerous micro- and nanoparticle delivery strategies. Such M cells are located in the Peyer's patches of the lower intestine, and its role is to "sample" potential antigens present in the intestinal track. Although M cells are the ideal portal for micro- and nanoparticle delivery, the problem with this approach is that these M cells represent typically less than 1% of the total intestine area, which makes the selective delivery to these sites more difficult. There is currently intense activity, in the pharmaceutical arena, on surface modification technologies to incorporate selective ligands for M cell delivery, including lectin-coated nanoparticles, and nanoparticles coated with exo polymers produced by toxic bacteria. The principle of this target uptake is that M cells, due to their antigen monitoring activity, have developed receptors that capture particles coated with glycoproteins such as those used in target delivery. Recent studies suggest that lectins, in particular, may be the key to improve the absorption of hydrophilic (and poorly absorbed) nutraceuticals such as isoflavones , however, there is little progress made, to date, in exploring those possibilities in nutrient and nutraceuticals applications. Another approach to promote nanoparticle uptake by M cells and enterocytes is the use of coatings to modify the surface chemistry of the nanoparticle systems. It has been found that increasing the hydrophilicity of the surface of

the nanoparticle typically promotes the translocation of nanoparticles across cellular cytoplasm. Another popular alternative to introduce hydrophilic moieties to the surface of the nanoparticle is the use of polyethylene glycol coatings. Perhaps one of the most effective methods of increasing the adhesion of nanoparticles to the mucin layer that line the surface of the intestines is the use of strong cationic coatings produced with synthetic polymers such as Various Eudragit grades polymers or cationic surfactants such as alkyl trimethyl ammonium salts. However, if the interaction between the cationic nanoparticle and the mucin proteins is too strong, the particle will remain adhered to the mucin and will not permeate through the epithelial tissue[9].

2.5 Polymers and their use to prepare nanoparticle

A number of polymers have been evaluated for the development of oral formulations, including naturally occurring polymers (e.g. starch, alginates and gelatin) and synthetic polymers (e.g poly(lactide-co-glycolide), polyanhydrides, phthalates, Eudragit (methacrylic acid derivatives). Toxicity, irritancy and allergenicity are the factor of primary concern and hence there is a need of biodegradable and soluble coating. The advantage of using natural polymers includes low cost, biocompatibility and aqueous solubility. However, the natural polymers may also be limited in their use due to presence of impurities, batch-to-batch variability and mainly low hydrophobicity. In comparison, synthetic polymers are more reproducible and can be prepared with the desired degradation rates, molecular weights and copolymer composition. Mainly studies have suggested that synthetic polymers exist in amorphous form and exhibit high permeability at body temperature. Such synthetic polymers are used for immediate as well as sustained release formulations.

Natural Polymers

The use of colloidal carriers made of hydrophilic polysaccharides like chitosan (CS) is increasing as a promising alternative for improving the transport of drugs and macromolecules, such as peptides, proteins, oligonucleotides and plasmids across biological membranes. CS has been shown to increase the paracellular permeability of mannitol across

Caco-2 intestinal epithelia. These findings attributed the property of transmucosal absorption enhancement. CS is soluble only in solutions at pH values below 6.5, and only protonated CS can open the tight junctions. Thereby, facilitating the paracellular transport of hydrophilic compound. CS can enhance insulin absorption across human intestinal epithelial cells without injuring them. CS is very interesting excipient for vaccine delivery also. Use of cyclodextrins in nanoparticulate drug delivery systems has been studied now a days. Mainly they are used to produce nanospheres as well as nanocapsules.

Synthetic Polymers

The foremost area of concern for these polymers is their biocompatibility and biodegradability. Polyesters and polyanhydrides are the most important class of polymers for drug delivery applications. Particle uptake is dependent on size, but was not exclusive to PP tissues. These synthetic polymers are mostly preferred for nanoparticle preparation because of its ease of preparation, commercial availability, versatility, biocompatibility and hydrolytic degradation into harmless products. Eudragits are now a days used as synthetic polymer for nanoparticulate drug delivery systems. Various grades of Eudragit are used as stabilizers door the preparation of nanoparticles. Because of the self-emulsifying properties of the methacrylic acid copolymers, it was possible to prepare aqueous dispersions of colloidal size containing Eudragit grades.

Polymers are also used as stabilizers, generally to prevent the aggregation of particles by conferring a surface charge. Higher surface charge shows greater stability. At high stabilizer concentrations, well above of the plateau of the adsorption isotherm, electrostatic stabilizers can cause a decrease in the diffuse layer leading to a decreased zeta potential and a decreased physical stability. Electrolytes are present in the gastrointestinal tract and the contact of the nanocrystals with these electrolytes cannot be avoided. Electrostatic stabilization is reduced in its efficiency in an electrolyte containing environment. Therefore it is important to find the optimal concentration for a stabilizer. The main functions of a stabilizer are too wet the drug particles thoroughly, and to prevent Ostwald's ripening and agglomeration of

nanosuspensions in order to yield a physically stable formulation by providing steric or ionic barriers[10].

2.6 Approaches to enhance permeability of BCS Class III drugs

1. **Novel Drug Delivery System**
    - Particulate Drug delivery System
    - Solid Lipid nanoparticles
    - Polymeric Nanoparticles
    - Solid Lipid Nanocarriers
    - Microparticles
    - Vesicular Drug Delivery System
    - Polymeric Micelles
    - MicroEmulsion/ NanoEmulsion
    - SMEDDS/ SNEDDS
    - Nanosuspension
2. **Use of Permeation Enhancer**
    - Transcellular Permeation Enhancer
    - Paracellular Permeation Enhancer
    - Polymeric Permeation Enhancer
3. **Use of Efflux Pump Inhibitors**
    - P-gp inhibitors
    - MDRP inhibitors
4. **Use of Enzyme Inhibitors**
    - Protease inhibitors
    - Peptidase inhibitors

2.7 Introduction To Nanosuspension

Nanosuspensions are submicron colloidal dispersions of nanosized drug particles stabilized by surfactants. It consists of the drug without any matrix material suspended in dispersion. They are the biphasic system, consisting of pure drug particles dispersed in an aqueous vehicle in which the diameter of the suspended particle is less than 1μm in size. This approach is useful for molecules with poor solubility, poor permeability, or both, which poses a significant challenge for the formulators. The reduced particle size renders the possibility of intravenous administration of poorly soluble drugs without any blockade of the blood capillaries. The suspensions can also be lyophilized and into a solid matrix.

Advantages:

- Enhance the solubility and bioavailability of drugs
- Suitable for hydrophilic drugs
- Higher drug loading can be achieved
- Dose reduction is possible
- Enhance the physical and chemical stability of drugs
- Provides a passive drug targeting

There are two main approach for formulating a nanosuspension i.e. top down and bottom up technology. The bottom up technology involves dissolving drug in a solvent which is then added to non-solvent to precipitate the crystals. The top down approach relies on mechanical attrition to render large crystalline particles into nanoparticles. The 'Top Down Technologies' include Media Milling (Nanocrystals®), High Pressure Homogenization in water (Dissocubes®), High Pressure Homogenization in nonaqueous media (Nanopure®) and combination of Precipitation and High-Pressure Homogenization (Nanoedege®). While the 'Bottom Up Technologies' include melt emulsification, microemulsion, precipitation methods[11].

2.7.1 Method of Preparation

1. Precipitation Method:

Precipitation method is a general method used to prepare submicron particles of poorly soluble drugs. In this method, drug is dissolved in solvent and then solution is mixed with solvent to which drug is insoluble in the presence of surfactant. Continuous addition of solution to such solvent (mainly water) leads to rapid super saturation of drug and produces ultrafine amorphous or crystalline particles. This method involves nuclei formation and crystal growth which are mainly dependent on temperature. High nucleation rate and low crystal growth rate are primary requirements for preparing a stable suspension with minimum particle size[12].

2. Milling Techniques:

(1) Media milling

In this technique, drugs are subjected to media milling for nanoparticle formation. Effect of impaction between the milling media and drugs gives essential energy for disintegration of the microparticulate system into nanoparticles. In this process, the chamber of milling is charged with the milling media involving drug, stabilizer, and water or suitable buffer, which is rotated at a very high shear rate to generate suspension. The major disadvantage of this method is the residues, which left behind the end product[13].

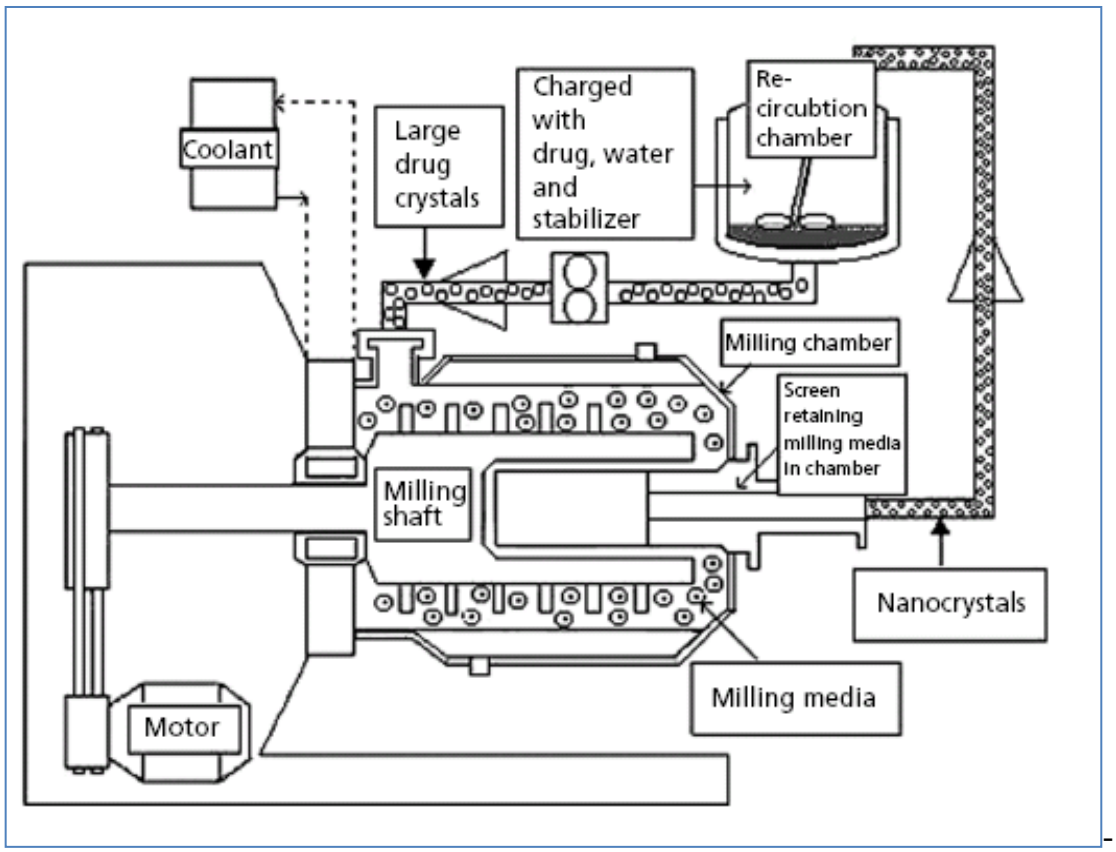


Figure 2.3: Media milling technique[13]

(2) Dry co-grinding

Nanosuspensions are prepared through wet grinding processes by using pearl ball mill, Silicon- Zerconium ball mill etc, since many years. Nowadays, nanosuspensions can be prepared by dry milling methods. Stable nanosuspensions are prepared by using dry grinding of poorly soluble drug with soluble polymers and copolymers after dispersing in liquid medium.

(3) Lipid emulsion/microemulsion template

The emulsion technique is applicable for drugs which are either partially water miscible or soluble in volatile organic solvents. Nanosuspensions are also obtained by diluting the emulsion, formed by using a partially water-miscible solvent as the dispersed phase. Microemulsion templates are one of the newer approaches used to produce nanosuspensions. They are dispersions of two immiscible liquids like water and oil and

stabilized thermodynamically by surfactant or cosurfactant. The drug is either loaded into preformed or internal phase of microemulsion and can be saturated by intimate mixing of drugs.

(4) Microprecipitation – High-pressure homogenization (Nanoedge)

Nanoedge is a combination of microprecipitation and high-pressure homogenization techniques. It includes precipitation of friable materials followed by fragmentation under high shear or thermal energy.

(5) Melt emulsification method

Solid lipid nanoparticles are mainly prepared by melt emulsification method. Here, the drug is first added to aqueous solution having stabilizer. The solution is heated at temperature higher than the melting point of the drug and then homogenized by high-speed homogenizer for the formation of emulsion. The temperature is maintained above the melting point of the drug during overall process. Finally, the emulsion is cooled to precipitate the particles. The particle size of nanosuspension mainly depends on parameters like drug concentration, concentration and type of stabilizers used, cooling temperature, and homogenization process.

3. High-Pressure Homogenization:

This technique involve the following three steps: First, drug powders are dispersed in a stabilizer solution to form presuspension; after that, presuspension is homogenized by high pressure homogenizer at a low pressure sometimes for premilling; and finally homogenized at a high pressure for 10 to 25 cycles until the nanosuspensions are formed with desired size.

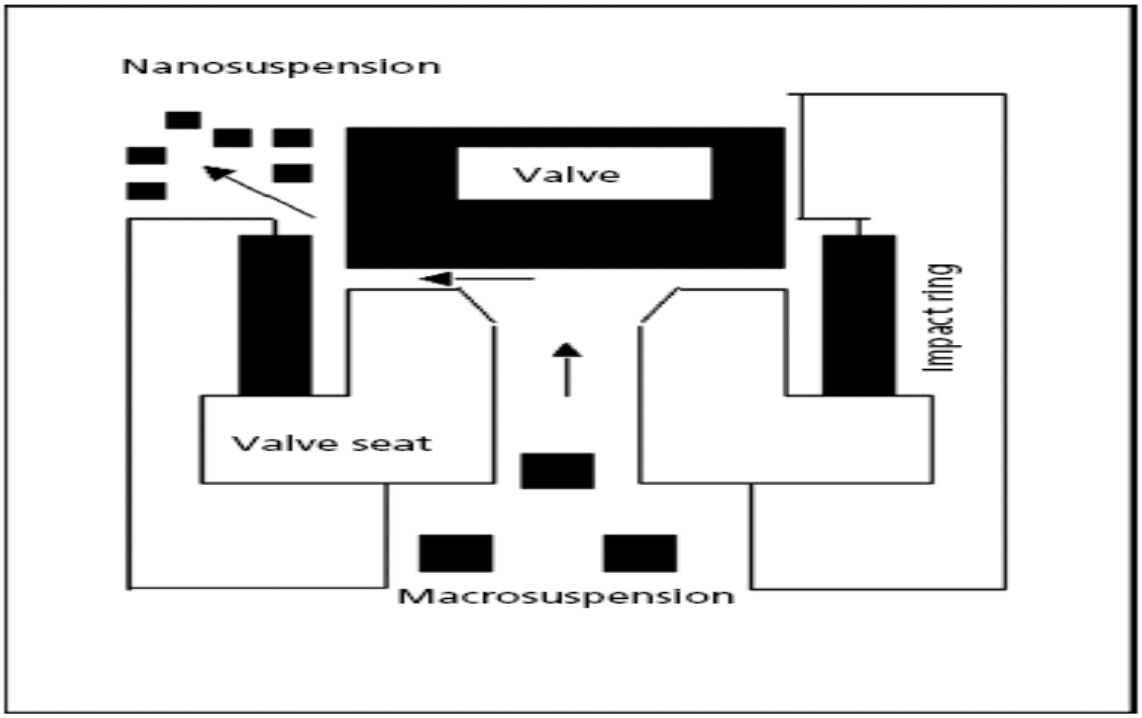


Figure 2.4: High pressure Homogenization technique

(1) Homogenization in Aqueous Media (Dissocubes)

The instrument can be operated at pressure varying from 100 to 1500 bars (2800-21,300 psi) and up to 2000 bars with volume capacity of 40 ml (for laboratory scale). For preparation of nanosuspension, it is essential to prepare a presuspension of the micronized drug in a surfactant solution using high-speed stirrer. According to Bernoulli's Law, the flow volume of liquid in a closed system per cross section is constant. The reduction in diameter from 3 cm to 25 µm leads to increase in dynamic pressure and decrease of static pressure below the boiling point of water at room temperature. Due to this, water starts boiling at room temperature and forms gas bubbles, which implode when the suspension leaves the gap (called cavitation) and normal air pressure is reached. The size of the drug nanocrystals that can be achieved mainly depends on factors like temperature, number of homogenization cycles, and power density of homogenizer and homogenization pressure.

(2) Homogenization in Nonaqueous Media (Nanopure)

Nanopure is suspension homogenized in water-free medium. It is "deep-freeze" homogenization where the drug suspensions in nonaqueous medium are homogenized at 0°C or sometimes below the freezing point. Because of very high boiling point and low vapor pressure of water, oils, and fatty acids, the drop of static pressure is not enough to begin cavitation in nanopure technology[14].

4. Supercritical fluid methods

Various methods like rapid expansion of supercritical solution (RESS) process, supercritical antisolvent process, and precipitation with compressed antisolvent (PCA) process are used to produce nanoparticles. In RESS technique, drug solution is expanded through a nozzle into supercritical fluid, resulting in precipitation of the drug as fine particles by loss of solvent power of the supercritical fluid. By using RESS method, Young *et al*. prepared cyclosporine nanoparticles having diameter of 400 to 700 nm. In the PCA method, the drug solution is atomized into the CO_2 compressed chamber. As the removal of solvent occurs, the solution gets supersaturated and finally precipitation occurs. In supercritical antisolvent process, drug solution is injected into the supercritical fluid and the solvent gets extracted as well as the drug solution becomes supersaturated[12].

5. Sonocrystallization method

Sonocrystallisation is the novel approach for particle size reduction. It is based on ultrasound technology. Ultrasound power is characterized by frequency range of 20- 100 khz. It enhances particle size reduction and controls the size distribution of pharmaceutical active ingredient. It involves mainly two processes, Nucleation and Crystal growth[15].

6. Emulsification Solvent Evaporation process

In a typical emulsification solvent evaporation process to produce nanoparticles, drug and polymer dissolved in a water-immiscible solvent are added drop wise to aqueous phase containing a surface stabilizer. A high shear is provided using a homogenizer, which reduces the droplet size of the organic dispersed phase. The evaporation of solvent hardens the nanoparticles. Formed nanoparticles are harvested from the aqueous slurry by lyophilization.

In a variation of the above process, the solvent removal is done by adding a large quantity of aqueous phase, which induces the rapid diffusion of the solvent from the internal into the external phase. In yet another variation, a water-miscible solvent such as acetone is added to the organic phase, which influences the droplet hardening process.

Various parameters in the emulsification solvent evaporation process that affect particle size, zeta potential, hydrophilicity and drug loading include

1. Homogenization intensity and duration
2. Type and amount of emulsifier, polymer and drug
3. Particle hardening (solvent removal) profile[10].

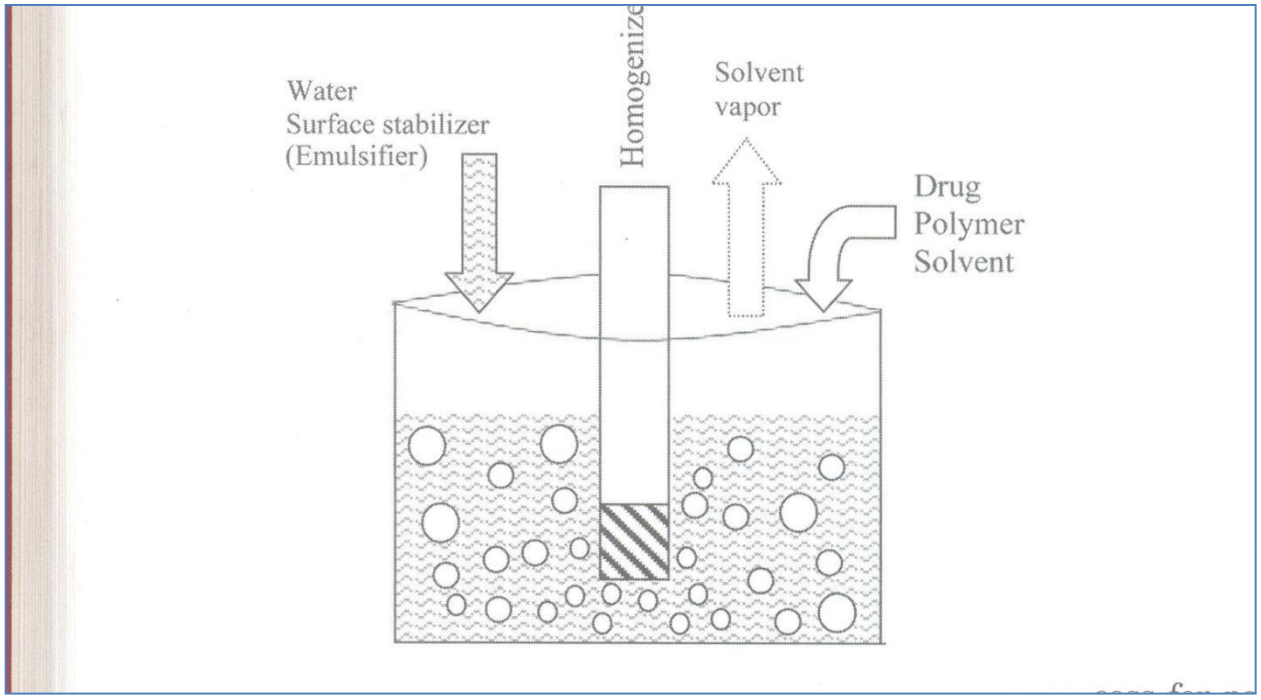


Figure 2.5: Emulsification Solvent Evaporation technique[10]

2.8 Marketed formulation of nanosuspension

Table 2.1: List of Marketed formulation of nanosuspension

Product	Active Pharmaceutical Ingredient	Manufacturing company
Rapamune	Sirolimus	Wyeth
Emend	Aprepitant	Merck
Tricor	Fenofibrate	Abbott
Megace	Megestrol acetate	Par pharmaceuticals
Triglide	Fenofibrate	Skye pharmaceuticals

2.9 Characterization of nanosuspension

1. Mean Particle Size and Particle Size Distribution

The mean particle size and particle size distribution affects saturation solubility, dissolution rate, physical stability, and *in vivo* performance of nanosuspensions. The particle size distribution and its range named Polydispersity index (PDI) can be determined by laser diffraction (LD), photon correlation spectroscopy, microscope, and coulter counter. With the help of PI, the physical stability of nanosuspensions can be determined and it should be as low as possible for the long-time stability of nanosuspensions. The PDI value in between 0.1 to 0.25 shows a fairly narrow size distribution, while PDI value more than 0.5 indicates a very broad distribution. LD can detect and quantify the drug microparticles during the production process. It also gives a volume size distribution and can be used to measure particles ranging from 0.05 up to 2 000 μm. The coulter counter gives the absolute number of particles per volume for the different size classes[16].

2. Crystalline State and Particle Morphology

Due to the impact of High Pressure Homogenization on crystalline particles, the change in their structure into amorphous or other polymorphic form occurs. Such kind of Alteration in the solid state of the drug particles and the extent of the amorphous portion is determined by X-ray diffraction analysis and supplemented by differential scanning calorimetry analysis[16].

3. Zeta potential (Surface Charge)

Zeta potential determines the physical stability of nanosuspension. Zeta potential is an indirect measurement of the thickness of the diffusion layer, i.e. can be used to predict long term stability. A minimum zeta potential of ±30 mV is required for electrostatically stabilized nanosuspensions and a minimum of ±20 mV for steric stabilization. The zeta potential values are commonly calculated by determining the particle's electrophoretic mobility and then converting the electrophoretic mobility to the zeta potential[17].

4. Saturation solubility and dissolution velocity

Nanosuspension increases the dissolution velocity and saturation solubility. Size reduction leads to increase in the dissolution pressure. An increase in solubility that occurs with relatively low particle size reduction may be mainly due to a change in surface tension leading to increased saturation solubility[17].

5. Differential Scanning Colorimetery analysis

Thermal properties of the powder samples were investigated with a differential scanning calorimeter Approximate 10mg of sample was analyzed in an open aluminium pan, and heated at scanning rate of 10^0C/min between 0^0C and 400^0C. Magnesia was used as the standard reference material to calibrate the temperature and energy scale of the DSC apparatus[16].

6. X-ray Powder Diffraction (XRPD) measurements

The crystalline state of the samples was estimated by an X-ray diffractometer. The experiments were performed in symmetrical reflection mode with a Cu line as the source of radiation. Standard runs using a 40 kV voltage, a 40mA current and a scanning rate of 0.02° min^{-1} over a 2θ range of $5–40^\circ$ were used. The samples analyzed were the same as the DSC experiments[16].

7. Drug entrapment efficiency

The percentage of incorporated drug (entrapment efficiency) was determined spectrophotometrically at particular wavelength. After centrifugation of the aqueous suspension, amount of the free drug was detected in the supernatant and the amount of incorporated drug was determined as the result of the initial drug minus the free drug[18].

The entrapment efficiency (EE %) could be achieved by the following equation:

Entrapment efficiency (%) =

$$\frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100$$

8. In vitro drug release studies

The *in vitro* release of Acyclovir from the formulation was studied through Dialysis membrane-110 (cut-off: 3500 Da) using Franz diffusion cell. The diffusion medium used was freshly prepared 0.1 N HCl solution (pH 1.2). Dialysis membrane-110, previously soaked overnight in the diffusion medium was placed between the donor and receptor compartments of the diffusion cell. Five ml of formulation was accurately placed into this assembly. The cylinder was attached to a stand and suspended in 30 ml of diffusion medium maintained at 37^0C so that the membrane just touched the receptor medium surface. The diffusion medium was stirred at low speed using magnetic stirrer. Aliquots, each of 2 ml volume were withdrawn at regular intervals and replaced by an equal volume of the receptor medium. The aliquots were suitably diluted with the receptor medium and analyzed by UV-Vis spectrophotometry at 254 nm[17].

2.10 Application of the nanosuspension

1. Enhanced oral bioavailability by nanoparticle uptake

Direct nanoparticle uptake is yet another method of improving the bioavailability of active ingredients, especially for compounds that are soluble in water but that have low permeability. Studies have suggested that direct nanoparticle uptake, especially for systems in the 10–100 nm range is a viable route for the delivery of active ingredients. There are three main routes of nanoparticle uptake: paracellular uptake, transcellular (transcytosis) uptake by enterocytes (which represent 90–95% of the epithelial cells of the intestine) and transcellular uptake by M (microfold) cells. The paracellular uptake (uptake through the interstitial space between epithelial cells) is considered to be the least effective of the three mechanisms because the space between cells ranges between 0.3 and 1 nm, which is too small for most nanoparticles to permeate. There are two methods of nanoparticle transcytosis, one is passive in which case the nanoparticle diffuses through the epithelial cell and the second method of involves the presence of receptors on the surface of the cells that capture nanoparticles with specific surface chemistry. The same basic mechanisms of transport apply to enterocytes and M cells. However the M cells are more permeable than enterocytes, and therefore M cells have been used as the target for numerous micro- and nanoparticle delivery strategies. Such M cells are located in the Peyer's patches of the lower intestine, and its role is to “sample” potential antigens present in the intestinal track. So by this way they directly go to the systemic circulation and by pass the first pass effect and enhance the oral bioavailability[10].

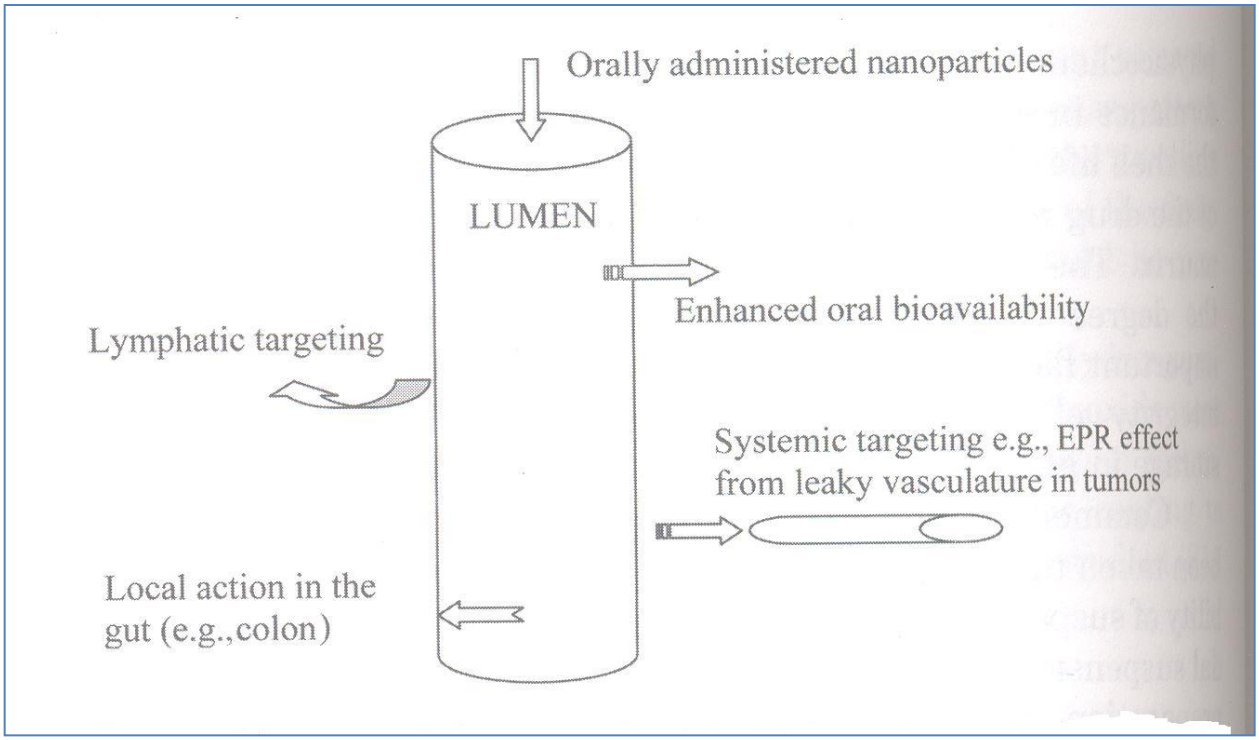


Figure 2.6: Oral uptake of nanoparticle[10]

2. Local delivery- colon specific targeting

Numerous drugs are inactivated in the GIT, because of the stomach pH, the presence of proteolytic enzymes, and the hepatic first pass effect. For drugs presenting a narrow intestinal absorption window, bioadhesive solid dosage forms offer an interesting approach to prolong the residence time at or before this absorption window. Targeting a drug directly to the colon offers many advantages like it has a near neutral pH, longer transit time, reduced digestive enzymatic activity and greater response to absorption enhancers.

Polymeric nanoparticulate carrier systems can target the inflamed tissue in intestinal bowel diseases. As no sedimentation occurs with colloidal drug carriers, they might be affected to a lesser extent because of higher diffusion rates. An important advantage of this strategy is direct contact of the carriers with the inflammation site, which in principle can provide higher drug concentration[19].

3. Parental Drug Delivery

The present approaches for parental delivery include micellar solutions, salt formation, solubilization using cosolvents, cyclodextrin complexation, and more recently vesicular systems such as liposomes and niosomes. But these methods have limitations like solubilization capacity, parental acceptability, high manufacturing cost, etc. To solve the above problems, the nanosuspension technology is used. Nanosuspensions are administered through various parental routes such as intraarticular, intraperitoneal, intravenous, etc. Additionally, nanosuspensions increase the efficacy of parenterally administered drugs. For example, Nimodipine i.v. nanosuspension and Omeprazole i.v. nanosuspension.

4. Pulmonary Drug Delivery

For pulmonary delivery, nanosuspensions can be nebulized through mechanical or ultrasonic nebulizers. Due to the presence of many small particles, all aerosol droplets contain drug nanoparticles. Aqueous suspensions of the drug can be easily nebulized and given by pulmonary route as the particle size is very small. Different types of nebulizers are available for the administration of liquid formulations.

5. Ocular Drug Delivery

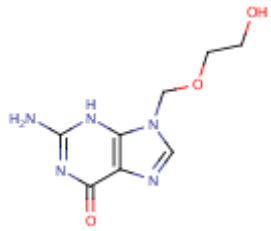
Nanosuspensions are used in ocular delivery of the drugs for sustained release. Now a days nanosuspension is prepared for ocular delivery using various Eudragit grades. Experiment showed higher availability of drug in aqueous humor of rabbit eye. Thus, nanosuspension formulation offers a promising way of improving the shelf-life and bioavailability of drug after ophthalmic application. Using an ultrasonic nebulizer, Budenoside drug nanoparticles were nebulized and the pharmacokinetics showed comparable AUC, higher Cmax and lower Tmax as that of the pulmicort respules.

6. Topical formulations

Drug nanoparticles can also be incorporated into water free ointments and creams, which have an increased saturation solubility and enhanced diffusion of drug into the skin[19].

2.11 Introduction To Drug

Table 2.2: Introduction to drug Acyclovir

Structure	
Chemical name	Acyclovir
Description	Solid state
Water Solubility	2.56 mg/ml
Log P	-1.56
Half life	2.5-3.3 hours
Melting point	256.5 - 257°C
IUPAC Name	2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]- 6H-purin-6-one, or 9-[(2-hydroxyethoxy)methyl]- guanine
Synonyms	Aciclovir, Acycloguanosine and ACV
Molecular formula	$C_8H_{11}N_5O_3$
Molecular weight	225.21g/mol
pKa	Aciclovir is an ampholyte with both weak acid and basic groups. Common literature pKa values for aciclovir are 2.27 and 9.25.
Salts, Esters,	Aciclovir is commonly used as the free acid form in solid oral

Polymorphs,Hydrates	dosage forms, whereas the sodium salt is used in parenteral dosage forms. Valaciclovir, the L-valyl ester of aciclovir, has been used orally to increase its Bioavailability. Several dipeptide ester prodrugs are being tested to assess their usefulness in therapeutics as well as some bile acid conjugate prodrugs and a phospholipid prodrug. Aciclovir is normally present in a hydrated form consisting of three aciclovir molecules to two molecules of water, corresponding to a theoretical water content of about 5%, but dose and solubility are normally expressed in units of anhydrous aciclovir. A stable anhydrous form can be obtained by drying hydrated aciclovir at temperatures above 150^0C. Although only slight and insignificant differences in solubility values exist between these two forms, the anhydrous form of aciclovir possesses poorer dissolution properties than the hydrated form.
Dosage strengths	200mg, 400mg, 800mg
Marketed formulations	Zovirax 400 mg tablet, Zovirax 800 mg tablet, Zovirax 200 mg capsule,Acyclovir 800 mg tablet, Zovirax 5% cream[21].

2.11.1 Therapeutic Indications, Therapeutic Index and Toxicity:

Aciclovir and its sodium salt are active against herpes simplex viruses (HSV-1 and HSV-2), varicella-zoster infections, and Epstein-Barr virus. Aciclovir is an acyclic nucleoside analogue, and it is incorporated into viral DNA inside an infected cell where it interferes with viral replication. Aciclovir is used intravenously in the treatment of severe initial and recurrent mucocutaneous infections caused by HSV-1, HSV-2 and varicella-zoster virus (chickenpox virus) in adults and children. It is also the drug of choice for treatment of herpes simplex encephalitis. Aciclovir is frequently given orally in the management of first and recurrent episodes of mucocutaneous herpes in selected patients, for the acute treatment of

herpes zoster (shingles) and for the treatment of chickenpox in adults and children. Aciclovir is also used topically in the treatment of mucocutaneous HSV infections, although it is substantially less effective than systemic therapy. The oral doses of aciclovir for adults range from 200 mg every 4 hour (while awake) to 800 mg three times a day for 5–10 days. For chronic suppression of recurrent infections, the dose is 400 mg twice a day. The oral dose for treatment of chickenpox and herpes zoster is 800 mg aciclovir every 4 hour for 5–10 days. Topical treatment of the affected skin or mucous membrane (not conjunctival) with 5% ointment or cream is given up to every 3 hour. For ocular herpes simplex keratitis, a 3% ointment may be applied five times daily up to every 4 hour until 3 days after healing. In young children, aciclovir is given intravenously at 250–500 mg/m^2 of body surface area every 8 hour. In older children and adults, intravenous injections are given at 5–10 mg/kg between every 8 hour. When aciclovir is given orally, the doses are typically low and serious adverse events are extremely rare, however, it is given at higher doses intravenously to individuals with serious illnesses, and is associated with more frequent toxic effects. The commonest adverse effects include nausea (2.7–8%), vomiting (2.5%), diarrhoea (1.5–2.4%), inflammation at the injection site (9%) and headache (0.6–5.9%). The commonest serious effects are neurotoxicity and nephrotoxicity[22].

2.11.2 Pharmacokinetic Properties:

1. Permeability and Absorption

A permeability study employing 3H-aciclovir indicated an apparent permeability coefficient (Papp) of about 1.19×10 cm/s. As drugs with a permeability in the range 70– 100% absorbed usually have a Papp value greater than 10×10^{-6} cm/s. Most of these data suggest that permeability of aciclovir is low. Aciclovir's absolute BA following oral administration has been reported to be in a range of 10–30% in humans. This poor systemic Bioavailability is considered to be a result of the characteristics of the drug itself and not its delivery vehicle. Its absorption occurs mainly by passive diffusion mechanism and it is slow, variable and incomplete. Maximum plasma concentrations are reached within 1.5–2.5 hours. Aciclovir shows a two compartment pharmacokinetic behavior, regardless of the dosage, duration of

treatment or frequency of administration. After multiple dose administration, steady-state concentrations are reached in 1–2 days. Some studies suggest that increasing doses result in decreasing Bioavailability or less than proportional increases in Cmax and it has been suggested that this behavior may be due to a saturable carrier system or a limited area for absorption in the gastro-intestinal (GI) tract or to the low solubility of this API. Other studies found near proportional increases in AUC with increasing doses in the dose range used clinically of 100–800 mg. Food does not appear to affect the rate and extent of absorption.

2. Distribution

Aciclovir is widely distributed into most body tissues, including the brain, kidney, lung, liver, heart tissue, muscle, spleen, placenta, uterus, vaginal mucosa and secretions, semen, saliva, amniotic fluid, aqueous humor and cerebrospinal fluid. Aciclovir demonstrates minimal protein binding (9–33%) at therapeutic plasma concentrations.

3. Metabolism and Excretion

Most of a single aciclovir dose (62–91% of an intravenous dose) is excreted unchanged in urine via glomerular filtration and tubular secretion, in adults with normal renal function. Aciclovir is metabolized in the liver, partially to 9-(carboxymethoxy) methyl]guanine (CMMG) and minimally to 8-[(hydroxy-9-(2-hydroxiethoxy)methyl]- guanine. The only known urinary metabolite is CMMG. Plasma concentrations of acyclovir appear to decline in a biphasic manner. In adults with normal renal function, $t_{1/2\,\dot{\varrho}}$ averages 0.34 hour and $t_{1/2\,\beta}$ averages 2.1–3.5 hours.

Acyclovir is a synthetic deoxyguanosine analog and it is the prototype antiviral agent that is activated by viral thymidine kinase.

Acyclovir is used orally for the treatment and prophylaxis of initial and recurrent episodes of genital and labial herpes and for the acute treatment of herpes zoster, for the treatment of varicella (chickenpox) in immunocompetent individuals[23].

2.11.3 Mechanism of action:

Viral (HSV-1, HSV-2 and VZV) thymidine kinase converts acyclovir to the acyclovir monophosphate, which is then converted to the diphosphate by cellular guanylate kinase, and finally to the triphosphate by phosphoglycerate kinase, phosphoenolpyruvate carboxykinase, and pyruvate kinase. Acyclovir triphosphate competitively inhibits viral DNA polymerase and competes with the natural deoxyguanosine triphosphate, for incorporation into viral DNA. Once incorporated, acyclovir triphosphate inhibits DNA synthesis by acting as a chain terminator[24].

2.12 Introduction To Excipients

1. Eudragit EPO

1) Nonproprietary Names

BP: Methacrylic Acid–Methyl Methacrylate Copolymer

EP: Methacrylic Acid–Methyl Methacrylate Copolymer

USP-NF: Amino Methacrylate Copolymer

2) Synonyms

Eastacryl, Kollicoat MAE, polymeric methacrylates

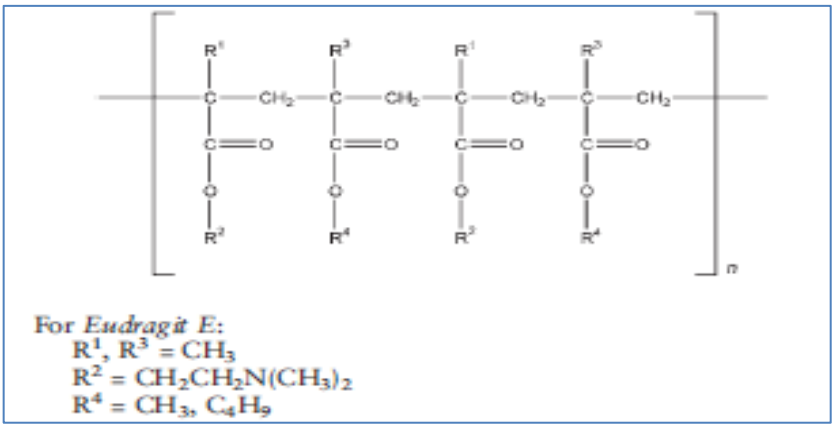
3) Chemical Name and CAS Registry Number

Poly(butyl methacrylate, (2-dimethylaminoethyl) methacrylate, methyl methacrylate) 1 : 2 : 1 and CAS Registry number-[24938-16-7]

4) Empirical Formula and Molecular Weight

$C_{18}H_{34}O_2$ 282.47

5) Structural Formula



6) Functional Category

Film-forming agent; tablet binder; tablet diluent, stabilizer

7) Applications in Pharmaceutical Formulation or Technology

Polymethacrylates are primarily used in oral capsule and tablet formulations as film-coating agents. Eudragit E is used as a plain or insulating film former. It is soluble in gastric fluid below pH 5.

8) Description

Eudragit E is a cationic polymer based on dimethylaminoethyl methacrylate and other neutral methacrylic acid esters. It is soluble in gastric fluid as well as in weakly acidic buffer solutions. Eudragit E PO is a white free-flowing powder with at least 95% of dry polymer.

9) Stability and Storage Conditions

Dry powder polymer forms are stable at temperatures less than 30^{8}C. Above this temperature, powders tend to form clumps, although this does not affect the quality of the substance and the clumps can be readily broken up. Dry powders are stable for at least 3 years if stored in a tightly closed container at less than 30^{0}C.

10) Incompatibilities

It doesn't show any visual incompatibility with other polymers or drugs.

11) Safety

Polymethacrylate copolymers are widely used as film-coating materials in oral pharmaceutical formulations. They are also used in topical formulations and are generally regarded as nontoxic and nonirritant materials. Based on relevant chronic oral toxicity studies in rats and conventionally calculated with a safety

factor of 100, a daily intake of 2 mg/kg body-weight of Eudragit EPO may be regarded as essentially safe in humans.

12) Regulatory Status

Included in the FDA Inactive Ingredients Database (oral capsules and tablets). Included in nonparenteral medicines licensed in the UK. Included in the Canadian List of Acceptable Non-medicinal Ingredients.

2. Ethanol

1) Nonproprietary Names

BP: Ethanol (96%)

JP: Ethanol

PhEur: Ethanol (96 per cent)

USP: Alcohol

2) Synonyms

Ethanolum (96 per centum); ethyl alcohol; ethyl hydroxide; grain, alcohol; methyl carbinol.

3) Chemical Name and CAS Registry Number

Ethanol [64-17-5]

4) Empirical Formula and Molecular Weight

C_2H_6O 46.07

5) Structural Formula

6) Functional Category

Antimicrobial preservative, disinfectant, skin penetrant, solvent.

7) Applications in Pharmaceutical Formulation or Technology

Ethanol and aqueous ethanol solutions of various concentrations are widely used in pharmaceutical formulations and cosmetics. Although ethanol is primarily used as a solvent, it is also employed as a disinfectant, and in solutions as an antimicrobial preservative. Topical ethanol solutions are used in the development of transdermal drug delivery systems as penetration enhancers. Ethanol has also been used in the development of transdermal preparations as a co-surfactant.

8) Description

In the BP 2009, the term 'ethanol' used without other qualification refers to ethanol containing 599.5% v/v of C_2H_6O. The term 'alcohol', without other qualification, refers to ethanol 95.1–96.9% v/v. Where other strengths are intended, the term 'alcohol' or 'ethanol' is used, followed by the statement of the strength. In the PhEur 6.0, anhydrous ethanol contains not less than 99.5% v/v of C_2H_6O at 208C. The term ethanol (96%) is used to describe the material containing water and 95.1–96.9% v/v of C_2H_6O at 20^0C.

9) Stability and Storage Conditions

Aqueous ethanol solutions may be sterilized by autoclaving or by filtration and should be stored in airtight containers, in a cool place.

10) Incompatibilities

In acidic conditions, ethanol solutions may react vigorously with oxidizing materials. Mixtures with alkali may darken in color owing to a reaction with residual amounts of aldehyde. Organic salts or acacia may be precipitated from aqueous solutions or dispersions. Ethanol solutions are also incompatible with aluminum containers and may interact with some drugs.

11) Safety

Ethanol and aqueous ethanol solutions are widely used in a variety of pharmaceutical formulations and cosmetics. It is also consumed in alcoholic beverages. Ethanol is rapidly absorbed from the gastrointestinal tract and the vapor may be absorbed through the lungs; it is metabolized, mainly in the liver, to acetaldehyde, which is further oxidized to acetate. Ethanol is a central nervous system depressant and ingestion of low to moderate quantities can lead to symptoms of intoxication including muscle in coordination, visual impairment, slurred speech, etc. Ingestion of higher concentrations may cause depression of medullary action, lethargy, amnesia, hypothermia, hypoglycemia, stupor, coma, respiratory depression, and cardiovascular collapse. The lethal human blood-alcohol concentration is generally estimated to be 400–500 mg/100 mL.

12) Regulatory Status

Included in the FDA Inactive Ingredients Database (dental preparations; inhalations; IM, IV, and SC injections; nasal and ophthalmic preparations; oral capsules, solutions, suspensions, syrups, and tablets; rectal, topical, and transdermal preparations). Included in the Canadian List of Acceptable Non-medicinal Ingredients. Included in nonparenteral and parenteral medicines licensed in the UK.

3. Tween 80

1) Nonproprietary Names

BP: Polysorbate 80

JP: Polysorbate 80

PhEur: Polysorbate 80

USP-NF: Polysorbate 80

2) Synonyms

Armotan PMO 20, Capmul POE-O, Cremophor PS 80, Crillet 4, Crillet 50, Drewmulse POE-SMO, Drewpone 80K, Durfax 80, Durfax 80K, E433, Emrite 6120, Eumulgin SMO, Glycosperse O-20, Hodag PSMO-20, Liposorb O-20, Liposorb O-20K, Montanox 80, polyoxyethylene 20 oleate, polysorbatum 80, Protasorb O-20, Ritabate 80, (Z)-sorbitan mono-9-octadecenoate poly(oxy1,2-ethanediyl) derivatives, Tego SMO 80, Tego SMO 80V

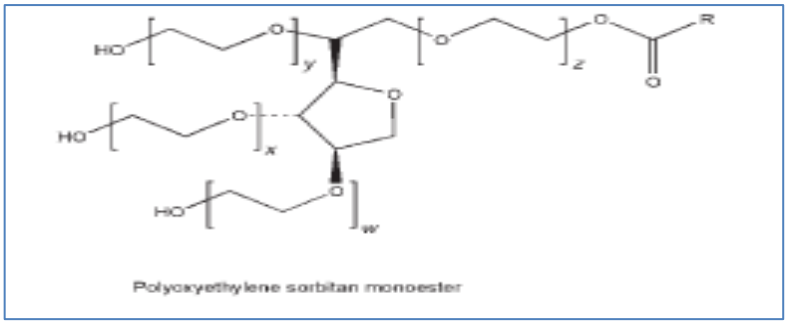
3) Chemical Names and CAS Registry Numbers

Polyoxyethylene 20 sorbitan monooleate [9005-65-6]

4) Empirical Formula and Molecular Weight

$C_{64}H1_{24}O_{26}$ 1310

5) Structural Formula



Polyoxyethylene sorbitan monoester

$w + x + y + z = 20$ (Polysorbates 20, 40, 60, 65, 80, and 85)

6) Functional Category

Dispersing agent; emulsifying agent; nonionic surfactant; solubilizing agent; suspending agent; wetting agent.

7) Applications in Pharmaceutical Formulation or Technology

Emulsifying agent-Used alone in oil-in-water emulsions 1–15, Used in combination with hydrophilic emulsifiers in oil-in-water emulsions-1–10, Used to increase the water-holding properties of Ointments-1–10 Solubilizing agent-For poorly soluble active constituents in lipophilic Bases-1–15 Wetting agent-For insoluble active constituents in lipophilic bases- 0.1–3

8) Description

Polysorbates have a characteristic odor and a warm, somewhat bitter taste. Tween 80 is Yellow oily liquid, although it should be noted that the absolute color intensity of the products may vary from batch to batch and from manufacturer to manufacturer.

9) Stability and Storage Conditions

Polysorbates are stable to electrolytes and weak acids and bases; gradual saponification occurs with strong acids and bases. The oleic acid esters are sensitive to oxidation. Polysorbates are hygroscopic and should be examined for water content prior to use and dried if necessary. Also, in common with other polyoxyethylene surfactants, prolonged storage can lead to the formation of peroxides. Polysorbates should be stored in a well-closed container, protected from light, in a cool, dry place.

10) Incompatibilities

Discoloration and/or precipitation occur with various substances, especially phenols, tannins, tars, and tarlike materials. The antimicrobial activity of paraben preservatives is reduced in the presence of polysorbates.

11) Safety

Polysorbates are widely used in cosmetics, food products, and oral, parenteral and topical pharmaceutical formulations, and are generally regarded as nontoxic and nonirritant materials. There have, however, been occasional reports of hypersensitivity to polysorbates following their topical and intramuscular use. Polysorbates have also been associated with serious adverse effects, including some deaths, in low-birthweight infants intravenously administered a vitamin E preparation containing a mixture of polysorbates 20 and 80. When heated to decomposition, the polysorbates emit acrid smoke and irritating fumes.

12) Regulatory Status

Polysorbates 80 is GRAS listed. Polysorbate 80 is accepted as food additives in Europe. Polysorbate 80 is included in the FDA Inactive Ingredients Database (IM, IV, oral, rectal, topical, and vaginal preparations). Polysorbates are included in parenteral and nonparenteral medicines licensed in the UK. Polysorbate 80 is included in the Canadian List of Acceptable Non-medicinal Ingredients[25].

CHAPTER 3
LITERATURE REVIEW

3.1 LITERATURE REVIEW ON PERMEATION ENHANCEMENT AND ROLE OF NANOPARTICLES

1. Edgar Acosta suggested that nanoparticles in the 100–1000 nm range are capable of producing substantial improvement in the bioavailability of the active ingredients. In most cases, this improvement in bioavailability seems to be linked to the direct uptake of the nanoparticle. Furthermore, direct nanoparticle uptake is controlled by the size and surface chemistry of the nanoparticle system. The use of this direct nanoparticle uptake, in particular for soluble but poorly absorbed ingredients, is one of the areas that needs to be explored in the future, as well as the potential side effects of these nanoparticle carriers.

2. A. Christy Hunter et al reported that the oral route for delivery of pharmaceuticals is the most widely used and accepted. Nanoparticles and microparticles are increasingly being applied within this arena to optimize drug targeting and bioavailability. Frequently the carrier systems used are either constructed from or contain polymeric materials. Examples of these nanocarriers include polymeric nanoparticles, solid lipid nanocarriers, self nano-emulsifying drug delivery systems and nanocrystals. The purpose of such studies is to describe these cutting edge technologies and specifically focus on the interaction and fate of these polymers within the gastrointestinal system.

3. Shaikh MS I et al suggested that Need for an oral replacement to parenteral delivery has led to renewed attentiveness in excipients like intestinal permeation enhancers which improve oral drug bioavailability. Delivery of a drug by oral route is predominantly restricted by pre-systemic degradation and poor penetration across the

gut

wall.

The major challenge in the oral drug delivery is the development of novel dosage forms to endorse absorption of poorly permeable drugs across the intestinal epithelium.

4. María José Cano-Cebrián et al reported that Hydrophilic drugs usually present low bioavailability after oral administration. One of the causes of this low bioavailability is their poor intestinal permeation through the paracellular pathway. This pathway is actually restricted by the presence of tight junctions at the apical side of the enterocytes. In the last few years, great interest has been focused on the structure and cellular regulation of tight junctions, materializing in more in-depth knowledge of this intestinal barrier. The *in vitro* and *in situ* studies involving the most promising paracellular permeation enhancers (e.g., medium chain fatty acids and chitosan and its derivatives), analyzing the degree of drug absorption enhancement achieved, as well as the potential associated toxicity.

5. M. Thanou et al studied that Chitosan, when protonated (pH<6.5), is able to increase the paracellular permeability of peptide drugs across mucosal epithelia. Tri methyl chitosan chloride (TMC) has been synthesized at different degrees of quaternization. This quaternized polymer forms complexes with anionic macromolecules and gels or solutions with cationic or neutral compounds in aqueous environments and neutral pH values. TMC has been shown to considerably increase the permeation and/or absorption of neutral and cationic peptide analogs across intestinal epithelia. It reversibly interacts with components of the tight junctions, leading to widening of the paracellular routes.

6. Lichen Yin et al developed that the mucoadhesion and the permeation enhancing effects of Tri methyl chitosan (TMC) and thiolated polymers for oral delivery of insulin. TMC-Cys with various molecular weights (30, 200, and 500 kDa) and quaternization degrees (15 and 30%) was allowed to form polyelectrolyte nanoparticles with insulin through self-assembly, which demonstrated particle size of

100–200 nm, zeta potential of +12 to +18 mV, and high encapsulation efficiency. TMC-Cys/insulin nanoparticles (TMC-Cys NP) showed a 2.1–4.7-fold increase in mucoadhesion compared to TMC/insulin nanoparticles (TMC NP), which might be partly attributed to disulfide formation between TMC-Cys and mucin as evidenced by DSC measurement.

7. A. Bernkop-Schnurch et al reported that Thiolated polymers (=thiomers) in combination with reduced glutathione (GSH) were shown to improve the uptake of hydrophilic macromolecules from the GI tract. The mechanism responsible for this permeation enhancing effect seems to be based on the thiol groups of the polymer. These groups inhibit protein tyrosine phosphatase, being involved in the closing process of tight junctions, via a GSH-mediated mechanism. By utilizing poly(acrylic acid)–cysteine/GSH as carrier matrix, an absolute oral bioavailability for low molecular weight heparin of 19.9±9.3% and a pharmacological efficacy—calculated on the basis of the areas under the reduction in serum glucose levels of the oral formulation versus subcutaneous (s.c.) injection—for orally given insulin of 7% could be achieved.

8. Mandal Surjyanarayan et al prepared the solid lipid nanoparticles of Flunarizine hydrochloride to improve its oral bioavailability by drug diffusion profile. Flunarizine Hydrochloride nanosuspension stabilized by poloxamer F 68, was prepared by using high speed homogenization technique and was lyophilized to obtain nanoparticles by using mannitol as cryoprotectant. Developed nanoparticle was characterized for particle size and size distribution, % drug content and drug entrapment efficiency. Ex vivo study was also carried out using rat ileum along with simulated intestinal fluid.

3.2 LITERATURE REVIEW ON DRUG AND POLYMER

1. J. Arnal et al reported that Literature data relevant to the decision to allow a waiver of in vivo bioequivalence (BE) testing (biowaiver) for the approval of immediate release (IR) solid oral dosage forms containing aciclovir are reviewed. Aciclovir therapeutic use and therapeutic index, pharmacokinetic properties, data related to the possibility of excipient interactions and reported BE/bioavailability (BA) studies were also taken into consideration in order to ascertain whether a biowaiver can be recommended. According to the Biopharmaceutics Classification System (BCS) and considering tablet strengths up to 400 mg, aciclovir would be BCS Class III. Hence, if: (a) the test product contains only excipients present in acyclovir solid oral IR drug products approved in ICH or associated countries, for instance as presented in this article; and (b) the comparator and the test product both are very rapidly dissolving, a biowaiver for IR aciclovir solid oral drug products is considered justified for all tablet strengths.

2. Praful Balavant Deshpande et al studied controlled release polymeric ocular delivery of acyclovir. Reservoir-type ocular inserts were fabricated by sandwiching hydroxypropyl methylcellulose (HPMC) matrix film containing acyclovir between two rate controlling membranes of cellulose acetate phthalate (CAP). The formulations were subjected to various physico-chemical evaluations. The in vitro release profile of all the formulations showed a steady, controlled drug release up to 20 h with non-Fickian diffusion behavior. A high correlation coefficient found between in vitro/in vivo release rate studies. Formation of acyclovir complex was confirmed by differential scanning calorimetry.

3. P. dandagi et al formulated a novel ophthalmic nanosuspension (ONS), an alternative carrier system to traditional colloidal carriers for controlled release (CR) of acyclovir (ACV). In the present study, ONS is employed to avoid some of major disadvantages of colloidal carriers systems such as instability in cul de sac and short half life by

increasing efficiency of drug encapsulation as well as by CR. A quassi-emulsion solvent evaporation method was used to prepare ACV loaded Eudragit RS 100 ONS with the aim of improved ocular bioavailability and distribution. Five different formulations were prepared and evaluated for pH of ONS, particle size, entrapment efficiency, differential scanning calorimetry (DSC), in vitro release profile, in vivo release studies and stability studies.

4. Cung An Nguyen et al studied that nanoparticulate dispersion were produced by an emulsification diffusion method involving the use of partially water miscible solvents and the mutual saturation of the aqueous and organic phases prior to the emulsification in order to reduce the initial thermodynamic instability of the emulsion. Because of the self-emulsifying properties of the methacrylic acid copolymers, it was possible to prepare aqueous dispersions of colloidal size containing up to 30% wt/vol of Eudragit RL, RS, and E using 2-butanone or methyl acetate as partially water miscible solvents, but without any surfactant.

5. Le Thi Mai Hoa et al reported that Polymeric drug nanoparticles were prepared by emulsion solvent evaporation method. In this study, prepared the polymeric drug nanoparticles consist of drug and Eudragit E 100. The morphology structure was investigated by scanning electron microscopy (SEM). The interactions between the drug and polymer were investigated by Fourier transform infrared spectroscopy (FTIR). The size distribution was measured by means of Dynamic Light Scattering. The nanoparticles have an average size of about 150 nm. The incorporation ability of drugs in the polymeric nanoparticles depended on the integration between polymer and drug as well as the glass transition temperature of the polymer.

6. Sanjay dey et al developed different formulations containing carbopol 934 P, acyclovir and selected concentrations of DMSO to evaluate drug content, spreadability, viscosity, pH and in-vitro permeation through mouse epidermis and

porcine skin. A maximum permeation flux of acyclovir through porcine skin was observed with an enhancement ratio of 1.55, when DMSO was incorporated at a concentration of 10%w/v in gel system. From histopathological and FTIR studies it was evident that the permeation of acyclovir, across mouse and porcine skin, were increased in the presence of DMSO which can be attributed to the partial extraction of lipids in the stratum corneum.

7. Bothiraja chellampillai et al studied the andrographolide-loaded pH-sensitive nanoparticles were prepared by nanoprecipitation technique using Eudragit® EPO (cationic poly methacrylate copolymer). The 3^2 factorial design was used to optimize the amount of polymer and stabilizer (Pluronic® F-68). The optimized batch obtained using 0.45% w/v of Eudragit® EPO and 0.6% w/v of Pluronic® F-68 showed high-encapsulation efficiency of 93.8 ± 0.67% with particle size of 255 ± 9 nm and zeta potential of 29.3 ± 3.4 mV. The bioavailability of andrographolide from optimized nanoparticles and pure andrographolide was assessed in male Wistar albino rats at a dose of 10 mg/kg. The improved dissolution rate owing to its reduced particle size, increased surface area and reduced diffusion layer thickness may have contributed to oral bioavailability.

8. P. Khachane et al reported that Eudragit EPO nanoparticles (EPO NP) are used for improving therapeutic efficacy of meloxicam (MLX). MLX loaded EPO NP were prepared by nanoprecipitation method and were characterized for particle size, encapsulation efficiency and for morphology. The *in vitro* dissolution profile of MLX loaded EPO NP and MLX suspension was evaluated. MLX loaded EPO NP had particle size of ~100 nm and the encapsulation efficiency of MLX was ~90%. The EPO NP significantly improved anti-inflammatory activity of MLX ($P < 0.01$) as compared to that of MLX suspension. The enhanced anti-inflammatory effect was maintained for a longer duration (6 h) in case of MLX loaded EPO NP. Oral administration of MLX loaded EPO NP also resulted in lesser ulcerogenicity as

compared to that of MLX suspension indicating that nanoparticles can also decrease the adverse effects associated with MLX treatment.

9. I. Garay et al developed a model process to obtain microparticles of an acrylate–methacrylate copolymer (Eudragit L100® and Eudragit EPO®) using supercritical carbon dioxide (SC-CO_2) as antisolvent (GAS). After studying the behaviour of the copolymers in SC-CO_2 at different operation conditions (pressure, temperature and presence of ethanol (EtOH)), efforts were invested in the optimization of Eudragit EPO® precipitation from an organic solution using carbon dioxide as antisolvent in batch mode. After loading the precipitation vessel with a fixed quantity of the copolymer dissolution, the SC-CO_2 has been added until the pressure of operation has been reached. Three process parameters, namely, solution nature, presence of surfactants and organic solvent removal step, have been evaluated. Microparticles with mean diameter from about 2 to 12 μm are obtained.

10. Rouslan Moustafine et al formulated oral colon drug delivery systems, swelling and release behavior of synthesized interpolyelectrolyte complexes (IPEC) between sodium alginate and Eudragit® EPO were investigated. The micro environmental changes in IPECs structure as a function of pH during swellability testing were investigated using FT-IR spectroscopy and elementary analysis.

3.3 LITERATURE REVIEW ON NANOSUSPENSION TECHNOLOGY

1. V.B. Patravale et al reported that Techniques such as media milling and high pressure homogenization have been used commercially for producing nanosuspensions. Recently, the engineering of nanosuspensions employing emulsions and microemulsions as templates has been addressed in the literature. The unique features of nanosuspensions have enabled their use in various dosage forms, including specialized delivery systems such as mucoadhesive hydrogels. Rapid strides have been made in the delivery of nanosuspensions by parenteral, peroral, ocular and pulmonary routes.

2. Y.K. Agrawal et al reported that Over the last decades, nanoparticle engineering has been developed and reported for pharmaceutical applications. Nanotechnology can be used to solve the problems associated with various approaches like solubility, permeability, stability at room temperature, compatibility with solvent, excipient, and photostability. Nanotechnology is defined as the science and engineering carried out in the nanoscale that is 10^{-9} m. The drug microparticles/micronized drug powder is transferred to drug nanoparticles by techniques like Bottom-Up Technology and Top-Down Technology. This approach is useful for molecules with poor solubility, poor permeability, or both, which poses a significant challenge for the formulators.

3. Prasanna Lakshmi et al suggested that Stability and bioavailability of the drugs can be improved by the Nanosuspension technology. Preparation of nanosuspension is simple and applicable to all drugs which are aqueous insoluble. Nanosuspensions are prepared by using wet mill, high pressure homogenizer, emulsion-solvent evaporation, melt emulsification method and super critical fluid techniques. Nanosuspensions can be delivered by oral, parenteral, pulmonary and ocular routes.

Nanosuspensions can also be used for targeted drug delivery when incorporated in the ocular inserts and mucoadhesive hydrogels.

4. Dhananjay S. Singare et al studied that nanosuspension manufacturing process was carried out on bead mill. Formulation factors evaluated were ratio of polymer to drug and ratio of surfactant to drug, whereas process parameters were milling time and milling speed. Responses measured in this study include zeta potential and, particle size distribution d(90). The ANOVA test reveals that ratio of polymer to drug and milling speed has significant effect on zeta potential whereas milling time and milling speed has significant effect on the particle size distribution of nanosuspension. The derived polynomial equation and contour graph aid in predicting the values of selected independent variables for preparation of optimum nanosuspension formulations with desired properties.

5. Ruolan Xiong et al developed that nimodipine nanosuspension was prepared by high-pressure homogenization (HPH). The effects of the production parameters such as pressure, cycle numbers and crushing principles on the mean particle size, 99% diameter and polydispersity of the nanosuspension were investigated. Characterization of the product was performed by scanning electron microscope (SEM) and differential scanning calorimeter (DSC). The safety of the nimodipine nanosuspension was discussed with special attention to contamination by microparticles and the increase in saturation solubility *C*s. Irritability study in rabbits showed that this formulation provided less local irritation and phlebitis risks than the commercial ethanol product, which represented a promising new drug formulation for intravenous therapy of subarachnoid hemorrhage (SAH)-related vasospasm.

6. Jan Mo schwitzer et al studied that the feasibility of omeprazole stabilization using the DissoCube technology was carried out and also various optimal production parameters for a stable, highly concentrated omeprazole nanosuspension were found. The high performance liquid chromatography analysis has proved the predominance

of the nanosuspension produced by high pressure homogenization in comparison to an aqueous solution. Even 1 month after production no discoloration or drug loss was recognizable when the nanosuspension was produced at 0 8C. As a result it can be stated that the production of nanosuspensions by high pressure homogenization is suitable for preventing degradation of labile drugs.

7. Wei Li et al suggested that the particle size reduction effect of RH on dissolution and absorption, three suspensions that containing different sized particles were prepared by high pressure homogenization and in vitro/in vivo evaluations were carried out. DSC and powder X-ray diffraction were used to study crystalline state of freeze dried powder of RH suspensions and the results showed that particles of RH microsuspension and nanosuspension remained in the same crystalline state as coarse suspension, but had lower lattice energy. The findings revealed that particle size reduction can influence RH absorption in gastrointestinal tract and nanosuspension can enhance oral bioavailability of RH in rats.

CHAPTER 4
EXPERIMENTAL WORK

4. EXPERIMENTAL WORK

4.1 Materials and Equipment Used

Materials used:

Table 4.1: List of Materials used

Materials	Company name
Acyclovir	Kindly provided by Cadila Healthcare Pvt Ltd (Ahmedabad, India)
Eudragit EPO	Kindly provided by Evonik India Pvt Ltd (Mumbai, India)
Tween 80	Central Drug House Pvt Ltd (Ahmedabad, India)
Ethanol AR	Ureca consumers co. op. stores Ltd (Ahmedabad, India)
HPMC 5 CPS	Central Drug House Pvt Ltd (Ahmedabad, India)
HPC	Central Drug House Pvt Ltd (Ahmedabad, India)
PVP K 30	Central Drug House Pvt Ltd (Ahmedabad, India)
Poloxamer 188	Kindly provided by Torrent Research Centre (Ahmedabad, India)
Sodium Lauryl Sulphate	Central Drug House Pvt Ltd (Ahmedabad, India)
Hydrochloric acid AR	S.D Fine Chemical Ltd (Mumbai, India)
Potassium hydrogen phosphate AR	Central Drug House Pvt Ltd (Ahmedabad, India)
Sodium Hydroxide AR	Central Drug House Pvt Ltd (Ahmedabad, India)
Distilled water	Freshly prepared in the Research laboratory

Equipment Used:

Table 4.2: List of Equipments used

Equipments	Company name
Electronic balance	Electronic balance, BL-220 H, Shimadzu corporation, (India)
Ultrasonicator bath	EIE Instruments Pvt Ltd, (India)
UV Visible Spectrophotometer	UV 1800 Shimadzu, Scientific Instruments, (Japan)
Magnetic stirrer with hot plate	EIE Instruments Pvt Ltd, (India)
Probe sonicator	Trans-O-Sonic Pvt Ltd, (India)
Refrigerated centrifuge	Bio Lab Pvt Ltd, (India)
Franz diffusion cell	Durga Glass Works, (India)
Hot Air Oven	EIE Instruments Pvt Ltd, (India)
Fourier transform Infrared Spectrophotometer	Jasco FTIR 6100, (Japan)
Differential Scanning Colorimetry	DSC 60 Shimadzu, Asia pacific, (Japan)
Zeta Sizer	Malvern Instruments Ltd (United Kingdom)
XRD	Xpert MPD, Phillips (Holland)
TEM	Tecnai 20, Phillips (Holland)

4.2 IDENTIFICATION OF ACYCLOVIR:

4.2.1 Melting Point Determination:

Melting point is the temperature at which the pure liquid and solid exist in the equilibrium. In the practice it is taken as equilibrium mixture at an external pressure of 1atmosphere. The thiel's tube method of melting point determination in liquid paraffin was used in the present study. Melting point was found to be 257°C[27].

Table 4.3: Melting point determination

	Standard	Observed
Melting point	256.5 - 257°C*	257°C

*USP-2007

Result: The melting point of Acyclovir was found to be 257°C.

Conclusion: The melting point determined is within the range of standard value, hence, it is concluded that the drug sample having intimate physical property as standard drug.

4.2.2 IR Spectra:

IR spectra of drug in KBR pellets at moderate scanning speed between 4000-400 cm^{-1} was carried out using FTIR (Jasco FTIR 6100 TYPE A, Japan). All the powder samples were dried under vacuum prior to obtaining any spectra in order to remove the influence of residual moisture[28].

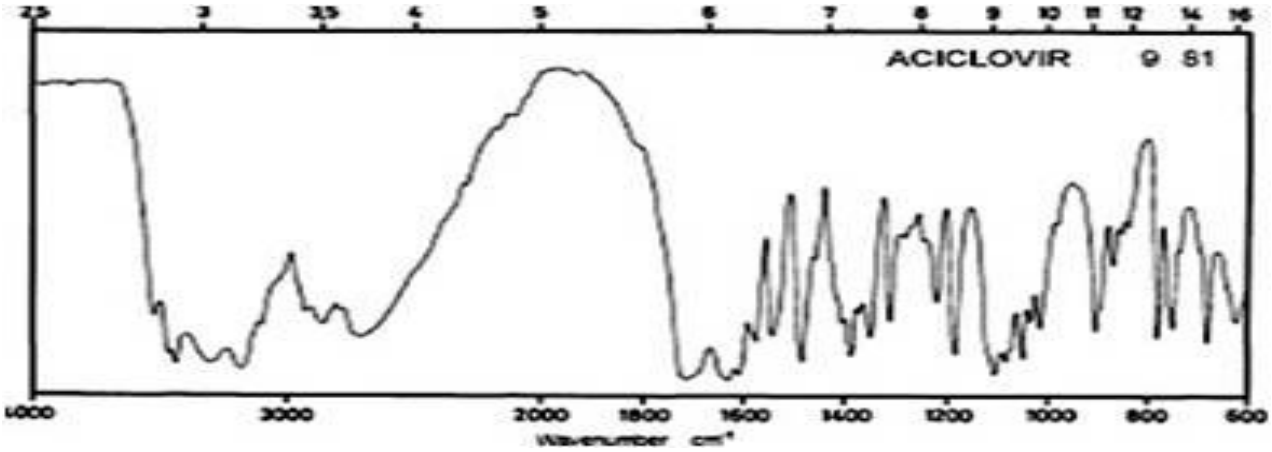


Figure 4.1: Reference FTIR spectra of Acyclovir

*BP-2010

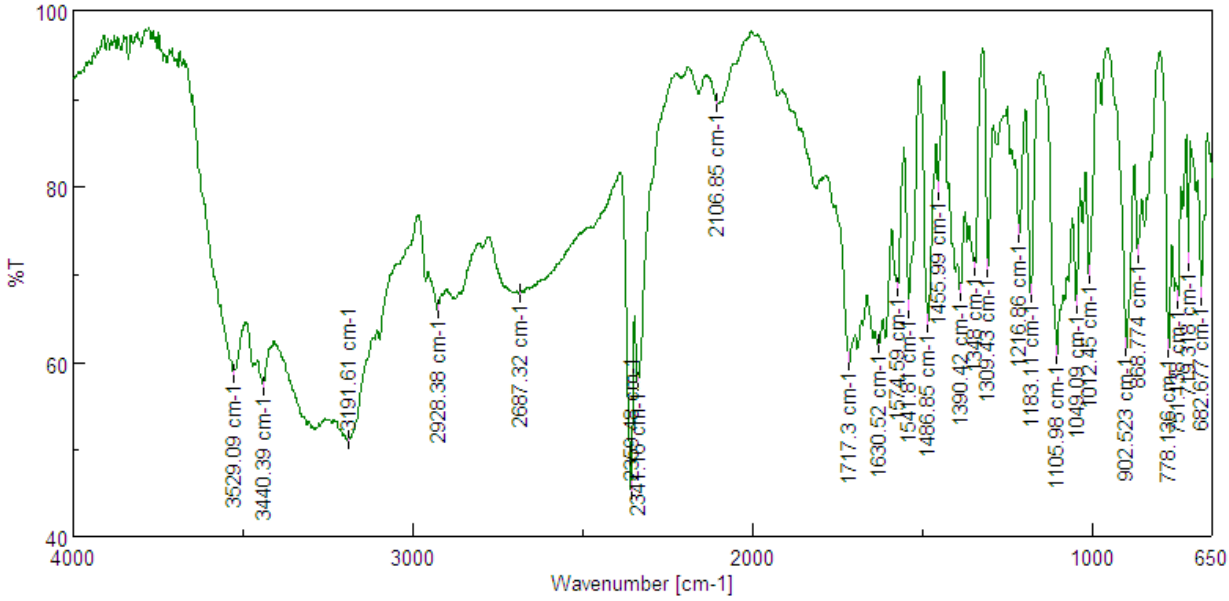


Figure 4.2: Observed FTIR spectra of Acyclovir

Table 4.4: Comparison of Reference and Test frequencies of Acyclovir

Functional Group	Standard Frequency	Observed Frequency
N-H stretching	3600-3200 cm^{-1}	3529.09 cm^{-1}
C-H of Aromatic ring	3100-3000 cm^{-1}	2928 cm^{-1}
O-H stretching	3200 cm^{-1}	3191 cm^{-1}
C-H of Alkenes	3100-3000 cm^{-1}	2928 cm^{-1}
C-H of Alkanes	3000 cm^{-1}	2928 cm^{-1}

Discussion: The sample spectrum of Acyclovir was compared with standard one and both spectra were found similar in peak values representing wave numbers. Thus, it can be concluded that procured Acyclovir sample was a pure drug.

4.2.3 UV absorption maxima of Acyclovir:

a) UV absorption maxima of Acyclovir in 0.1 N HCl:

UV scanning was done for 100 µg/ml drug solution from 200-800 nm in 0.1 N HCl as a blank using Shimadzu UV 1800 double beam UV/Visible spectrophotometer. The absorption maxima was found to be at 254 nm.

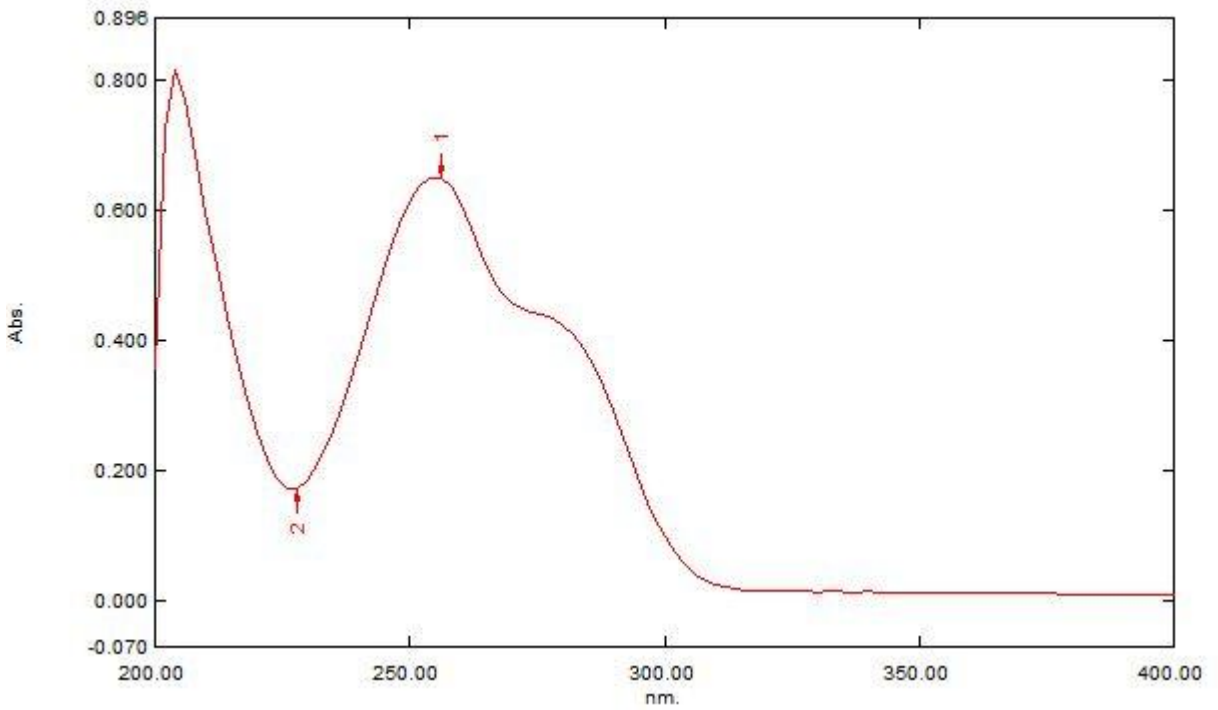


Figure 4.3: UV absorbance spectra of Acyclovir in 0.1 N HCl

Table 4.5: Acyclovir maximum absorbance and concentration

Concentration	Λmax	Absorbance
16µg/ml	254 nm*	0.8182

*IP-2010

Conclusion:

The above UV spectra of Acyclovir shows the maximum absorption at 254 nm, which was constant after dilution and similar to the reported standard value (254 nm). This also indicates identity and purity of the drug sample[26].

4.3 ESTIMATION OF ACYCLOVIR:

4.3.1 Standard curve of Acylovir in 0.1 N HCl (pH 1.2):

Preparation of stock solution:

10 mg of Acylovir was accurately weighed and transferred into 100 ml volumetric flask. It was dissolved in 0.1 N HCl (pH 1.2) and volume was made up to the mark with 0.1 N HCl (pH 1.2) to get 100 µg/ml solution.

Preparation of Standard Curve:

A primary stock solution was prepared by weighing accurately 100 mg of Acyclovir on an analytical balance. The drug was transferred into 100 ml volumetric flask and then 25 ml of 0.1 N HCl (pH 1.2) was added and sonicated for 15 mins. The final volume was made up to 100 ml with 0.1 N HCl (pH 1.2) and was mixed well. A series of dilutions were prepared by withdrawing required amount of volume like 0.6, 0.8, 1, 1.2, 1.4, 1.6 ml from stock solution (100 µg/ml) and were transferred to a 10 ml volumetric flask. The final volume was made up to 10 ml with 0.1 N HCl (pH 1.2) to get concentration in the range of 6-16 µg/ml respectively[26].

Table 4.6: Standard curve of Acyclovir in 0.1 N HCl **(pH 1.2)**

Concentration (µg/ml)	Absorbance
0	**0**
4	**0.2136**
6	**0.3082**
8	**0.3996**
10	**0.4952**
12	**0.6106**
14	**0.7103**
16	**0.8182**

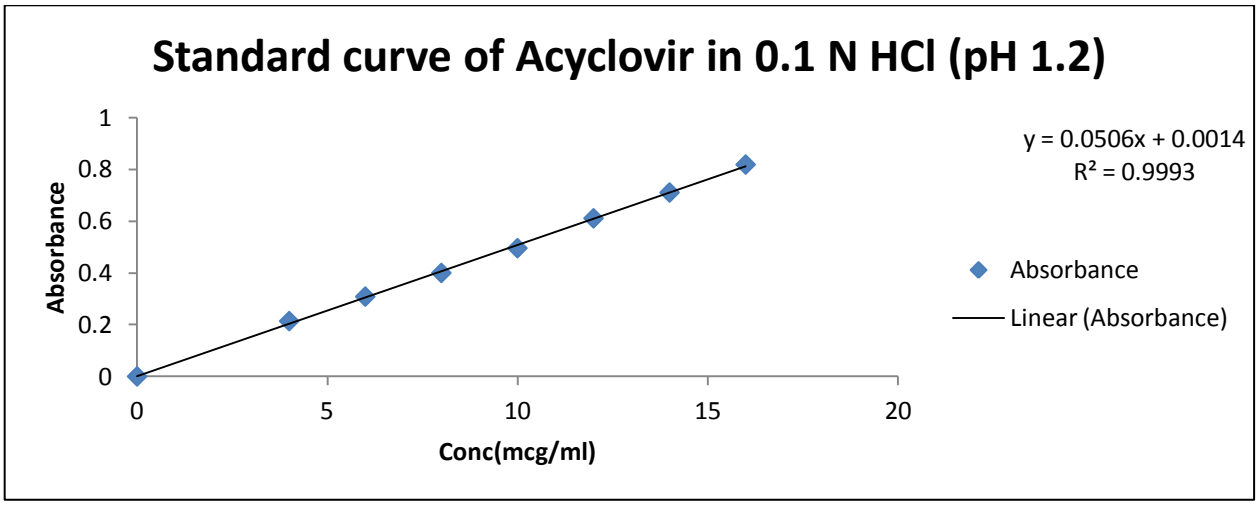


Figure 4.4: Standard curve of Acyclovir in 0.1 N HCl (pH 1.2)

Regression Analysis

Regression analysis for standard curve of acyclovir in 0.1 N HCl

Table 4.7: Regression analysis for standard curve of acyclovir in 0.1 N HCl

Regression parameter	Value
Correlation coefficient	0.9995
Slope	0.0509
Intercept	0.0029

4.3.2 Standard curve of Acylovir in Phosphate buffer (pH 6.8):

Preparation of stock solution:

10 mg of Acylovir was accurately weighed and transferred into 100 ml volumetric flask. It was dissolved in Phosphate buffer (pH 6.8) and volume was made up to the mark with Phosphate buffer (pH 6.8) to get 100 µg/ml solution.

Preparation of Standard Curve:

A primary stock solution was prepared by weighing accurately 100 mg of Acyclovir on an analytical balance. The drug was transferred into 100 ml volumetric flask then 25 ml of Phosphate buffer (pH 6.8) was added and sonicated for 15 mins. The final volume was made up to 100 ml with Phosphate buffer (pH 6.8) and was mixed well. A series of dilutions were prepared by withdrawing required amount of volume like 0.4, 0.6, 0.8, 1, 1.2, 1.4 ml from stock solution (100 µg/ml) and were transferred to a 10 ml volumetric flask. The final volume was made up to 10 ml with Phosphate buffer (pH 6.8) to get concentration in the range of 4-14µg/ml respectively[26].

Table 4.8: Standard Curve of Acyclovir in Phosphate buffer (pH 6.8)

Concentration (µg/ml)	Absorbance
0	**0**
2	**0.1135**
4	**0.2264**
6	**0.3842**
8	**0.5127**
10	**0.6228**
12	**0.7443**
14	**0.8279**

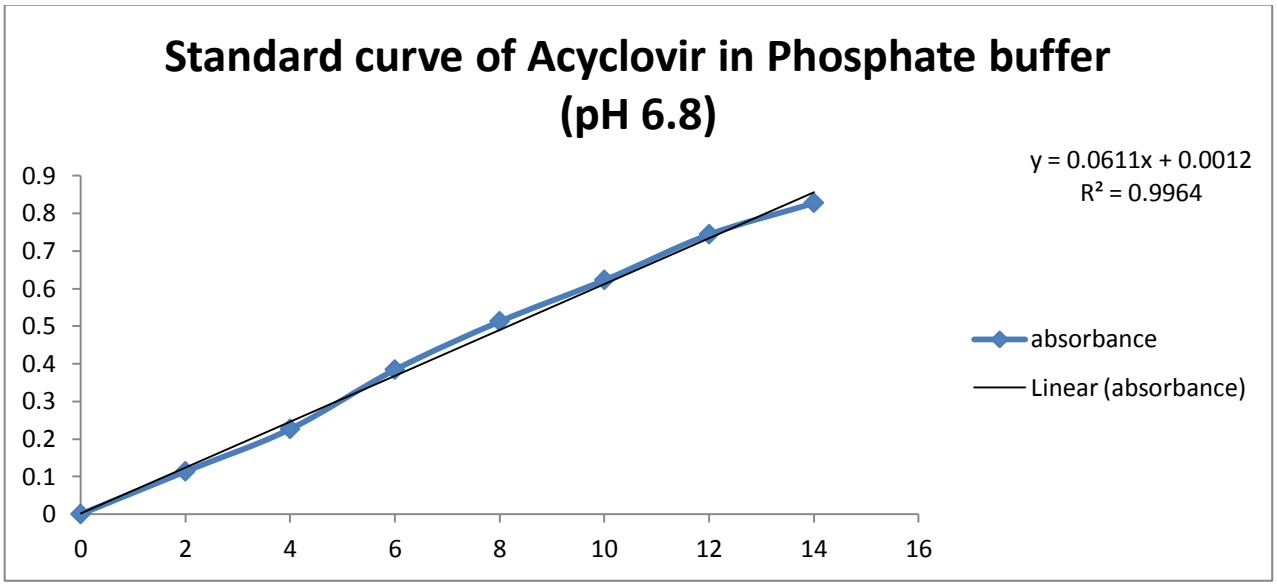


Figure 4.5: Standard curve of Acyclovir in Phosphate buffer (pH 6.8)

Regression Analysis

Regression analysis for standard curve of acyclovir in Phosphate buffer (pH 6.8)

Table 4.9: Regression analysis for standard curve of acyclovir in Phosphate buffer (pH 6.8)

Regression parameter	Value
Correlation coefficient	0.9958
Slope	0.0607
Intercept	0.0057

4.4 PREFORMULATION STUDY:

DRUG-EXCIPIENT COMPATIBILITY STUDY:

Compatibility studies were performed using FT-IR spectrophotometer. The IR spectrum of pure drug and physical mixture of drug and polymer were studied by making a KBr disc. The characteristic absorption peaks of Acyclovir were obtained at different wave numbers in different samples. The peaks obtained in the spectra formulation correlates with the peaks of drug spectrum. This indicates that the drug is compatible with the formulation components. The spectra for all formulations are shown below.

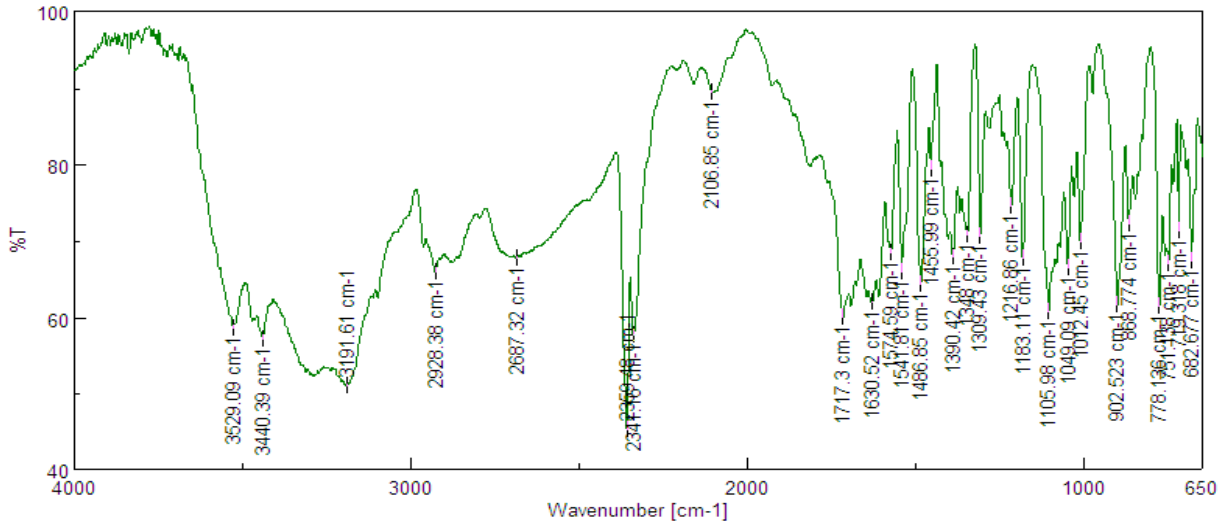


Figure 4.6: FTIR spectra of Acyclovir

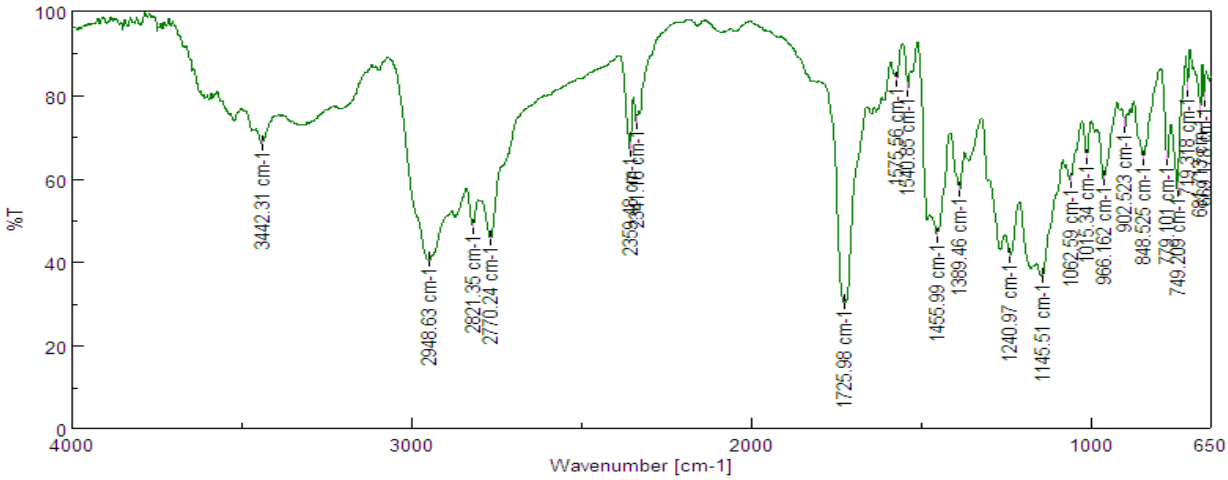


Figure 4.7: FTIR spectra of Eudragit EPO

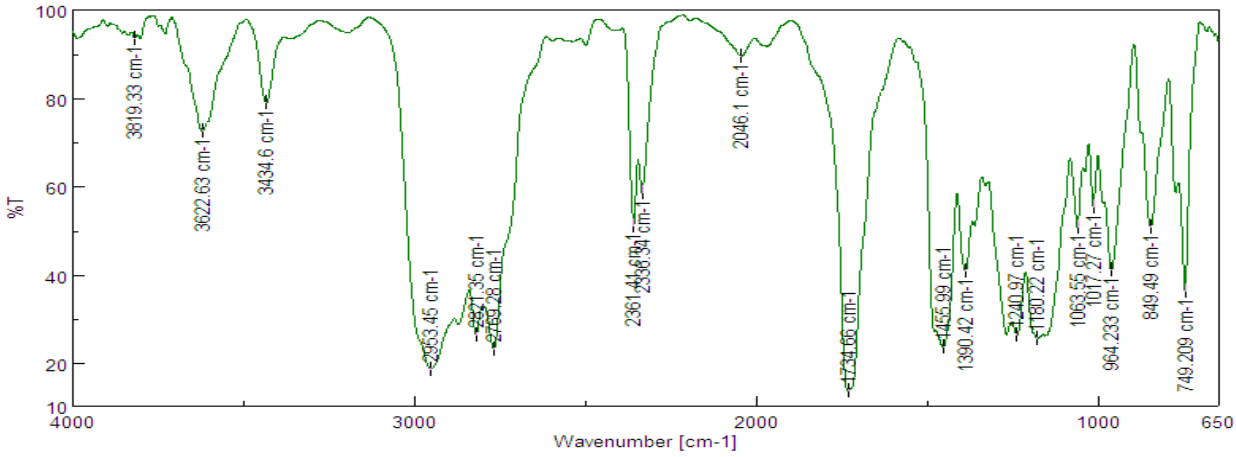


Figure 4.8: FTIR spectra of Acyclovir + Eudragit EPO

Table 4.10: FTIR Drug-Excipient Compatibility study

Sample	Peaks	Indication
Drug (Acyclovir)	3191 cm^{-1}	N-H stretching
Polymer (Eudragit EPO)	2361 cm^{-1}	C-N bending
Drug + Polymer Mixture	3200 cm^{-1} 2361 cm^{-1} 2953 cm^{-1}	N-H stretching, C-N bending CH of alkane

Conclusion: Preformulation study for the drug and excipients was conducted using FTIR spectrophotometer. Acyclovir and various polymer mixtures showed the respective characteristic bands of Acyclovir at 3191, 2361 and 2953 cm^{-1}. The results confirmed that there was no chemical interaction between drug and excipients.

4.5 Characterization of nanosuspension:

1. Mean Particle Size and Particle Size Distribution

   The particle size of nanosuspension was measured using Malvern Zetasizer ZS200. Each sample was measured at least three times. The average values were employed for the calculations of the response surfaces[29].

2. Zeta potential (Surface Charge)

   The zeta potential of nanosuspension was measured using Malvern Zetasizer ZS200 at 25±0.5^{0}C. Each sample was measured at least three times. The average values were employed for the further calculations[29].

3. Drug entrapment efficiency

   The percentage of Acyclovir (entrapment efficiency) was determined spectrophotometrically at 254 nm. After centrifugation of the aqueous suspension, amount of the free drug was detected in the supernatant and the amount of incorporated drug was determined as the result of the initial drug minus the free drug. The entrapment efficiency (EE %) could be achieved by the following equation:

   Entrapment efficiency (%) =

$$\frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100$$

4. In vitro drug release studies

   The *in vitro* release of Acylovir from the formulation was studied through Dialysis membrane-110 (cut-off: 3500 Da) using Franz diffusion cell. The diffusion medium used was freshly prepared 0.1 N HCl solution (pH 1.2). Dialysis membrane-110, previously soaked overnight in the diffusion medium was placed between the donor and receptor compartments of the diffusion cell. Five ml of formulation was accurately placed into this assembly. The cylinder was attached to a stand and suspended in 30 ml of diffusion medium maintained at 37^{0}C so that the membrane just touched the receptor medium surface. The diffusion medium was stirred at low speed using magnetic stirrer. Aliquots, each of 2 ml volume were withdrawn at regular intervals and replaced by an equal volume of the receptor medium. The

aliquots were suitably diluted with the receptor medium and analyzed by UV-Vis spectrophotometry at 254 nm.

5. Differential Scanning Colorimetery (DSC) analysis

Thermal properties of the powder samples were investigated with a differential scanning calorimeter Approximate 10mg of sample was analyzed in an open aluminium pan, and heated at scanning rate of 10^0C/min between 0^0C and 400^0C. Magnesia was used as the standard reference material to calibrate the temperature and energy scale of the DSC apparatus. To evaluate the internal structure modifications of the drugs before and after nanosizing process, thermal analysis was performed on Acyclovir, Eudragit EPO and their physical mixtures.

6. X- Ray Diffraction study

X-ray powder diffraction analysis was performed on Xpert MPD Instrument. The sample was prepared by spreading powder samples on a PMMA specimen holder rings from Bruker and were scanned from 2 to 40◦ 2^{θ} at the rate of 2◦/min with 0.02◦ step size and 0.6 s/step at 40KV and 40 mA. The divergence and anti-scattering slits were set to 1◦ and the stage rotated at 30 rpm. Data analysis was performed using "EVA Part 11" version 14.0.0.0.

7. Surface Morphology Study/Transmission Electron Microscopy

Due to the impact of Homogenization on crystalline particles, the change in their structure into amorphous or other polymorphic form occurs. Such kind of Alteration in the solid state of the drug particles and the extent of the amorphous portion is determined by Transmission Electron Microscopy (TEM) studies.

8. Ex-vivo drug release study

It was carried out using rat ileum in a modified diffusion apparatus equilibrated at 37^0C. one end of the rat ileum was tied and 0.2 ml of nanosuspension was put into it and the other end was sealed and tied. The rat ileum was immersed in 250 ml of

diffusion medium i.e. simulated intestinal fluid and the process was carried out at 50 rpm along with constant aeration. 2 ml of sample was withdrawn at each time interval and the same amount of diffusion medium was replaced and was analyzed spectrophotometrically at 254 nm after required dilutions[31].

9. Stability study

Stability is defined as the extent to which a product remains within specified limits throughout its period of storage and use. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under influence of variety of environmental factors such as temperature, humidity and light, and to establish a re-test period for drug substance or a shelf life for the drug product and recommended storage conditions. It was carried out at 40^0C and 75% RH. A drug formulation is said to be stable if it fulfills the following requirements:

- It should contain at least 90% of the stated active ingredient
- It should contain effective concentration of the added preservatives, if any
- It should not exhibit discoloration or precipitation, nor develops foul odor
- It should not develop irritation or toxicity.

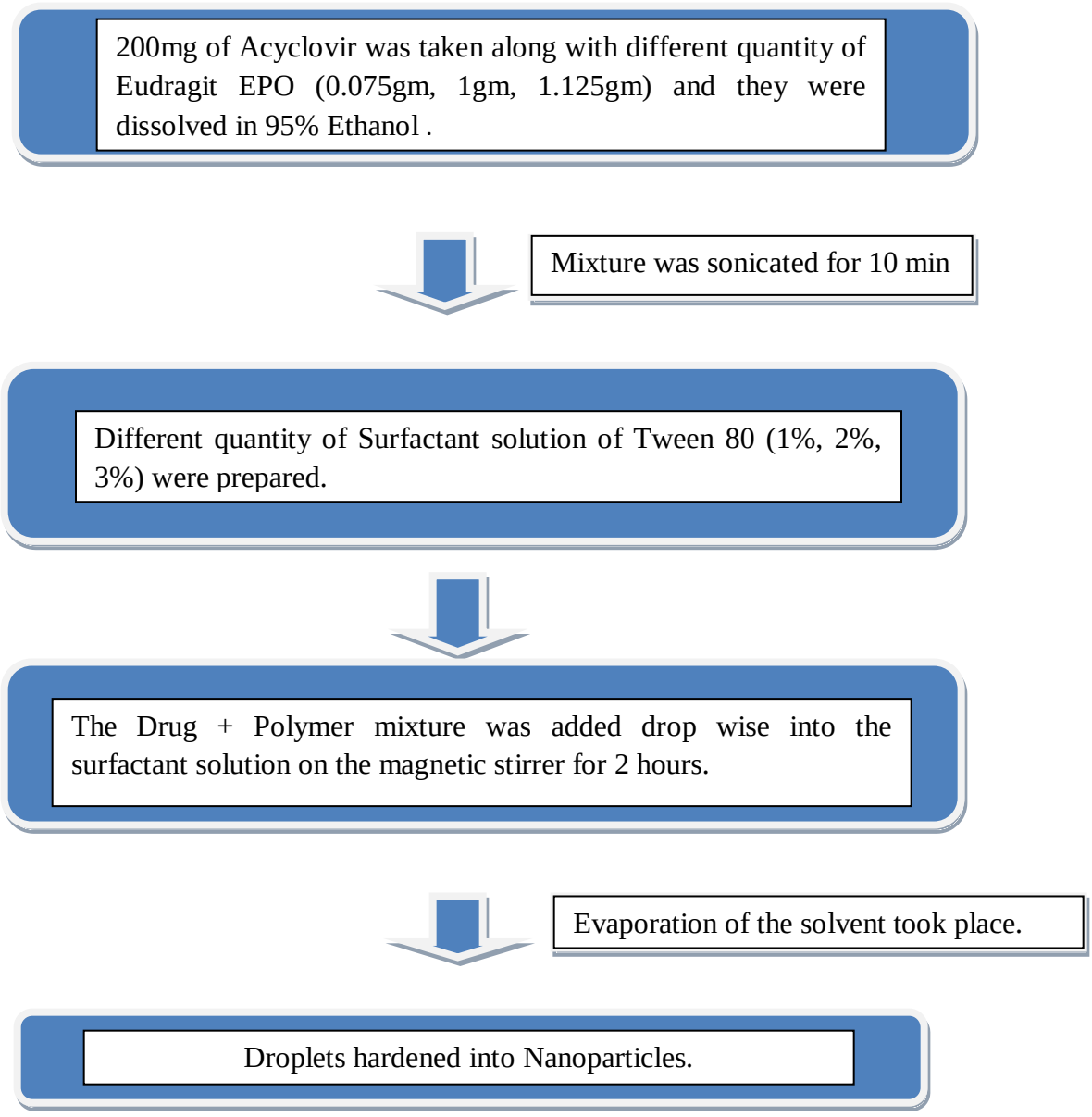
4.6 Method of Preparation

4.6.1 Ultrasonication method

Initially, the nanosuspension was prepared by Ultrasonication method. Here the drug (Acyclovir) along with various excipients like HPMC 5 CPS, HPC, PVP K 30, Poloxamer 188, SLS, Tween 80 dispersed in the aqueous media. After that the above mixture of drug and excipients was allowed to get sonicate for different time intervals like 5, 10 and 15 minutes. But, we couldn't get satisfactory result with the help of Ultrasonication method. So, another method was applied to prepare nanosuspension[32].

4.6.2 Emulsification Solvent evaporation method

An emulsion is a metastable dispersion of two or more immiscible liquids in the presence of surfactant. Emulsion can be used to produce nanoparticles by dissolving a drug and polymer in a water immiscible solvent and adding a mixture drop wise to an aqueous solution containing surfactant. Shear is applied through homogenization or sonication to decrease droplet size to nanoscale. The droplet hardens into nanoparticles after evaporation of the solvent. Here, we have taken polymeric stabilizer Eudragit EPO along with two different surfactant stabilizers like Tween 80 and SLS[33].



4.7 PRELIMINARY TRIALS:

4.7.1 Preliminary trials for the selection of Polymeric stabilizer and Surfactant stabilizer:

4.7.1.1 Ultrasonication method

In this method, various Polymeric stabilizers like HPMC 5 cps, HPC, PVP K 30 and Poloxamer 188 were used. To prevent the aggregation between particles, such Polymeric stabilizers were used in nanosuspension. Along with them, Surfactant stabilizers like Tween 80 and SLS were used and the role of such stabilizers was to decrease the surface tension and by this way decrease in the particle size occurred.

Table 4.11: Trial Batch using HPMC 5 CPS and SLS

Concentration of HPMC 5 CPS (%)	Concentration of SLS (%)
0.25	0.5, 1, 1.5, 2, 2.5
1	0.5, 1, 1.5, 2, 2.5
2	0.5, 1, 1.5, 2, 2.5
3	0.5, 1, 1.5, 2, 2.5
4	0.5, 1, 1.5, 2, 2.5

Table 4.12: Trial Batch using HPC and SLS

Concentration of HPC (%)	Concentration of SLS (%)
1	0.5, 1, 1.5, 2, 2.5
2	0.5, 1, 1.5, 2, 2.5
3	0.5, 1, 1.5, 2, 2.5
4	0.5, 1, 1.5, 2, 2.5
5	0.5, 1, 1.5, 2, 2.5

.

Table 4.13: Trial Batch using PVP K 30 and SLS

Concentration of PVP K 30 (%)	Concentration of SLS (%)
1	0.5, 1, 1.5, 2, 2.5
2	0.5, 1, 1.5, 2, 2.5
3	0.5, 1, 1.5, 2, 2.5
4	0.5, 1, 1.5, 2, 2.5
5	0.5, 1, 1.5, 2, 2.5

Table 4.14: Trial Batch using Poloxmer 188 and SLS

Concentration of Poloxamer 188 (%)	Concentration of SLS (%)
1	0.5, 1, 1.5, 2, 2.5
2	0.5, 1, 1.5, 2, 2.5
3	0.5, 1, 1.5, 2, 2.5
4	0.5, 1, 1.5, 2, 2.5
5	0.5, 1, 1.5, 2, 2.5

Table 4.15: Trial Batch using HPMC 5 CPS and Tween 80

Concentration of HPMC 5 CPS (%)	Concentration of Tween 80 (%)
0.25	1, 5, 8, 12, 15
1	1, 5, 8, 12, 15
2	1, 5, 8, 12, 15
3	1, 5, 8, 12, 15
4	1, 5, 8, 12, 15

Table 4.16: Trial Batch using HPC and Tween 80

Concentration of HPC (%)	Concentration of Tween 80 (%)
1	1, 5, 8, 12, 15
2	1, 5, 8, 12, 15
3	1, 5, 8, 12, 15
4	1, 5, 8, 12, 15
5	1, 5, 8, 12, 15

Table 4.17: Trial Batch using PVP K 30 and Tween 80

Concentration of PVP K 30 (%)	Concentration of Tween 80 (%)
1	1, 5, 8, 12, 15
2	1, 5, 8, 12, 15
3	1, 5, 8, 12, 15
4	1, 5, 8, 12, 15
5	1, 5, 8, 12, 15

Table 4.18: Trial Batch using Poloxamer 188 and Tween 80

Concentration of Poloxamer 188 (%)	Concentration of Tween 80 (%)
1	1, 5, 8, 12, 15
2	1, 5, 8, 12, 15
3	1, 5, 8, 12, 15
4	1, 5, 8, 12, 15
5	1, 5, 8, 12, 15

4.7.12 Emulsification-Solvent evaporation method

Here, polymeric stabilizer Eudragit EPO along with two different surfactant stabilizers like Tween 80 and SLS were used.

Table 4.19: Trial Batch using Eudragit EPO and Tween 80

Concentration of Eudragit EPO (gm)	Concentration of Tween 80 (%)
0.05	1, 2, 3
0.1	1, 2, 3
0.15	1, 2, 3
0.2	1, 2, 3
0.25	1, 2, 3
0.5	1, 2, 3

Table 4.20: Trial Batch using Eudragit EPO and SLS

Concentration of Eudragit EPO (gm)	Concentration of SLS (%)
0.05	1, 1.5, 2
0.1	1, 1.5, 2
0.15	1, 1.5, 2
0.2	1, 1.5, 2
0.25	1, 1.5, 2
0.5	1, 1.5, 2

Discussion: On the basis of clarity, the combination of Eudragit EPO and Tween 80 was found better than the combination of Eudragit EPO and SLS. And also, the particle size of Eudragit EPO and Tween 80 combination was found to be of 224 nm and for Eudragit EPO and SLS was found to be of 677 nm. So, further optimization study was carried out with the help of Eudragit EPO and Tween 80 combination.

4.8 Optimization of the nanosuspension

In order to obtain best formulation and process of nanosuspension, the relationship between controllable variable and quality variable must be understood. The traditional method used to study this relationship involves "changing one variable at a time, while keeping others as constant". This approach has been proved to be expensive, laborious and also unfavorable to fix errors that are unpredictable and at times even unsuccessful. On the other hand Design of experiment (DoE) can serve as an efficient and economical method of obtaining the necessary information to understand relationship between variables. DoE provides not only efficient use of resources, but also provides a method of obtaining a mathematical model which can be used to characterize and optimize formulation and or process.

For the optimization purpose, here 3^2 full factorial design has been used. Concentration of polymeric stabilizer (X_1) and concentration of surfactant stabilizer (X_2) were selected as independent variables, while Particle size, % Entrapment efficiency and % Drug release were selected as dependent variables[34].

Table 4.21: 3^2 full factorial design

Independent variables	Design level	
	Coded level	Uncoded level
1. Concentration of Eudragit EPO (gm)	-1	0.075
	0	0.1
	+1	0.125
2. Concentration of Tween 80 (%)	-1	1
	0	2
	+1	3

Table 4.22: Application of 3^2 full factorial design

BATCH CODE	**Variable level in Coded Form**		**Actual Value**	
	X1	X2	X1	X2
B1	-1	-1	0.075	1
B2	-1	0	0.075	2
3	-1	+1	0.075	3
B4	0	-1	0.1	1
B5	0	0	0.1	2
B6	0	+1	0.1	3
B7	+1	-1	0.125	1
B8	+1	0	0.125	2
B9	+1	+1	0.125	3
B10	0	0	0.1	2
B11	0	0	0.1	2

CHAPTER 5
RESULT AND DISCUSSION

5. RESULT AND DISCUSSION:

For the preparation of nanosuspension, two methods were tried simultaneously and they were Ultrasonication method and Emulsification solvent evaporation method. The main criteria was to achieve particle size in the range of 1-300 nm[35].

5.1 Results of Preliminary trials

Discussion: from visual inspection and on the basis of clarity, the optimum concentration of polymeric and surfactant stabilizer were found to be HPMC 5 CPS- 2% and SLS- 1.5%, and it was showing particle size of 583 nm.

Discussion: from visual inspection and on the basis of clarity, the optimum concentration of polymeric and surfactant stabilizer were found to be HPC - 1% and SLS- 1.5%, but it was not showing particle size in range.

Discussion: from visual inspection and on the basis of clarity, the optimum concentration of polymeric and surfactant stabilizer were found to be PVP K 30- 5% and SLS- 1.5%, but it was not showing particle size in range.

Discussion: from visual inspection and on the basis of clarity, the optimum concentration of polymeric and surfactant stabilizer were found to be Poloxamer 188- 3% and SLS- 1.5% and it was showing particle size of 358 nm.

Discussion: from visual inspection and on the basis of clarity, the optimum concentration of polymeric and surfactant stabilizer were found to be HPMC 5 CPS- 1% and Tween 80- 5% and it was showing particle size of 456 nm.

Discussion: from visual inspection and on the basis of clarity, the optimum concentration of polymeric and surfactant stabilizer were found to be HPC- 1% and Tween 80- 5%, but it was not showing particle size in range.

Discussion: from visual inspection and on the basis of clarity, the optimum concentration of polymeric and surfactant stabilizer were found to be PVP K 30-5% and Tween 80- 5%, but it was not showing particle size in range.

Discussion: from visual inspection and on the basis of clarity, the optimum concentration of polymeric and surfactant stabilizer were found to be Poloxamer 188- 3% and Tween 80- 5% and it was showing particle size of 306 nm.

Conclusion: After applying Ultrasonication method, we couldn't get satisfactory result in terms of particle size. So we have tried another method Emulsification-Solvent evaporation method for the preparation of nanosuspension.

5.12 Emulsification-Solvent evaporation method

In this method, Eudragit EPO was used as Polymeric stabilizer and it is also having self emulsifying property. Along with it, Tween 80 and SLS were used as Surfactant Stabilizers.

Discussion: On the basis of clarity, the combination of Eudragit EPO and Tween 80 was found better than the combination of Eudragit EPO and SLS. And also, the particle size of Eudragit EPO and Tween 80 combination was found to be of 224 nm and for Eudragit EPO and SLS was found to be of 677 nm. So, further optimization study was carried out with the help of Eudragit EPO and Tween 80 combination.

5.2 Optimization of the Formulation

Table 5.11: 3^2 full factorial design

BATCH CODE	Variable level in Coded Form		Actual Value	
	X1	X2	X1	X2
B1	-1	-1	0.075	1
B2	-1	0	0.075	2
B3	-1	+1	0.075	3
B4	0	-1	0.1	1
B5	0	0	0.1	2
B6	0	+1	0.1	3
B7	+1	-1	0.125	1
B8	+1	0	0.125	2
B9	+1	+1	0.125	3
B10*	0	0	0.1	2
B11*	0	0	0.1	2

***=Check point batches**

Discussion: From the result obtained after 3^2 full factorial design it was found that the batch B7, containing low amount of surfactant stabilizer and moderate amount of polymeric stabilizer showed lesser particle size, better entrapment efficiency and more than 90% of drug release within 3 hours. So, B7 batch was selected for further ex vivo diffusion study.

5.3 Evaluation of the nanosuspension

Discussion: From the Particle size and zeta potential study, we found out that Batch B4 was showing lesser particle size, which is 91.79nm and Zeta potential is -3.07 mV.

5.3.2 % Entrapment efficiency:

$$\text{Entrapment efficiency (\%)} = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100$$

Discussion: The drug content in eleven batches of Acyclovir nanosuspension was studied. The amount of drug bound per 1 ml of nanosuspension was determined in each batch. It was observed that the entrapment efficiency has been increased with the increase in concentration of polymer in the formulations. As the concentration of polymer increased, the % Entrapment efficiency also increased.

5.3.3 In vitro Diffusion study:

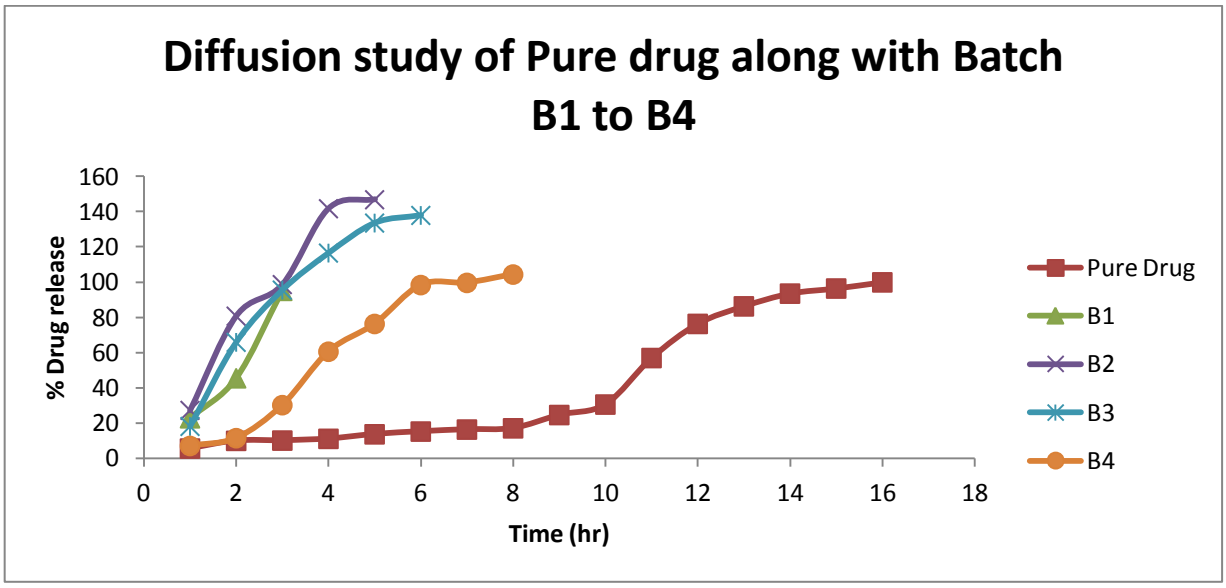


Figure 5.23: Diffusion study of pure drug along with Batch 1 to Batch 4

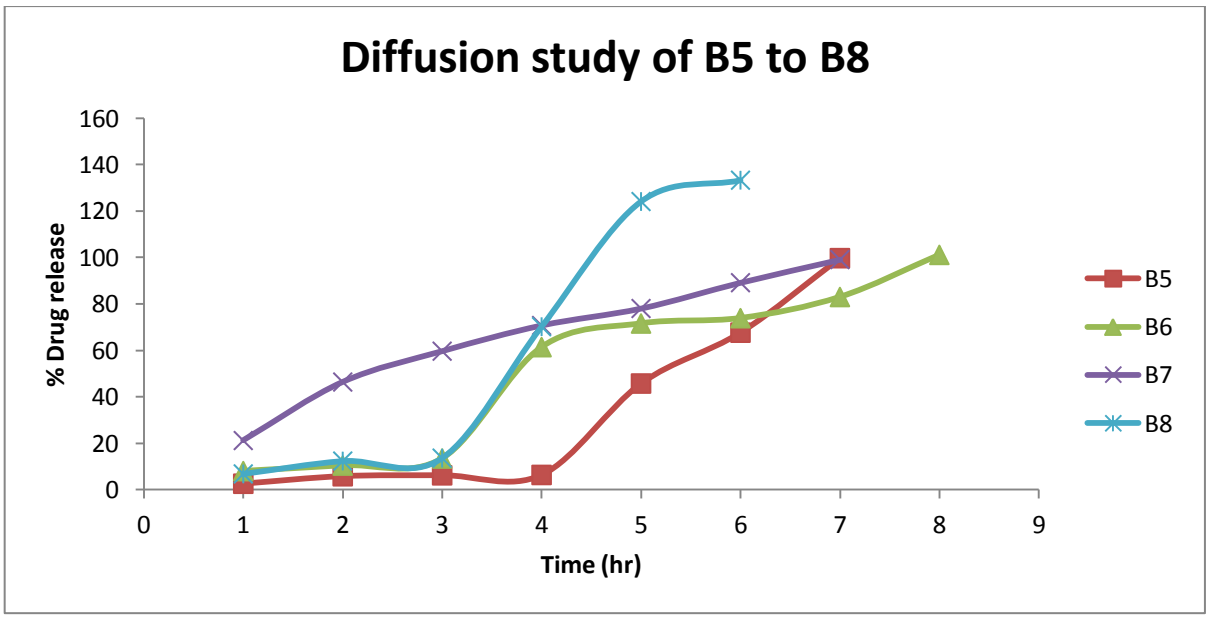


Figure 5.24: Diffusion study of Batch B5 to B8

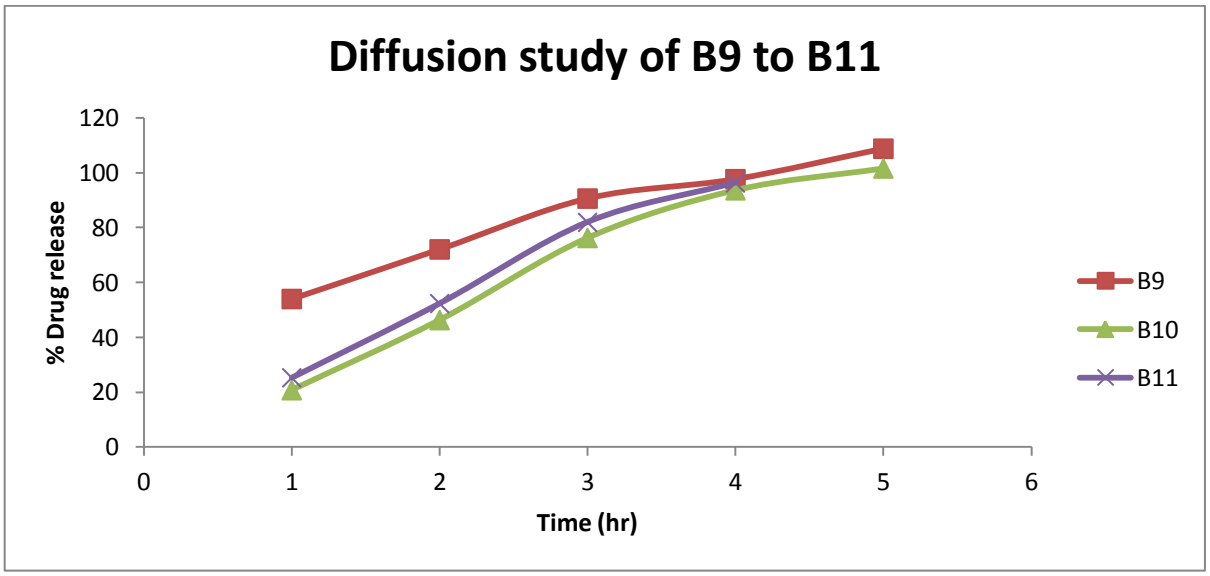


Figure 5.25: Diffusion study of Batch B9 to B11

Discussion: From the diffusion study, we have found out that Batch B7 was showing 99.79% release in 3 hours. So, we have taken Batch B7 for further comparison with marketed formulation.

5.3.4 Differential Scanning Colorimetry (DSC)

Discussion: From the DSC study, we can conclude that crystalanity of the drug has been reduced due to shortening of the peak. But further XRD study is required to confirm the amorphous nature.

5.3.5 X-Ray Diffraction study (XRD)

Discussion: To confirm the crystalline state of drug, polymer and Nanosuspension, X- Ray diffraction study was performed. All kinds of energy input during production did not change their crystalline state, either the attendance of stabilizer. However, distinctions in relative peak intensity can be detected among the samples. That was probably caused by particle size reduction. Usually, drugs with lower crystallinity and smaller size have higher dissolution rate and bioavailability. Therefore, particle size reduction and the change in crystallinity of Acyclovir powder were expected to increase its dissolution rate and bioavailability.

5.3.6 Transmission Scanning Microscopy (TEM)

Discussion: Transmission Electron photographs show that the particle size of the optimized batch B7 was found to be 60.88 nm and there were presence of discrete, smooth and spherical particles. Along with sphericity, drug particles were surrounded by the Eudragit EPO layer.

5.3.7 Results of the 3^2 full factorial design:

1. Particle size

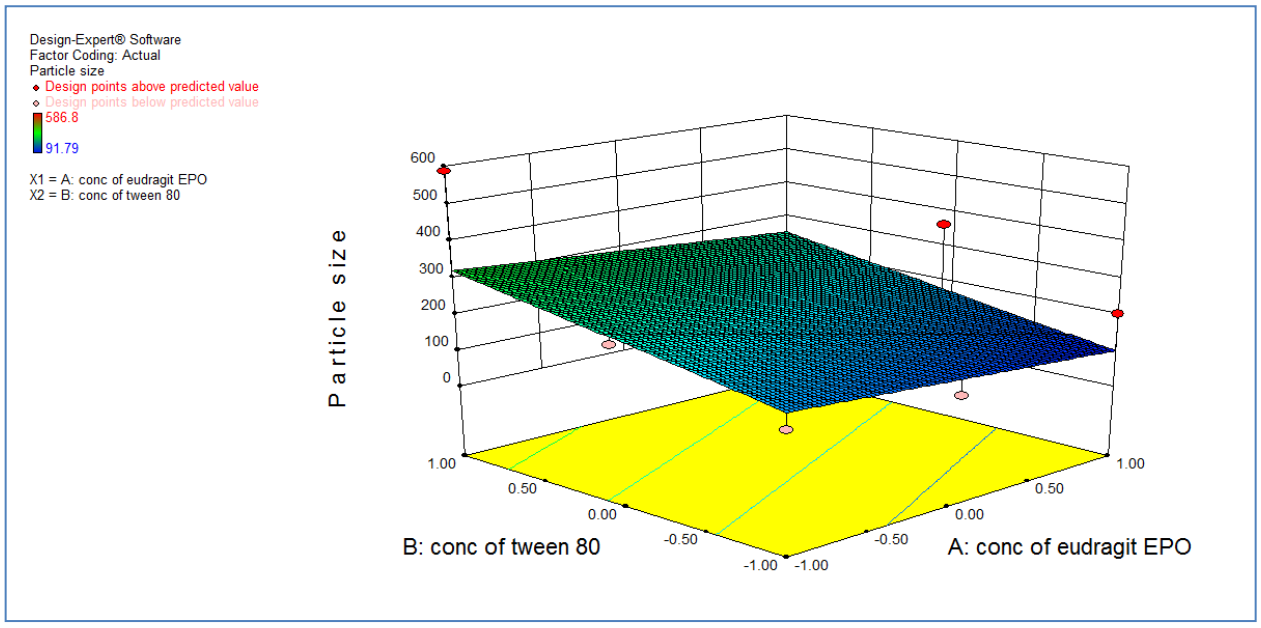


Figure 5.32: 3-D Surface plot of Particle size

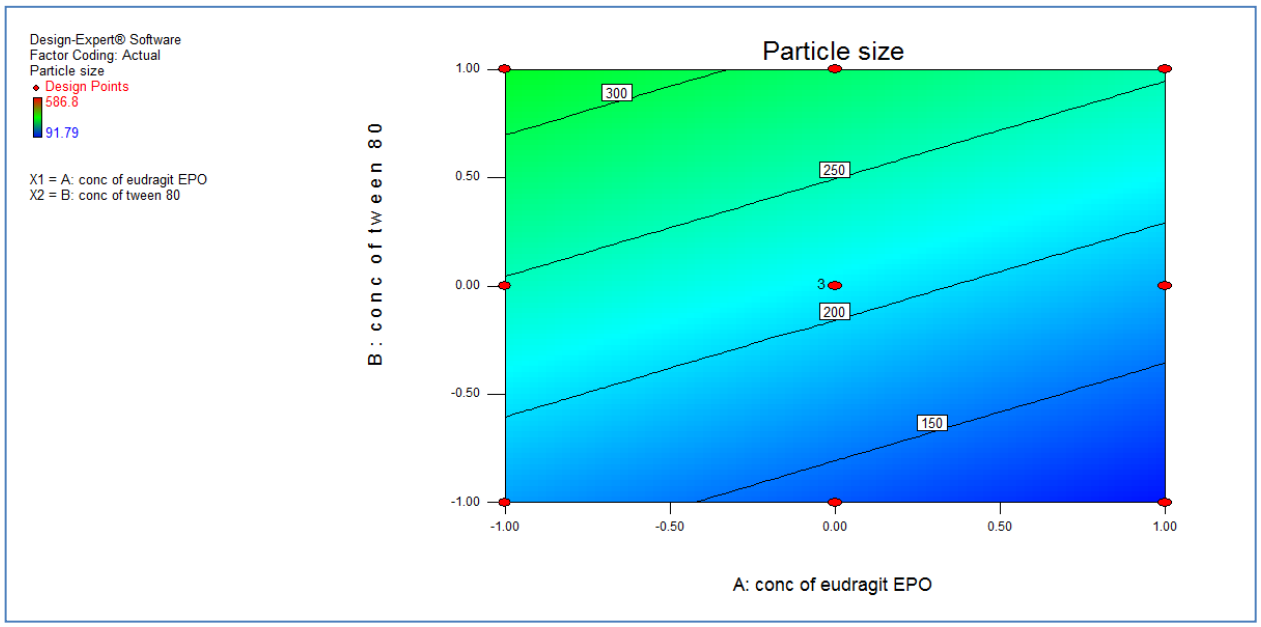


Figure 5.33: Contour plot of Particle size

Final equation:

Particle size= +143.90 - 34.50 * A +76.79 * B -126.85 * A * B +152.44 * A^2 -27.62 * B^2 (Full model) (R^2=0.9996)

Particle size = +143.90 -126.85 * A * B +152.44 * A^2 (Reduced model) (R^2=0.9764)

Discussion: The "Model F-value" of 4.08 implies the model is not significant. But the P value of Conc of tween 80 (0.0915) is smaller than the P value of Conc of Eudragit EPO (0.3920). Hence, the conc of surfactant plays important part for the particle size reduction.

2. % Entrapment efficiency

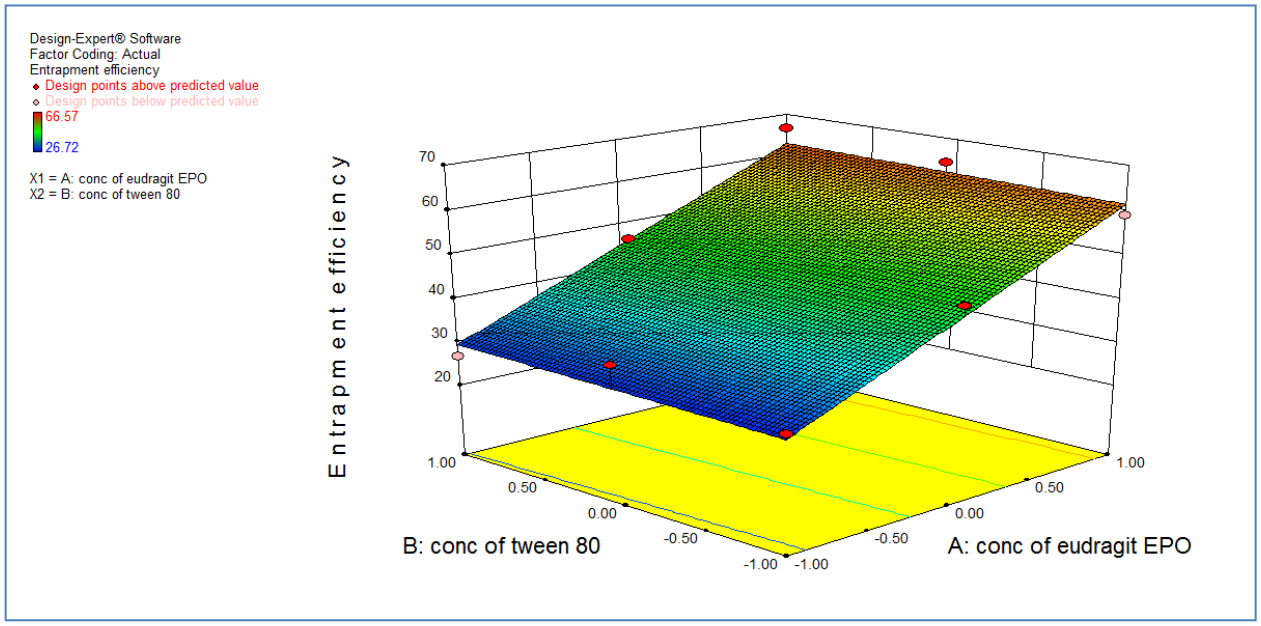


Figure 5.34: 3-D Surface plot of % Entrapment efficiency

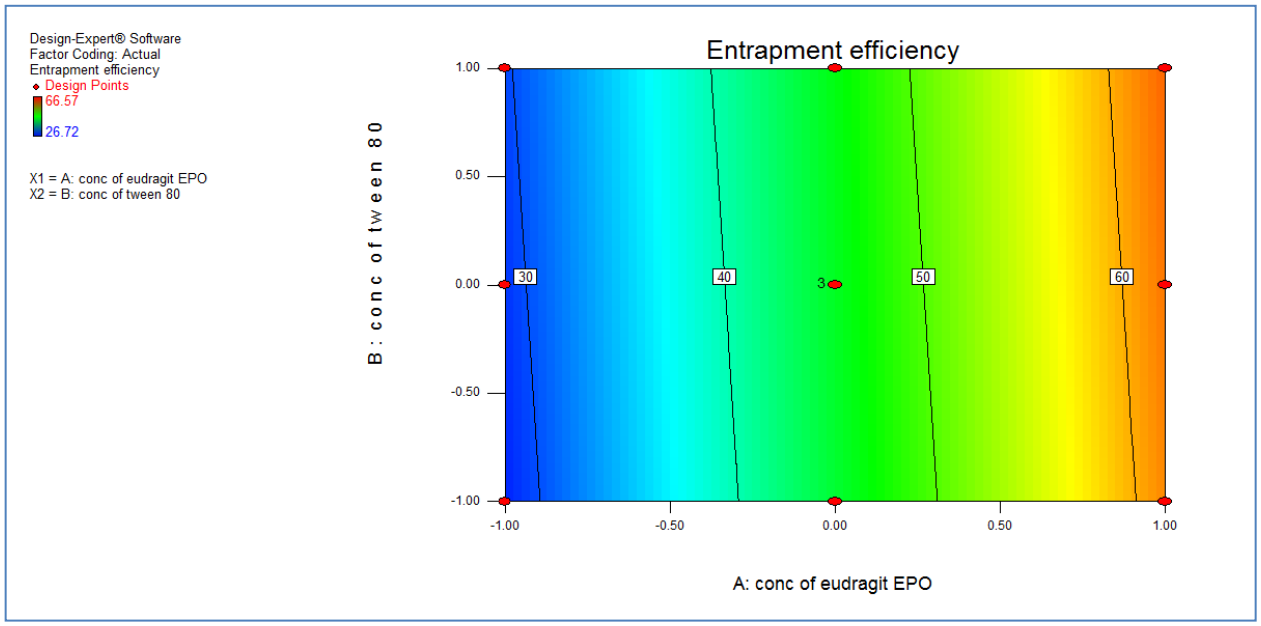


Figure 5.35: Contour plot of % Entrapment Efficiency

Final equation:

Entrapment efficiency = +44.22 +16.62* A +0.70 * B +2.55*A*B +2.48 *A^2 -0.022 * B^2 (Full model) (R^2=0.9687)

Entrapment efficiency = +44.22 +16.62 * A +2.55 * A (Reduced model) (R^2=0.9663)

Discussion: The Model F-value implies that the model is significant. And it also shows that the as the conc of polymer increased, its entrapment efficiency also increased.

3. % Drug release

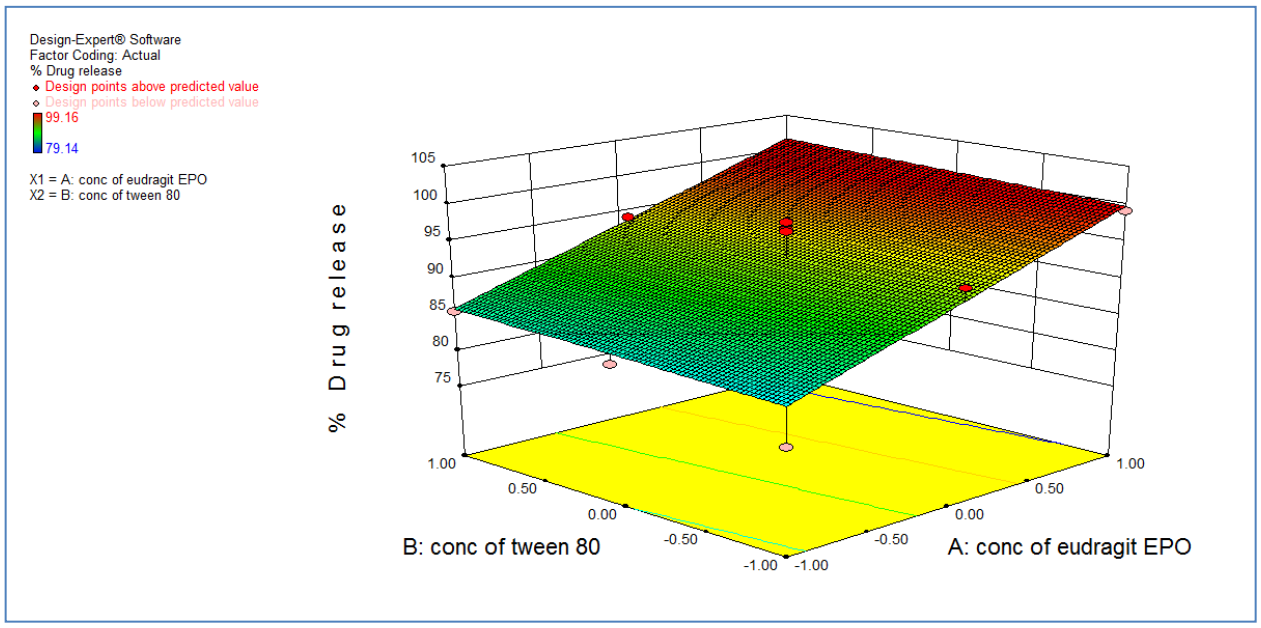


Figure 5.36: 3-D Surface Plot of % Drug release

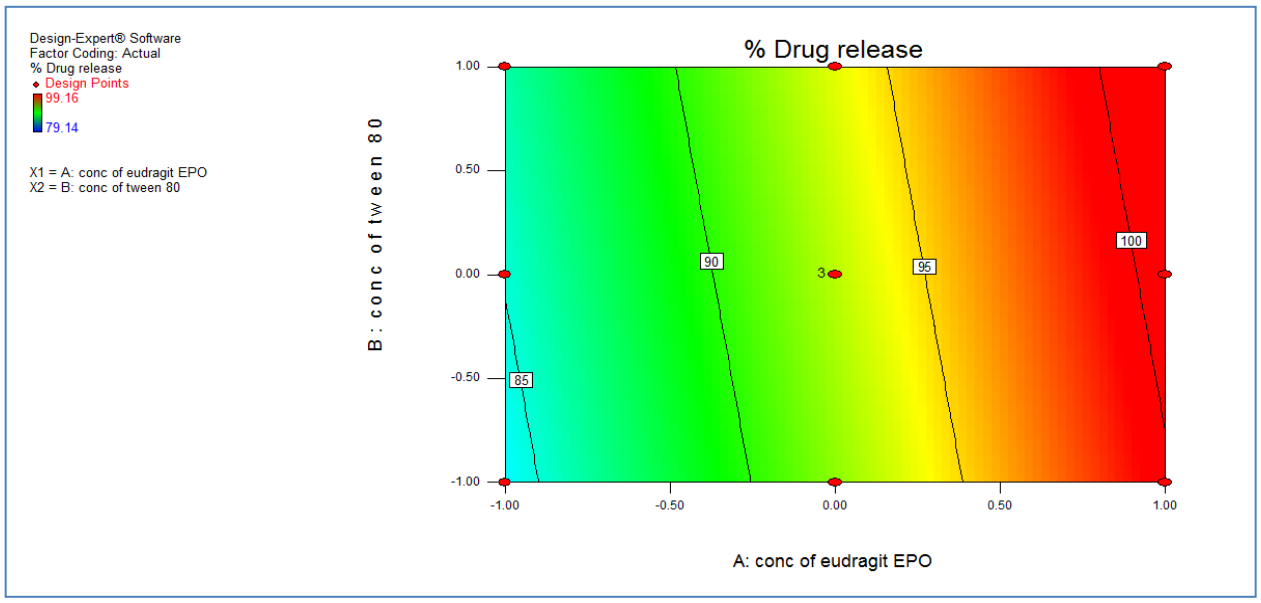


Figure 5.37: Contour Plot of % Drug release

Final equation:

% Drug release = +96.33 +7.79 * A +0.89 * B -1.98 A * B - 4.60 * A^2 -1.73 * B^2 (Full model) (R^2=0.9858)

% Drug release = +96.33 +7.79 * A -1.98 A -4.60 * A^2 (Reduced model) (R^2=0.9736)

Discussion: The model F-value implies that the model is significant. Eudragit EPO is an immediate release polymer. So it also showed that as the conc of polymer increased, the % drug release also increased.

Conclusion: From the optimization study, we found out that Batch B7 was the best batch amongst all the batches, as it was showing particle size of 206.8 (PDI- 0.408), along with zeta potential of 7.23 mV, Entrapment efficiency of 59% and 99.16% release at the end of 2 hours.

5.3.8: Comparison between Marketed formulation and optimized Batch B7

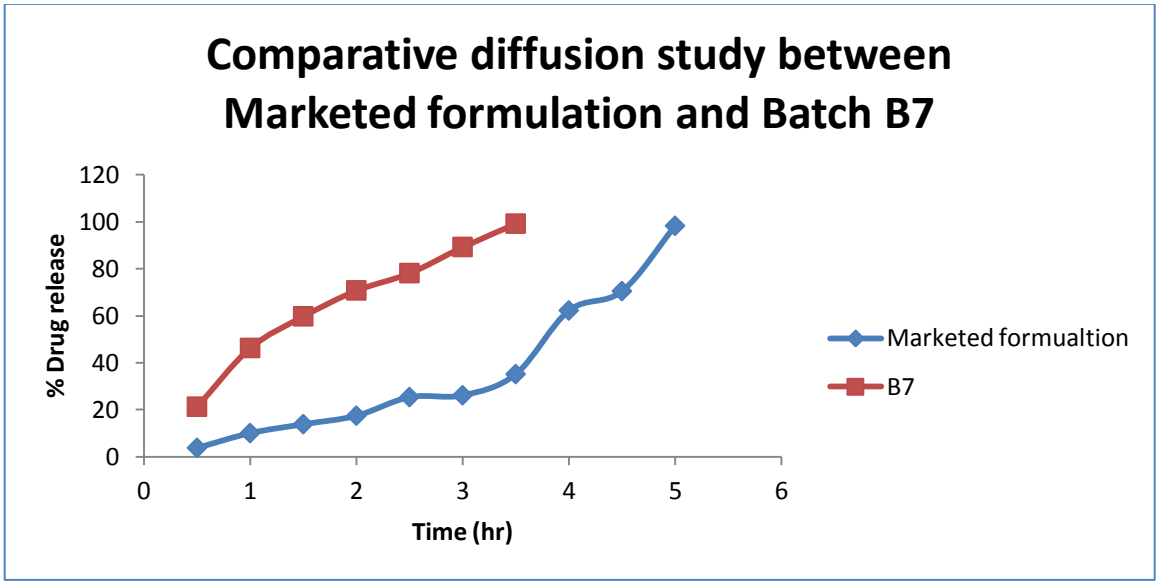


Figure 5.38: Diffusion study of Marketed formulation and Batch B7

Discussion: From the comparison study of marketed formulation along with Batch B7, we can conclude that our nanosuspension was showing better release in lesser time than the marketed formulation.

5.3.9: Ex vivo Diffusion study

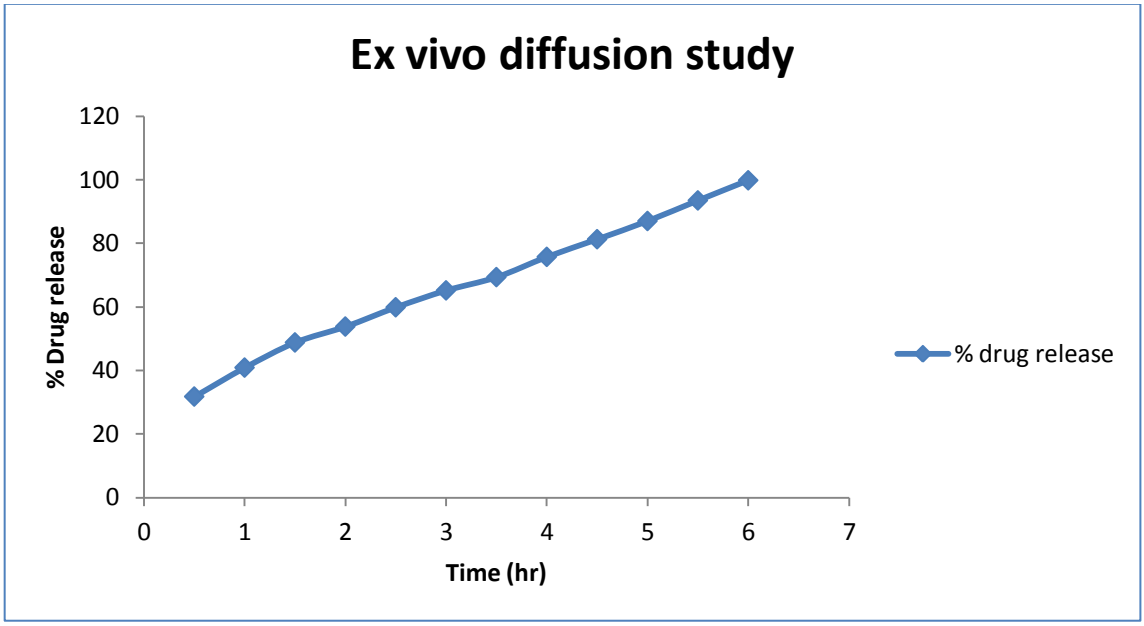


Figure 5.39: Ex vivo diffusion study of Batch B7

5.3.10: Stability study

Table 5.32: Stability study of Batch B7

Condition	Time	Particle size (nm)	In vitro diffusion study	Ex vivo study
40 ^{0}C/ 75% RH	Initial	206.8 nm	At 3 hrs, 99.16%	At 6 hrs, 99.79%
	1 month	On going	On going	On going
	2 months	On going	On going	On going

CHAPTER 6
SUMMARY

From all the route of administration, Oral administration has been the most common and favorable route for delivery of the drugs. Drugs with high solubility and low permeability are classified as BCS class III drugs. Due to their inauspicious physicochemical and chemical properties which are difficult to change many drug molecules show poor permeability. Ordinarily, BCS class III drugs are not adapted to oral formulations. Acyclovir, a class III drug shows only 20% bioavailability.

In the present study, Nanosuspension of Acyclovir was successfully developed in the form of permeation enhancement which offers a suitable and practical approach in serving desired objective of increase in the permeability, faster drug release with increase in bioavailability by the administration through oral route.

Nanosuspension was formulated using Eudragit EPO, HPMC 5 cps, HPC, Poloxamer 188, PVP K 30 as Polymeric stabilizer and along with them tween 80 and SLS were used as surfactant stabilizers. Two methods were used simultaneously for the preparation of nanosuspension and they were Ultrasonication method and Emulsification solvent evaporation method. Optimization of polymeric stabilizers and surfactant stabilizers were done based on the preliminary trials conducted. Preformulation study for the drug and excipients was conducted using FT-IR spectrophotometer. No drug-excipients interaction was observed. On the basis of Preliminary trials, Eudragit EPO and Tween 80 were selected for further optimization study and Emulsification solvent evaporation method was selected as with the use of this method, the particle size was found within the range. 3^2 full factorial design has been applied for the optimization process. Here, the conc of Eudragit EPO and conc of Tween 80 were selected as independent variables, While particle size, % Entrapment efficiency and % drug release were selected as dependent variables.

From the surface morphology study, It has been found that as the conc of surfactant was increased, the particle size was decreased. For % Entrapment efficiency, as the conc of polymer was increased, its entrapment efficiency was also increased. Due to the immediate

release property of Eudragit EPO, the % Drug release of the drug was increased, with the increase in the conc of polymer.

After the Optimization process, the optimized batch was B7 as it was having particle size of 206.8 nm, along with PDI of 0.408 and Zeta potential of 7.23 mV. The % Entrapment efficiency was found to be of 59 % and it was showing 99.16 % release at the end of 3 hrs. Further TEM (Transmission Electron Microscopy) study was carried out to check the surface morphology of the B7 formulation and it was found that the particles were of spherical shape and particle size was of 60.88 nm and the drug was surrounded by the Eudragit EPO layer. DSC and XRD study was done to check the loss of crystallinity and from those studies, the amorphous nature of the formulation was found. Further ex vivo study was carried out using rat ileum and it was showing 100% drug release in 6 hrs.

It can be concluded that the nanosuspension of Acyclovir was prepared using Emulsification solvent evaporation method. Also, the dosage form showed immediate penetration across the GIT mucosa. The permeation enhancement has been achieved along with increase in the Bioavailability. Hence, nanosuspension containing acyclovir provides higher bioavailability as compared to the marketed product and can be used as best alternative as antiviral agent.

CHAPTER 7
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