FORMULATION DEVELOPMENT AND IN VITRO CHARACTERIZATION OF GASTRORETENTIVE FLOATING ACYCLOVIR TABLETS

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A RESEARCH DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF PHARMACY IN INDUSTRIAL PHARMACY OF THE UNIVERSITY OF NAIROBI.

DECLARATION OF ORIGINALITY

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TABLE OF CONTENTS

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This project is dedicated to my family; my wife Angel, and sons; Davis and Jefferson whose moral support and prayers were evident through the entire course. Your sacrifices are truly appreciated

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
AOR	Angle of Repose
AUC	Area Under the Curve
AIDS	Acquired Immune Deficiency Syndrome
BCS	Biopharmaceutical Classification System
CI	Carr's Index
CMV	Cytomegalovirus
DNA	Deoxyribonucleic Acid
EBV	Epstein Barr Virus
FTIR	Fourier Transformed Infrared
GRDDS	Gastroretentive Drug Delivery Systems
HAART	Highly Active Antiretroviral Therapy
HPLC	High-Performance Liquid Chromatography
HIV	Human Immunodeficiency Virus
HPMC	Hydroxypropyl Methyl Cellulose
HHV-3	Human Herpes virus 3
HHV-5	Human Herpes Virus5
HHV-6	Human Herpes Virus 6
HHV-7	Human Herpes Virus 7
HHV-8	Human Herpes Virus 8
HR	Hausner's Ratio
HZ	Herpes Zoster
IR	Infrared
MCC	Microcrystalline Cellulose
MRT	Mean Residence Time
PEO	Polyethylene Oxide
PHN	Postherpetic Neuralgia
QbD	Quality by Design
SI	Swelling Index

$t_{1/2}$	Half-Life
USP	United States Pharmacopoeia
UV	Ultra Violet

ABSTRACT

***Background*:**Acyclovir is the prototype DNA polymerase inhibitor used in management of herpes zoster infections. The bioavailability of the drug is beset by low solubility and low permeability at doses above 400mg. Consequently, high dosages of the drug need to be administered in multiple doses to maintain the therapeutic concentrations required to inhibit viral replication. The high pill burden occasioned leads to suboptimal patient compliance, untoward effects and poor treatment outcomes. The objective of this project was to formulate and characterize gastroretentive floating tablets of acyclovir to increase the mean gastric residence time thereby improving the bioavailability of the drug. A unit dosage of 200mg was selected as it fits into BCS III thus requiring only modification of the drug's permeability to improve the bioavailability.

***Methods*:**A laboratory experimental study design was employed for this study. Preformulation studies were conducted to determine the compatibility of the drug and the excipients using attenuated FTIR spectroscopy. The simplex lattice mixture design was utilized to guide the variation of polymers proportions to investigate the individual contributions of each polymer and their combined effects towards the observed dependent variables. The independent variables were polymers (Hydroxypropyl methyl cellulose K 100M (X1), Hydroxypropyl methyl cellulose K4M (X2) and Carbopol (X3)) whose proportions were varied as per the experimental design.

The dependent variables were the floating lag time (Y1), total floating time (Y2), and the cumulative drug released at 3,6, and 8 hours (Y3, Y4, Y5) respectively. The resulting data was keyed into the Design expert software® to generate the polynomials describing the contribution of each independent variable to the outcomes listed above. Validation of the formulation model was done using the optimized formulations for which checkpoint formulation was fabricated and the accuracy of the model established. In addition, the prescribed pharmacopoeial tests for tablets were performed as appropriate.

Results and Discussion:Statistical analysis was conducted using the Design expert, STATA and MS Excel as appropriate. All formulations complied with the required pharmacopoeial specifications for weight uniformity, dimensions, friability and assay. Formulation F2 depicted the most desirable profile as all experimental parameters were within the required range for the optimization criterion. F2 depicted 142s, 14h,38.30%,66.02% and 81.20% for floating lag time, total floating time, cumulative drug release at 3,6 and 8 hours respectively.

Conclusion:The findings point to the feasibility of fabricating a commercially viable tablet dosage of floating acyclovir that exhibits a controlled drug release profile therefore affording a more convenient dosing schedule.

CHAPTER 1: INTRODUCTION

1.1 Background

Herpesviridae is a group of Deoxyribonucleic acid (DNA) viruses that infect both animals and humans. There exists over 100 species of this virus of which eight of them are known to infect humans. These include herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), varicella-zoster virus (VZV/HHV-3), Epstein Barr virus (EBV/HHV-4), Cytomegalovirus (CMV/HHV-5), human herpesvirus 6 (HBLV/HHV-6), human herpesvirus 7(HHV-7) and the Kaposi's sarcoma virus (HHV-8)[1]. According to a report published by the World Health Organization (WHO), it is estimated that more than 3.7 billion people are infected with HSV-1 globally. The highest burden of HSV-1 is in the African continent with an estimated 350 million women and 355 million men being infected. The second highest HSV-1 burden is found in South East Asia and the Pacific.HSV-1 virus is transmitted through oral-oral contact and clinically manifests as oral-labial herpes also known as cold sores around the mouth[2]. The global burden of HSV-2 is estimated to be around 417 million in the year 2012 with the highest burden being found in Africa. HSV-2 is predominantly transmitted through sexual intercourse. The disease manifests clinically as sores or blisters in the genital area. There is a strong correlation between the transmission of HIV and HSV-2[3,4].The herpes simplex viruses are highly infectious and are incurable.

Varicella-zoster virus is an exclusively human virus with worldwide distribution. It is highly infectious with the primary infection leading to acute varicella otherwise termed as chickenpox. Transmission occurs following direct contact with a skin lesion and or through airborne droplets. The primary infection presents with fever and a vesicular pruritic rash that mainly affects the face and the trunk. The primary infection is followed by a latent phase in the cranial nerve and dorsal root ganglia. Orofacial acute herpes zoster (HZ) is the clinical manifestation of reactivation of varicella-zoster virus infection. In the United States ,the annual incidence of the diseases ranges between 1.5 to 3 people per 1000 persons[5]. Notable complications include secondary bacterial infections, post-herpetic neuralgia and occasionally chronic neuropathic pain at the infection site [6].

Epstein Barr virus is widespread in human populations and primarily affects B-Lymphocytes and epithelial cells. It is transmitted through body fluids and clinically manifests as infectious mononucleosis characterized by fever, pharyngitis and cervical lymphadenopathy[7]. Cytomegalovirus infections are not obvious in immunocompetent individuals; it, however, may

cause generalized systemic infections such as encephalitis, retinitis, hepatitis, nephritis, splenomegaly, and colitis. After a primary infection, it exhibits a latent phase that can be reactivated in the immunocompromised state [8]. HHV-6 is associated with roseola infantum that involves rapid onset of fever that lasts for 3-5 days, a rash on the torso, limbs, and face follows as the fever subsides[9]. HHV-7 is also known to cause some cases of roseola infantum as it is closely associated with HHV-6. The Human Herpes virus type 8 is associated with connective tissue cancers. The highest prevalence is in central Africa and is most severe in immunocompromised patients, resolving with the restoration of the immune system. The incidence of KS has significantly decreased since the introduction of High Antiretroviral Therapy (HAART)[10].

The drug of choice for the treatment of herpes infections is acyclovir, the drug is very effective against HSV-1 and HSV-2. Its efficacy against VZV and HHV-6 is less compared to HSV-1 and HSV-2[11]. The drug was discovered in 1974 by Schaeffer et al in the search of inhibitors of adenosine deaminase. It is a structural analogue of the nucleoside thymidine[12]. The first formulation was registered in the year 1981 as a topical formulation. It is currently available in intravenous, oral solid and topical formulations. The innovator product is marketed by GlaxoSmithKline by the trade name Zovirax®. The oral dosage forms include tablets and powder for reconstitution. Tablets are presented with the strengths available being 200mg and 800mg. Generic versions of the same also include a 400mg tablet. The powder for reconstitution is developed for paediatrics, geriatrics and any other individuals that have difficulties in swallowing the tablet formulation. It comes with the strength of 200mg/5ml. The intravenous formulation is reserved for patients who cannot take the drug orally, each vial contains 250mg or 500mg of acyclovir.

The drug exhibits a poor and unpredictable bioavailability profile when administered orally owing to its low solubility in aqueous media(2.5mg/ml) and a narrow absorption window with the drug being predominantly being absorbed in the proximal duodenum [13]. The drug is classified as a BCS III molecule when administered in doses up to 400mg above which the drug is a BCS IV drug due to limitations of both solubility and permeability [14].From a review of literature , the oral bioavailability is reported to be between 15-30% [11,15]. Several studies in animals and humans observed that the bioavailability decreases with increasing dosage indicating that the drug is absorbed through an active saturable process [13]. Absorption of acyclovir predominantly occurs in the stomach and the upper part of the small intestine[16].The drug also exhibits rapid elimination with a serum half-life of 1.5-3 hours in

adults and 3-4 hours in neonates with unaltered renal function [13]. As a result, the oral dosage of the drug in adults with herpes zoster infections is extremely high requiring administration of 800mg of the drug five times daily (~ every four hours) for seven days [17–20]. In most developing countries, the formulations available are the 200mg and 400mg tablets necessitating administration of 20 and 10 tablets respectively. This pill burden complicates compliance, as the disease prevalence is higher among immunocompromised patients already burdened by a cocktail of Highly Active Antiretroviral Therapy (HAART) or chemotherapy drugs.

Strategies to improve drug bioavailability are numerous. Approaches include physical and or chemical modifications of the active drug substance as well as employing various formulation strategies. Formulation approaches may include solubility enhancement using size reduction including micronization and nanomization, use of cosolvents, solubilizing agents, use of complexation and employing use of solid dispersions among others. Where bioavailability is limited due to extensive pre-systemic metabolism, controlled drug release systems have been employed. With regard to acyclovir, bioavailability may be improved by prolonging the gastric mean residence time (MRT) that addresses the narrow absorption window that occasions limited absorption [21]. A matrix system offering a controlled drug release profile also prevents saturation of the carriers observed in high dosages. To enhance solubility, numerous studies have highlighted the value of co-crystals and solid dispersions as approaches to enhance the dissolution of acyclovir [22].

Many studies have been done to establish methods of enhancing the bioavailability of drugs exhibiting a narrow absorption windows, among the approaches are gastroretentive formulations. Gastroretentive approaches that have been described in the literature include high-density systems ,floating systems, mucoadhesive systems, and swelling devices all geared towards prolonging the mean gastric residence time [23–25]. A review of the literature has not yielded any research that combines approaches to enhance the bioavailability of acyclovir by addressing both the solubility and absorption issues that ultimately lead to the high dosages administered to patients.

1.2 Problem statement

Herpes zoster is a disease caused by human Herpes zoster3 [17]. The primary infection is termed chickenpox and is transmitted through contact and inhalation of contaminated air. The disease is highly infectious and has an incubation period of between 12 and 16 days. The onset of the disease is preceded by a mild fever and generalized malaise two days before the onset of

a rash. The rash predominantly presents on the face and trunk and is followed by pruritic vesicles. The primary infection is self-resolving with conventional management involving supportive treatment to contain the symptoms [26]. Lifelong immunity against the virus is conferred to the individual following the primary infection with the latent infection remaining in the dorsal root and the cranial nerve ganglia. The disease arises following the reactivation of this latent varicella zoster and spreads from the ganglion to the surrounding neural tissue and the corresponding cutaneous dermatome [27].The reactivation is characterized by a painful unilateral vesicular rash that lasts between 2-4 weeks with high morbidity [17,28]. With advanced age, intensive chemotherapy, malignancies and immunosuppression due to HIV and or post-transplantation medication, the latent herpes zoster virus reactivates causing shingles [29]. The disease occurs in over 30% of persons aged above 70 years in Europe [30]. The incidence of herpes zoster in the developed world is 4 persons per 1000 annually with this increasing to 10-11 per 1000 in those aged 70 years and above and is higher among women [31–33]. The incidence of herpes zoster rose significantly in the early 1980s in sub-Saharan Africa in tandem with the spread of the HIV infection [34]. The main complication observed these patients is postherpetic neuralgia that ensues following the curation of the vesicles. It is accompanied by a debilitating pain that is refractory to conventional analgesics and requires the use of tricyclic antidepressants or anticonvulsants to manage. The neuralgia may be acute or chronic [6,18,35].

Conventional treatment of herpes zoster includes the eradication of the virus and management of the acute pain that presents with the rash. Acyclovir is the prototype deoxyguanosine analogues of synthetic compounds that are used in the treatment of herpes zoster [36]. Other derivatives include valaciclovir, which is a prodrug of acyclovir and converted to the latter in vivo [15]. The other drug registered for this indication is famciclovir. Acyclovir exhibits poor oral bioavailability that is associated with its poor aqueous solubility and incomplete absorption [15]. The bioavailability has been shown to decrease with increasing dosage inferring to a saturable active absorption mechanism. The drug exhibits a very narrow absorption window predominantly occuring in the stomach and proximal duodenum with 50-60% of the drug being excreted in the faeces following oral administration [37]. All these factors conspire to lower the bioavailability of the drug to between 15-30% of the administered dose [38].

As a result, the management of herpes zoster requires the administration of an extremely high dosage of the drug; with adults receiving 800mg five-time daily (roughly every four hours) [36]. The innovator product; Zovirax® comes at strengths of 200mg and 800mg and is largely

unavailable to most of the patients as they cannot afford it. The generic versions available come with strengths of 200mg and 400mg requiring the patients to swallow 20 and 10 tablets daily respectively. Consequently, the compliance to treatment instruction has been reported to be suboptimal [37]. Many patients default on acyclovir treatment as well as HAART due to the extremely high pill burden which subsequently predisposes them to the development of drug resistance. Those who faithfully take their medication are confronted with potential drug adverse effects arising from the cocktail of drugs administered. The risk of drug-drug interactions also increases significantly. The overall outcome is poor treatment outcomes and reduced quality of life for the patient.

This project aimed to develop and characterize a gastroretentive floating tablet formulation of acyclovir. This formulation design aspired to increase the mean residence time in the gastric environment, while providing controlled release of acyclovir in a fashion that can increase the bioavailability and therefore afford a more convenient dosing regimen. The formulation approach employed the use of hydrophillic and gelling polymers as well as gas producing excipients to effect buoyancy of the unit formulation. The project aimed to achieve a product with the desired qualitative, quantitative characteristics and pharmacokinetic profile on the laboratory scale which can subsequently be scaled up thereby offering patients and clinicians a superior alternative to existing regimens. The findings will be published in a relevant and reputable journal to the advancement of science.

1.3 Objectives

1.3.1 The broad objective

The project goal was to formulate and characterize gastroretentive acyclovir floating tablets with enhanced biopharmaceutical properties.

1.3.2 Specific objectives

1. To design the formulation using the Quality by Design approach
2. To perform preformulation studies and determine drug excipient compatibility
3. To formulate gastroretentive floating tablets using the DoE
4. To characterize the finished pharmaceutical product *in vitro*
5. To optimize the formulation and validate the formulation model

1.4 Significance and anticipated outcome

Acyclovir is a safe and effective drug in the management of herpes zoster infection. The oral formulation is beset by poor bioavailability as a consequence of its limited aqueous solubility and low permeability which is attributable to its narrow absorption window and a saturable active carrier-mediated absorption mechanism. These undesirable pharmacokinetic profiles complicate the oral administration of the drug and require high dosage strengths administered frequently (800mg five times daily). This dose strength and dosing frequency is fraught with poor patient compliance due to the high pill burden occasioned. The formulation of 200mg gastroretentive floating tablets of acyclovir aspires to address the permeability shortcoming as the drug exhibits high solubility at this dosage strength. The unit dosage of 200mg was this fit into BCS III thus requiring only modification of the drugs permeability to improve the bioavailability.

CHAPTER 2: LITERATURE REVIEW

2.1 Pharmacological classification of Acyclovir

Acyclovir is a synthetic deoxyguanosine analogue and is the prototype in the class of antiviral agents that target viral thymidine kinase. It exhibits selective affinity for binding to the enzyme thymidine kinase expressed by HSV-1, HSV-2, VZV and other members of the Herpesviridae family of viruses. Based on its mechanism of action it is classified as a DNA polymerase inhibitor[11].The drug is considered a prodrug as it requires activation by viral thymidine kinase to the metabolite acyclovir monophosphate (Figure 1). The metabolite is further phosphorylated by cellular enzyme guanylate kinase to yield a diphosphate moiety with final phosphorylation by phosphoenolpyruvates carboxykinase and pyruvate kinase to yield acyclovir triphosphate. The triphosphate competitively inhibits viral DNA polymerase by competing with the natural substrate deoxyguanosine triphosphate for incorporation into the DNA chain. Upon incorporation, it induces DNA chain termination[39].

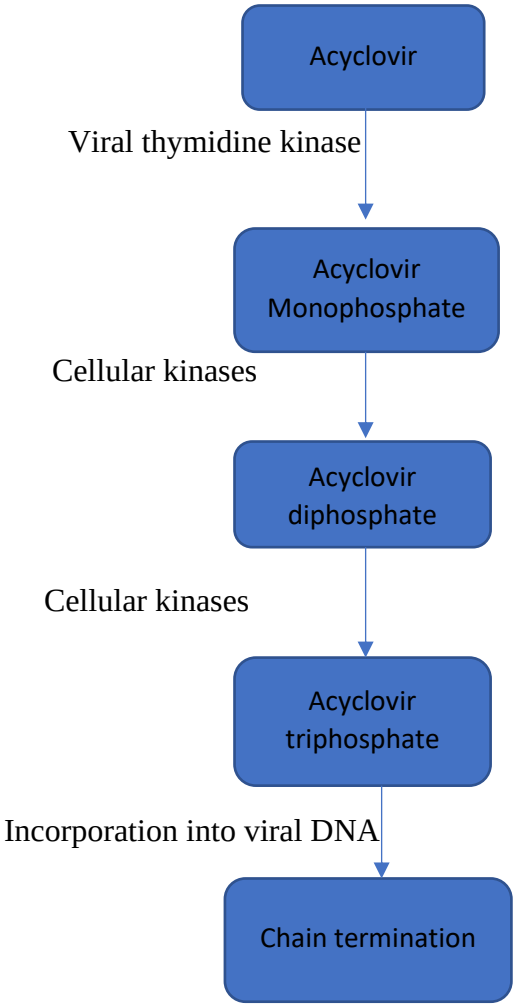


Figure 1: Mechanism of action of Acyclovir and other deoxyguanosine analogues [40]

2.2 Physicochemical properties

The drug chemical name is 9-(2-hydroxyethoxymethyl)guanine, a synthetic acyclic nucleoside analogue of guanosine [39]. The chemical formula is $C_8H_{10}N_5O_3$,with the structural formula as depicted in Figure 2 below.

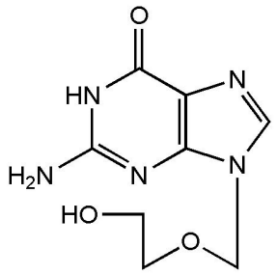


Figure 2: Structural formula of Acyclovir.

Acyclovir has a molecular weight of 225.308g/mol. The molecule is compliant with the Lipinski rule of 5 as it has a molecular weight < 500, <5 hydrogen bond donors <10 hydrogen bond acceptors and a Log P of -1.56 (highly lipophilic). It exhibits two pKas at pH 2.27 and 9.25 and aqueous solubility of 2.5mg/ml at 37°C and pH 7.4 [41–44].

The drug exists as a white crystalline solid with a melting point range of 255-256°C[45].

2.3 Pharmacokinetic profile

2.3.1 Absorption

Due to its low aqueous solubility (2.5mg/ml) and the saturable absorption mechanism, the bioavailability of orally administered acyclovir is between 12-30%. The drug has a very narrow absorption window with absorption predominantly occurring in the gastric region and the upper part of the duodenum. The bioavailability of acyclovir following oral administration has been observed to decrease with increasing dosage inferring to a saturable active transport mechanism [12,13,46]. Absorption of acyclovir is not affected by food. Studies to establish the transepithelial permeation of acyclovir on CaCO2 cell lines indicate that it has low permeability that can be enhanced by using penetration enhancing excipients [47,48]. The pharmacokinetic parameter indicating the extent of absorption, AUC, increases linearly when the drug is administered using dose strengths between 200mg and 400mg five times daily. Nonlinear kinetics are observed above this dosing regimen indicating a saturable active absorption process [38].

2.3.2 Distribution

Following oral administration, acyclovir exhibits a two-compartment model of distribution like that observed when it is administered intravenously. Studies indicate that it binds to plasma proteins, at range 20-33%, indicating a low potential for drug interactions. Data on the volume of distribution is unavailable but studies indicate that the drug is extensively distributed in the tissues including the brain. The highest concentration is observed in the renal system where the drug concentration are 10-fold those observed in plasma [49]. Drug concentrations in the heart, lung, and liver were found to be equal to the plasma concentration. Transplacental permeation has been demonstrated in rats and rabbits and in clinical trials [50]. The levels of acyclovir in breast milk were 3.2-fold higher than that observed in plasma indicating active transportation, but constitute around 1% of the dose required to cause toxicity [51,52].

2.3.3 Metabolism

Acyclovir undergoes limited biotransformation in the body. Minor hydroxylation and oxidation occur on the molecule giving rise to 8-hydroxyacyclovir and 9-carboxyacyclovir accounting for 9-14% of the administered dose. No studies have indicated the cleavage of acyclovir in any species [50].

2.3.4 Excretion

Acyclovir is primarily excreted via the kidneys through active secretion and glomerular filtration. Following intravenous administration, between 71-99% of the administered dose is excreted through urine while 2% is excreted in faeces. This is in contrast with the disposition in oral administration where between 50-60% of the drug is unabsorbed thus is eliminated in faeces [38,53,54].

2.4 Biopharmaceutical classification profile of Acyclovir

The biopharmaceutical classification system (BCS) is an approach that categorizes orally administered drugs based on two physicochemical properties; solubility and permeability. Acyclovir fits into the BCS class III drug category as it exhibits a poor permeability and high solubility profile at low dosages of up to 400mg. When administered at doses of 800mg as occurs in herpes zoster treatment, the drug falls into BCS IV exhibiting low solubility and permeability [14,55]. As such efforts to improve its bioavailability may be directed at enhancing solubility while others seek to improve the permeability. Novel formulation approaches that can enhance both attributes are more likely to achieve significant improvements in bioavailability compared to those addressing individual problems.

2.5 Bioavailability

Bioavailability refers to the rate and extent to which the active moiety of the drug substance becomes available at the site of action following drug administration via extravascular routes. It's a critical parameter that determines many elements of the formulation design including the route of administration, the selection of the salt form, the dosage form, the unit dosage and frequency of dosing among others. Bioavailability of a drug is a function of its aqueous solubility, dissolution rate, permeability across the intestinal mucosal barrier, chemical and metabolic stability profile [56,57]. The bioavailability of a drug can, therefore, be described as dissolution limited or permeability limited based on the solubility and permeability respectively [58,59]. The formulation team developing a drug is charged with the responsibility of addressing the factors contributing to poor bioavailability of a drug while considering other critical attributes of the molecules including the potency, selectivity, and stability. Bioavailability of the drug is therefore established during the lead optimization process and through the entire preformulation process to inform whether further physicochemical modifications are required or determine whether special formulation approaches need to be adopted to enhance it. The major approaches include formulation technologies to enhance the solubility of highly lipophilic molecules while others are directed at enhancing the permeability through the intestinal mucosal barrier [22,60]. Figure 3 below depicts the BCS classification of drugs and the approaches employed to enhance their bioavailability.

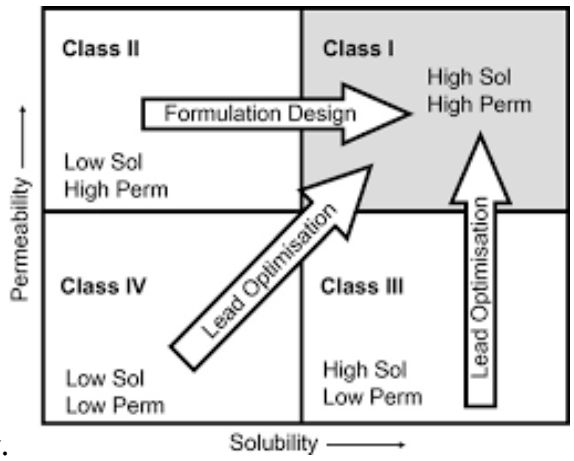


Figure 3: BCS classification of drugs and approaches to improve bioavailability[61].

2.6 Approaches to enhance drug solubility

The dissolution of poorly soluble drugs intended for solid dosage forms can be addressed through various methods. The major approaches include preparation of solid dispersions, formulation of cocrystals, crystal engineering to reduce crystallinity, inclusion complexes,

particle size reduction, nanomization and careful selection of polymorphs and formulation as salts [22,56,57,61–64]. Numerous studies have attempted to enhance the solubility of acyclovir using formulation of cocrystals with various tartaric chemicals as well as co-grinding [65–67].

2.6.1 Solid dispersions

Solid dispersions refer to formulations consisting of two or more components commonly including a hydrophilic matrix usually polymers and a hydrophobic drug [68,69]. The solid can be either crystalline or amorphous forms. The solid dispersions are aimed at enhancing the solubility profile of poorly soluble drugs by distributing it in the hydrophilic polymer matrix either molecularly or as amorphous or crystalline particles[70]. The polymers used in carrier systems include hydroxypropyl methylcellulose, polyvinyl pyrrolidone, polyethylene glycol, methylcellulose, urea, lactose, citric acid among others [71,72]. The requirement for selection is that they should be inactive pharmacologically and be compatible with the active substance whose solubility the formulation scientist seeks to be improved. Solid dispersions can be classified into various categories and include simple eutectic mixtures, solid solutions (can be continuous or discontinuous) [64].

Upon solubilization of the carrier molecules, a fine suspension of the drug particles is exposed to the dissolution medium. The smaller particle size affords higher wettability contributing to a faster dissolution rate. The drug is molecularly dispersed in solid solutions and exists in the amorphous form [57,73]. Following the dissolution of the solid the drug is presented in a supersaturated solution. Increased wettability and the effect of cosolvency also contributes to the enhanced solubility. The drug release properties are dependent on the carrier drug combinations with various polymers exhibiting different release profiles. To establish the value of solid dispersions, dissolution profiles of the pure drug, physical mixture, and the solid solutions are compared [71].

Solid preparations can be prepared through different methods. These include hot melt approach whereby the drug and the carrier are melted in the same vessel and the resulting liquid cooled off using appropriate technologies [71,74]. The resulting solid eutectic is then milled to effect particle size reduction. The drug becomes entrapped within the carrier matrix. The miscibility of the drug and the carrier system in the molten state is a prerequisite for the preparations of solid solutions. Further considerations include the thermostability of the drug and the carrier. Solid dispersions may also be prepared by the solvent method [75]. The process involves dissolving the drug and the carrier in a common solvent followed by evaporation of the solvent

under vacuum. The product is a solid solution of the drug dispersed in the highly soluble carrier system. The drug carrier ratio determines the rate of drug release from the solid dispersion. To use the solvent method, the drug, and the carrier must both be soluble in the chosen solvent system. The solvent used is then removed by evaporation or freeze drying. It must be emphasized that all the solvent should be removed from the mixture. Hot melt extrusion is currently the main technique employed in the fabrication of solid dispersions whereby the drug and the carrier are thoroughly mixed in an extruder with the technology avoids the use of organic solvent and high temperatures that preclude the preparation of thermolabile drugs [71,76].Spray drying is also employed in the formulation of solid dispersions with several marketed products using this technology [72].

Solid dispersions are characterized and analyzed using various methods including infrared spectroscopy, X-ray diffraction, and thermogravimetric analysis. The drug release studies may also be included [73,77]. Lack of crystallinity can be used to distinguish solid solutions from solid dispersions. Differential scanning calorimetry is the most preferred method in that gives quantitative information and aids in the determination of the crystallinity of the resulting solid dispersion [71].

2.6.2 Nanotechnology application in the enhancement of the solubility of BCS II and BCS IV drugs

Nanomization has been one of the frontier technologies applied to enhance the solubility of drugs coming through the drug discovery pipeline [64]. The technology was developed in the early 1990s with the first product employing this technology, Rapamune® being approved by the Food and Drug Authority (FDA) in the year 2000 [78]. Nanoparticles are defined as materials whose size is below 1µm [79]. They include nanosuspensions, nanoemulsions and nanocrystals based on the states they exist in. Nanomization has the capacity to increase the solubility of poorly soluble drugs through surface area amplification therefore rapid dissolution [80]. The resulting particles also do exhibit higher stability profile as they are lighter, therefore less susceptible to sedimentation if formulated as suspensions.

2.6.2.1 Fabrication of nanoparticles.

Nanoparticles manufacturing is broadly classified into two categories; top-down and bottom-up approaches. The top-down process entails comminution of the micronized drug particles using planetary ball mills or using high-pressure homogenization. The starting material comprises of solid particles much larger than the resulting nanoparticles. The size reduction is

achieved through mechanical processes. The wet milling process is done by incorporating liquids such as water or polyethylene glycol and stabilizers; wet milling achieves higher milling efficiency compared to dry milling. The primary mechanism of size reduction in planetary ball mills is attrition and shearing [79–82].

High-pressure homogenization is widely used in the formulation of nanosuspensions that contain the drug material and stabilizers. The process entails incorporation of the stabilizer in a dispersion medium (usually water) in proportions of 0.2- 2.0% w/w. The required concentration of the drug, usually 5% w/w of the drug material is then added to the dispersion medium containing the stabilizer under magnetic stirring after which the resulting suspension is transferred to the high-pressure homogenizer. Initial size reduction occurs rapidly due to the existence of defects in the crystal lattice of the particles as well as bigger cracks which enable quick crack propagation. Subsequent milling achieves slower size reduction and a plateau is achieved after comminution has been optimized. Homogenization effects size reduction through high shear, cavitation, and interparticle collisions [83–85].

The stabilizers are required to prevent aggregation of the nanoparticles leading to size enlargement. They achieve stabilization through two mechanisms; Steric stabilization and electrostatic stabilization. Steric stabilizers adsorb onto the surface of the drug particles with their hydrophilic tails protruding into the bulk liquid phase. These presents steric hindrance for the particles tending to approach each other [79]. The steric stabilizers are mainly polymers of cellulosic origin including hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC), polyvinyl pyrrolidone (PVP)and polyvinyl alcohol (PVA). Pluronic's are also used and include F68 and F127. Electrostatic stabilizers are usually surfactants, and these adsorb onto the surface of the nanoparticles repelling other approaching particles to prevent flocculation. The most commonly used surfactants are the anionic ones including sodium lauryl sulphate. Stabilizer drug ratios cited in literature range from 0.1: 1 to 0.2:1 [78].To optimize the stability of nanosuspensions and nanoemulsions a combination of the two stabilizing mechanisms is often explored [78,80].

In the bottom-up approach, the solid particles are dissolved in an appropriate solvent system after which controlled precipitation is done through the addition of an antisolvent usually water. The stabilization is then effected in a manner similar to the one described above [82].

2.6.2.2 Solidification of the resulting nanosuspensions

Solidification of liquid nanoparticles can be achieved using freeze drying, spray drying or lyophilization. To obtain the solid nanocrystals the nanosuspensions or nanoemulsions are loaded into a freeze drier where the dispersion fluid is evaporated. A redispersant is usually added to prevent aggregation as the nanoparticles become concentrated upon drying. Materials used as redispersants are usually sugars like sucrose, lactose, and mannitol. The drying process is done at temperatures between -20°C and -70°C with the duration reducing at the lower temperatures. The risk of drug destruction is higher at lower temperatures [80,86–88].

2.6.2.3 Characterization of nanoparticles

The morphology, size and zeta potential of the resulting nanoparticles is often evaluated using microscopy and a Zetasizer respectively. The Zetasizer employs laser diffraction to calculate the particle size. The shape can be described as spherical, cuboid or cylindrical with the optimal surface area being realized with the spherical particles [89].

2.7 Enhancing drug permeability

Enhancing the permeability of drugs with poor permeation presents a bigger hurdle compared to efforts geared towards addressing poor solubility. Approaches include the use of excipients that distort the structural integrity of the intestinal barrier; these include EDTA and chitosan [90–92]. Approaches to prolong the gastric residence time can also be employed in enhancing the permeability of drugs impaired by a narrow absorption window [25]. The use of controlled release formulations would also contribute to improved permeability for drugs whose low permeability is due to saturable absorption mechanism. Use of agents that inhibit efflux transporters has also been investigated [21,23–25,93].

2.8 Gastroretentive drug delivery systems (DDDS)

Gastroretentive drug delivery systems are formulation approaches that are designed to improve the bioavailability of drugs with a narrow absorption window. These include drugs that are destined to act locally in the gastric environment like antacids and antiulcer drugs. Drugs that are predominantly absorbed from the gastric mucosa and the upper part of the duodenum also benefit significantly from the extension of the gastric residence time [23,94]. Other candidates include drugs that are unstable in the alkaline environment of the small intestines. Acyclovir exhibits a narrow absorption window with the drug absorption predominantly occurring in the gastric mucosa and the upper part of the duodenum[95]. Strategies employed to prolong the

gastric residence time include mucoadhesive systems, swelling systems, high-density systems, floating systems and combinations thereof [24,93].

2.8.1 Expandable systems

These are formulations that are designed to swell upon contact with gastric fluid affording them a large volume that prevents them from being pushed through the pyloric sphincter, therefore, prolonging their residence in the stomach. They exploit the swelling properties of hydrophilic polymers that have high swelling indices thus afford significant gain in volume by the tablet core[96]. The volume of the unit tablet decreases as the drug is consistently released and the drug core is eventually evacuated from the stomach. These formulations use polymer swelling and unfolding to achieve the volume increase. Hydrophilic polymers commonly employed in the formulation of the expandable systems include hydropropylmethyl cellulose (HPMC), Polyethylene oxide and Carbopol. Advances in research have enabled development of intelligent polymers that alter their volume and drug release properties depending on the prevailing physiological environmental factors including pH temperature, and solvent concentration [97–99].

2.8.2 Mucoadhesive systems

These are formulations designed to adhere to the gastric mucosa thereby increasing the time that the unit dosage form remains in the gastric region. This approach employs the properties of polymers that have bioadhesive properties including, HPMC, alginic acid, chitosan, polyacrylic acid, dextran, xanthum gum, and Carbopol. The duration of adhesion is dependent on the polymer characteristics. The intimate contact of the polymer and the mucin proteins localizes the drug core and prolongs gastric residence significantly and affords a controlled drug release profile in a predictable manner. Drawbacks for this approach include the risk of sticking to the oesophagus causing ulceration, high turnover of the gastric mucosa and constant erosion of the bioadhesive layer.

2.8.3 High-density systems

This approach utilizes high-density excipients that make the tablets denser than the gastric milieu (1.004g/ml) causing them to sink to lowest part of the stomach, the antrum and rugae where they can resist peristaltic expulsion. Studies have established that the densities required to use this approach range between 2.4 and 2.8g/ml [100,101]. Potential excipients for high-density systems include Zinc oxide, Barium sulphate, Iron oxide and Titanium oxide

[25,93,94]. The main disadvantage of this approach is that the large size of the unit tablets due to the excipients used [102].

2.8.4 Floating systems

This has been the most widely researched approach among the GDDS. It utilizes polymers and technologies that make the tablets buoyant in the gastric environment [103]. Formulation approaches include effervescent and non-effervescent technologies. Effervescent formulations include a gas producing excipients such as $NaHCO_3$, $CaCO_3$, and citric acid that produce carbon dioxide upon interaction with the hydrochloric acid in the stomach. The gas generated becomes entrapped in the polymeric matrix thus reducing the tablets density to less than 1.004g/ml effecting the buoyancy. Others include raft forming systems[104].

Non-effervescent formulation utilizes the swelling and gelling potential of polymers. Upon contacting the gastric fluids, the polymers swell outwards and form a gel layer that entraps gas inside their matrices core. The drug diffuses through the polymeric matrix in a controlled fashion prolonging the release time the rate of which is determined by the matrix viscosity and the length of the diffusion path.

2.8.5 Combined approaches

Other formulations include combined approaches mainly; swelling and floatation, swelling and bio adhesion, high-density systems and bio-adhesion. The utilization of all these approaches improves bioavailability, therefore, reducing the amount of drug required for treatment.The stability of acyclovir in low pH was established through literature search with publications indicating that the drug is stable at low pH levels (1.2) over time period of up to seven days [105].

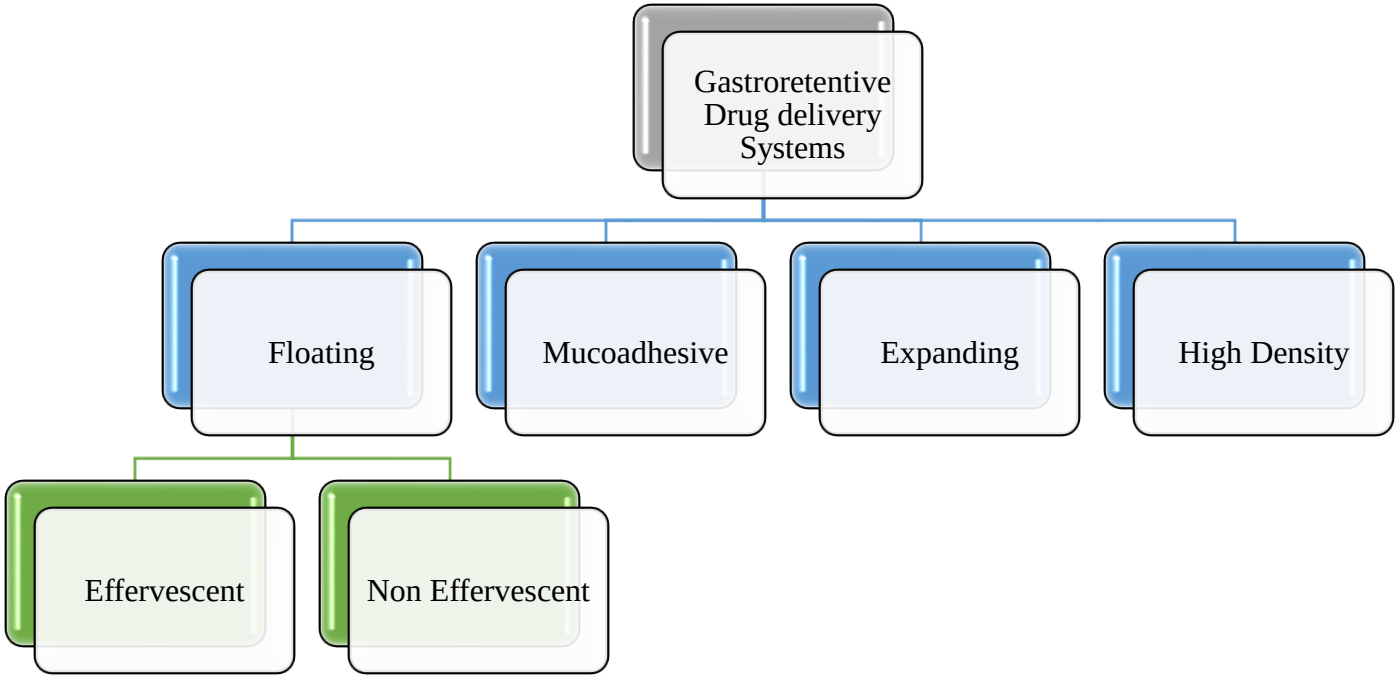


Figure 2.8: Gastroretentive drug delivery systems formulation approaches

CHAPTER 3: MATERIALS AND METHODS

3.1 Study location

The formulation and analysis were carried out in the Department of Pharmaceutics and Pharmacy Practice, and the Drug Analysis and Research Unit in the Department of Pharmaceutical Chemistry in the School of Pharmacy, University of Nairobi.

3.2 Materials

The active pharmaceutical ingredient Acyclovir and the reference standard was a kind donation by Universal corporation, Nairobi Kenya.

The excipients include hydroxypropyl methylcellulose, (HPMC K100 and HPMC K4M) Carbopol 934P, Polyvinyl pyrrolidone (PVP), magnesium stearate sodium bicarbonate. These materials were procured from Research Lab Fine Chem Industries, Mumbai India; a GMP certified pharmaceutical excipients manufacturer. The analytical reagents including sodium hydroxide, Acetic acid, hydrochloric acid was procured from Loba Chemie, India.

All the reagents used in the analysis were of analytical grade.

3.3 Equipment

A single station tablet press(Inweka India), Analytical balance(Sartorius Gmbh, Germany), Fourier transformed Infrared Spectrophotometer(Shimadzu IR Prestige 2.1,Tokyo Japan, HPLC(Shimadzu,Tokyo Japan), Ultra violet spectrophotometer(Genesys 10S V 4UV-VIS), Dissolution Apparatus II(Electrolab, India),Stability chamber(Binder Gmbh) Electronic tablet hardness tester(Scheuniger and Co, Germany), and Friability tester(Erweka Germany) ,pH meter(Jenway)were used in the study.

3.4 Preformulation studies

3.4.1 Identification of the active ingredient

The identity of the acyclovir was established using FTIR employing the attenuated total reflectance technology. Powder samples were prepared and scanned in the FTIR to generate the respective spectra over the range 500-4000cm^{-1}

The absorption peaks were matched with those of the reference spectra obtained from the chemical reference standard under similar experimental conditions. Further, validation of the identification was done while conducting the assay where the retention time of the sample were compared to those of the reference standard assayed under similar experimental conditions.

3.4.2 Drug-excipient compatibility tests

The excipients were selected based on the target product profile backed by an extensive literature review of formulations that utilized floating and swelling polymers. Binary mixtures of the active substance and excipient were accurately weighed and mixed, stored at the accelerated environmental conditions (40°C± 2, 75± 5% RH) for thirty days. The FT-IR spectra of the pure drug substance, drug-excipient binary mixtures were obtained for the range 400-4000 cm^{-1} prior and after the month storage in the accelerated conditions. The resulting spectra was analyzed for peak disappearance, displacement, or any form of variation indicative of chemical and/or physical incompatibility.

3.4.3 Micromeritics

The angle of repose, bulk density, tapped density, Carr's index and Hausner's ration were determined to establish the flow properties of the powder blend. Forty grams of the powder blend were accurately weighed andcarefully transferred to a clean glass beaker; the angle of repose was determined using the funnel method using a height of 4.0 cm. The powder was then gradually transferred into a graduated 100ml graduated measuring cylinder to determine the bulk density. To obtain the tapped density, the cylinder was fixed on a loop and subjected to tapping until no further change in volume was recorded. The tests were done in triplicate and the average and standard deviation recorded. The formulas for angle of repose, Carr's Index and Hausners ratio are given below

$$\mathbf{A = Tan^{-1}(h/r)} \qquad \textit{Equation 1}$$

Where **A** is the angle of repose, **h** refers the height of the powder pile and **r** refers the radius of the powder pile in centimetres.

$$\mathbf{Bulk\ density\ (BD) = Mass\ of\ powder(g)/Volume(cm^3)} \qquad \textit{Equation 2}$$

$$\mathbf{Tapped\ density\ (TD) = Mass\ (g)/\ Tapped\ volume(cm^3)} \qquad \textit{Equation 3}$$

$$\mathbf{Carr's\ index = (TD-BD)/\ TD * 100} \qquad \textit{Equation 4}$$

Where TD is the tapped density and BD is the bulk density of the powder blend respectively.

$$\mathbf{Hausner's\ ratio = TD/\ BD} \qquad \textit{Equation 5}$$

Where TD, BD refer to the tapped density and the bulk density of the powder blend respectively.

3.5 Design of experiment (DOE)

The experimental design for this project was fashioned to the simplex lattice mixture design, a modification of the response surface methodology of experimental designs [106]. This design allows the evaluation of specific individual and combined interactive effects of the independent variables on the dependent variables [107,108]. Three factors were investigated; HPMC k100M(X_1), HPMC k4M (X_2) and Carbopol 934P and constituted the independent variables in this study. Their relative proportions in the formulation were varied as per the simple lattice mixture design model to a total polymer concentration of 180mg. A total of ten combinations was generated by the Design expert® software [106,109]. Five dependent variables were selected include. These included floating lag time (Y_1), Total floating time(Y_2), Cumulative drug release at 3 hours, 6 hours and 8 hours (Y_3, Y_4, and Y_5) respectively. The amount of all other ingredients was held constant in all the formulations; Acyclovir 200mg, $NaHCO_3$ 110mg, Polyvinyl pyrrolidone (PVP) 45mg and Magnesium stearate 5mg, making a total tablet weight of 540mg. Other process variables including the mixing process were also held constant to minimize the compounding effects that would cause alterations in the dependent variables. As such, the observed differences in the observed outcomes could be attributed to the effects of the polymer variations. Table 1 below details the actual composition of each formulation.

The data generated from the floating tablets was keyed into the Design expert software and subsequently used to generate the polynomials describing the relationship between the input and outcome variables. The software guided the selection of the best models to explain the relationship between the independent and outcome variables based on the significant p values, observed and adjusted R^2 in the respective models. Response surface plots and contour plots were generated to detail the relationships graphically. Ultimately the software was used to generate the optimized formulation with the objectives of minimizing the floating lag time, maximizing the floating time and obtaining a controlled drug release profile with maximal cumulative drug release target being achieved at 8 hours. A final formulation F_0 was fabricated to validate the proposed optimized formula.

The second order polynomial described by the equation below was envisioned.

$$Y=\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3 \quad \textit{Equation 6}$$

Where Y is the dependent variable, β_1, β_2 and β_3 are the regression coefficients of pure independent variable while β_{12}, β_{13} and β_{23} represent the regression coefficients for the respective interaction components for the independent variables.

Table1.Composition(mg) of formulations in the simplex lattice mixture design(non-coded)

Formulation	**ACV**	**HPMC K100M**	**HPMC K4M**	**Carbopol 934**	**$NaHCO_3$**	**PVP**	**Mg. St.**	**Total**
F_1	200	180	0	0	110	45	5	540
F_2	200	0	180	0	110	45	5	540
F_3	200	0	0	180	110	45	5	540
F_4	200	120	60	0	110	45	5	540
F_5	200	60	120	0	110	45	5	540
F_6	200	0	120	60	110	45	5	540
F_7	200	0	60	120	110	45	5	540
F_8	200	120	0	60	110	45	5	540
F_9	200	60	0	120	110	45	5	540
F_{10}	200	60	60	60	110	45	5	540

3.5.1 The rationale for the selection of polymers and other excipients

Fabrication of effervescent gastroretentive tablets includes hydrophilic gel-forming polymers that entrap the carbon dioxide released when the gastric acid interacts with the effervescent ingredients[110]. The gas entrapping causes the density of the tablet to fall below that of gastric contents, therefore, effecting the buoyancy of the unit formulation. The so formed matrix also facilitates the controlled release of the drug molecules from the core with the rate being dependent on the viscosity of the gel matrix. HPMC has been extensively used in controlled release oral formulations as it exhibits these gelling properties [111]. In floating drug delivery systems, the polymer swells and gels around the dry central core polymer matrix entrapping the carbon dioxide generated by the effervescent ingredients. In addition, it is available in a variety of grades that yield different viscosity profiles upon interacting with water. HPMC k4M and HPMC k100M were selected based on the wide range of viscosity viz 4,000mPa and 100mPa respectively [21,93,104,112]. Blending different proportions of the two affords variation of the gel viscosity to optimize drug release profile. Another key component in floating tablets is a hydrophilic polymer that achieves high swellability to achieve the desired buoyancy as well as restrict the expulsion of the unit formulation through the pylorus [113]. Carbopol 934 has been extensively employed in the fabrication of floating tablets and grade 934 was selected due to its wide availability, safety, and cost. Carbopol also swells in aqueous solutions.

The proportions of the three polymers were varied to accurately determine the contribution of each in the dependent variable; floating lag time, total floating time and cumulative drug release. The proportions of the formulations were guided by the combinations of an augmented, non- replicated simplex lattice mixture design (Table 2). This experimental design allowed the generation of a second order mathematical model (quadratic and above) to accurately detail the correlation between the independent variables and the observed outcomes [114]. Being a three factor, three level design, a total of ten combinations will be formulated and evaluated [106]. The specific polymer ratios are detailed in the table below and in Figure 4 [115].

Polyvinyl pyrrolidone and microcrystalline cellulose have been included as binders. Their use in direct compression with favourable tablet hardness profile has been cited widely in the literature. Magnesium stearate was incorporated as a lubricant. Sodium bicarbonate is the effervescent moiety that generates the carbon dioxide required for effecting the buoyancy.

Table 2: Coded proportions of polymers in the experimental design

	HPMC k100 LV (X_1)	**HPMC k4M (X_2)**	**Carbopol 934(X_3)**
1	1	0	0
2	0.667	0.333	0
3	0.333	0.667	0
4	0	1	0
5	0	0.667	0.333
6	0	0.333	0.667
7	0	0	1
8	0.333	0	0.667
9	0.667	0	0.333
10	0.333	0.333	0.333

NB: The order of the ratio combinations is random and were generated by the software.

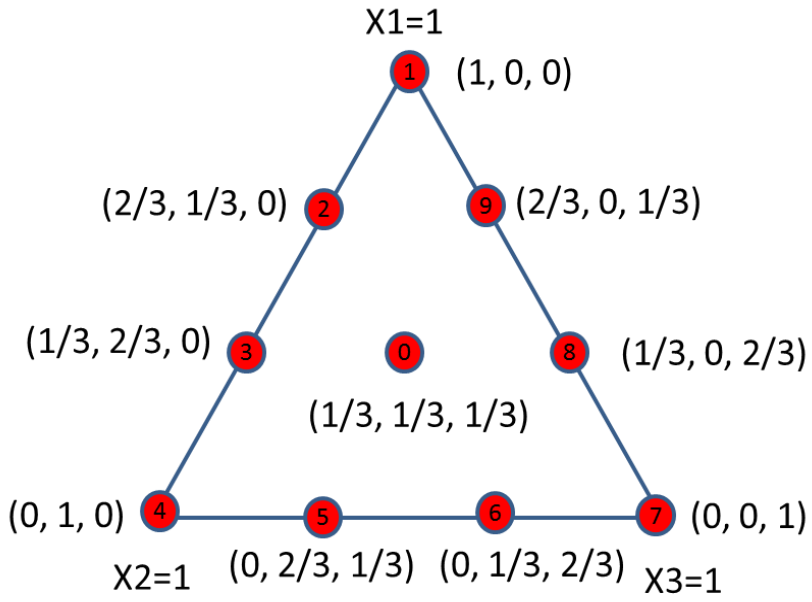


Figure 3.5: Proportions of variables in the augmented simplex lattice design.X1(HPMC K100M), X2(HPMC K4M), X3(Carbopol)

The augmented design comprises of ten formulations containing 3 pure substances(1,0,0) (0,1,0) and (0,0,1), six binary mixtures (2/3,1/3,0) , (1/3,2/3,0), (0,2/3,1/3), (0,1/3,2/3) (1/3,0,2/3) and (2/3,0,1/3) and one ternary mixture comprising (1/3,1/3,1/3) of each polymer.

3.6 Formulation

Different batches comprising of varying ratios of the polymers were prepared by direct compression employing the single punch tablet press (Inweka Gmbh). The drug, polymers, $NaHCO_3$ and PVP were accurately weighed individually using a calibrated analytical balance (Sartorious GMBH) according to the DoE and passed through a mesh size 50 to obtain a fine powder. The powders were then be blended for 15 minutes using a plastic container and a spatula employing the geometric mixing approach. Magnesium stearate was then added, and mixing continued for an additional 4 minutes. The resulting mixture was then compressed using a single station tablet press (Inweka GMBH having a die diameter of 10mm) after the precompression parameters had been obtained.

3.7 Post compression quality assurance tests

3.7.1 Tablet friability test.

The friability of the floating acyclovir tablets was established using the USP 2018 guidelines. Twenty tablets were randomly selected and dedusted. These were accurately weighed using the analytical balance and be loaded into the tablet friability testing apparatus (Electrolab)and set at 100 rotations (25rpm for 4minutes). The weight of the tablets is taken after removing any loose dust.

The friability is calculated as the percentage weight loss using the formula

$$\mathbf{F\% = (W_1 - W_2)/W_1 * 100} \qquad \textit{Equation 7}$$

Where F, W1, and W2 represent percentage weight loss, initial weight, and final weight respectively.

Triplicate tests were done with the friability being the average value of the three tests. A value of less than 1% is acceptable.

3.7.2 Tablet thickness and diameter

The thickness and diameter of ten randomly selected tablets were taken using a Vernier calliper. The average values and standard deviation were then calculated from the data obtained.

3.7.3 Tablet hardness test

The mechanical integrity of the tablet was established by measuring the force required to break them diametrically on a specific plane. Ten tablets were randomly selected and individually placed between the plates so that the force is applied across the diameter. The breaking force was recorded in Newtons with the acceptable range being between 60 and 80N [116].

3.7.4 Uniformity of weight test

From each batch, a total of twenty tablets were randomly selected and their individual weights determined. The individual weights were compared to the average tablet weight. Compliance was achieved where no more than two tablets exceed or fell below the pharmacopoeial specifications of NMT 5% and NMT 10% for a single tablet for tablets weighing more than 250mg and 325 mg for the British Pharmacopoeia and United states pharmacopoeia respectively [117,118].

3.8. Drug release properties and modelling of drug release profiles

The rate of dissolution of acyclovir was evaluated using the USP dissolution apparatus II (Electrolab, India). The dissolution medium was 0.1 N hydrochloric acid, a volume of 900ml was filled for each vessel and the stirrer speed set at 50 RPM for the duration of 8 hours. The bath temperature was set at 37±0.5°C

Automated sampling was done where 10ml aliquots of the fluid was drawn at specified timings (1,2,3, 4,6, and 8hours). Six samples were drawn for every batch. The samples were filtered using a filter membrane;45microns. After sample withdrawal, a similar volume of the dissolution media maintained at the same temperature was added to the dissolution vessel. Samples were diluted as appropriate and then analysed using UV spectroscopy at 254nm and the average absorbance and standard deviation recorded. The cumulative amount of acyclovir released is calculated at each sampling instant. Scatter plots of the time versus cumulative percentage drug release was plotted from the data.

3.8.1 Description of the dissolution profiles

The cumulative percentage of drug released was calculated at each sampling instant. The ensuing data was be subsequently fitted to zero order kinetics, first-order kinetics, Higuchi and Korsemeyers-Peppas kinetics using the DDSolver dissolution kinetic modelling software[119–121]. Equations for plotting the kinetic data are as follows[119].

Zero-order

$$\mathbf{Qt = Q_0 + K_0 t} \qquad \textit{Equation 8}$$

Where $Q_{t\ and}$ Q_0 is the cumulative percentage of drug release at time t and time zero respectively and K_0 is the zero-order release constant.

First Order

$$\mathbf{lnQ_t = lnQ_0 + k_1 t} \qquad \textit{Equation 9}$$

Where Q_t and Q_0 is the cumulative percentage of drug release at time t and time zero respectively and K_1 is the first order release constant.

Higuchi model

$$\mathbf{Q = K_H t^{0.5}} \qquad \textit{Equation 10}$$

Where Q is the cumulative drug release at time t and K_H is the Higuchi dissolution constant.

Korsemeyers-Peppas model

$$\mathbf{Mt/\ M\infty = Kt_n} \qquad \textit{Equation 11}$$

Where Mt/ M∞ refers to the fraction of drug dissolved at time t and k is the release constant while n is the drug release exponent that describes the mechanism of drug release. The interpretation of the release mechanism is as per Table 3 [122,123].

Key parameters to be recorded include the dissolution constants and R^2 for each model. The model with the highest R^2 values will be selected for explaining the release profile for each formulation [124,125].

Table 3. Interpretation of the drug release mechanism

Release exponent(n)	**Drug release Mechanism**
n≤ 0.5	Fickian diffusion
0.5<n<0.89	Non-fickian
0.89	Case II
n> 0.89	Super case II

Fickian drug release mechanism describes a situation whereby drug release is predominantly driven by diffusion of drug molecules through the polymer matrix, non fickian release describes the situation where drug release occurs through both diffusion and swelling of the matrix. While in super case II, drug release is driven by complex mechanisms involving diffusion, matrix erosion and swelling all occurring concomitantly.

3.9 Assay of active ingredient

3.9.1 Preparation and assay of test solution

Twenty tablets of acyclovir floating gastroretentive tablets were pulverised using a mortar and pestle and thoroughly mixed to homogeneity. Samples of the resulting powder were accurately weighed in triplicate to obtain a powder weight equivalent to 10mg of acyclovir. 10mg sample for the reference acyclovir was also weighed.

The samples were dissolved in 30ml using freshly prepared 0.1N NaOH with the aid of a sonicator for a duration of 15 minutes. The volume was gradually made up to 100ml using distilled water with consistent shaking to ensure homogenous distribution. The process yielded a clear solution containing a concentration of approximately 0.1mg/ml of the drug. The solutions were left to settle for 5 hours after which 2ml was pipetted into glass after filtration through a 0.45µm filter paper.

Analysis was done using an Agilent 1260 (Agilent Technologies, California, USA) HPLC machine throughout the study. It consisted of a quaternary pump (DEAB818623), an autosampler DEAC37011, a column thermostat DEACN39585 column oven with a block heating mechanism. A diode array UV detector was employed. The stationary phase employed was a Xterra reverse phase C_{18} 5µm (dimensions 250nm*4.6mm.

The mobile phase was freshly prepared 0.02N glacial acetic acid. The operational parameters included a sampling volume of 20µl, a flow rate of 1.5ml/min and a column temperature of 40°C. The method of detection utilized was UV set at a wavelength of 254nm.

The system suitability test was done to confirm that the equipment would sufficiently analyse the samples. Six replications of the standard solution at a concentration of 0.1mg/ml were analyzed with the parameters of interest being the retention time, peak asymmetry and peak resolution. Evaluation and quantification were done on Open Lab CD (EZ Chrom edition) Version A.04.07 chemstation software which controlled all aspects of the system.

The percentage label claim of acyclovir was determined using the formula in the equation 12

$$L.C = (R_u/R_s * C_s/C_u) \times 100 \qquad \textit{Equation 12}$$

Where L.C, R_u, R_s, Cs, C_u represents percentage labelled claim, peak area of test solution, peak area of standard solution, concentration of standard solution and concentration of sample solution respectively.

3.10 In vitro buoyancy test

In vitro buoyancy of the formulation was established by measuring the floating lag time and the total duration that they remain afloat. The test was performed using a volumetric flask containing 200ml of 0.1 N HCl. Tablets randomly selected of each batch were loaded into the vessels and visually observed. The time taken for each tablet to float to the surface was recorded as floating lag time (FLT) while the total time duration during which the tablet remain afloat was recorded as the total floating time. The experiment was done for six tablets per batch with the average time and corresponding standard deviation and coefficient of variation being recorded.

3.11 Swelling index

Six tablets per batch were accurately weighed and individual loaded into a volumetric flask containing 100ml of 0.1 N HCl. The tablets were removed every hour for eight hours and excess fluid removed using a blotting paper. Their weights were recorded after which they were placed back into the volumetric flask. The swelling index was calculated from the data using the following equation;

$$\mathbf{SI = \{(W_t - W_0)/ W_0 * 100\}} \qquad \textit{Equation 13}$$

Where SI is the swelling index, W_0 is the initial tablet weight and W_t is the tablet weight after time t.

3.12 Statistical analysis

The data obtained from these experiments was analyzed using the Software for Statistical and Data Science (STATA) version 14. Dissolution data analysis was done using DDSolver, while the effect of individual polymers and the combined effects on floating lag time (FLT), total floating time (TFT) and the cumulative drug release and swelling index was determined using Design Expert Software Version -12 Statease®.The significance level was set at an an α value <0.05 giving 95% confidence level for the results findings.

3.13 Optimization of formulation

The optimization of the formulation was done in accordance with the target pharmacokinetic profile using the numerical optimization procedure. The concentration of the independent variables was kept within the range employed in the experimental design. It was found desirable to target a floating lag time of 15 seconds as this minimal floating lag time reduces the risk of the dosage unit being expulsed from the gastric environment. Further the maximum total floating time was set at 12 hours to enhance the controlled drug release profile envisioned while ensuring that the unit dosage form do not dwell in the gastric region inordinately.

The target cumulative drug release profile was set as 30%, 60% and 80% at 3,6 and 8 hours respectively. The highest desirability proposed by the design expert was selected and the corresponding polymer composition used to fabricate the optimized formulation in triplicate.

The optimization criteria set is enumerated in the table below.

Table 3.13 Optimization criteria for floating acyclovir tablets

Variable	**Optimization criteria**
HPMC K100M	Within range
HPMC K4M	Within range
Carbopol	Within range
Floating lag time	Target NMT 15s
Total floating time	Target 12 hours
Drug release (3 h)	Target 30%
Drug release (6h)	Target 60 %
Drug release (8h)	Target (80%)

NMT; No more than

CHAPTER 4. RESULTS AND DISCUSSION

4.1.1 Identification of active ingredient

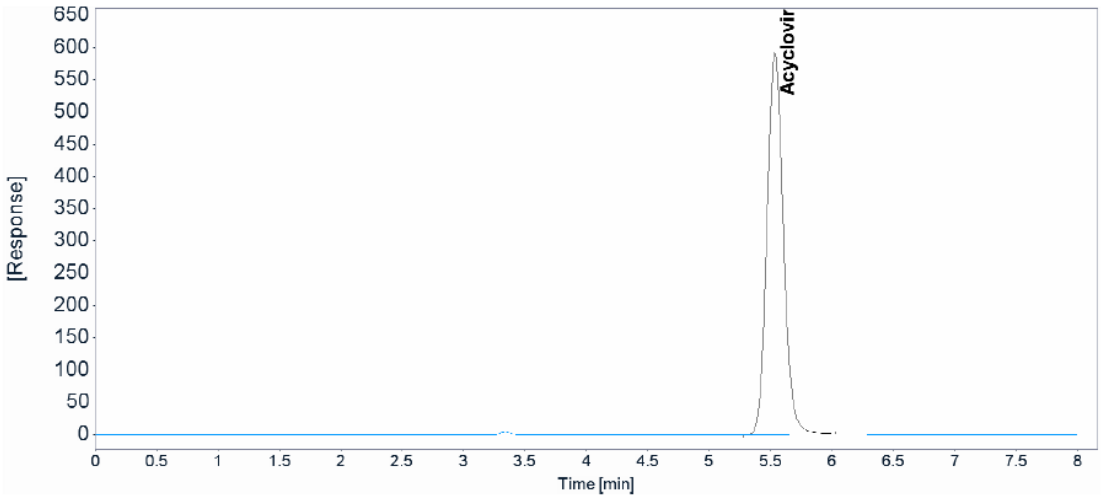
The FTIR spectra of the active ingredient was concordant with that of the reference standard and that of the Reference Infrared absorption spectra of acyclovir in the Japanese pharmacopoeia. The spectra depicted principal peaks that can be ascribed to the respective functional groups as detailed in Table 4.1

Table 4.1: Wave numbers and corresponding functional groups

Wave number(cm^{-1})	**Functional group**
3433	N-H stretch
3182	O-H Stretch
2972	Aliphatic C-H stretching
2895	Aliphatic C-H stretching
1700	C=O stretch
1483	C=N stretch
1180	C-N

4.1.2 Chromatographic identification

The identity of the acyclovir was further validated during the quantitative analysis of the floating acyclovir tablets using HPLC. The retention time of the API and the standard were the same (5.5 minutes) under similar experimental conditions therefore indicating identity as shown in figures 4.1.2a and 4.1.2b.



Name	RT	Area	Peak_AreaPercent	Peak_Asymmetry	Peak_TheoreticalPlates_USP	Peak_Resolution_USP	Peak_RelativeRetTime
Acyclovir	**5.5**	**11517249**	**100**	**1.1**	**8206**	**0.0**	

Figure 4.1.2a Chromatogram of acyclovir standard in 0.02N glacial acetic acid

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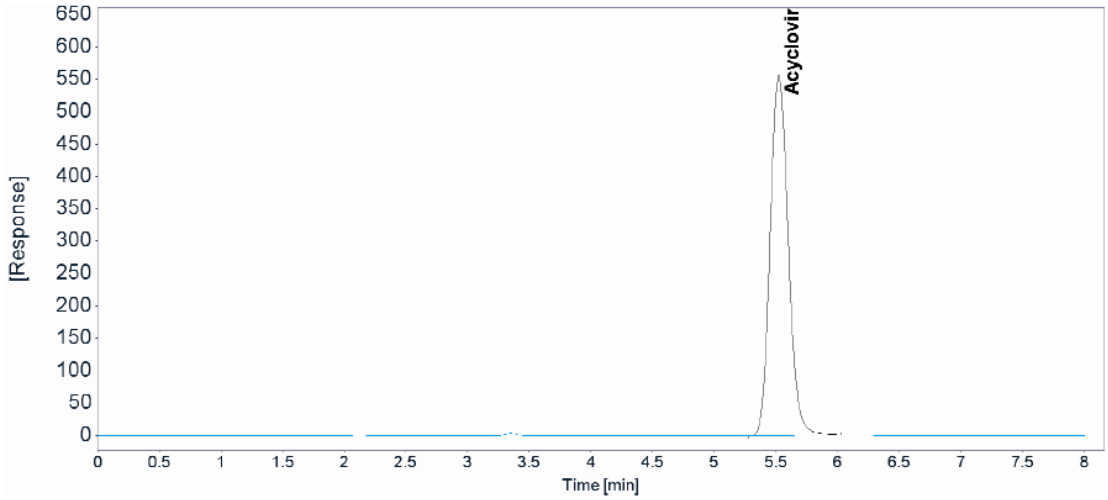


Name	RT	Area	Peak_AreaPercent	Peak_Asymmetry	Peak_TheoreticalPlates_USP	Peak_Resolution_USP	Peak_RelativeRetTime
Acyclovir	**5.5**	**11861415**	**100**	**1.1**	**6772**	**0.0**	

Figure 4.1.2b Chromatogram of the test solution of acyclovir in 0.02N glacial acetic acid

4.2 Drug excipient compatibility studies

The FTIR spectra of the binary mixtures did not reveal any incompatibilities and all the principal peaks observed in the pure active substance were conspicuously evident in the spectra of the binary mixtures. Minor changes in the spectra could be attributed to overlapping of peaks of the specific excipient and those of acyclovir. The FTIR spectra of the binary mixtures are annexed.

4.3 Micromeritics

Precompression tests were conducted to evaluate the powder blend flow properties. The parameters of interest were the angle of repose (AOR), bulk density (BD), tapped density (TD), Carr's index (CI) and the Hausners ratio (HR)

Formulations F1, F2, F4, F5 had angles of repose between 40.40° and 41.32° which is described as passable. This is consistent with other studies of formulations consisting of acyclovir powder which is very cohesive thus exhibits poor flow properties.

Formulation F3 containing Carbopol as the only polymer exhibited an angle of repose of 36.02° which is described as fair flow. The other formulations containing Carbopol and HPMC K100M and HPMC K4M hand angles of repose between 39.22° and 40°. There was better flow commensurate to increasing proportion of Carbopol in the polymer mix inferring that Carbopol promotes better flow compared to the other polymers used.

The bulk densities of the formulations differed depending on the polymer composition. F1 and F2 had bulk densities of 0.45 and 0.40 g/cm^3 respectively while F3 containing pure Carbopol had a lower density of 0.35g/cm$^{3.}$ F4 and F5 that contained alternating proportions of HPMC K100M and HPMC K4M had densities of 0.408 and 0.412g/cm^3 respectively.

The tapped density of HPMC containing blends was between 0.512 and 0.571 g/cm^3 while those containing Carbopol exhibited a tapped density between 0.408 and 0.444 g/cm^3. The results predict that Carbopol has a higher compressibility compared to the grades of HPMC used in this study. The Carr's index ranged between 14.28 % to 22.43 with the lower values corresponding to increasing proportions of Carbopol 934. The largest values were observed for F4; the binary mixture of HPMC k100m and HPMC K4M.The Hausners ratios ranged from 1.16 to 1.267 with the lowest value being that of F 6, a binary blend containing HPMC K4M and Carbopol.

It can be concluded that the presence of Carbopol slightly improved the flow properties of the powder blends as evidenced by the precompression parameters; angle of repose, Carr's Index and Hausner's ratio for F3, F6, F7, F8, F9 and F10 in comparison with those of those containing pure grades of HPMC. The findings indicate that Carbopol 934 has better flow properties compared to HPMC K100M and HPMC K4M.This observation may be attributed to the lower bulk density of the Carbopol compared to the other polymers[126].The precompression parameters are summarized in table 4.3 below.

Table 4.3: Precompression characterization of powder blend properties

Formulation	**Angle of Repose**	**Bulk Density (BD) g/cm^3**	**Tapped Density (TD)**	**Carr's Index (CI) %**	**Hausners ratio (HR)**
F1	41.00	0.459	0.571	19.69	1.244
F2	41.32	0.404	0.512	21.25	1.267
F3	36.02	0.3478	0.408	14.795	1.173
F4	40.40	0.408	0,526	22.43	1.28
F5	41.01	0.412	0.533	22.76	1.294
F6	39.22	0.381	0.444	14.28	1.16
F7	38.10	0.380	0.444	14.41	1.16
F8	40.1	0.4081	0.4762	15.62	1.19
F9	39.22	0.3883	0.434	10.52	1.117
F10	39.8	0.400	0.5063	20.99	1.266

4.4 Post compression parameters

The direct compression of the powder blends yielded round and shiny flat plain tablets devoid of any defects. The post compression parameters of interest in this study were; weight uniformity, friability, tablet thickness, hardness and assay.

The uniformity of weight is an indication as to the consistency of the die filling process and an indirect indicator that the manufacturing process will achieve dosage units with equal amounts of the drug substance. The weights complied with the USP pharmacopoeia requirements for tablets that no more than two tablets deviate from the average weight of twenty randomly

selected units by 5% with none deviates by more than twice this percentage for tablets weighing more than 325mg [118]. All the batches complied with this requirement.

Friability refers to the tendency of the tablet to lose surface material due to mechanical attrition during storage and transport. The friability test is thus done to determine the extent of mass lost when the tablets are subjected to mechanical agitation and require that no more than 1% should be lost after 100 revolutions [127]. All the formulations were complying to this requirement with friability ranging from 0.392% to 0.846% for F3 and F4 respectively. Formulations containing Carbopol were found to be less friable compared to those containing HPMC only.

The thickness of the tablet is the only dimensional variable related to the pharmaceutical manufacturing process of tablets. It is indicative of the compressibility of the powder blend and should be constant for each batch. The thickness of the floating tablets was uniform for all batches ranging between 0.5 to 0.53cm.

The hardness of solid dosage forms is paramount as it has a direct bearing on the friability and disintegration profiles of the unit dosage form. Tablets with low hardness tend to be friable in nature thus easily lose surface material. On the other tablets that are too hard may impact on the dissolution and disintegration of the unit formulation as the entry of the dissolution media is impended. This parameter is also critical for floating tablets as these formulations require the entry of the gastric fluid into the unit dosage form to react with the effervescent moieties to produce the carbon dioxide required to effect buoyancy. As such to achieve optimal buoyancy while also maintaining acceptable friability an optimal hardness profile must be selected. The floating acyclovir tablets had a hardness of between 65 and 80N which is consistent the range of tablet hardness of other floating formulations in literature[128,129]. The compressibility of tablets varied between batches with lower compression pressures being required in proportion with the amount of Carbopol present. The post compression experimental findings are enumerated in the table 4.4.

Table 4.4: Post compression characteristics of floating acyclovir tablets

Formulation	**Breaking Force(N)**	**Friability (%)**	**Average Tablet Weight**	**Thickness(cm)**	**Assay (%)**
F1	69.6 ±3.1	0.743	545.25 ±7.5	0.5	98.93±1.0
F2	67.9 ±6.6	0.682	548.6 ±4.5	0.5	102.43±2.6
F3	73.6±3.7	0.392	536.3±7.8	0.53	101.93±3.1
F4	75.1±4.7	0.846	546.61±6.3	0.5	101.49±1.9
F5	70.6±3.7	0.631	550.37±4.1	0.5	99.27±2.8
F6	71.6±3.8	0.579	546.28±4.5	0.5	101.05±8.9
F7	75.3±3.8	0.842	551.02±4.6	0.51	100.54±4.9
F8	74.2±2.2	0.640	545.99±6.6	0.5	103.64±8.2
F9	76.15±2.6	0.538	547.525±4.1	0.51	103.93±4.1
F10	75.33±4.2	0.539	547.66±4.8	0.52	99.65±4.9

4.5 Buoyancy of floating acyclovir tablets

Buoyancy of floating tablet occurs when the density of the unit dosage form falls below that of the media. This is achieved when the effervescent moiety reacts with 0.1N HCl to produce carbon dioxide that becomes entrapped by the gel formed by the polymers. During the preliminary runs, it was determined that the proportion of polymer in the formulation had a significant effect on the floating lag time as insufficient polymer proportions (24%w/w) failed to produce the gel viscosity required to entrap the carbon dioxide being generated. This therefore led to continuous bubbling from the unit dosage form and failure to float as depicted in figure 4.4. Intermediate polymer proportions (27%w/w) could achieve floatation; however, the floating behaviour was unstable with the tablet initially floating then sinking after reaching the surface of the fluid where the carbon dioxide was lost. These observations led to the selection of higher polymer proportions of was 33.33%w/w, which were used in the formulation of tablet batches F1-F10.

Figure 4.5: Continuous bubbling from a preliminary dosage unit containing 24%w/w polymer proportion

The hardness of the unit formulations also played a key role in effecting the buoyancy; tablets with hardness above 120N and containing HPMC K100M and Carbopol 934 failed to float completely. This could be attributed to the low porosity and high gel density that impended the entry of the acid to facilitate effervescence by reacting with the sodium bicarbonate. Tablets with a hardness below 55N achieved instant buoyancy, however they all failed the friability test as they were too soft. These observations made in the preliminary formulations allowed selection of the right tablet hardness range. To maintain tablet integrity and achieve rapid floatation the hardness was maintained at between 65 to 80N which exhibited acceptable friability and buoyancy properties.

All the formulated tablet batches achieved floatation within three minutes. The floating lag time ranged 2.8-142 s with the shortest lag time being observed in F10; the ternary mixture blend of HPMC K100: HPMC K4M: Carbopol 934 having the centroid proportions. F2 which is a pure blend of HPMC K4M had the longest floating lag time 142s. Formulations containing Carbopol showed shorter floating lag times compared to those containing pure or binary blends the HPMC grades. The longer floating lag time observed for F2 could be attributable to the lower viscosity that failed to entrap the carbon dioxide much earlier. F1, a pure blend of HPMC K100 yielded sufficiently high gel viscosity to entrap the gas produced hence the shorter floating lag time observed.

All formulations containing Carbopol had rapid floating times of less than 30s. This observation may be attributed to the superior gelling properties of Carbopol owing to the high hydration rates therefore achieving rapid entrapment of the carbon dioxide produced. The binary blends containing Carbopol had floating lag time ranging between 3.86-6.15s which could be explained by the varying viscosity of the resulting gels. The ternary blend F10 had the lowest floating lag time of 2.8s.

These results indicate that the right polymer proportion is required to achieve rapid buoyancy; low polymer concentrations fail to entrap the gas while too high polymer concentrations impair the entry of the acidic medium required to generate the effervescence required for buoyancy. An optimal viscosity is thus required to achieve buoyancy. Carbopol is paramount to achieve a reduction in the floating time due to its superior hydration properties thus rapid gelling. The buoyancy parameters are summarised in table 4.4 below.

The total floating time (TFT) ranged from 7 hours for F3 to 48 hours for F1. The TFT for the other formulations were 14, 26,12,13,14,30, 14 and 30 hours for F2, F4, F5, F6, F7, F8, F9 and F10 respectively. F3 containing pure Carbopol floated for only 7 hours with all the six tablets sinking to the bottom of the vessel, the matrix integrity of this batch was also not sustained with erosion being observed. All other batches maintained a total floating time beyond the targeted 12 hours. The matrix integrity was also maintained in these batches.

Table 4.5 Buoyancy of floating acyclovir tablets

Formulation	**Floating Lag Time (FLT) (s)**	**Total Floating Time (TFT) (hours)**
F1	40.0 ±3.2	48
F2	142 ±10.4	14
F3	30±0.9	7
F4	67±9.3	26
F5	120±6.4	12
F6	7±1.2	13
F7	3.86±0.3	14
F8	6.3±1.1	30
F9	2.37±0.3	14
F10	2.8±0.8	30

4.6 Preparation of acyclovir calibration curve

To generate the calibration curve for acyclovir, 10mg of the reference standard were accurately weighed and transferred into a 100ml volumetric flask. The powder was dissolved in 50ml of freshly prepared 0.1N HCl and made up to a volume of 100ml using the same solvent. The solution was sonicated for 15 minutes to homogenize it at a temperature of 37°C and was then allowed to stand for 30 minutes. This yielded a stock solution containing 100µg per ml.

Serial dilutions were done to obtain concentrations of 2, 5,8, 10,15,16 and 20µg/ml. The absorbance of these solutions was determined using Genesys 10 UV-Vis V4 set at a wavelength of 254nm. The tests were done in triplicate with the average and standard deviation being recorded. The results are summarized in table 4.5.

Table 4.6: Absorbance values of acyclovir reference(n=3)

Concentration (µg/ml)	**Absorbance ± SD**
5	0.275± 0.014
8	0.350±0.009
10	0.539±0.023
15	0.818±0.018
16	0.842±0.015
20	1.093±0.043

The obtained UV absorbances were plotted against their respective concentrations to generate a calibration curve. The two continuous variables depicted a positive linear relationship with the linear regression equation described by the formula in equation 10. The R^2 value of 0.9918approaches unity implying a strong positive correlation between absorbance and concentration of acyclovir in the solution at the concentrations used. A scatter plot depicting the same is shown in figure 4.5

Y=0.0553X-0.0258 *Equation 14*

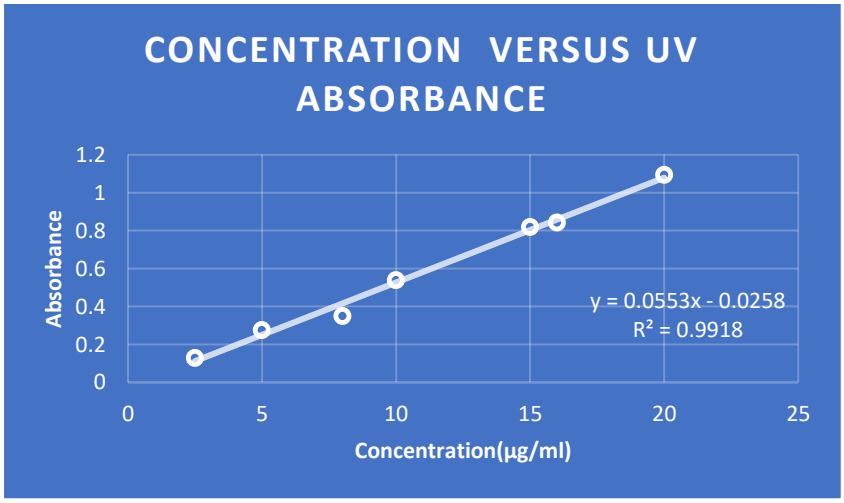


Figure 4.6: Calibration curve showing the relationship between concentration of acyclovir and UV absorbance.

4.7 Drug release characterization and modelling of dissolution profiles

The average value of the absorption and the standard deviation were calculated. Cumulative percentage release of the drug was then calculated using the calibration curve obtained earlier against the theoretical maximum release of 222µg/ml. The cumulative percentage drug release is summarized in table 4.5

Table 4.7: Cumulative percentage drug release as a function of time for different polymeric blends.

	Time in Hours							
Formulation	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
F1	3.9	6.27	12.36	15.2	19.74	22.83	27.57	31.05
F2	9.55	23.66	38.30	51.48	61.97	66.02	75.34	81.19
F3	2.80	7.22	16.07	31.56	43.57	54.48	60.64	65.23
F4	3.6	5.33	7.80	11.07	14.12	18.60	22.05	26.60
F5	6.1	11.58	12.53	15.42	19.52	23.06	28.14	29.43
F6	3.4	6.37	9.6	12.9	16.03	20.25	21.10	29.53
F7	4.85	10.69	19.68	27.72	37.8	44.31	52.29	58.77
F8	3.42	3.90	5.51	8.12	10.30	12.85	14.99	16.57
F9	3.1	5.67	7.66	9.89	12.95	15.06	19.11	22.01
F10	2.69	4.47	6.88	9.39	11.05	13.98	15.88	18.37

Formulations containing HPMC K100M exhibited the highest extent of retardation for the release of acyclovir with the cumulative percentage release of 31%, 27%, 30%, 17%, 22% and 18% being observed for F1, F4, F5, F8, F9 and F10 respectively after eight hours. Lower retardation effects were observed in formulations containing HPMC K4M except in F6. The lower retardation could be attributable to its low viscosity in aqueous solutions in comparison with Carbopol 934 and HPMC K100M.The cause of diminished release effect observed in formulation F6 was indeterminate. Carbopol 934 too had lower retardation effect than HPMC K100M on drug release depicting a 65% cumulative drug release at the eighth hour.

Combination of the different polymers yielded higher retardation effects as evidenced by the drug release profiles of binary mixtures F4, F5, F6, F8, F9 and the ternary mixture F10 where the drug release was slower than for pure blends F1, F2 and F3.

The drug release was found to be dependent on the viscosity of the polymers where the more viscous HPMC k100M exhibited pronounced retardation effects compared to HPMC k4M that has a lower viscosity. Diffusion is also dependent on the length of the diffusion path.

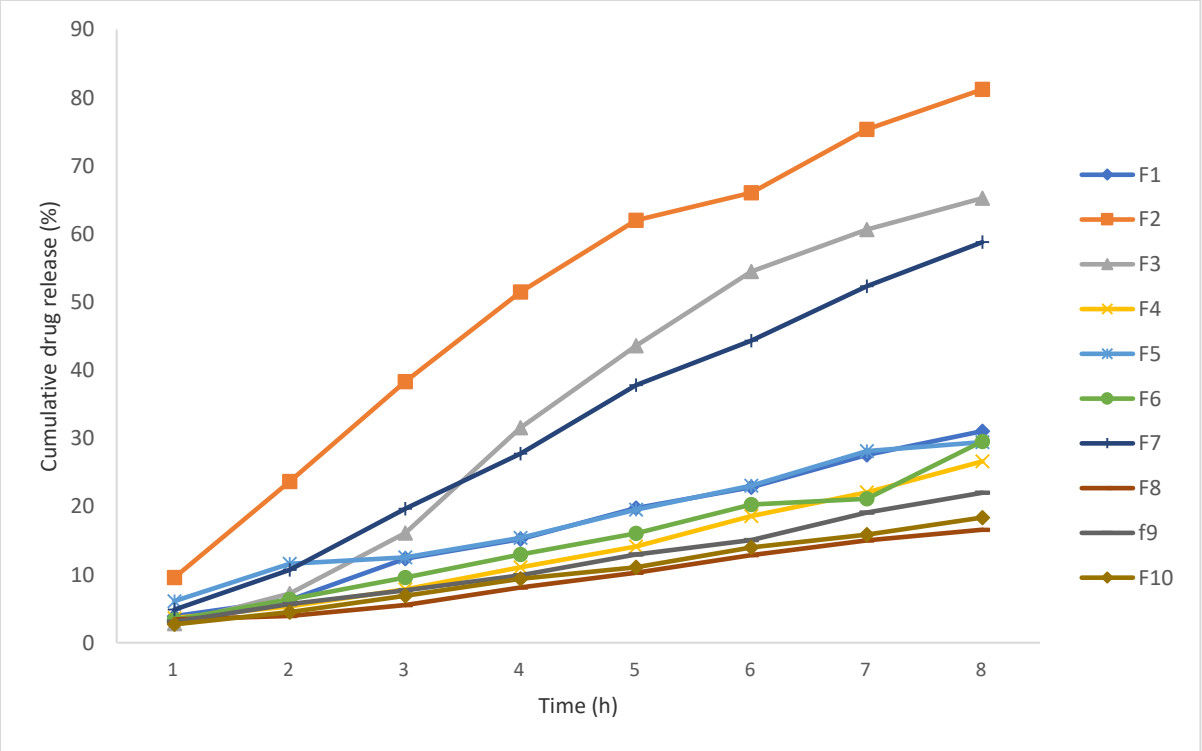


Figure 4.7: Cumulative drug release profile for floating acyclovir tablets in 0.1N HCl

4.7.1 Modelling of drug release kinetics

The cumulative percentage of acyclovir release data was keyed into the DDsolver modelling software to elucidate the kinetics through which the drug was being released from the various formulations. The data was fitted to zero order, first order, Higuchi and Korsemeyers Peppas release kinetics. The selection of the best fitting model was based on the highest value of the regression coefficient (R^2). The mechanism of drug release was also determined from the value of *n* as derived from the Korsemeyers Peppas equation.

All the formulations depicted zero order drug release kinetics where a constant amount of the drugs was released per unit time with the regression coefficient ranging between 0.9686 and 0.9981 for F2 and F10 respectively.

The predominant drug release mechanism was Super case II except for formulations F2 and F5 that depicted a non-fickian drug release mechanism.

Table 4.7. 1: Kinetics modelling of acyclovir release from floating acyclovir tablet matrices

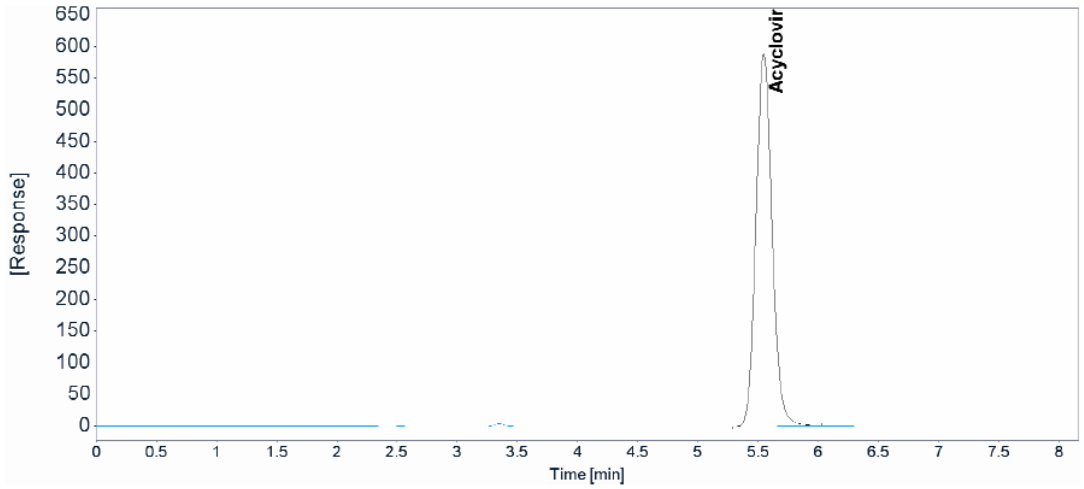
	Zero order		**First Order**		**Higuchi**		**Korsemeyers-Peppas**			**Release mechanism**
Formulation	K_0	R^2	K_1	R^2	K_H	R^2	k_{KP}	R^2	n	
F1	3.879	0.9950	0.045	0.9868	9.601	0.8002	3.689	0.9968	1.028	Super case II
F2	10.161	0.9686	0.18	0.9671	26.154	0.8640	14.992	0.9799	0.832	Non Fickian
F3	9.897	0.9789	0.111	0.8790	18.884	0.6962	5.179	0.9681	1.256	Super case II
F4	3.334	0.9846	0.034	0.9605	7.153	0.7424	2.149	0.9932	1.201	Super case II
F5	3.355	0.9839	0.045	0.9677	9.246	0.8760	5.516	0.9787	0.808	Non Fickian
F6	7.406	0.9747	0.037	0.9595	7.769	0.7632	2.788	0.9773	1.101	Super case II
F7	3.619	0.9972	0.095	0.9447	18.867	0.7627	5.786	0.9951	1.128	Super case II
F8	12.101	0.9851	0.022	0.9815	4.883	0.7997	2.056	0.9846	1.009	Super case II
F9	9.382	0.9913	0.029	0.9836	6.190	0.7921	2.418	0.9925	1.052	Super case II
F10	10.990	0.9981	0.025	0.9963	5.352	0.8181	2.340	0.9979	0.988	Super case II

4.8 Assay

Preliminary quantitative studies were done using UV spectroscopy at a wavelength of 254nm. The system suitability test was also found to be appropriate for the assay of acyclovir and depicted good peak resolution, peak asymmetry, theoretical plates and retention time All the batches were compliant with the pharmacopoeial specifications; USP 2019 which stipulated an acceptance criteria of acyclovir content range 90%-110%. All batches were within this range and varied between 98.9 to 103.9% of the label claim. These results were corroborated by definitive assay utilizing HPLC where the labelled claim was 101.9 to 105.3%. Table 4.7 summarises the quantitative values of acyclovir for the respective batches.

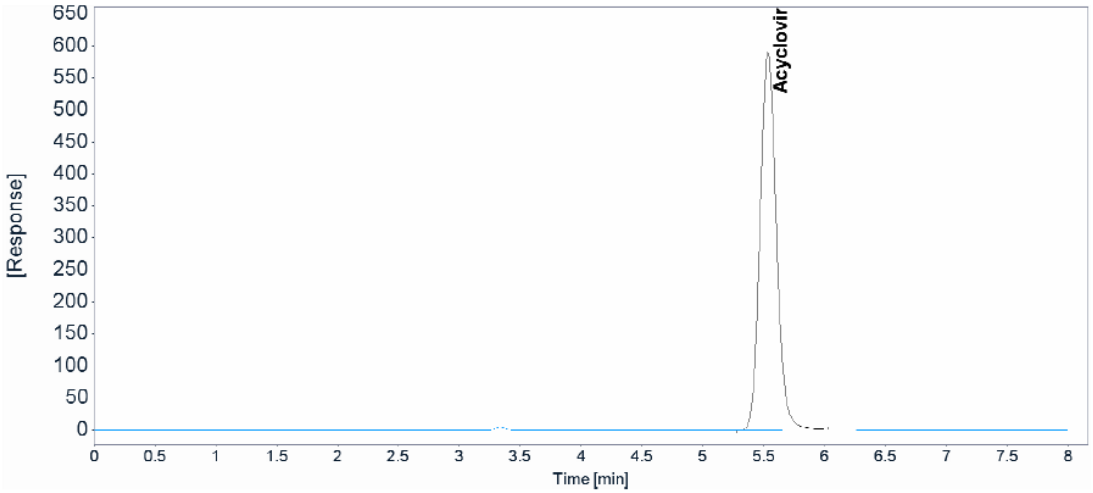
Table 4.8: Label claim (% n=6) of acyclovir in floating tablets.

Batch	**AV quantity (%)**	**SD (%)**
F1	101.9	1.5
F2	103.8	1.92
F3	102.0	0.95
F4	103.5	1.2
F5	102.7	1.08
F6	103.9	0.89
F7	103.3	0.04
F8	103.5	1.45
F9	105.3	1.03
F10	100.7	0.68



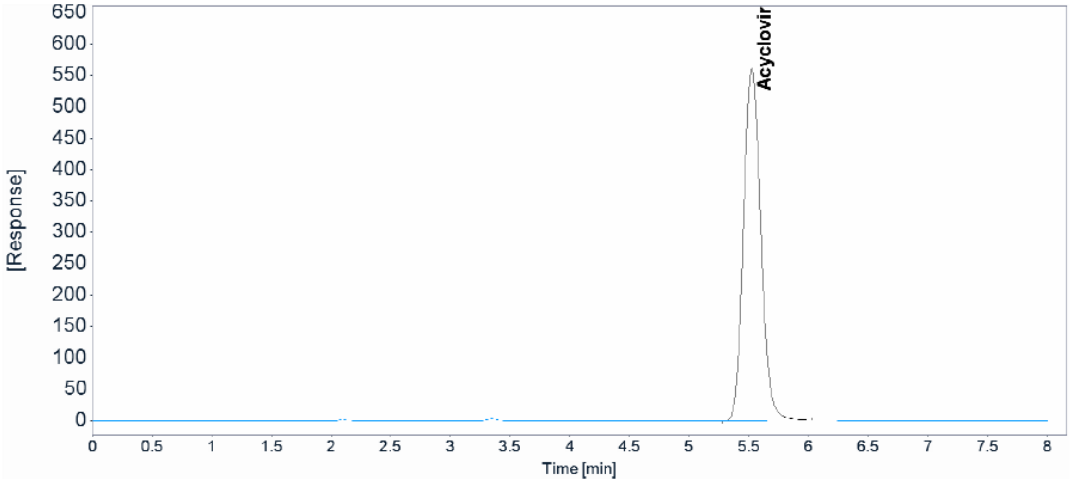
Name	RT	Area	Peak_AreaPercent	Peak_Asymmetry	Peak_TheoreticalPlates_USP	Peak_Resolution_USP	Peak_RelativeRetTime
Acyclovir	**5.5**	**11503746**	**100**	**1.1**	**8189**	**0.0**	

Figure 4.8.1: Chromatogram of acyclovir for determination of system suitability.



Name	RT	Area	Peak_AreaPercent	Peak_Asymmetry	Peak_TheoreticalPlates_USP	Peak_Resolution_USP	Peak_RelativeRetTime
Acyclovir	**5.5**	**11509646**	**100**	**1.1**	**8192**	**0.0**	

Figure 4.8.2: Chromatogram of the acyclovir standard solution



Name	RT	Area	Peak_AreaPercent	Peak_Asymmetry	Peak_TheoreticalPlates_USP	Peak_Resolution_USP	Peak_RelativeRetTime
Acyclovir	**5.5**	**11865110**	**100**	**1.1**	**6952**	**0.0**	

Figure 4.8.3: Chromatogram of F1 batch of acyclovir solution.

4.9 Swelling index

To prevent expulsion from the gastric region, the unit dosage must sufficiently expand and maintain a diameter above that of the pyloric sphincter;12mm. Six tablets were immersed in 200ml beakers containing 0.1N HCl after their average unit weights had been taken. The tablets were withdrawn at hourly intervals until the eight hours, blotted with absorbent paper to effect drying after which the weights taken. They were then carefully returned to the vessel. The average weight gain and corresponding standard deviation were recorded.

F3 exhibited the highest swelling index of all the formulations, it achieved a swelling index of 370.07±18.8 % by the eighth hour. These findings are consistent with other studies where Carbopol was used in gastroretentive formulations. The high swelling index can be attributed to its high hydration rate. The matrix integrity was sustained up until the sixth hour after which the cylindrical shape of the tablet was distorted. Formulation F2 containing HPMC k4M had the lowest swelling index of all the formulations, the weight gain increased gradually from 38.4% to maximize at 58.03% by the fourth hour. The tablets then started eroding from the surface with a swelling index of 46% being observed at the end of the eight hours. The size attained was above the diameter of the pylorus(12mm) thus sufficient to prevent peristaltic removal of the tablet from the stomach thus achieve gastroretention.

The swelling indices for F1, F4, F5, F6, F7, F8, F9 and F10 were 126, 167, 119,158,167,179,157 and 170 % respectively. The matrix integrity of these formulations remained intact throughout the eight hours with slight distortions being observed for formulations F7 and F9 that contained high proportions of Carbopol. Figure 4.9a shows a comparison of the relative tablet size in the dry and wetted state. The swelling indices are summarized in table 4.9 and figure 4.9b

Figure 4.9a: Photograph of F8 batch of acyclovir floating tablet in dry and wetted state after eight hours in 0.1N HCl

Table 4.9: Swelling indices of acyclovir floating tablets in 0.1N HCl as a function of time

Formulation	**Percentage swelling as a function of time(h)**							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
F1	58.38±0.04	75.52±4	87.25±6	95.21±7	109.37±3.1	114.64±4.15	118.43±5.4	126.71±6.9
F2	38.40±8.4	48.13+5	51.53+7.3	58.03+2	52.35+4.6	50.54+1.7	48.29+0.3	46.05+0.9
F3	139.9±7.92	183.0±7.70	242.23±11.80	267.84±13.74	308.49±13.28	325.22±12.44	363.49±15.43	370.07±18.8
F4	63.71±5.81	80.35±3.36	99.07±6.32	111.25±4.2	127±1.74	142.01±3.28	153.68±4.75	167.45±3.48
F5	57.43±4.926	78.34±1.81	90±3.85	102±5.40	110.64±6.1	118.46±6.56	126.28±4.81	119.69±7.72
F6	64.77±1.93	94.32±2.21	106±0.93	116±2.03	126.21±2.56	137.06±4.10	147.40±2.79	158.14±6.67
F7	74.63±4.92	98.28±5.3	109±5.5	122±7.03	133.34±3.23	142.52±5.22	154.90±7.06	167.12±8.64
F8	78.26±2.18	109.94±1.88	125.17±1.54	146.56±2.87	154.34±2.15	166.37±3.86	173.08±2.79	179.61±3.38
F9	77.88±6.69	100.52±6.59	116.24±8.39	131.25±8.1	135.99±7.60	145.20±5.96	151.12±5.84	157.32±6.88
F10	76.26±3.20	103.78±2.26	122.94±4.31	133.71±4.30	143.98±4.53	151.85±4.64	156.23±6.91	170.91±4.413

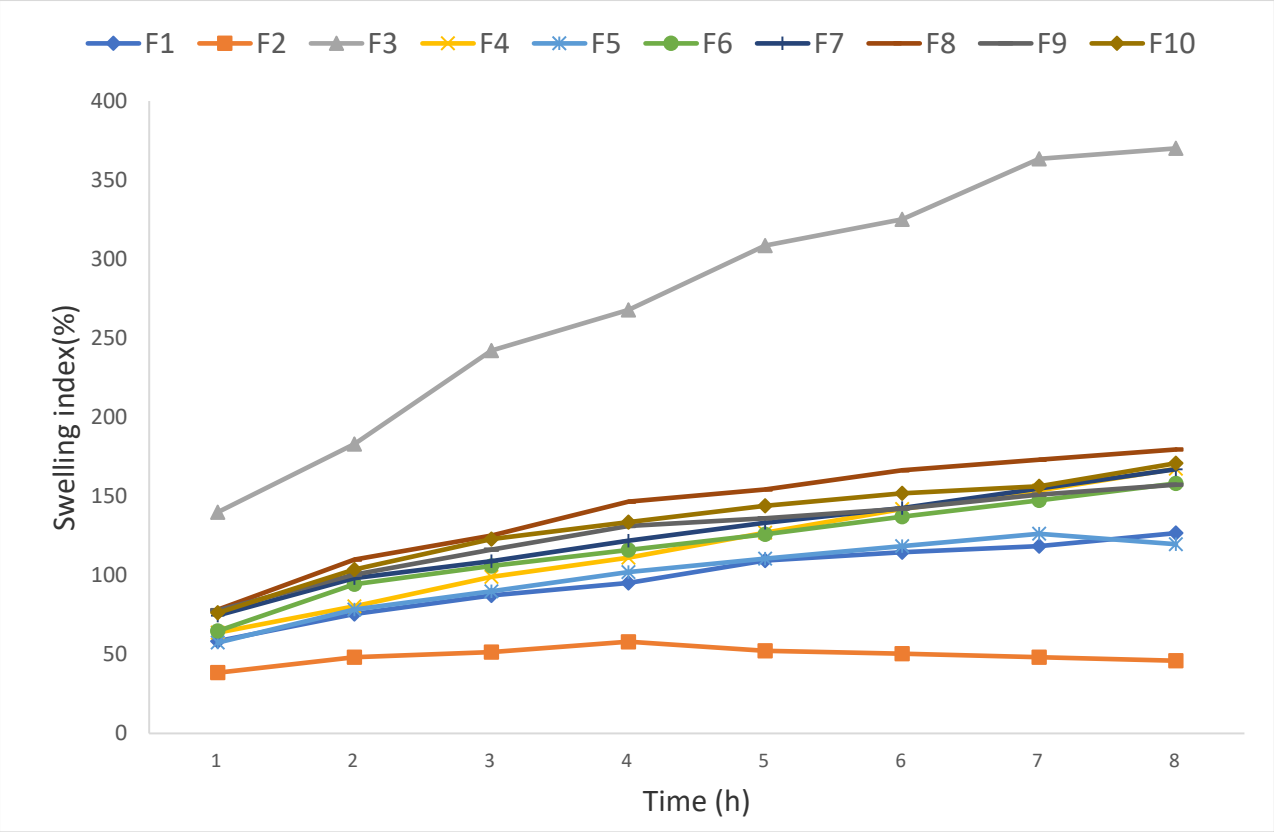


Figure 4.9b: Graphical representation of swelling indices of acyclovir tablets in 0.1N HCl as a function of time

4.10 Statistical analysis

The data obtained was keyed into design expert version 12. Analysis was conducted to determine the model that best described the relationship between the input variables and the selected outcomes based on the regression coefficient and a significant p value.

Floating lag time (FLT) and the total floating time (TFT) were best described by a quadratic model. The mathematical expressions for the two are given in the equations below. The two-dimensional contour plots and three-dimensional response surface graphs were also generated and are as shown in the figures 4.9.1 to 4.9.10 in annex 5.

$$FLT = 38.89X_1 + 141.33X_2 + 36.34X_3 - 160X_1X_3 - 360X_2X_3 \quad \text{Equation 15}$$

$$TFT = 48.57X_1 + 13.57X_2 + 7.14X_3 - 53.68X_1X_2 - 26.68X_1X_3 + 385.75X_1X_2X_3 \quad \text{Equation 16}$$

With respect to equation, the coefficients $\beta_2 > \beta_1 > \beta_3$ indicating that component 2(HPMC K4M) produced tablets with the longest floating lag time. Coefficients β_{13} and β_{23} are negative inferring to a synergistic effect in lowering the floating lag time.

The main component prolonging the total floating time was found to be component X_1(HMPC K100M).

Cumulative drug release also best fitted the quadratic model, the mathematical expressions are highlighted below.

$$Y_3 = 12.51X_1 + 34.97X_2 + 18.44X_3 - 59.29X_1X_2 - 38.22X_1X_3 - 52.58X_2X_3 \quad \text{Equation 17}$$

$$Y_4 = 24.95X_1 + 60.12X_2 + 56.78X_3 - 94.33X_1X_2 - 117X_1X_3 - 114.41X_2X_3 \quad \text{Equation 18}$$

$$Y_5 = 33.62X_1 + 74.02X_2 + 69.06X_3 - 114X_1X_2 - 142.39X_1X_3 - 121.53X_2X_3 \quad \text{Equation 19}$$

From the equations HPMC K100M had the highest retardation effect which can be attributed to the high viscosity of the gel formed. In contrast HPMC K4M had minimal retardation effect. Carbopol when combined with HPMC tended to further improve the retardation as evidenced by the higher values of the interaction coefficients.

4.11 Optimization

The objective of pharmaceutical formulations is to achieve a dosage form that has optimized parameters. The desirable characteristics of acyclovir included low floating lag time, total floating time enough to last till all the drug has been released and a complete drug release around twelve hours. The data obtained from analysis was analyzed for optimization using design expert where the above stated requirements were input. The best desirability obtained was 0.8 and corresponded to a formulation fitting that of F2, a pure blend containing HPMC K4M. This formulation was thus considered to be the optimized batch that would aid in achievement of the desired product parameters.

5.0 CONCLUSION

Gastroretentive floating tablets of acyclovir sodium were successfully formulated using a simple lattice mixture design. The QbD approach employed yielded high-quality tablets whose characteristics fitted or closely approached the quality target product profile defined on the onset. The critical role of employing design of experiments in the fabrication of pharmaceutical products was evident as this enabled the investigation of the effects of individual variables on the outcomes as well as the interactive effects of the same.

The buoyancy, controlled release and swelling properties envisioned in the experimental design were achieved.

The study findings indicate that formulating acyclovir tablets with prolonged gastric residence and achieving a controlled drug release profile is feasible. Such a formulation will result in decreased frequency of dosing with the optimized formulation pointing to an eight hourly dosing schedule. This would greatly improve patient compliance and consequently treatment outcomes.

6.0: RECOMMENDATIONS

Additional studies are required to characterize the *in vivo* performance of the floating acyclovir tablets in appropriate animal models. Further optimization efforts are also required to accentuate the desirable physicochemical properties of the formulation. The complete pharmacokinetic profiling of the formulation will be paramount.

Complementary formulation approaches that would enhance solubility and improve the permeability for higher dose units need to be explored. For instance, nanomization of the acyclovir drug substance could be used alongside the gastroretentive floating approaches to afford administration of higher dosage units above 400mg.

Buoyancy of gastroretentive solid dosage forms is in part dependent on the shape of the dosage unit. With respect to this attribute, this study only examined the floating properties of flat faced circular tablets. An ovoid shaped tablet of acyclovir would afford formulation of higher unit dosage forms of acyclovir.

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ANNEXES

Annex 1: Schematic workflow chart for fabrication and characterization of gastroretentive floating acyclovir tablets.

Annex 2: Project budget outline

PARTICULARS	QUANTITY/DETAILS	COST(USD)
1. Active pharmaceutical ingredient		
Acyclovir powder -CAS 59277-89-3	**1000 grams**	**1300**
2. Excipients and in process reagents		
• **Hydroxypropyl methyl cellulose (HPMC k100)**	**1000 grams**	**250**
• **Hydroxypropyl methyl cellulose K4M**	**1000 grams**	**250**
• **Carbopol 934**	**1000 grams**	**500**
• **Sodium Bicarbonate (pharmaceutical grade)**	**500 grams**	**250**
• **Polyvinlypyrollidone (PVP)**	**500 grams**	**400**
• **Citric acid**	**500grams**	**300**
• **Microcrystalline cellulose**	**500 grams**	**200**
3. Laboratory Analysis		
3.1 Reagents		
• **Acyclovir reference standard**	**300mg**	**500**
• **HCl**		**50**
• **NaOH**		**50**
• **KBr**	**100mg**	**100**
• **Acetic acid**	**5L**	**50**
3.2 Accessories		
• **C-18 column**	**1**	**600**
3.3 Services		
• **Service charge (Final formulation)**	**10 samples**	**1000**

4.Formulation		
• **Technologists**	**3**	**600**
5. Equipment		
• **Glassware**		**200**
• **Pharmaceutical powder screens (Set of six)**	**1**	**300**
6.Stationery		
• **Printing paper**	**4 rims**	**30**
• **cartridge**	**2**	**80**
• **Binding material**		**10**
7.Publishing costs		
• **Journal fee for manuscript submission**	**1**	**500**
8. Data acquisition and analysis		
• **Statistical software (STATA V 14)**	**1**	**525**
8. Contingency		
• **Shipping of materials**		**300**
• **Import duty**		**200**
• **Clearing**		**200**
9. TOTALS		**8245**

Annex 3: Workplan

ACTIVITY	Jan. 2019	Feb. 2019	March. 2019	Apr. 2019	May. 2019	Jun. 2019	Jul. 2019	Aug. 2019	Sept. 2019	Oct. 2019
Research Proposal Writing										
Presentation for correction and approval										
Sourcing of all materials and equipment										
Preformulation studies										
Formulation and evaluation studies										
Data analysis and report writing										
Presentation and Defence of project										

Annex 4: FTIR spectra

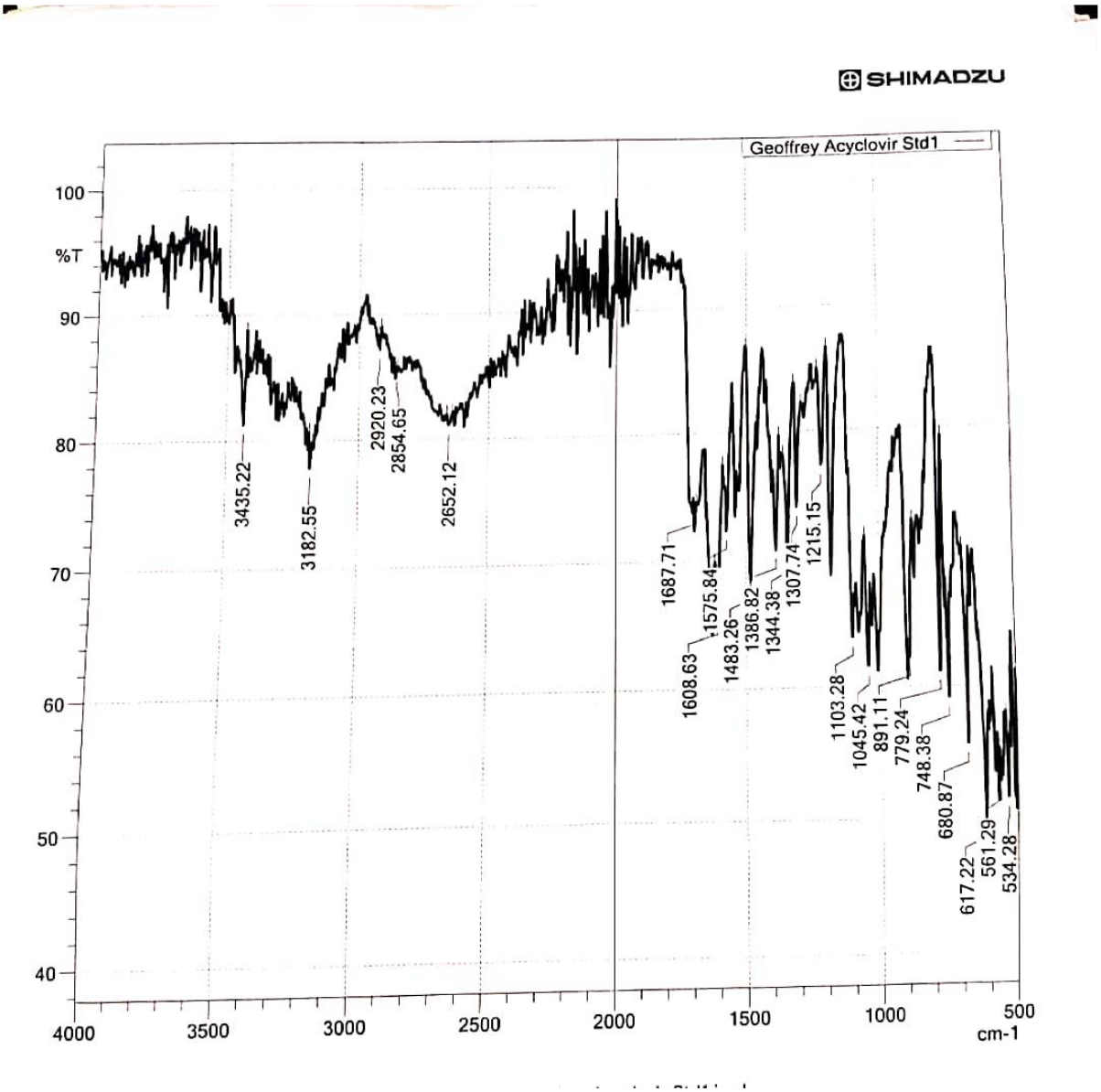


Figure 4.1.1: FTIR spectra of acyclovir standard

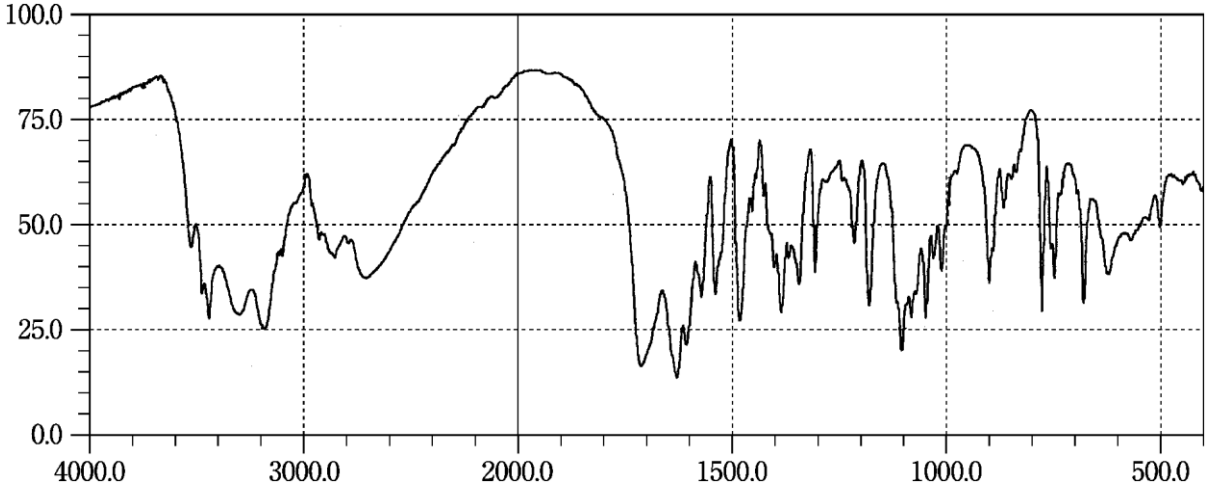


Figure 4.1.2: Reference IR spectra of acyclovir (Japanese pharmacopoeia)

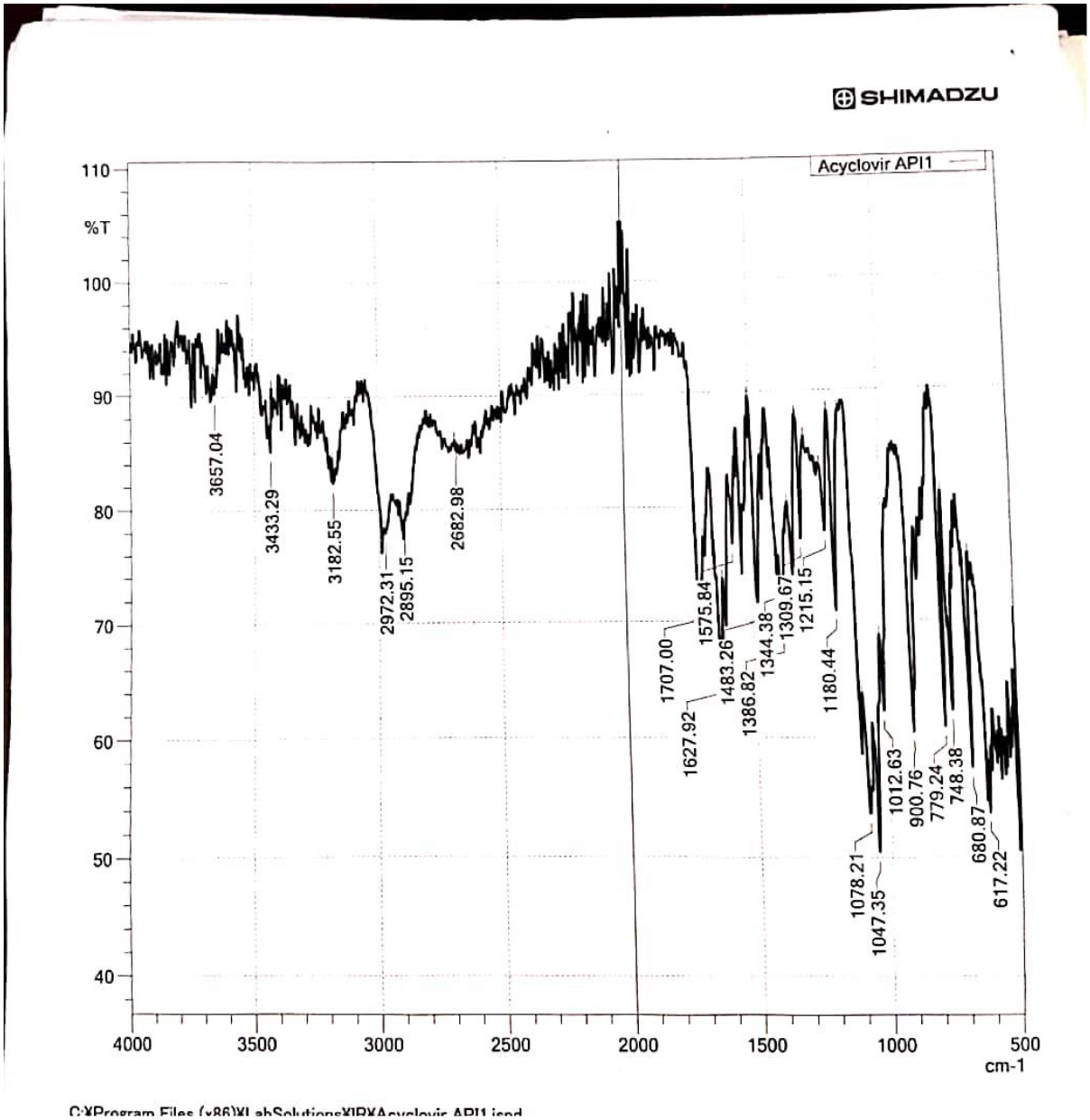


Figure 4.1.3: FTIR spectra of acyclovir active ingredient

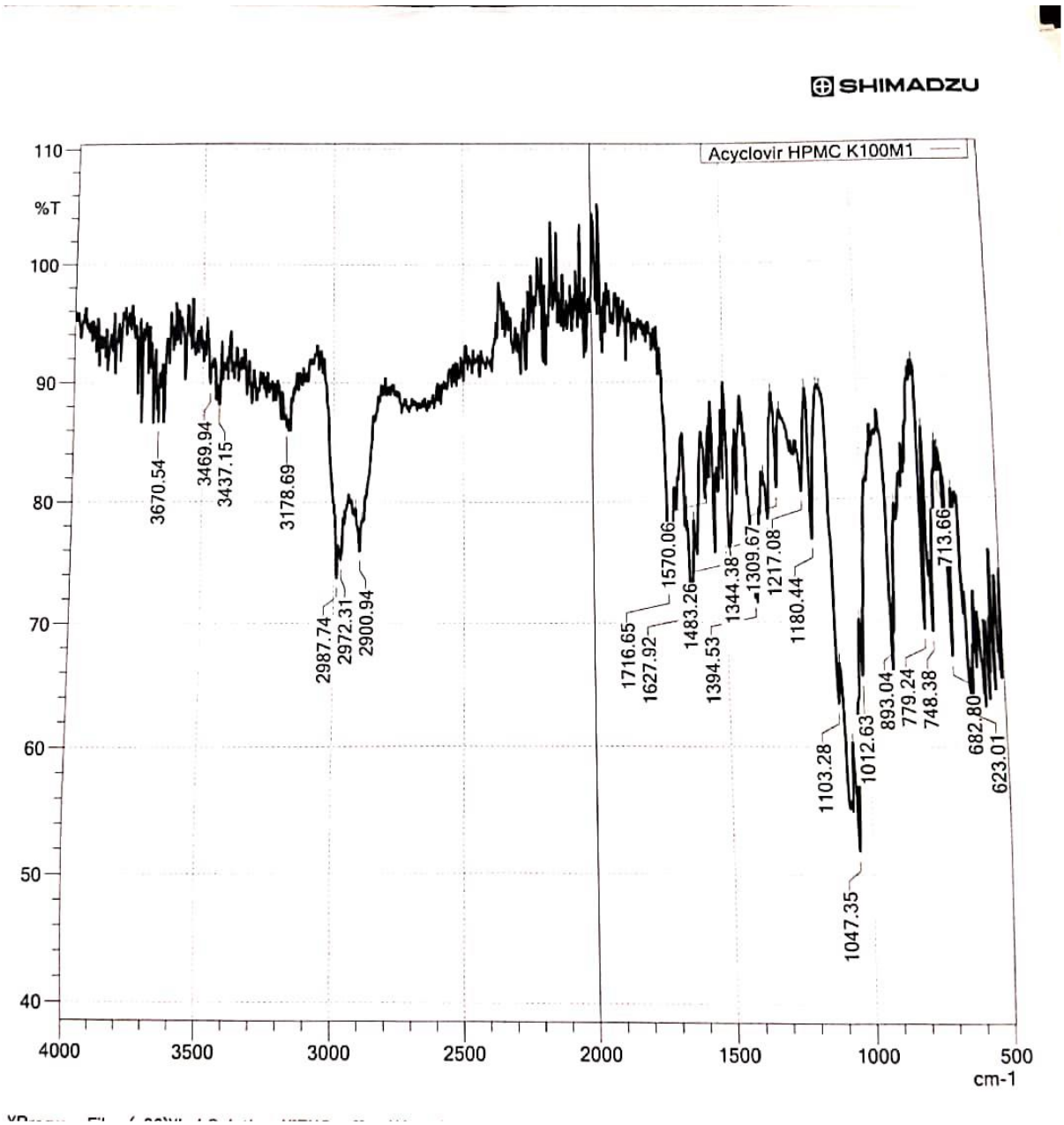


Figure 4.2.1: FTIR spectra of a binary mixture of acyclovir and HPMC K100M

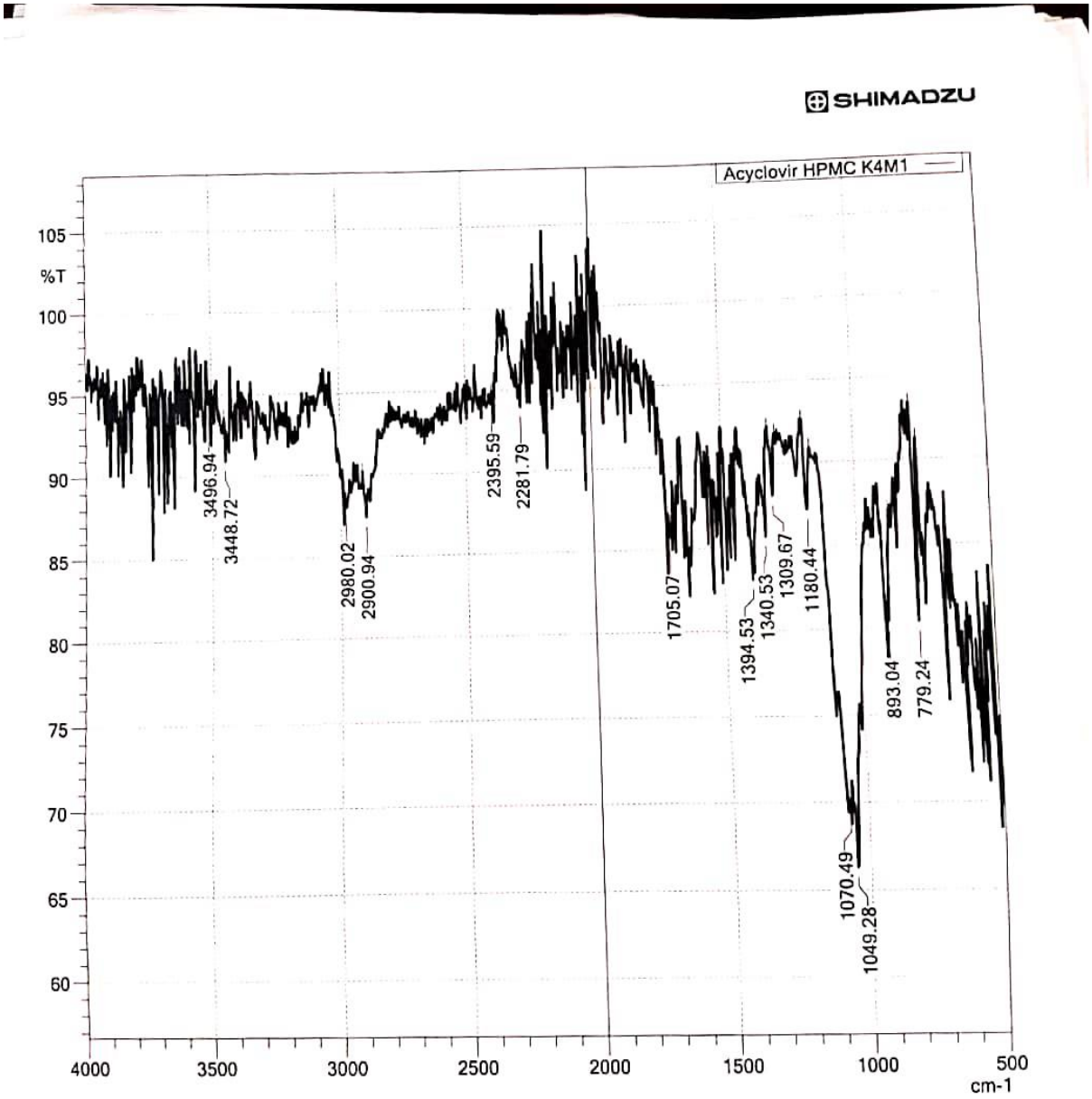


Figure 4.2.2: FTIR spectra of a binary mixture of acyclovir and HPMC K4M

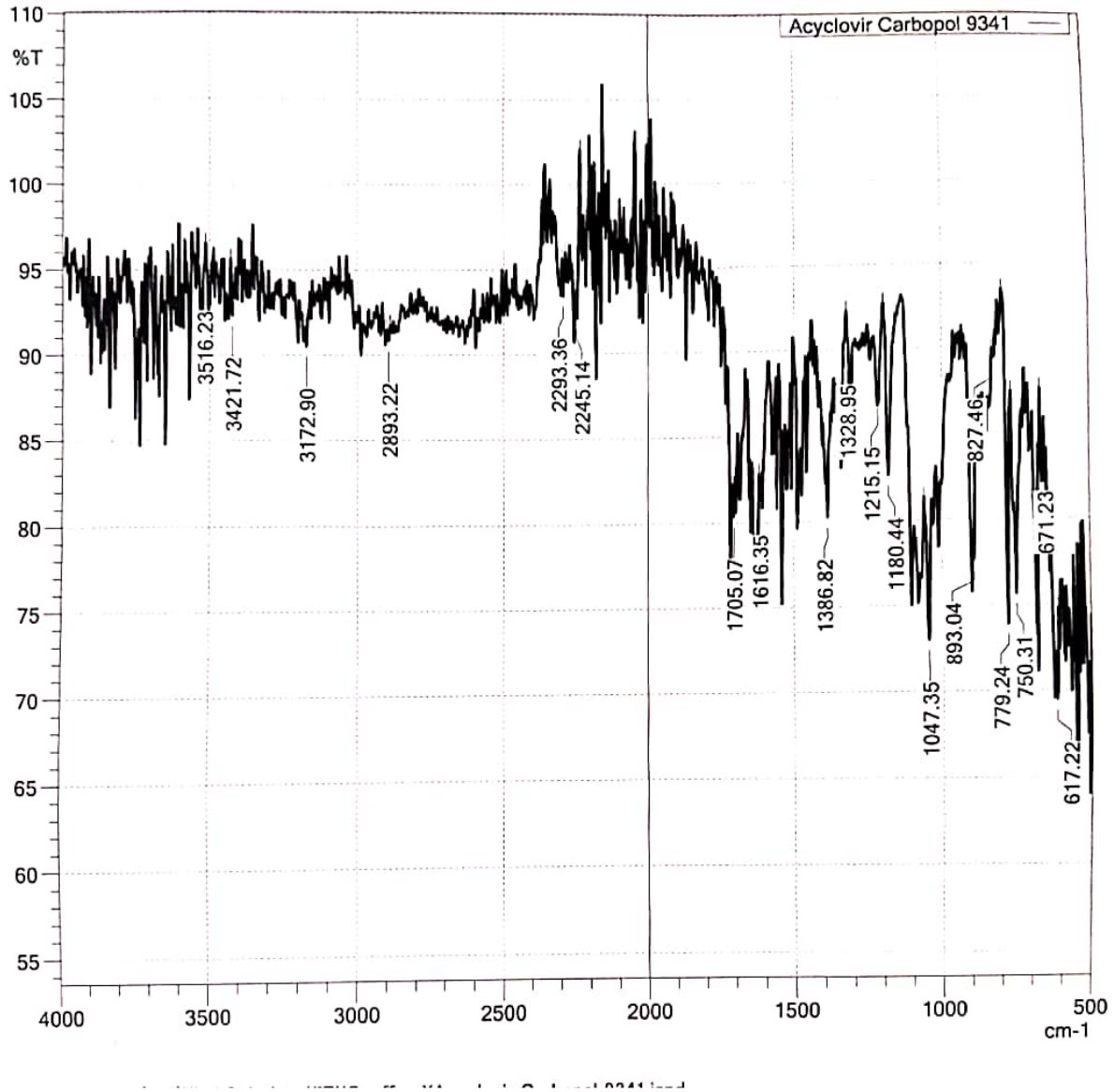


Figure 4.2.3: FTIR spectrum of a binary mixture of acyclovir and Carbopol 934

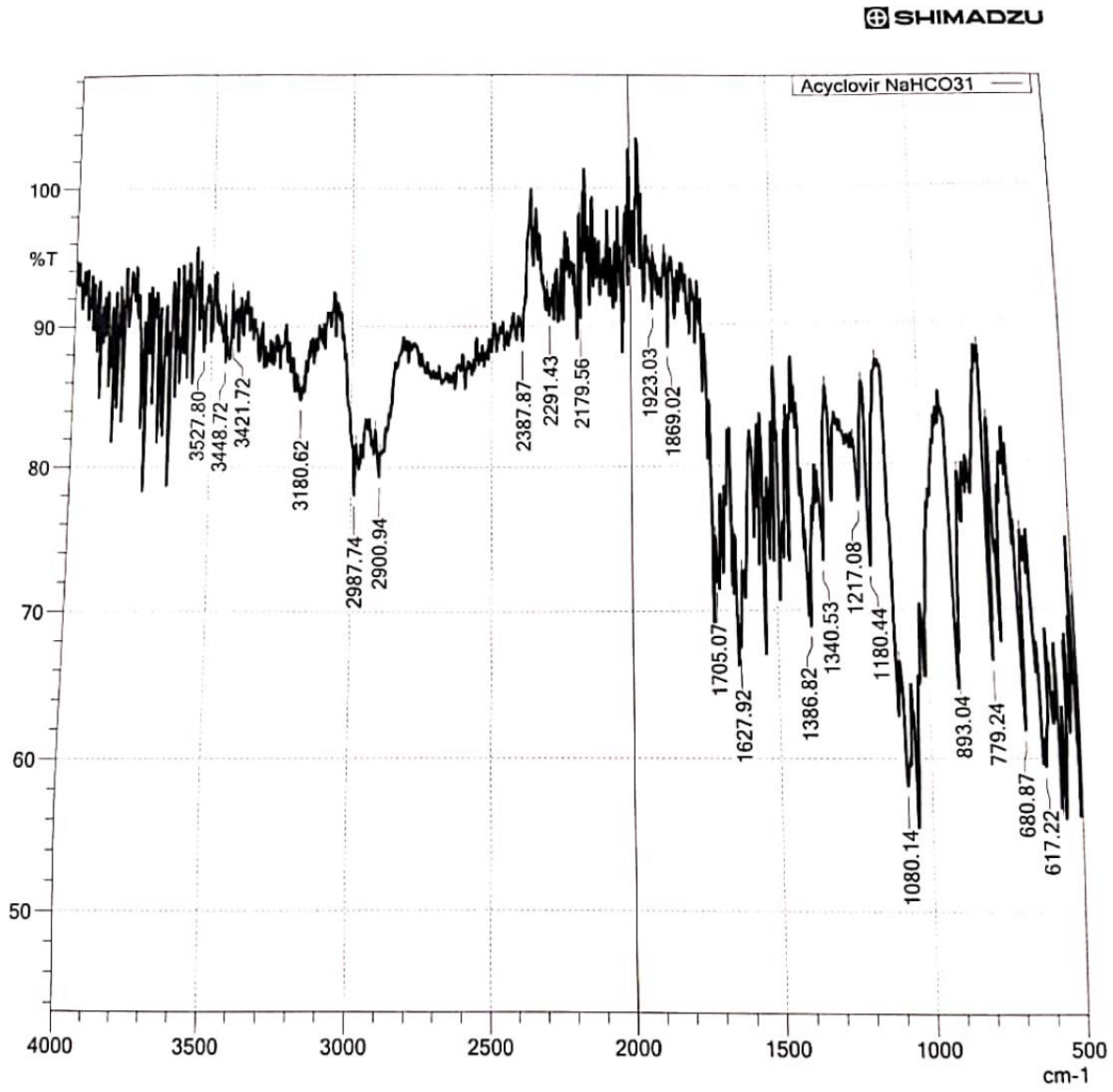


Figure 4.2.4: FTIR spectra of a binary mixture of acyclovir and sodium bicarbonate

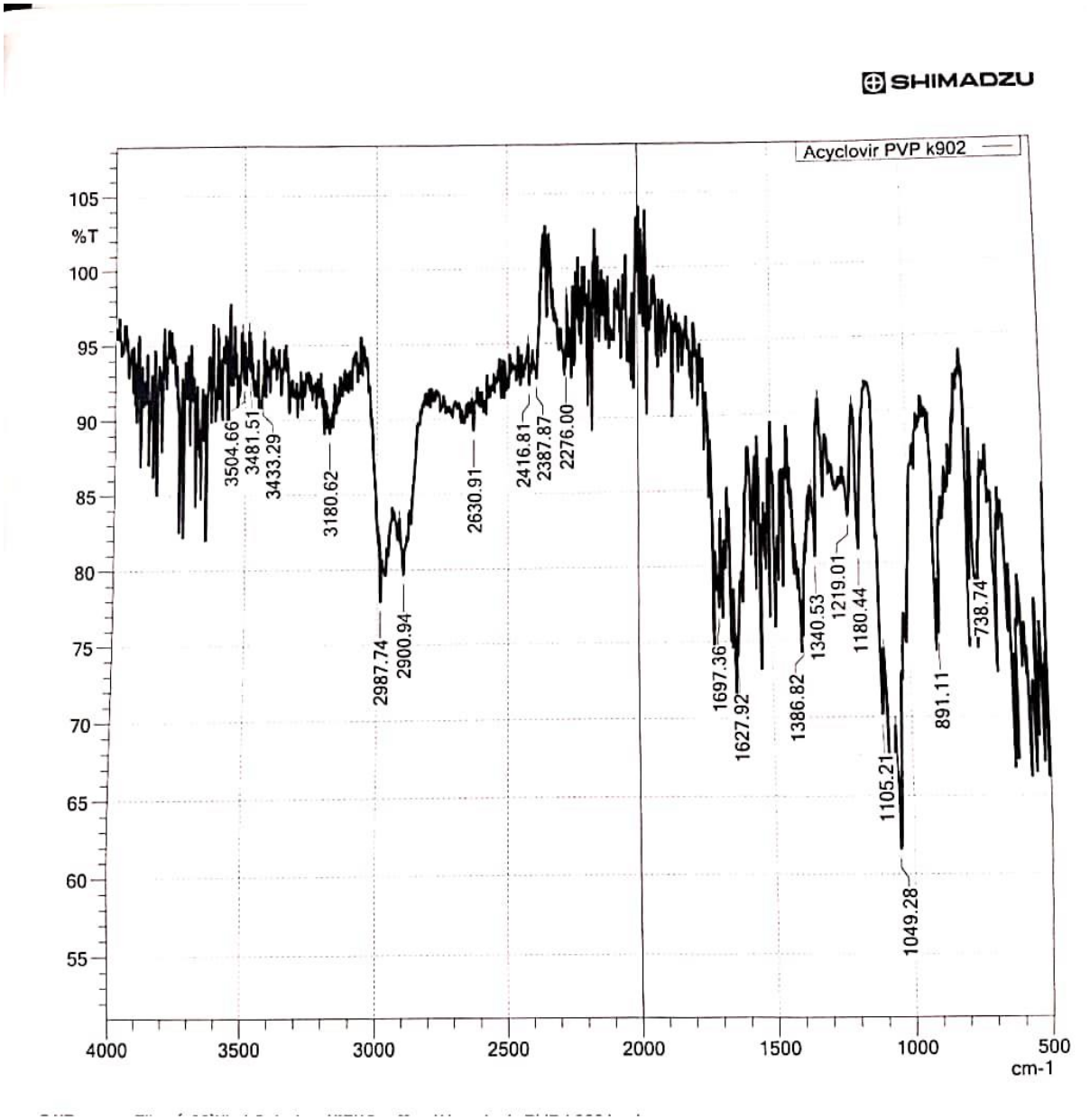


Figure 4.2.5: FTIR spectra of a binary mixture of acyclovir and Polyvinyl pyrrolidone K90

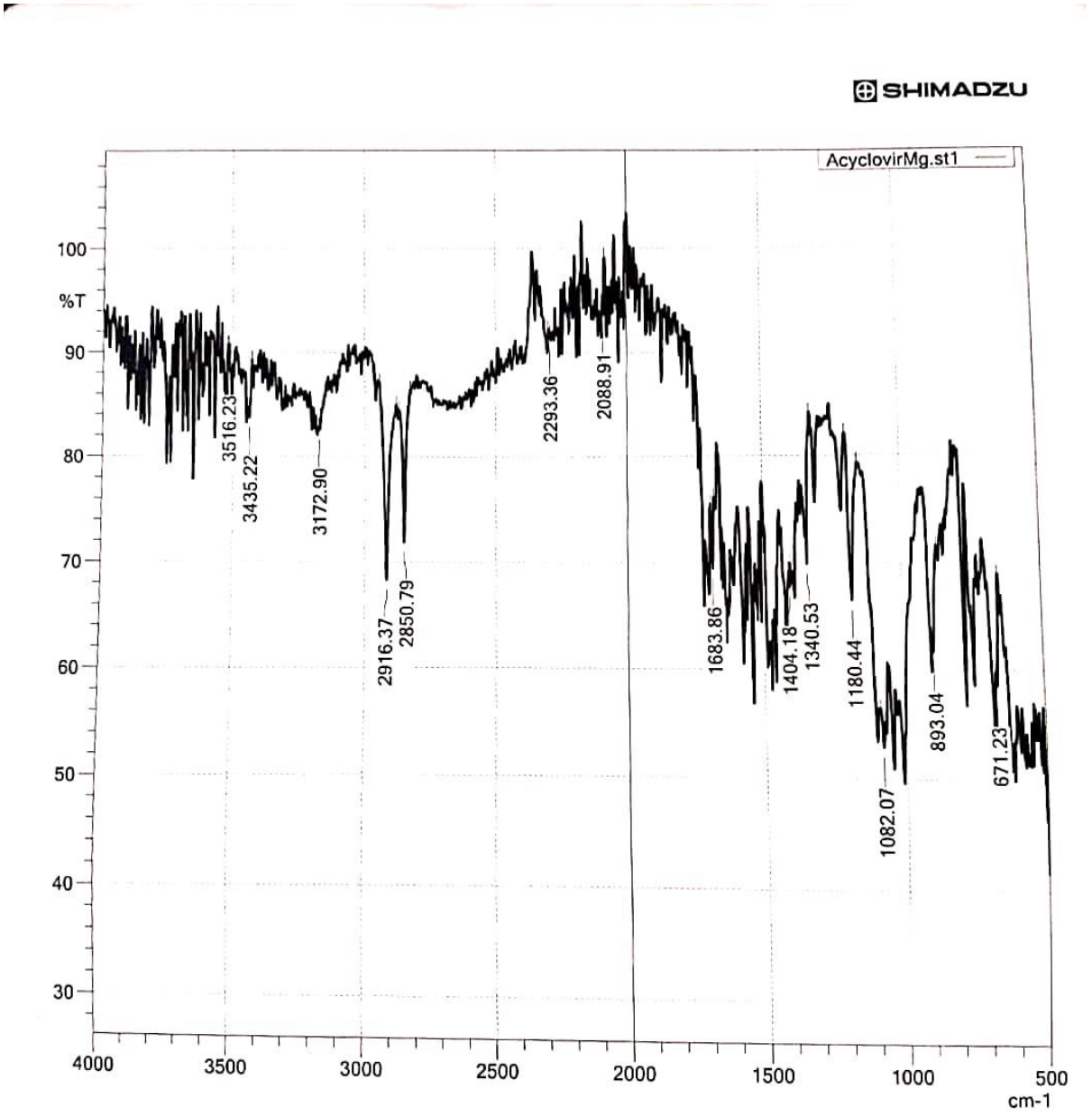


Figure 4.2.6: FTIR spectra of a binary mixture of acyclovir and Magnesium stearate

Annex 5: Contour plots and response surface graphs

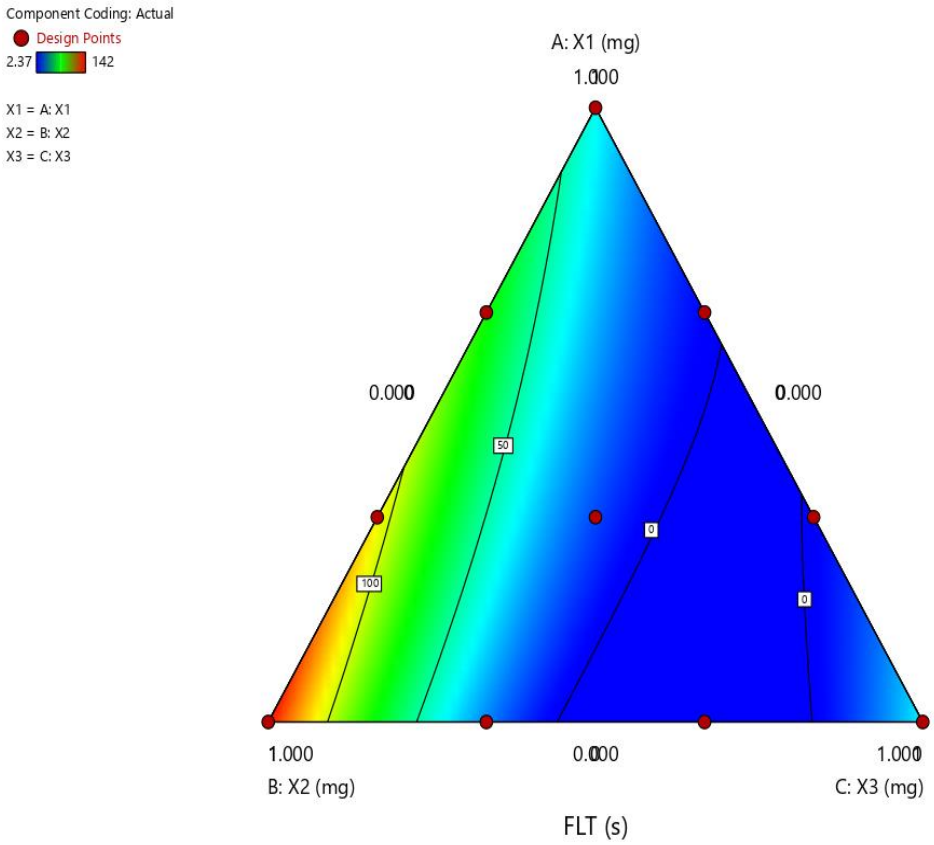


Figure 4.10. 1 Contour plot showing the effect of different polymers on the floating fat time (FLT)

Where X1, X2 and X3 represents HPMC K100M, HPMC K4M and Carbopol respectively.

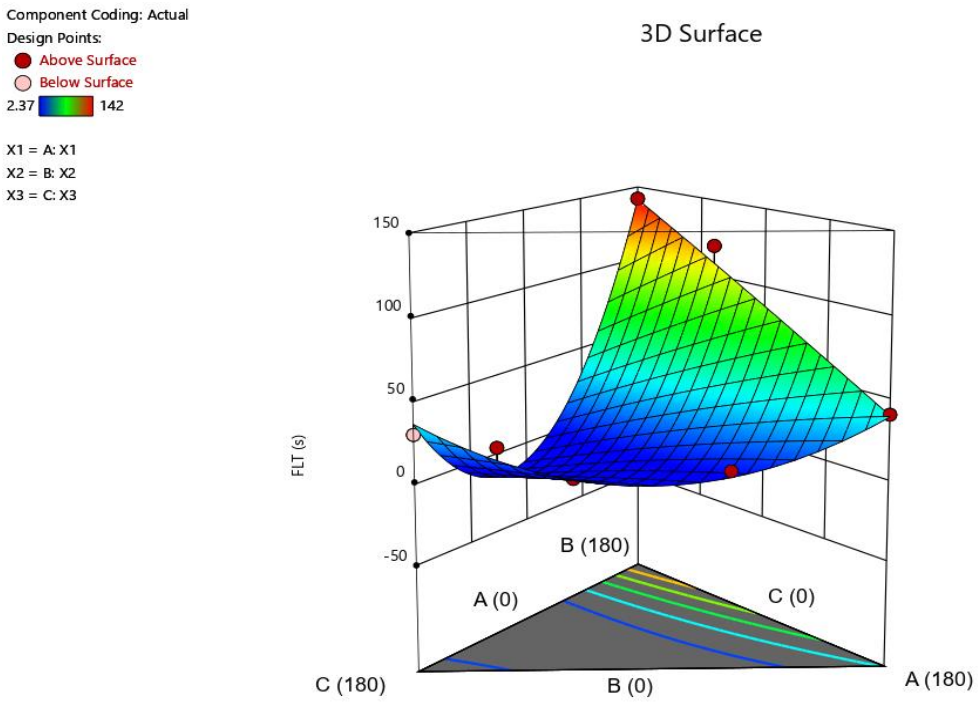


Figure 4.10.2: Response surface plot showing the effect of different polymers on the floating lag time Where A, B, C represents HPMC K100M, HPMC K4M and Carbopol respectively.

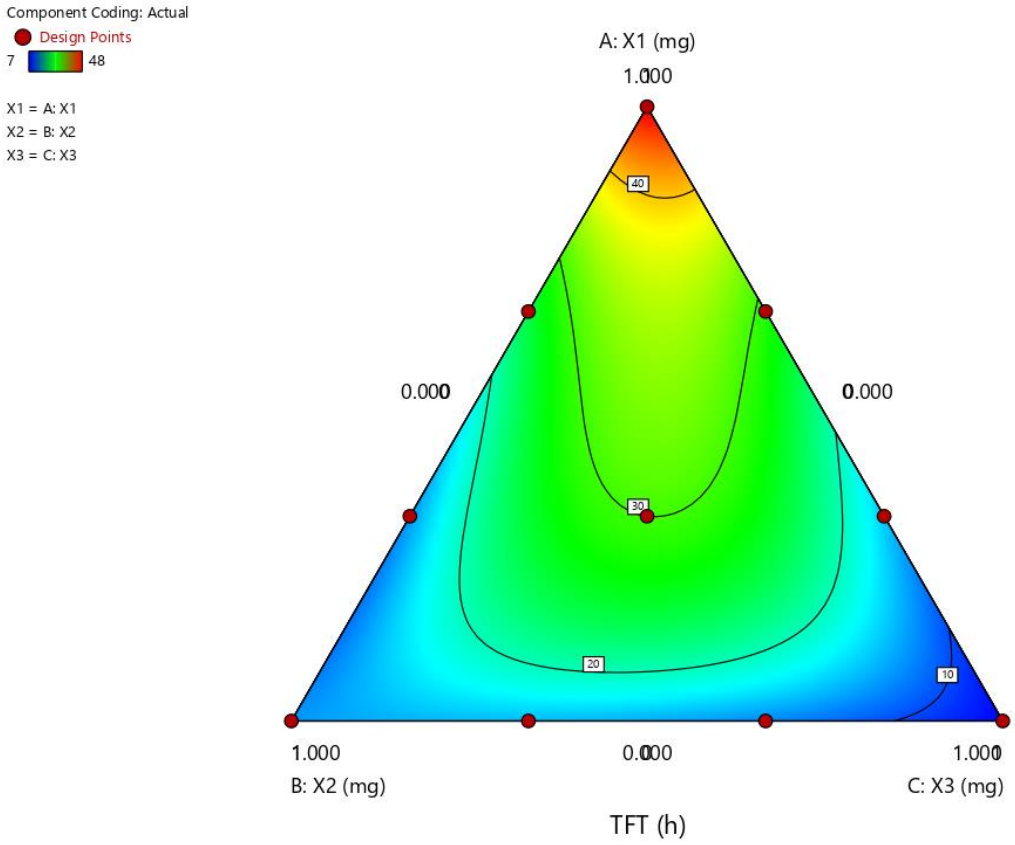


Figure 4.10.3: Contour plot showing the effect of polymers on total floating time (TFT), X1, X2, X3 represents HPMC K100M, HPMC K4M and Carbopol respectively

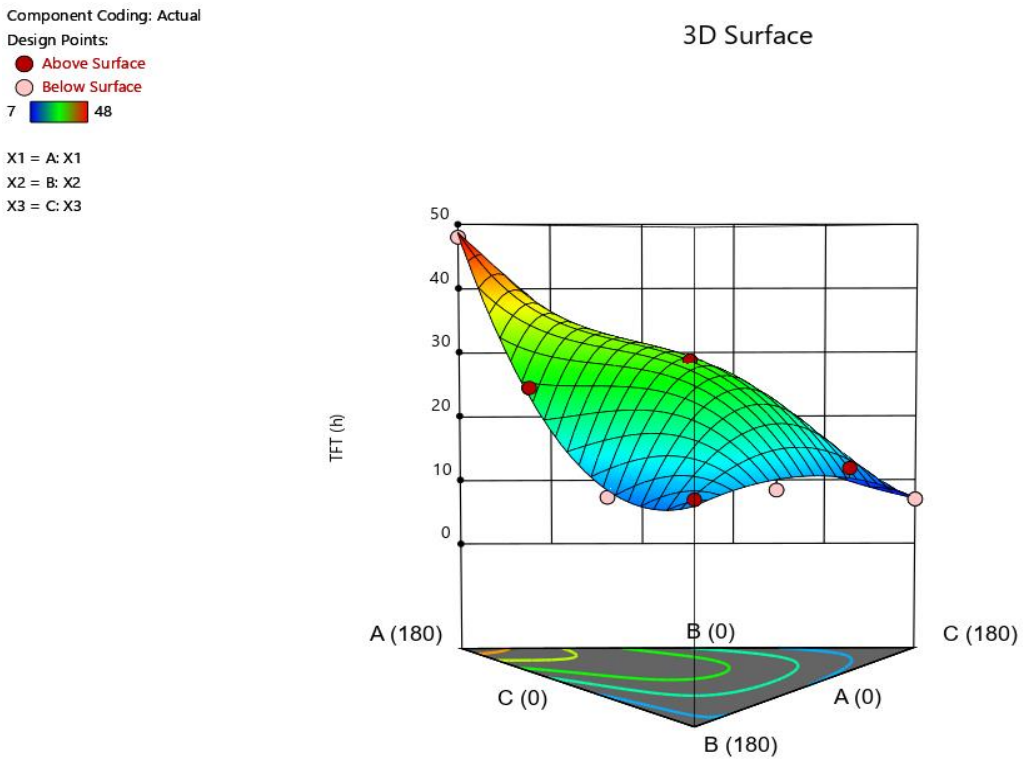


Figure 4.10.4: Response surface plot showing the effects of HPMC K100M (A), HPMC K4M (B) and Carbopol 934(C) on the total floating time of acyclovir tablets.

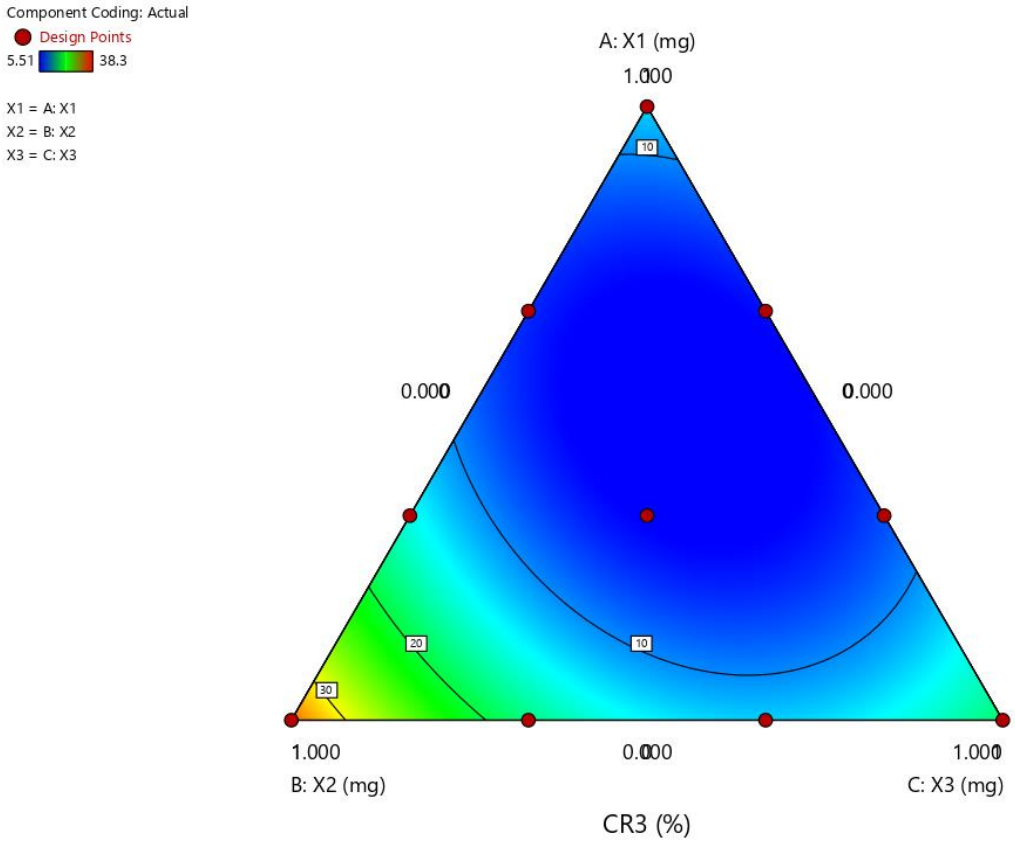


Figure 4.10.5: Contour plot showing the effect of the polymers HPMC K100 (X1), HPMC K4M (X2) and Carbopol 934 (X3) on the cumulative drug release at 3 hours.

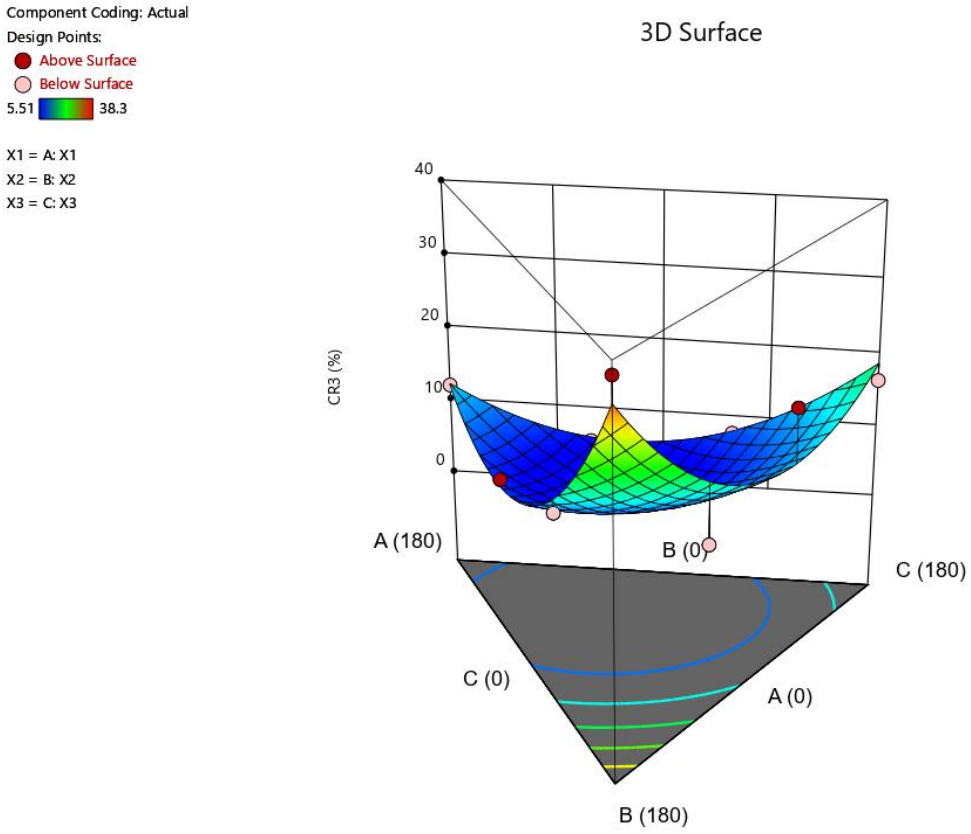


Figure 4.10.6: A response surface plot showing the effects of polymers HPMC K100M (A), HPMC K4M (B) and Carbopol 934 on the cumulative drug release at 3 hours

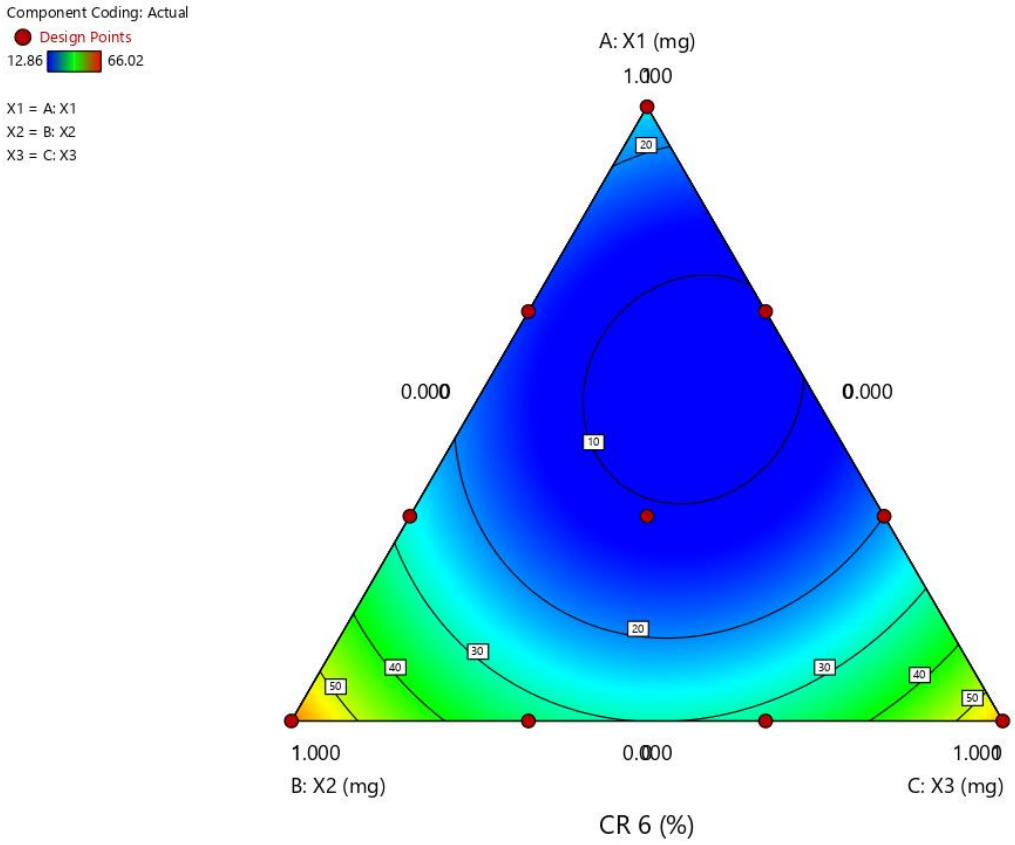


Figure 4.10.7 Contour plot showing the effects of polymers HPMC K 100M (X1) HPMC K4M (X2) and Carbopol 934 on the cumulative drug release at the 6th hour.

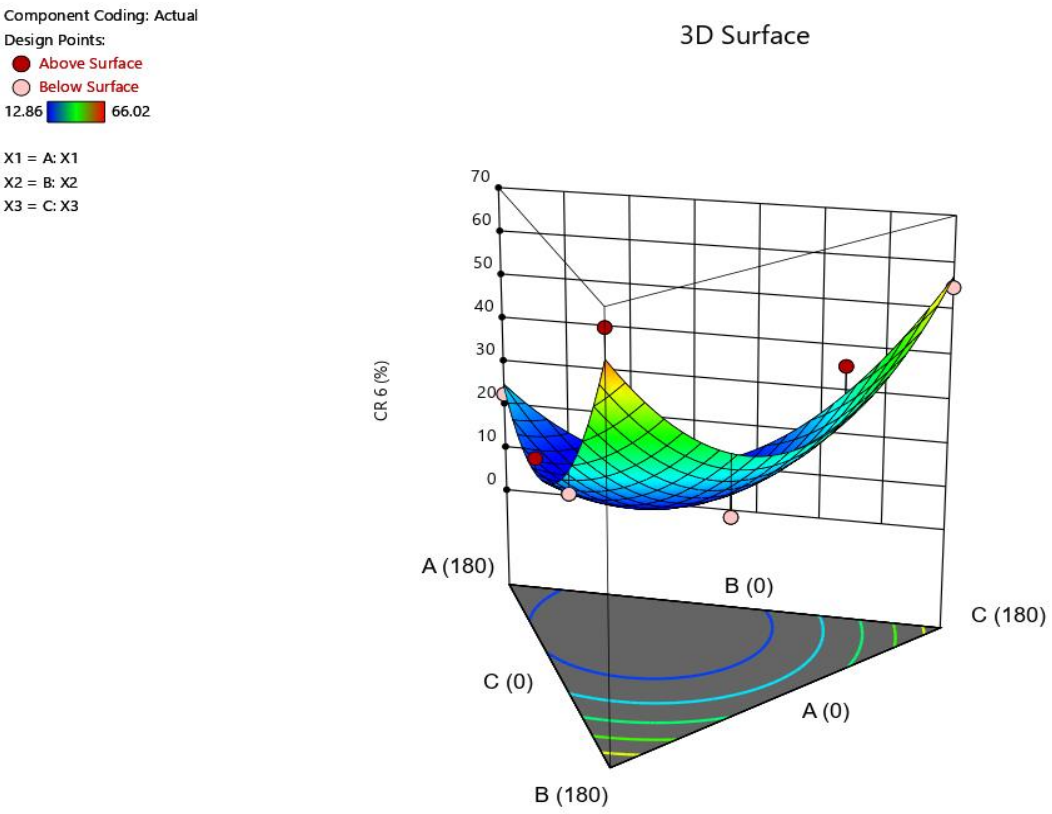


Figure 4.10.8: A response surface plot showing the effects of polymers HPMC K100M (A), HPMC K4M (B) and Carbopol on the cumulative drug release on the 6th hour.

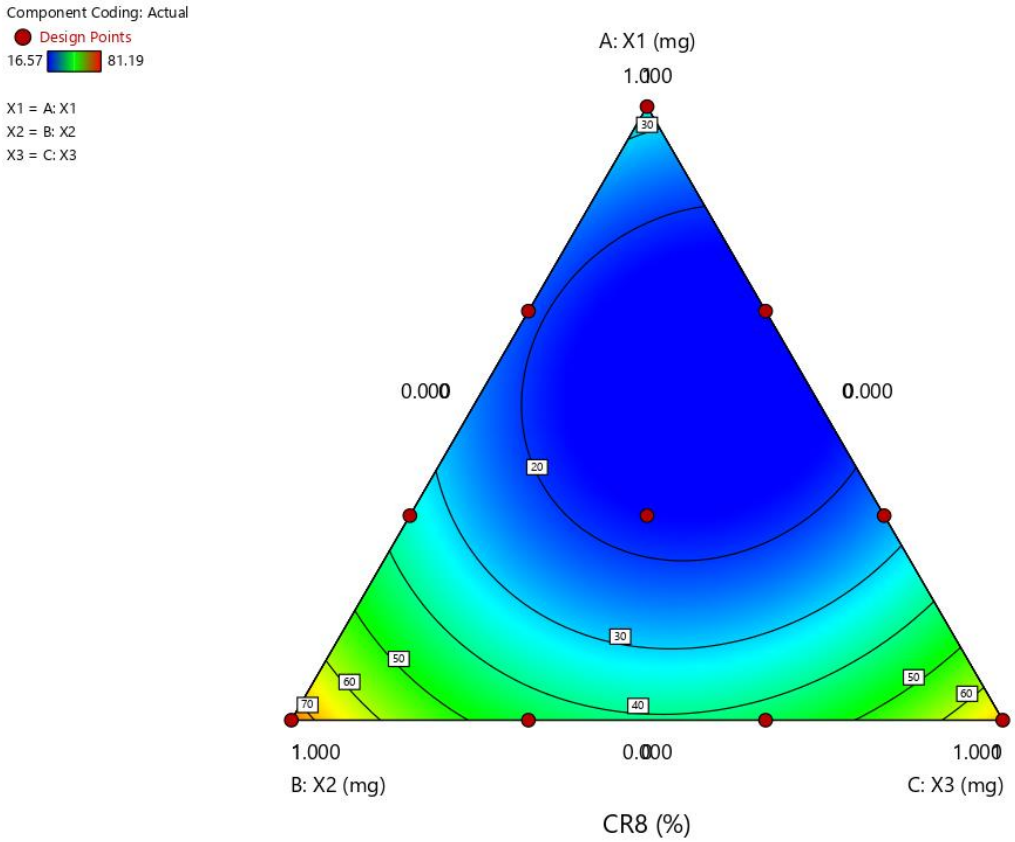


Figure 4.10.9: Contour plot showing the effects of polymers HPMC K100M (X1), HPMC K 4M (X2) and Carbopol 934 (X3) on the cumulative percentage drug release on the 8th hour.

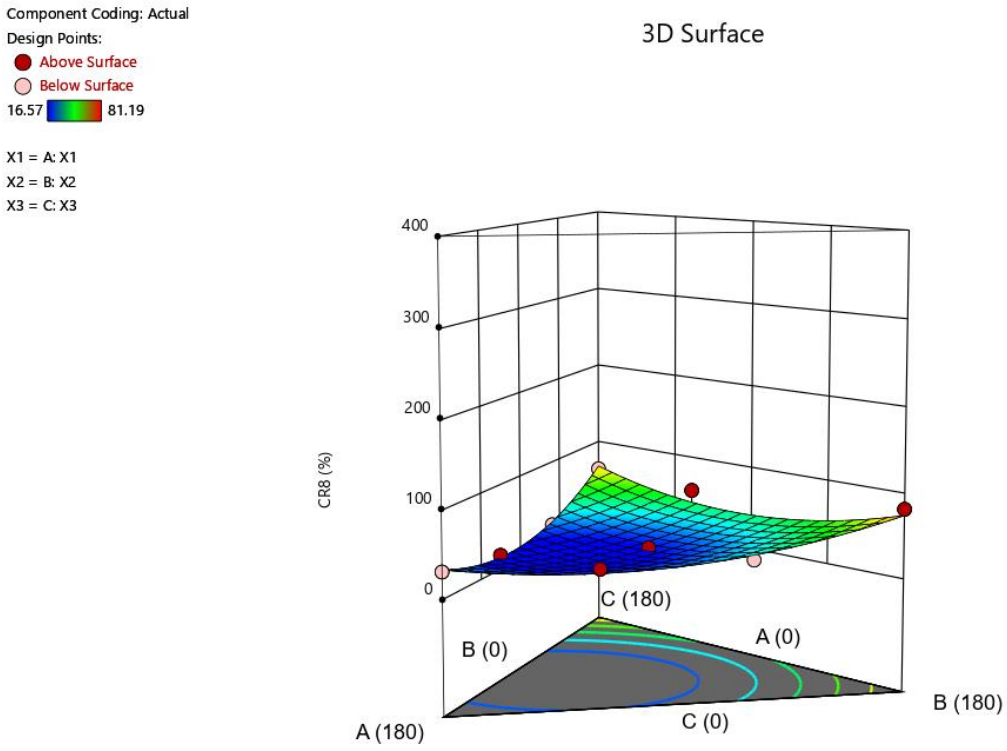
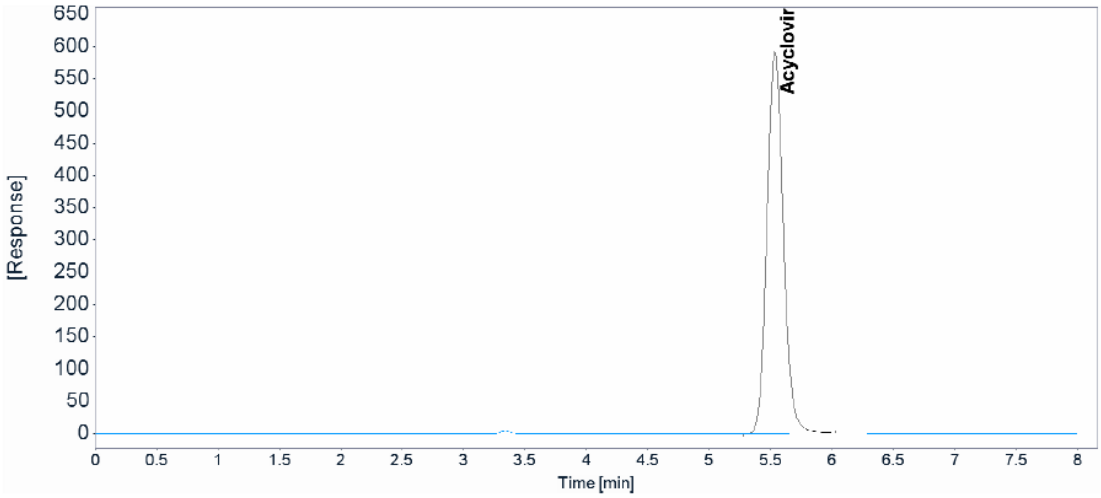


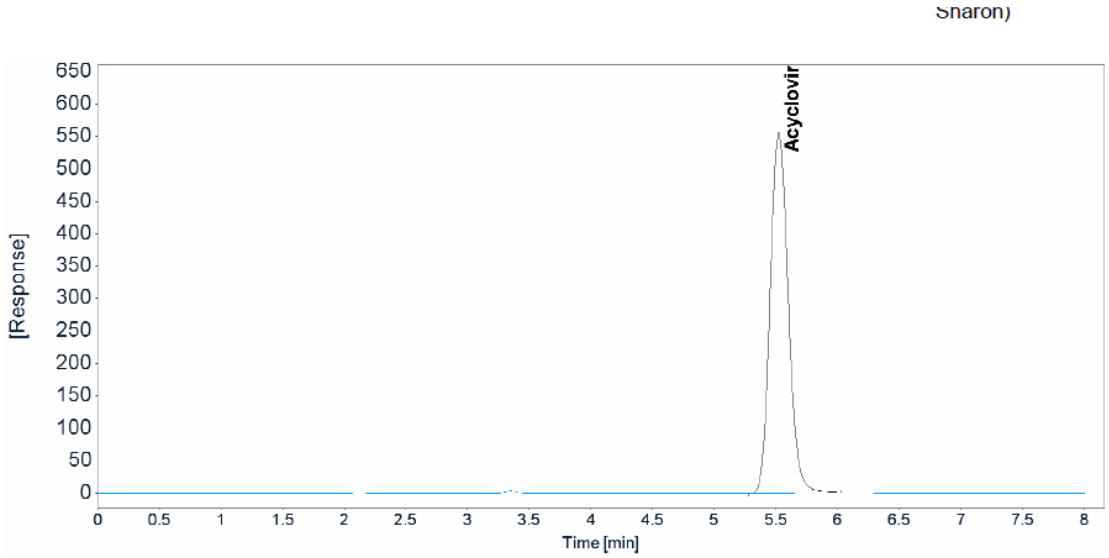
Figure 4.10.10: A response surface plot showing the effects of polymers HPMC K100M (A), HPMC K4M (B) and Carbopol 934 on the cumulative drug release on the 8th hour.

Annex 6: Chromatograms



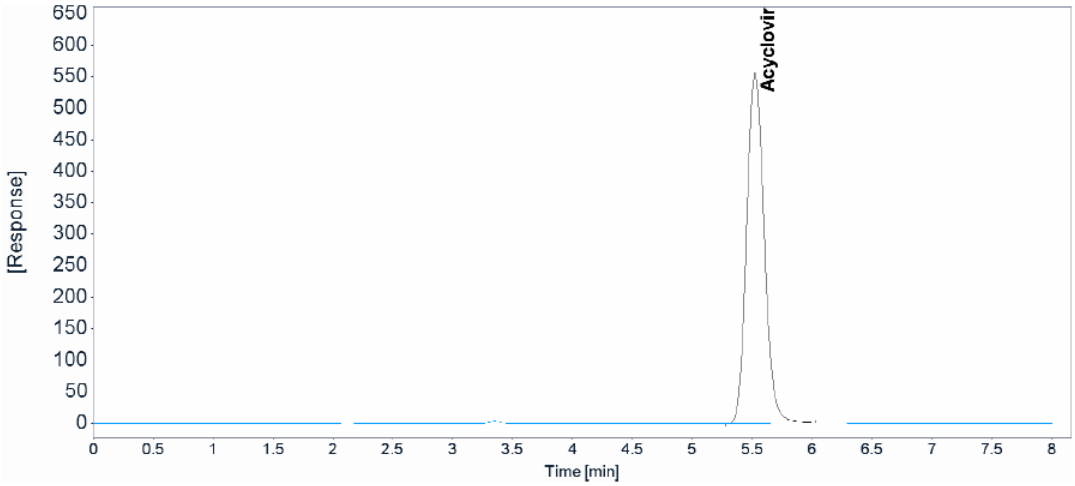
Name	RT	Area	Peak_AreaPercent	Peak_Asymmetry	Peak_TheoreticalPlates_USP	Peak_Resolution_USP	Peak_RelativeRetTime
Acyclovir	**5.5**	**11517249**	**100**	**1.1**	**8206**	**0.0**	

Figure 4.1.2a: Chromatogram of acyclovir standard



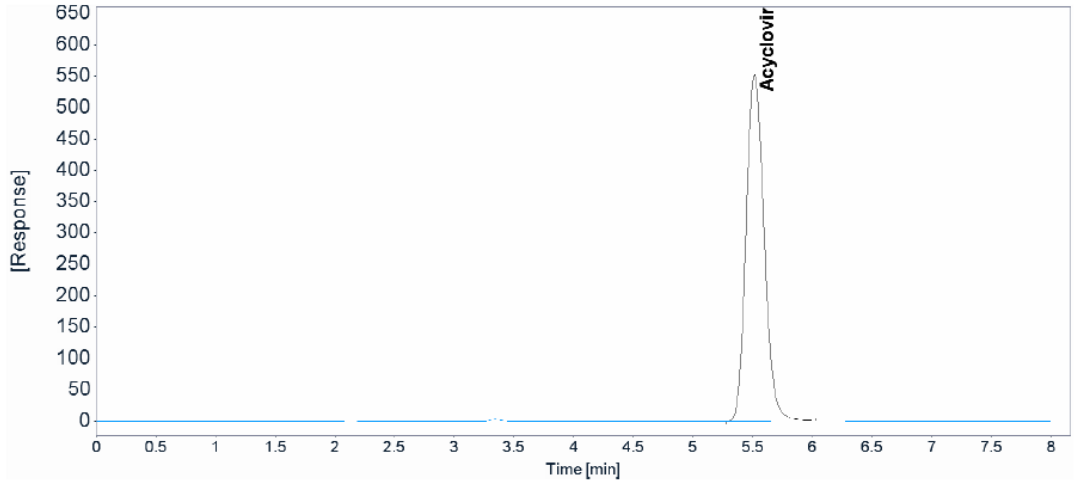
Name	RT	Area	Peak_AreaPercent	Peak_Asymmetry	Peak_TheoreticalPlates_USP	Peak_Resolution_USP	Peak_RelativeRetTime
Acyclovir	**5.5**	**11861415**	**100**	**1.1**	**6772**	**0.0**	

Figure .4.1.2b: Chromatogram of acyclovir active ingredient



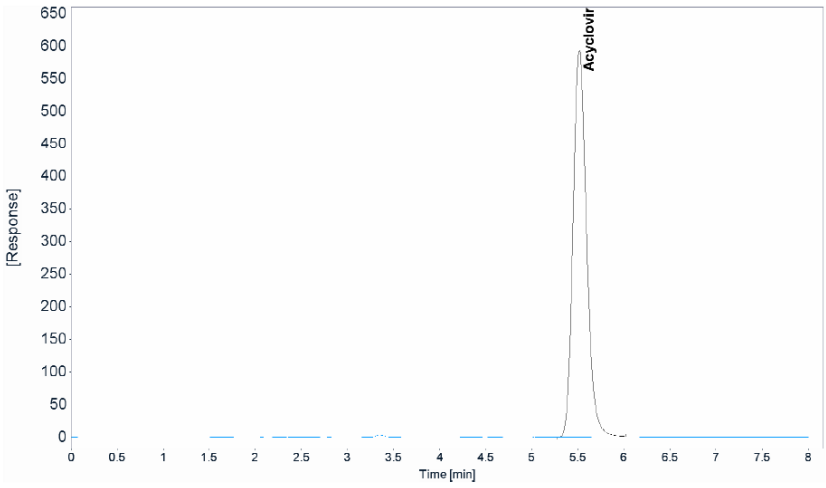
Name	RT	Area	Peak_AreaPercent	Peak_Asymmetry	Peak_TheorecticalPlates_USP	Peak_Resolution_USP	Peak_RelativeRetTime
Acyclovir	**5.5**	**11861415**	**100**	**1.1**	**6772**	**0.0**	

Figure 4.8a: Chromatogram of F1 formulation batch of acyclovir floating tablets



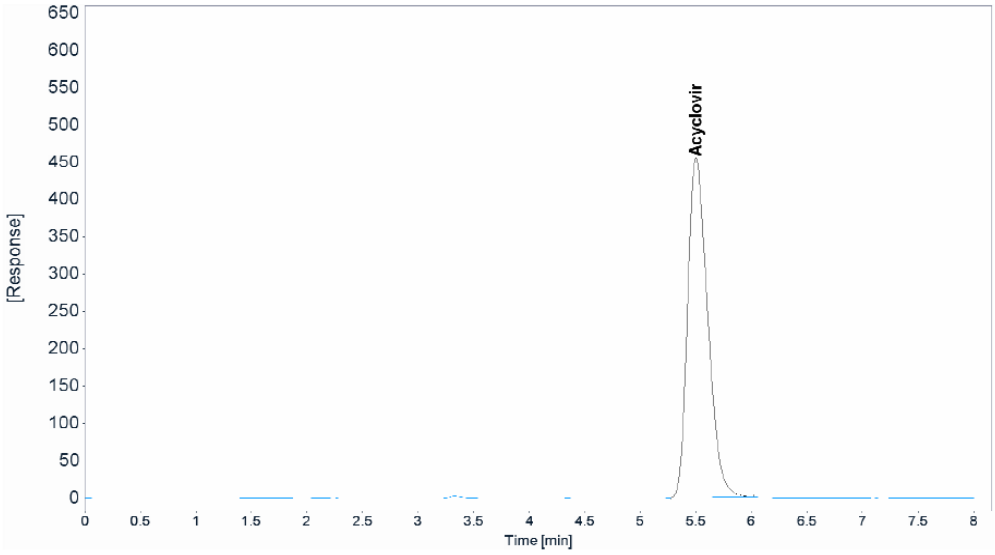
Name	RT	Area	Peak_AreaPercent	Peak_Asymmetry	Peak_TheorecticalPlates_USP	Peak_Resolution_USP	Peak_RelativeRetTime
Acyclovir	**5.5**	**12124328**	**100**	**1.2**	**6390**	**0.0**	

Figure 4.8b: Chromatogram of F2 formulation batch of acyclovir floating tablets



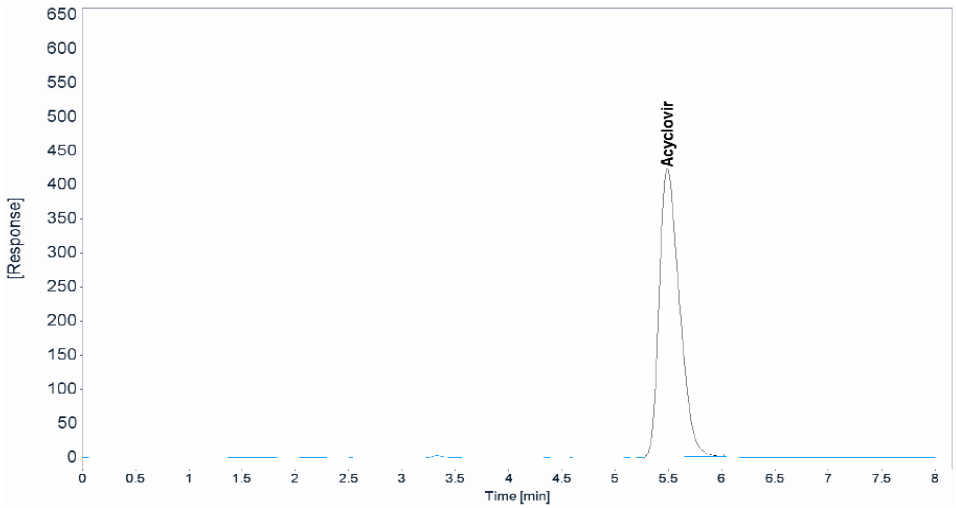
Name	RT	Area	Peak_AreaPercent	Peak_Asymmetry	Peak_TheoreticalPlates_USP	Peak_Resolution_USP	Peak_RelativeRetTime
Acyclovir	**5.5**	**12719005**	**100**	**1.2**	**6799**	**0.0**	

Figure 4.8c: Chromatogram of F3 formulation batch of floating acyclovir tablets



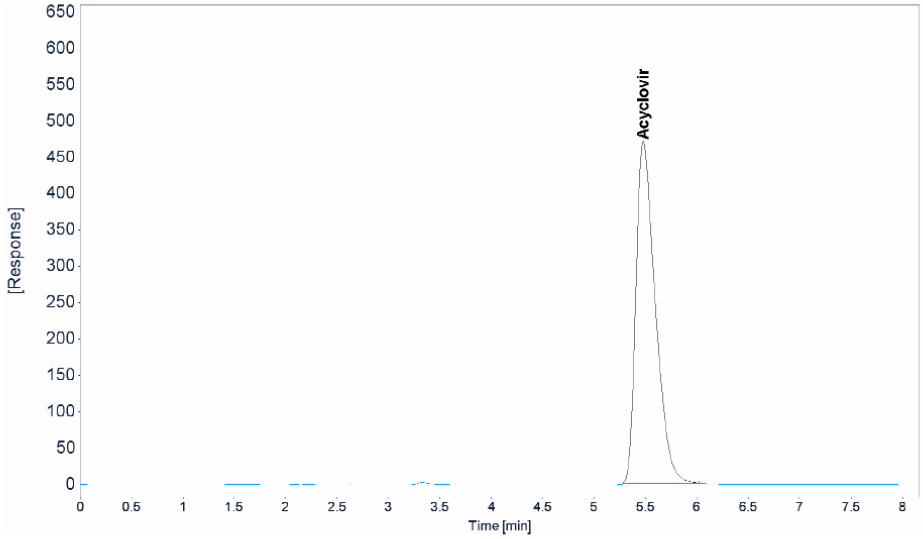
Name	RT	Area	Peak_AreaPercent	Peak_Asymmetry	Peak_TheoreticalPlates_USP	Peak_Resolution_USP	Peak_RelativeRetTime
Acyclovir	**5.5**	**11986953**	**100**	**1.4**	**4181**	**0.0**	

Figure4.8d: Chromatograph of F4 formulation batch of floating acyclovir tablets



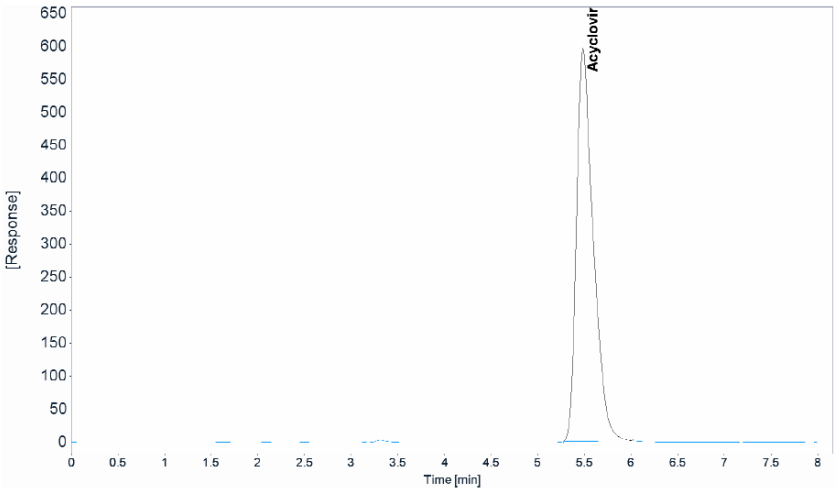
Name	RT	Area	Peak_AreaPercent	Peak_Asymmetry	Peak_TheoreticalPlates_USP	Peak_Resolution_USP	Peak_RelativeRetTime
Acyclovir	**5.5**	**11644092**	**100**	**1.4**	**3783**	**0.0**	

Figure 4.8e: Chromatograph of F5 formulation of acyclovir floating tablets



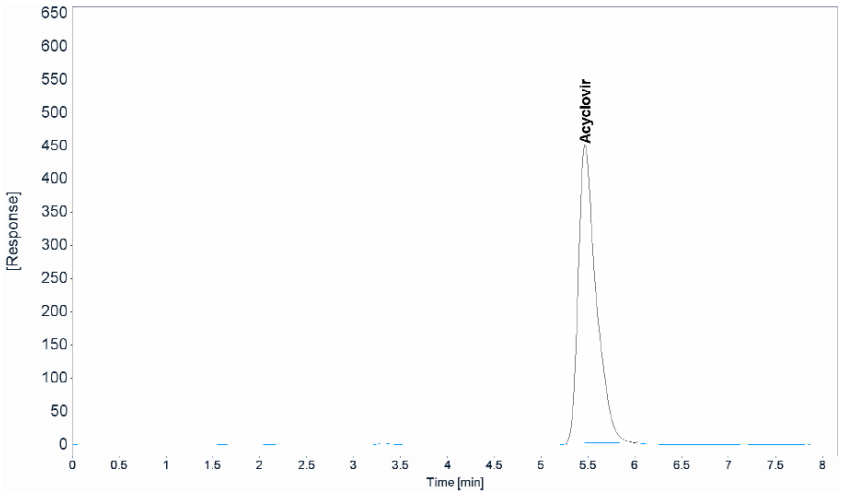
Name	RT	Area	Peak_AreaPercent	Peak_Asymmetry	Peak_TheoreticalPlates_USP	Peak_Resolution_USP	Peak_RelativeRetTime
Acyclovir	**5.5**	**12921901**	**100**	**1.5**	**3854**	**0.0**	

Figure 4.8f: Chromatograph of F6 formulation batch of acyclovir floating tablets



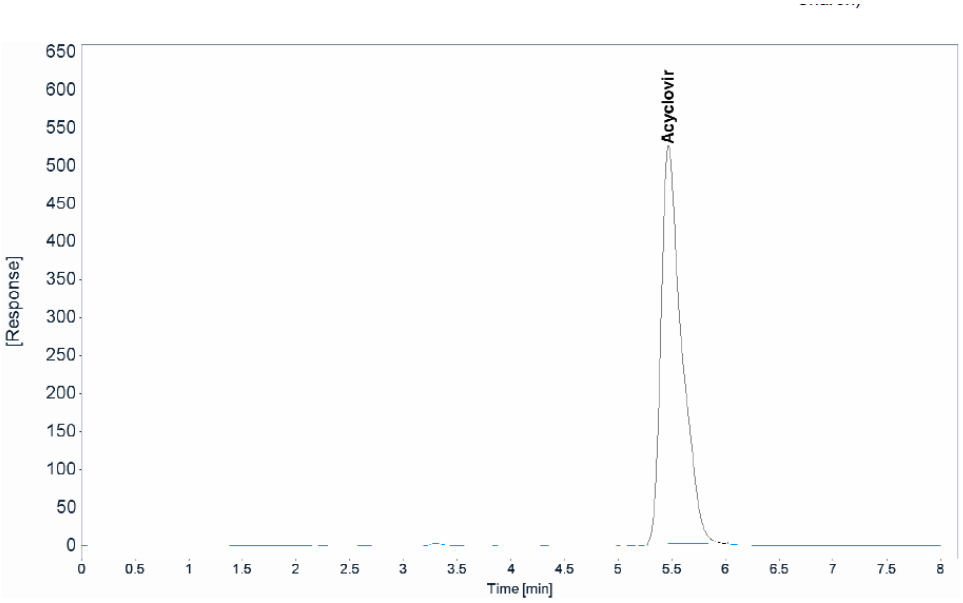
Name	RT	Area	Peak_AreaPercent	Peak_Asymmetry	Peak_TheoreticalPlates_USP	Peak_Resolution_USP	Peak_RelativeRetTime
Acyclovir	**5.5**	**15563571**	**100**	**1.4**	**4433**	**0.0**	

Figure 4.8g: Chromatograph of F7 formulation batch of acyclovir floating tablets



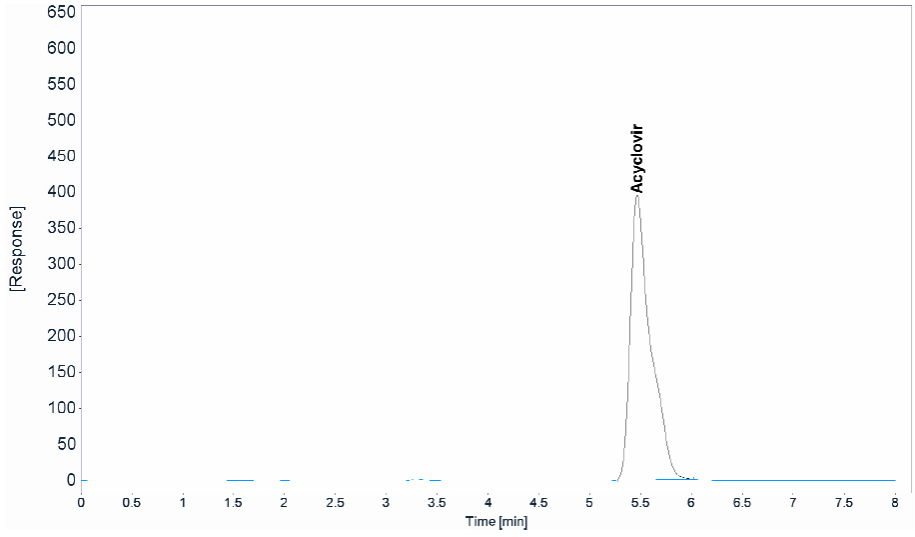
Name	RT	Area	Peak_AreaPercent	Peak_Asymmetry	Peak_TheoreticalPlates_USP	Peak_Resolution_USP	Peak_RelativeRetTime
Acyclovir	**5.5**	**12376749**	**100**	**1.5**	**4203**	**0.0**	

Figure 4.8h: Chromatograph of F8 formulation batch of acyclovir floating tablets



Name	RT	Area	Peak_AreaPercent	Peak_Asymmetry	Peak_TheorecticalPlates_USP	Peak_Resolution_USP	Peak_RelativeRetTime
Acyclovir	**5.5**	**14626822**	**100**	**1.6**	**4180**	**0.0**	

Figure 4.8i: Chromatogram of F9 formulation batch of acyclovir floating tablets



Name	RT	Area	Peak_AreaPercent	Peak_Asymmetry	Peak_TheorecticalPlates_USP	Peak_Resolution_USP	Peak_RelativeRetTime
Acyclovir	**5.5**	**11382436**	**100**	**1.7**	**4140**	**0.0**	

Figure 4.8j: Chromatogram of F10 formulation batch of acyclovir floating tablets