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Aproaches To Develop Acyclovir Gastro-Retentive Formulations Using Hot Melt Extrusion Technology

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APROACHES TO DEVELOP ACYCLOVIR GASTRO-RETENTIVE FORMULATIONS USING HOT MELT EXTRUSION TECHNOLOGY

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ABSTRACT

The aim of the current study was to prepare and compare acyclovir (ACV) floating gastro-retentive formulations synthesized via hot-melt extrusion (HME) techniques using P-CO_2 and sodium bicarbonate. Physical mixtures of ACV (20%, w/w) and HPC EF (70%, 60%, w/w) with HPC MF (10%, w/w) or HPMCAS-LG (10%, w/w) were prepared using a co-rotating twin-screw extruder with a screw configuration showed in **Figure 2**. P-CO_2 was injected into zone 9 of the extruder or $NaHCO_3$ was added (10% w/w) during the extrusion process. *In vitro* dissolution studies (0.1 N hydrochloric acid medium) demonstrated that the formulations floated on the medium because of their porosity and low density. Formulations with $NaHCO_3$ (F9, F10) showed better extended release potential than the counterparts with P-CO_2 (F7, F8). The *in vitro* drug release mechanism of the optimized formulation was found to best fit with the first-order (R^2=0.9686) and Higuchi models (R^2=0.9614). Accelerated stability studies demonstrated similar release profiles between fresh samples and stability samples after 3 months storage. Altogether, the ACV floating gastro-retentive formulations were successfully developed, which can prolong the gastric residence time to extend therapeutic effects.

DEDICATION

This thesis is dedicated to my dear parents, Mr. Guogang Zhang and Mrs. Hong Tian and to everyone who helped me and guided me through my own times of stress and anxiety. Also, I am grateful to my girlfriend, Sooyeon Chung, who supported me all the time.

LIST OF ABBREVIATIONS AND SYMBOLS

ACV Acyclovir

P-CO_2 Pressurized Carbon Dioxide

$NaHCO_3$ sodium bicarbonate

DSC Differential Scanning Calorimetry

HME Hot-Melt Extrusion

SEM Scanning Electronic Microscopy

FTIR Fourier Transform Infrared

Hydroxypropyl cellulose HPC

Hydroxypropyl methylcellulose acetate succinate HPMCAS

ACKNOWLEDGMENTS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Oral dosage forms, one of the commonly used routes of administration, have the following advantages: ease of dosing administration, patient compliance, and flexibility in formulation. However, conventional oral preparations have some restrictions; some drugs are absorbed in the upper part of the stomach or small intestine, possess short half-lives, and quickly disappear from systemic circulation. Hence, to achieve adequate therapeutic activity, frequent administration is required [1]. To improve such circumstance, oral sustained-controlled release formulations, which can slowly release to the gastrointestinal tract (GIT), should be developed to maintain effective systemic drug concentrations for long periods [2]. The gastro-retentive drug delivery system can prolong the retention time of a drug in the stomach, thereby extending its transport time throughout the gastrointestinal tract and increasing the degree of drug absorption, ultimately improving the clinical efficacy [3]. Many approaches for developing such gastro retentive systems have been reported in the literature, such as floating systems, muco-adhesion systems, swellable systems, high-density systems, modified shape systems, or the simultaneous administration of pharmacological agents that delay gastric emptying [4]. Based on current research, floating drug delivery systems (FDDS) possess the greatest advantages among the different types of gastric retention drug delivery systems [5]. This drug delivery system is suitable for drugs with an absorption window in the stomach or upper small intestine [6]. FDDS can be divided into two types: effervescent formulations and non-effervescent formulations [7]. In the present study, non-effervescent FDDS was selected. In this system, hydrophilic polymers are used as a matrix, which can swell to form a continuous gel layer that controls drug release from the matrix [8].

In the pharmaceutical industry, solid dispersions are one of the most promising strategies for improving the oral bioavailability of poorly water-soluble drugs. This strategy can significantly increase bioavailability by reducing particle size, improving wetting, and reducing agglomeration [9]. Processing technologies such as spray drying (SD), hot-melt extrusion (HME), freeze-drying (FD), and supercritical fluid drying (SFD) can be employed to prepare solid dispersions [10]. Among them, HME is one of the most promising processing technologies. HME is the process of converting raw materials with a rotating screw under controlled conditions (temperature, feed rate, and pressure) through a die, into a product of uniform shape [11]. In addition to being widely used in the preparation of amorphous solid dispersions, HME is also used in many other applications such as taste-masking, modified drug release, and stabilization of an active pharmaceutical ingredient (API) [12]. There are many advantages of using HME compared to conventional pharmaceutical processing methods. For example, it is a solvent-free processing method for solid dispersions, the equipment has a small carbon footprint, and it entails a fast-continuous manufacturing process [13].

In recent years, the combination of carbon dioxide (CO_2) and HME has attracted the interest of researchers. CO_2 is environmentally friendly, non-flammable, and has a low cost [14]. According to the literature, pressurized CO_2 (P-CO_2) can be used as a foaming agent [15]. Because of the expansion characteristics of P-CO_2 on the extrusion die during the HME process, the micro-morphology of the extrudate is transformed into a foam-like structure [16]. This morphological change could lead to a porous and low-density system, which causes the floating of extrudates in the medium.

Acyclovir (ACV, 9-(2-hydroxyethoxymethyl) guanine), an antiviral drug that can inhibit the action of viral DNA polymerase and DNA replication of different herpes virus [17], is primarily used to treat herpes simplex virus type 1 and 2 (HSV-1 and HSV-2),

varicella-zoster virus, chickenpox, and shingles [18,19]. The molecular weight of acyclovir is 225.2, the melting point of acyclovir is 256℃, logP of acyclovir is -1.59, water solubility of acyclovir is 1.2±0.07 mg/mL at 25°C, and the pKa of acyclovir is 2.34 and 9.23[20][21][22]. Although oral administration is the most convenient route for patients, oral absorption is not satisfactory for many drugs. ACV has pharmacokinetic limitations. Its oral bioavailability is 10-20%; however, this decreases with an increase in dose[23]. Further, 80% of an oral dose is not absorbed and can be detected in the feces. ACV has a relatively short elimination half-life of 3 h; thus, the frequency of administration of ACV is high, up to five times per day, thereby leading to patient incompliance [23]. ACV is soluble in acidic pH and is mainly absorbed from the upper gastrointestinal tract when administered orally [23]. Experiments show that the drug is only absorbed from the upper part of the gastrointestinal tract [24]. Thus, a gastro-retentive sustained release formulation of ACV should be developed.

In the present study, we aimed to prepare ACV gastro-retentive formulations via HME techniques using P-CO_2 and sodium bicarbonate. A subsequent goal of this study was to compare the products of P-CO_2 versus sodium bicarbonate to derive an appropriate approach for gastric retention; this will enable the establishment of a porous and low density floating multi-particulate system that can prolong gastric residence time, reduce the number of dosages, and improve bioavailability and therapeutic efficacy. Herein, hydroxypropyl cellulose (HPC) EF, HPC MF, and hydroxypropyl methylcellulose acetate succinate (HPMCAS) LG were used as the matrix polymers.

2. MATERIALS AND METHODS

2.1 Materials

ACV was purchased from Xinxiang Pharmaceutical Co. Ltd. (Xinxiang, Henan, China). AquaSolve™ HPMCAS LG, Klucel™ EF, and Klucel™ MF were donated by Ashland Specialty Ingredients (Wilmington, DE). Sodium bicarbonate (USP/NF standard) was purchased from Spectrum Chemical Mfg. Corp (Gardena, CA, USA). CO_2 was supplied in gas cylinders (pure clean) by Airgas (Tupelo, MS). All other chemicals and reagents used in the present study were of analytical grade.

2.2 Methods

2.2.1 Formulations

In this study, different formulations were investigated and separated into two different stages (**Table 1**). The first stage (stage one) was to examine the effect of sodium bicarbonate and P-CO_2 on a single polymer. In this process, 6 formulations of a single polymer with 20% w/w drug load were examined. The second stage (stage two) was based on the results of the dissolution study; thus, a binary mixture of polymers was blended to achieve sustained release of the drug. The physical mixture of the polymer and ACV (20% w/w) was uniformly blended for 20 min using a V-shell blender (Maxiblend, Globe Pharm). Thereafter, it was fed into the extruder.

Table 1. **ACV** formulations.

Formulation		ACV (w/w)	Excipients (w/w)	P-CO_2 (PSI)
Stage 1	F1	20%	80% HPC EF	100-250
	F2	20%	80% HPC MF	150-300
	F3	20%	80% HPMC-AS LG	150-300
	F4	20%	70% HPC EF, 10% $NaHCO_3$	-
	F5	20%	70% HPC MF, 10% $NaHCO_3$	-
	F6	20%	70% HPMC-AS LG, 10% $NaHCO_3$	-
Stage 2	F7	20%	70% HPC EF, 10% HPC MF	100-250
	F8	20%	70% HPC EF, 10% HPMC-AS LG	100-250
	F9	20%	60% HPC EF, 10% HPC MF, 10%$NaHCO_3$	-
	F10	20%	60% HPC EF 10% HPMC-AS LG 10% $NaHCO_3$	-

2.2.2 Hot-melt extrusion process

The gastro-retentive formulations were prepared using HME techniques employing a twin-screw extruder (16-mm Prism Euro Lab; Thermo Fisher Scientific, Waltham, MA). The extruder is divided into 10-barrel areas next to the gravimetric feeder. This study used a screw configuration consisting of four conveying zones and three mixing zones. For the formulations F1， F2, F3, F7 and F8, pressurized CO_2 was injected into the extruder using a high-pressure regulator connected to flexible stainless-steel hose with armor casing. The other end of the hose was connected to a four-way connection and fitted with a pressure

gauge, bleed valve, and check valve (ball type for a unidirectional flow of gas), with the latter connected to an injection port at zone 9 (conveying zone) of the extruder（**Figure 1**）. The regulator knob was used to regulate the P-CO_2 flow rate[25]. For the other formulations F4, F5, F6, F9 and F10, the uniformly blended physical mixture of polymer and ACV (20% w/w) and sodium bicarbonate (10% w/w) was fed into the extruder using the volumetric feeder without CO_2. The temperature of the barrel was 140℃ with 100 rpm for both the approaches.

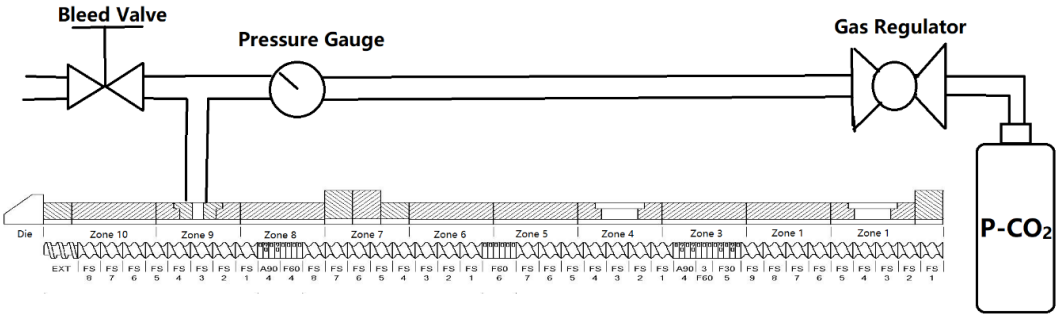

Figure 1. Schematic representation of the set-up for the combined HME/P-CO2 techniques; Injection of P-CO2 at Zone 9.

2.2.3 Differential scanning calorimetry (DSC)

DSC (TA Instruments, New Castle, DE) was used to investigate the polymorphic and thermal properties of the API, excipients, and extrudates. All samples were prepared in TA aluminum pans and lids (Tzero) with an average sample weight of 5–7 mg. All samples were kept at equilibrium via holding at a temperature of 25 °C followed by heating from 25 °C to 300 °C at a ramp rate of 10 °C/min under an inert nitrogen cell purge flow of 50 mL/min.

2.2.4 UV- Visible spectrophotometric analysis

UV- Visible spectrophotometric method was used (GENESYS 6 UV- Vis spectrophotometer, Thermo Scientific, Madison WI, US) to determine the ACV concentration at a wavelength of 252 nm[26]. The calibration curve was linear over the concentration range of 2 to 18 μg/mL (R^2 >0.9999).

2.2.5 Drug content

The extrudate was milled into a fine powder using a coffee blender. First, 50 mg of each formulation was weighed and transferred into a 250-mL volumetric flask. Methanol (60 mL) and 0.1 N hydrochloric acid (190 mL) were then added to the flask and sonicated for 15 min. The samples were centrifuged, and the supernatant was diluted to the appropriate concentration (40 times) and analyzed using a UV-Vis spectrophotometer at 252 nm.

2.2.6 *In vitro* drug release

In vitro drug release studies were conducted using a USP dissolution apparatus II equipment (Hanson Research SR8plus station). Each experiment was carried out in triplicate in 900 mL 0.1 N hydrochloric acid as a medium at 37±0.5 ℃ for 12 h. 2000mg of each formulation was weighted and put in the medium. The paddle speed was set at 50 rpm. Samples were analyzed at 0.5, 1, 2, 4, 6, 8, 10, and 12 h. Aliquots of 2 mL of dissolution media were collected and an equivalent volume of fresh dissolution media was added. The samples were filtered through 0.2-μm, 13 mm PTFE membrane filters (Whatman, Inc., Haverhill, MA, USA), diluted to the appropriate concentration (40 times) with dissolution

media, and analyzed using a UV-Vis spectrophotometer with the above method. The floating ability of the formulations was observed during the dissolution study.

2.2.7 Drug release Kinetics

Mathematical models (first order, zero order and Higuchi) of drug dissolution were used to assess the dissolution profiles [27,28].

Zero-order model:

$$W_0 - W_t = kt \qquad (1)$$

Where W_t is the amount of drug released at time t, W_0 is the initial concentration of drug at time t=0, and k is the zero-order rate constant.

First-order model:

$$ln(W_t / W_0) = k_1 t \qquad (2)$$

Where W_t is the amount of drug released at time t, W_0 is the initial concentration of drug at time t=0, and k_1 is the first-order rate constant.

Higuchi model:

$$W = kt^{\frac{1}{2}} \qquad (3)$$

Where W is the amount of drug released at time t and k is the Higuchi dissolution constant.

2.2.8 Fourier transform infrared (FTIR) spectroscopy analysis

FTIR studies were conducted with an Agilent Cary 660 FTIR Spectrometer (Agilent Technologies, Santa Clara, CA, USA) in the 4000–600 cm^{-1} spectral range. The bench top ATR (Pike Technologies, Madison, WI, USA) was equipped with a single bounce diamond-coated ZnSe internal reflection element. To study the interaction between the drug

and polymer, a small amount of the extrudate powder was placed on top of the diamond crystal and compressed with the MIRacle high-pressure Clamp.

2.2.9 Scanning electron microscopy (SEM)

Samples were mounted on aluminum stubs held with a carbon adhesive film, which were coated using a Hummer® 6.2 sputtering system (Anatech Ltd., Battlecreek MI, USA) in a nitrogen environment at 120 mm Hg and a current of 15 mA. The morphology of each sample was observed using a scanning electron microscope operated at an accelerating voltage from 1.0 kV to 5.0 kV (JEOL JSM-5600; JEOL, Inc., Peabody MA, USA).

2.2.10 Stability tests

A stability study of the stage two formulations was conducted at 40 °C and 75% relative humidity, which are accelerated stability test conditions, for three months. All formulations were stored in 20-mL clear glass scintillation vials. The samples were analyzed at time zero and after 3 months. Drug content determination and dissolution testing were performed and compared with the fresh samples. To compare the release profiles of the initial and stability samples, a similarity factor (f_2) was calculated [29]. The equation for the similarity factor is represented in Eqn. 4.

$$f_2 = 50\, log\left\{\left[1 + \frac{1}{n}\sum_{n=1}^{n}(R_t - T_t)^2\right]^{-0.5} \times 100\right\} \qquad (4)$$

Where R_t and T_t are % dissolved for the initial sample and stability sample, respectively, at each time point while n denotes the number of time points. If the f_2 value of the two drug release profiles is between 50 and 100, the initial and stable samples are considered similar [30].

3. RESULTS AND DISCUSSION

HPC and HPMC are commonly used as hydrophilic carrier materials in modified release formulations. Both HPC and HPMC can swell to form a viscous hydrogel that controls drug release from the matrix [31]. Additionally, because of the high melt viscosity of HPMC, processing of HME generates high torque and requires a high temperature [31]. Further, injection of CO_2 is difficult to achieve. HPC EF has a low molecular weight and low melt viscosity, it's easy to get the extrudates with P-CO_2. Therefore, HPC EF was selected as the primary polymer. However, because HPC EF dissolves very quickly to achieve extended release, some secondary polymers were required to be added to delay drug dissolution.

HPMCAS is insoluble in acidic aqueous solutions. Therefore, addition of HPMCAS can delay the dissolution of gastric floating tablets and gastro-retentive pellets. HPMCAS-LG has a higher concentration of succinyl groups than other grades (MG and HG), which show higher hydrophilicity [32]. Similarly, HPC MF has a higher molecular weight and viscosity; thus, HPMCAS-LG and HPC MF were selected as secondary polymers.

The injection of CO_2 during HME of a binary mixture of drug and polymer resulted in the synthesis of a porous low-density foaming structure, with floating characteristics that prolong the gastric residence time. After 50 °C, sodium bicarbonate gradually decomposes to produce CO_2, sodium carbonate, and water. Therefore, in the process of HME, the formulation with sodium bicarbonate can be synthesized into a foamed strand. When exposed to stomach acid, CO_2 is generated by reaction with sodium carbonate, and sodium bicarbonate with hydrochloric acid, which is entrapped in the polymer gel matrix to prolong buoyancy

[33]. CO_2 has been investigated as a temporary plasticizer, which can both increase the free volume between polymer chains and decrease chain entanglement, causing reduction in melt viscosity and the glass transition temperature [32,34]. Therefore, sodium bicarbonate and P-CO_2 were selected as foaming agents to prepare a porous and low density floating multi-particulate system.

3.1 Hot Melt Extrusion

All formulations were successful prepared with a Process 16 twin screw extruder (Thermo Fisher Scientific) except F3 and F4 because of the high melt viscosity and high molecular weight of HPC MF and HPMCAS-LG (**Table 2**). The Thermo Fisher standard screw configuration **(Figure 2)**, which consists of four conveying zones and three mixing zones, was also employed. To achieve extrudability of materials during the extrusion process, the extrusion temperature was set at 10-20 °C above the T_g of the polymer [32]. Further, HPC EF, HPC MF, and HPMC-AS LG were selected as controlled release polymers, all of which have a T_g near 120 °C. As sodium bicarbonate begins to dissociate at 110 °C, reaches its peak at approximately 150 °C, and finishes below 170 °C, the optimum processing temperature was selected as 140 °C to achieve the foaming effect of SBC. To ensure consistency of the test parameters, the temperature of all formulations was optimized at 140 °C. The screw speed of the extruder was set at 100 rpm, and barrel torque was controlled between 30% to 65%. In the processing of Formulation 6, the temperature was increased to 160 °C to reduce the high motor torque and facilitate the ease of extrusion because of the high melt viscosity of HPMC. During extrusion with P-CO_2, the pressure was maintained between 150-250 psi. When 4 or 6 zones were selected for CO_2 injection, the back-pressure phenomenon was very evident, ultimately spreading the materials out of the feeding zone. To avoid this spreading,

the injection zone was kept away from the feeding zone. Thus, the zone 9 was selected as the injection zone of P-CO_2. Extrudates of HPC MF or HPMC-AS LG (F2 and F3) with P-CO_2 failed owing to the high molecular weight and high melt viscosity, which increased the difficulty of CO_2 penetration into the matrix. Other formulations successfully produced foamy, porous extrudates.

Table 2. Result of formulations

Formulation		**Result**
Stage one	F1	Successful
	F2	Failed
	F3	Failed
	F4	Successful
	F5	Successful
	F6	Successful
Stage two	F7	Successful
	F8	Successful
	F9	Successful
	F10	Successful

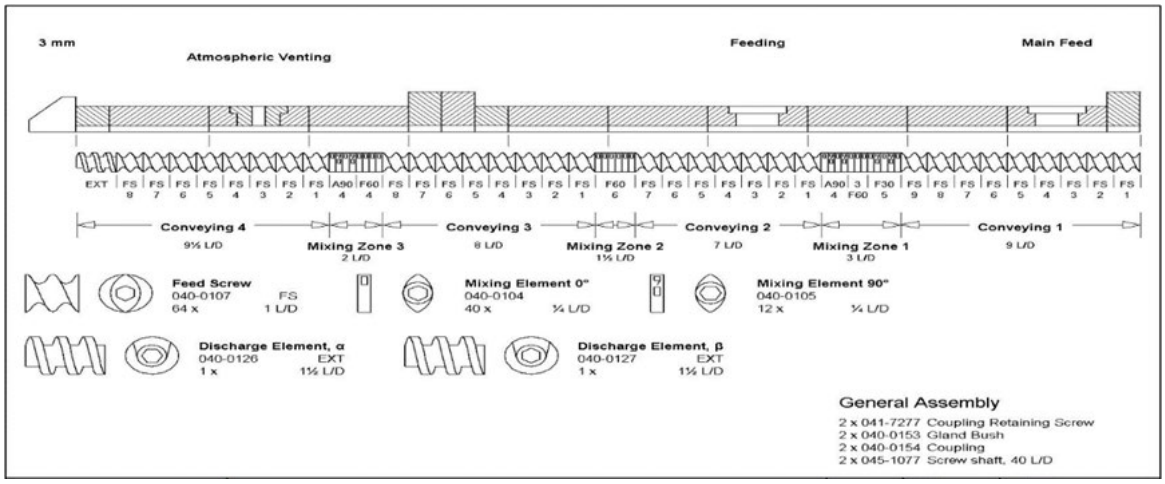

Figure 2. Thermo Fisher standard screw configuration

3.2 Differential scanning calorimetry (DSC)

A DSC image of pure API, pure polymers, and formulations in stage two is presented in **Figure 3**. DSC thermograms of pure ACV revealed decomposition at 257 °C with onset at 249.5 °C. The transformations between different crystal structures of ACV are shown in **Figure 4**. According to the literature, the commercial form of ACV is a 3:2 ACV/water solvate, termed Form V. Upon heating, two transitions of Form V are formed. First, Form V is dehydrated to Form III, which then changes to Form IV between 170 °C and 180 °C [35]. Hence, the small endothermic peak at approximately 177 °C is for the transformation of the crystal form of ACV. Reports reveal that Form V of ACV has two overlapping peaks between 148 °C and 179 °C [35], thereby aligning with the DSC image of pure ACV. In the DSC thermogram for F9 and F10, both have an endothermic peak at approximately 170 °C. The melting point of sodium bicarbonate is ~167 °C, indicating that there is residual sodium bicarbonate in F9 and F10. The thermograms of F7 and F8 revealed peaks at ~250 °C, which indicates that complete conversion of ACV in F7 and F8 into the amorphous state was not achieved after HME coupled with P-CO_2 processing. In contrast, in F9 and F10, the absence

of the peak indicated that F9 and F10 assumed an amorphous nature after HME with the addition of sodium bicarbonate. This phenomenon may be caused by the formation of a eutectic mixture of ACV and sodium bicarbonate. Eutectic mixtures tend to destroy intermolecular forces (reduce changes in enthalpy) or increase the disorder generated during melting (changes in entropy). As this leads to a reduction in melting temperature [36], transformation of the drug into an amorphous state can be easily achieved. However, this hypothesis should be further confirmed as it was not within the scope of the current study.

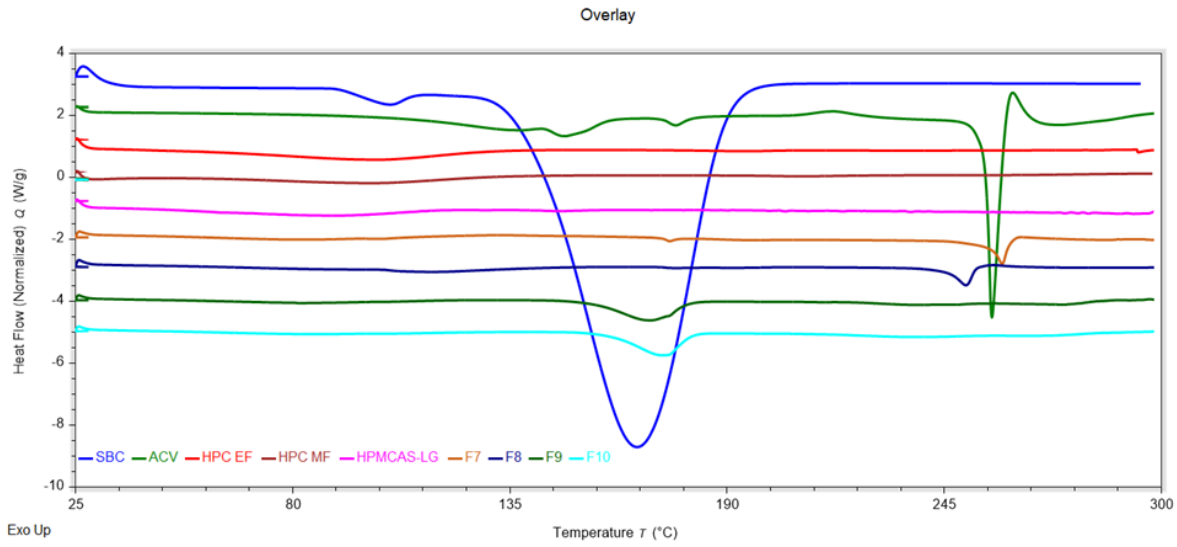

Figure 3. DSC thermograms of pure API, pure polymers, and acyclovir extrudates with P-CO_2 and addition of sodium bicarbonate.

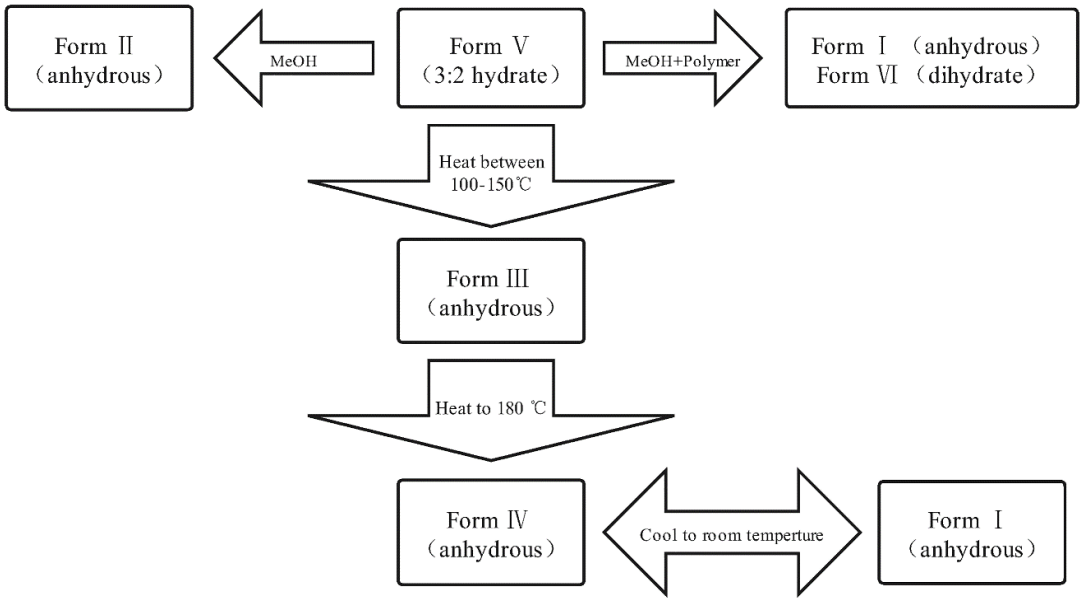

Figure 4. Transformations of acyclovir.

3.3 Drug content

UV-Visible spectrophotometric analysis confirmed that the drug content of ACV in all formulations is consistent with the theoretical value and it was within the range of 93.8–102.5%, which confirmed that the ACV was uniformly distributed in the extrudates. All results were listed in **Figure5.**

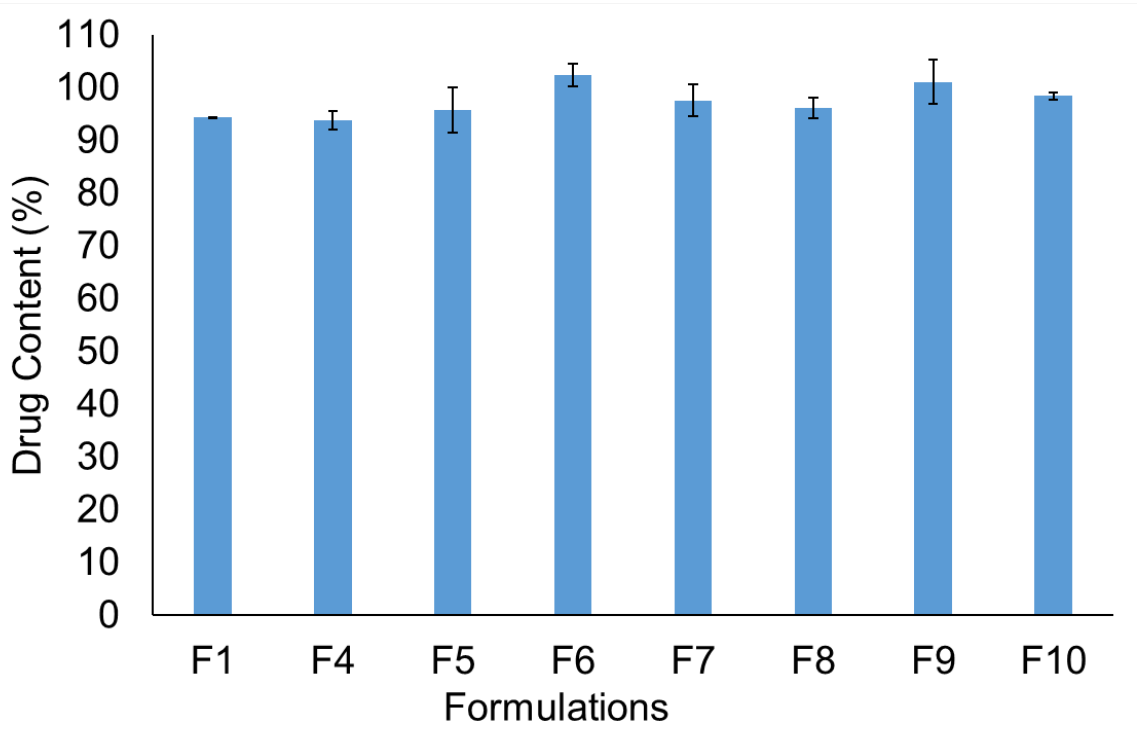

Figure 5. Drug content of each formulation

3.4 *In vitro* drug release

All formulations floated immediately and throughout the course of the dissolution study, when placed in the dissolution media, without any lag time, thereby demonstrating good buoyancy. In **Figure 6**, the formulations manufactured using the HPC EF polymer (F1, F4) exhibited faster drug release than those with HPC MF (F5), HPMCAS-LG (F6), and the combination of polymers. This is because HPC EF exhibited faster erosion than HPC MF and HPMCAS-LG. At 1 h, the drug release rate was 60.4±3.04% and 51.6±5.5%; however, at 4 h, the rate exceeded 90% in the F1 and F4 formulations, respectively. The ACV gastro-retentive formulations of HPC EF were successfully prepared using HME with P-CO_2 and the addition of sodium bicarbonate. However, the release rate was too fast; thus, high viscosity polymers were added as secondary polymers to achieve the sustained-release characteristics. The

dissolution profile of F5 and F6 demonstrated that HPC MF and HPMCAS-LG retarded drug release. HPC MF has a high viscosity, which contributed to high gel layer density, thereby retarding polymer dissolution [31]. HPMCAS-LG not only has a high melt viscosity [32], but is also insoluble in acidic aqueous solution. Therefore, formulation F6 showed drug release of 74% after the 12-h dissolution test. Some pellets were also evident in the dissolution medium after 12 h.

In the first hour, the dissolution rate of F7 and F8 was faster than that of F9 and F10 (**Figure 7**). This is because F7 and F8 have a higher porosity, which results in increased surface area contact with the solvent. The surface area of the substance accessible to the dissolution medium can affect the release rate [37].

The similarity factor (f_2) value of the F9 and F10 is 78.26. Both F9 and F10 gave comparable dissolution profiles. So, there is no significance different between F9 and F10. After 2 hours, F8 was found to exhibit a slower release than F7 as it had HPMCAS. However, in the first 2-hour, higher release rate was achieved with F8 than with F7, which may be due to the uneven application of pressure during the P-CO_2, resulting in higher porosity for F8 than F7.

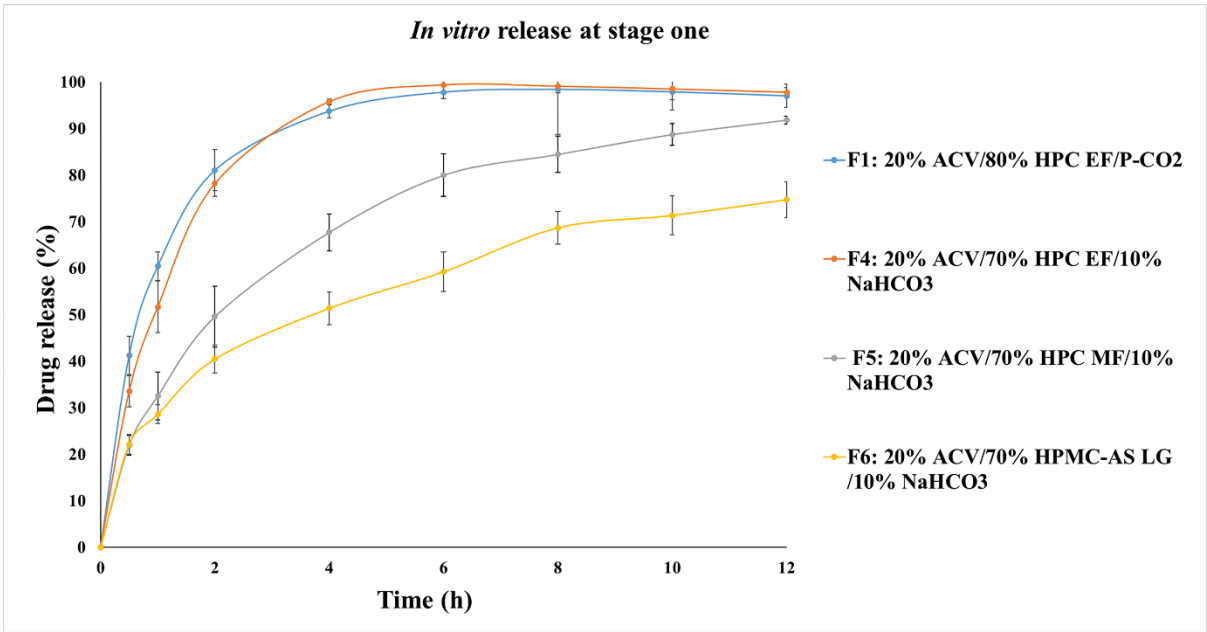

Figure 6. Dissolution profile of stage one formulations.

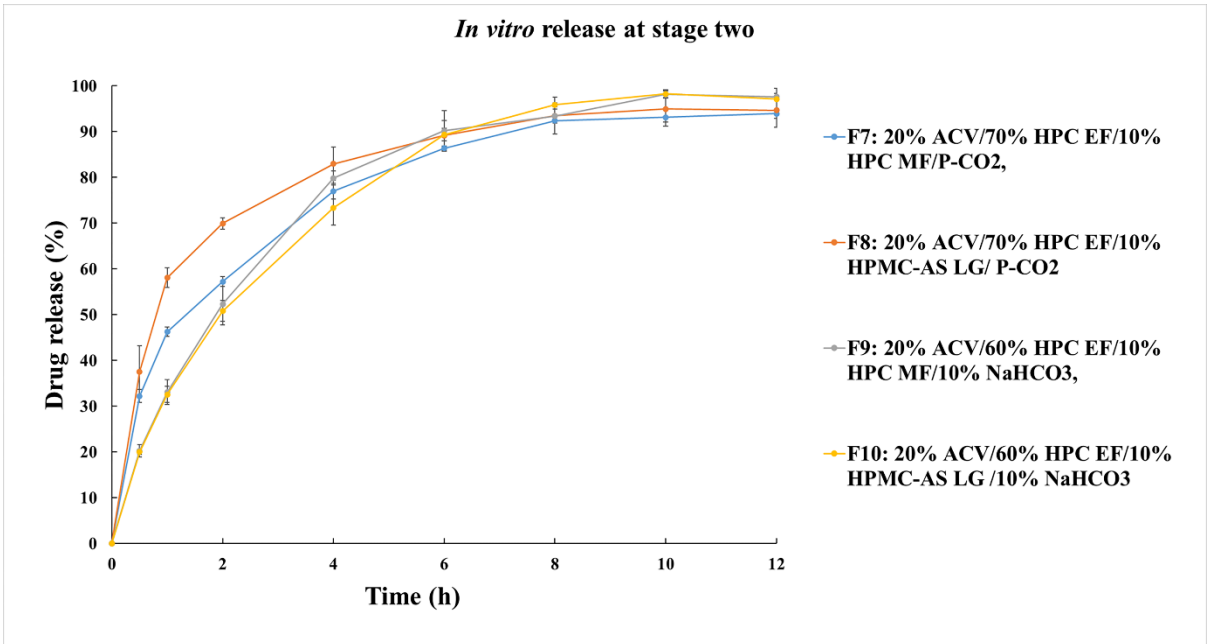

Figure 7. Dissolution profile of stage two formulations.

3.5 Drug release Kinetics:

Effects of various formulation parameters are presented in **Table 3.** The correlation coefficient (R^2) of each formulation was used as an indicator of the best fitting for each of the drug release models. The release mechanism for all formulations in this research could not be attributed to a zero-order mechanism according to the release kinetics data. Based on the correlation coefficient result, all formulations except F1 and F2, exhibited the best fit with the first-order and Higuchi models. If the correlation coefficient of the Higuchi model is high, the mechanism of drug release can be considered to be a diffusion-controlled release mechanism [27]. Hence, all formulations, except F1 and F2, had diffusion mechanisms. F9, with in combination with HPC EF, HPC MF, and SBC, had a similar correlation coefficient in the Higuchi model to F5 in combination with HPC MF and SBC. F10, in combination with HPC EF, HPMCAS-LG, and SBC, had a similar correlation coefficient in the Higuchi model to F10, in combination with HPMCAS-LG and SBC. This finding demonstrates that viscosity of HPC MF and HPMCAS-LG was responsible for controlling drug release. F4, in combination with HPC EF and SBC, could not form a strong hydrogel around the pellets, indicating that HPC EF has a lower viscosity. According to the dissolution profile and the dissolution kinetics, F9 and F10 were considered the optimized formulations.

Table 3. *In vitro* release Kinetics of Acyclovir Gastro-Retentive Formulations

Formulation code	Zero-order	First-order	Higuchi
	Correlation coefficient (R^2)		
F1	0.5648	0.7807	0.8024
F4	0.6065	0.7059	0.8315
F5	0.8128	0.979	0.956
F6	0.8484	0.9535	0.9804
F7	0.7396	0.9467	0.9267
F8	0.6447	0.9179	0.8625
F9	0.7983	0.9605	0.9472
F10	0.8278	0.9686	0.9614

3.6 Fourier transform infrared (FTIR) spectroscopy analysis

FTIR spectra of the API, polymer and formulations of stage two are presented in **figure 8**. All HPC EF, HPC MF and HPMCAS-LG spectra showed a significant absorbance peak at 1,046 cm^{-1}, which represented C-O functional groups. The infrared spectra of ACV showed a characteristic peak, for carbonyl, at 1650-1750 cm^{-1}; the C=N stretching band appears at 1631 cm^{-1} in the infrared spectra of ACV, the peak at 1150-1050 cm^{-1} indicated C-O-C; the peak at 3437 cm^{-1} represents the aromatic secondary stretching vibration [38]. All formulations showed evident peaks of the API and polymer in their formulations, which indicated that there was no obvious chemical change between the API and polymers under the studied processing conditions.

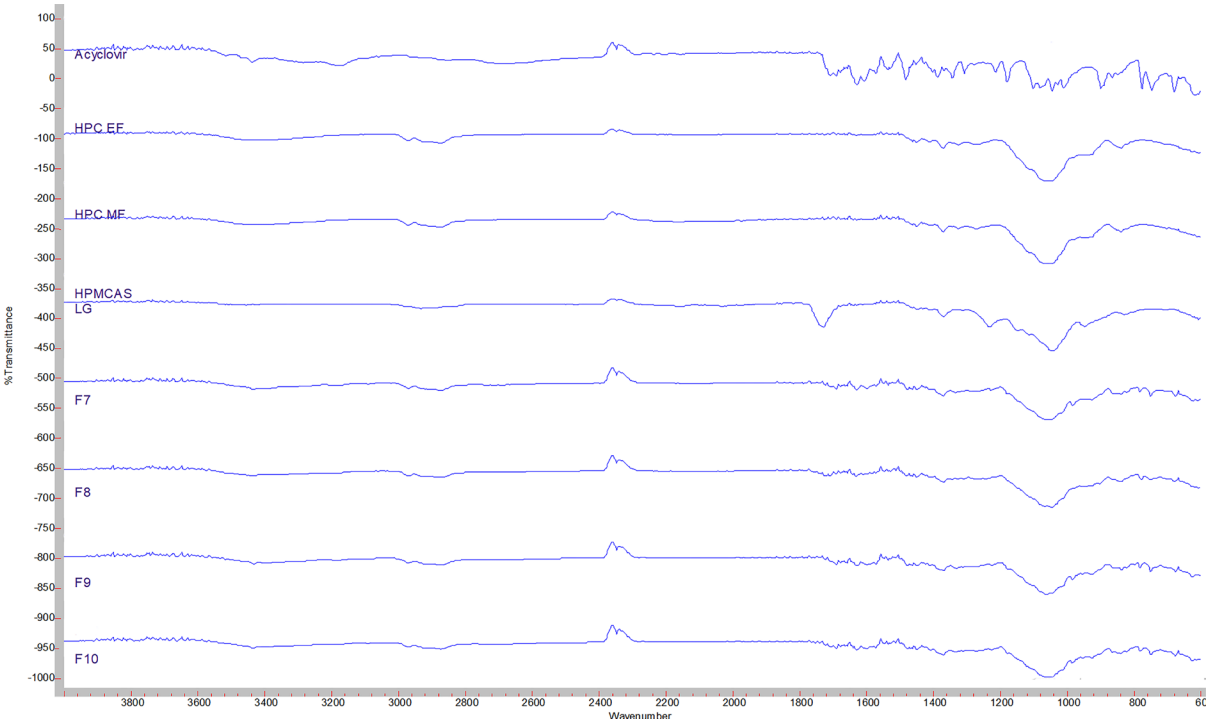

Figure 8. FTIR spectra of the formulations, pure $NaHCO_3$, and pure acyclovir

3.7 Scanning electron microscopy (SEM)

The SEM images of the stage two (F7, F8, F9, F10) processed extrudates are shown in **Figure 8.** The microscopic morphology of the extrudates with P-CO_2 or addition of sodium bicarbonate was transformed to the foam-like framework owing to the expanded CO_2, which increased their inner surface area and porosity [25]. Extrudates with CO_2 injection produced a regular, round, and well-distributed porous structure, while extrudates with addition of sodium bicarbonate did not display uniformity as CO_2 release may not be consistent during the extrusion process. Nonetheless, the extrudate with P-CO_2 (F7, F8) had a better foam structure than the extrudate with sodium bicarbonate (F9, F10). As a result, it can be inferred that P-CO_2 readily changes the morphology of the extrudate.

.

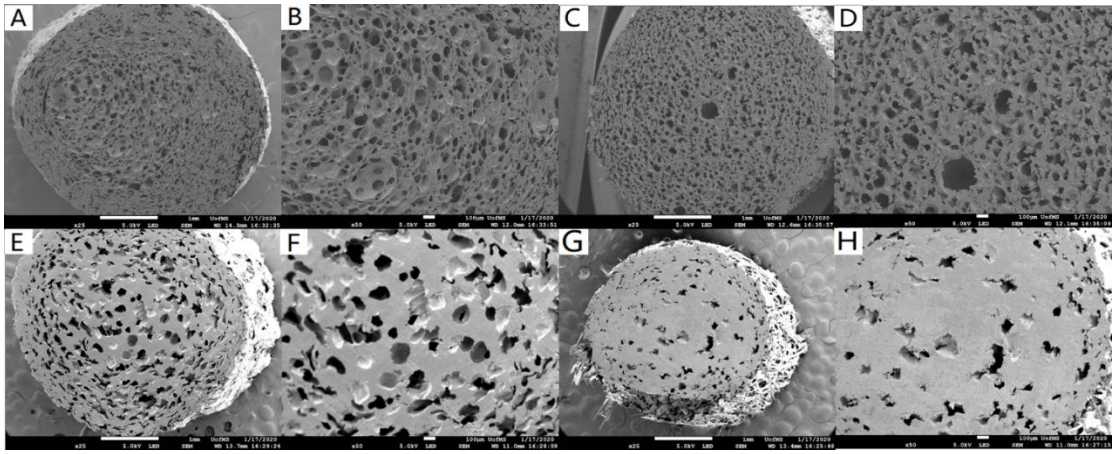

Figure 8. SEM images of acyclovir extrudates with P-CO_2 and the addition of sodium bicarbonate. A and B: F7; C and D: F8; E and F: F9; G and H: F10.

3.8 Stability tests

Figure 9 shows the drug content results after stability testing under the conditions of 40 °C and 75% relative humidity for three months (accelerated stability test conditions). The dissolution date showed in **Tablet 4.** The similarity factor (f_2) value of the dissolution study was 64.46 for F7, 73.61 for F8, 65.67 for F9, and 73.38 for F10, which are all greater than 50. Therefore, the stability test results indicated that the formulations showed no significant change relative to the fresh samples regarding drug content and the dissolution study. Such finding suggests that the developed formulations were stable over 3 months.

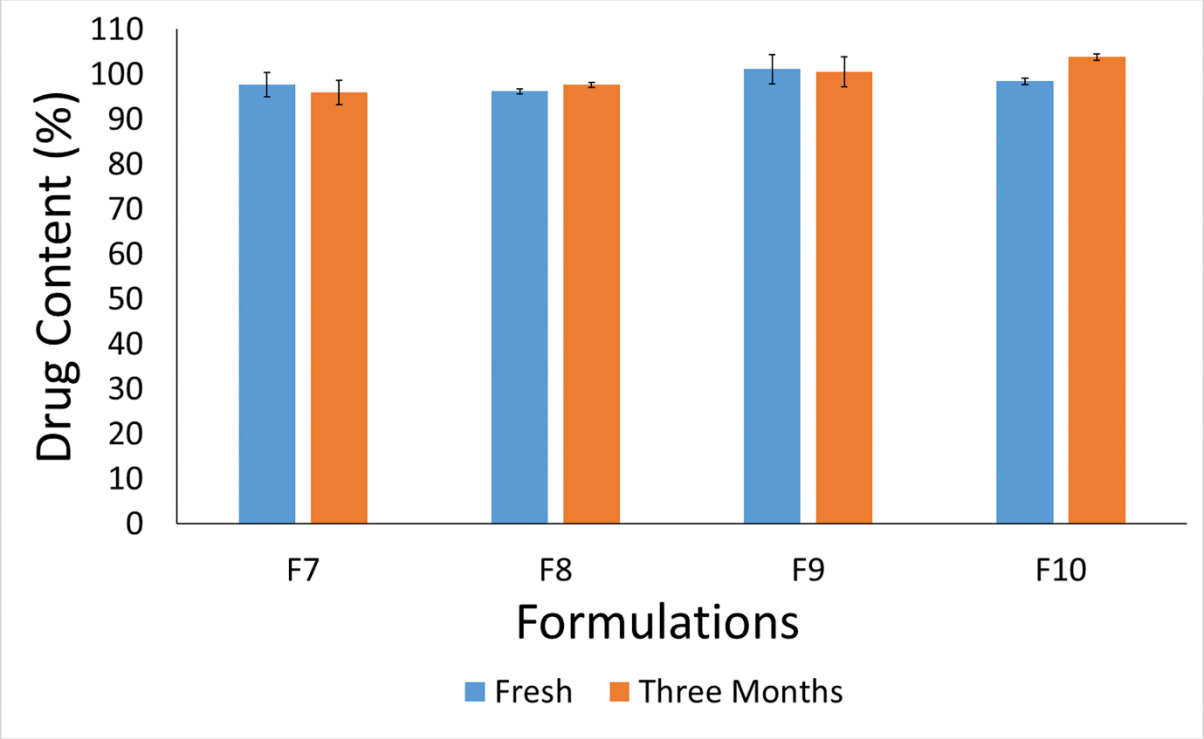

Figure 10. Drug content data of the initial ACV extrudates and the 3-month stability samples stored at 40 °C/75% RH.

Table 4. Dissolution data of stage two of the initial and the 3-monthc ACV extrudates

Drug release (%)	Formulations							
	F7		F8		F9		F10	
Time (h)	Fresh	3 months	Fresh	3 months	Fresh	3 months	Fresh	3 months
0	0	0	0	0	0	0	0	0
0.5	27.10±1.41	32.24±2.23	37.60±5.66	44.06±3.32	20.27±1.38	20.63±2.10	20.01±0.52	24.83±2.17
1	37.94±1.01	46.25±1.98	58.10±2.16	59.45±5.53	33.08±2.70	27.98±5.17	32.57±1.78	33.22±1.99
2	54.55±1.10	57.24±1.60	69.97±1.24	75.54±2.77	52.36±4.59	41.80±6.56	50.83±2.30	47.21±3.02
4	68.89±1.72	76.99±2.12	82.98±3.64	82.01±0.55	79.86±3.64	76.94±3.95	73.40±3.82	77.11±3.53
6	81.83±0.42	86.40±0.92	89.14±3.30	89.01±1.93	90.20±3.30	86.20±4.56	89.34±1.33	86.38±2.22
8	91.62±0.09	92.40±0.33	93.48±4.02	93.37±1.39	93.43±4.02	90.40±3.89	95.85±0.46	92.67±3.48
10	91.27±1.92	93.12±1.73	94.98±2.85	93.20±2.30	98.15±2.85	95.99±2.32	98.23±0.90	96.01±1.23
12	90.75±1.03	93.93±1.33	94.68±3.67	92.67±0.94	97.63±3.67	95.47±0.82	97.13±2.37	93.90±3.82

4. CONCLUSION

Based on the findings of this study, floating pellets can be used as a platform for manufacturing a viable gastro-retentive formulation. In stage one, HPC MF or HPMC-AS LG extrudates with sodium bicarbonate showed good buoyancy and extended release owing to their high molecular weight and high melt viscosity. ACV gastro-retentive formulations of HPC EF were successfully formulated using HME with P-CO_2 and the addition of sodium bicarbonate. In stage two, the ACV gastro-retentive formulations with a binary mixture of polymers were successfully produced using HME with P-CO_2 and the addition of sodium bicarbonate. The formulations showed extended release for 8 hours with over 90% release of the drug at the end of the dissolution study. Of all the formulations, F10 (20% ACV/60% HPC EF/10% HPMC-AS LG /10% $NaHCO_3$) was considered to be optimized formulation based on its dissolution profile and dissolution kinetics. Compared to the formulations containing sodium bicarbonate, the formulations with P-CO_2 had a faster release owing to the larger porosity. Compared to HPC EF, HPMCAS-LG showed better retardation of drug release. The morphology of the extrudates was changed to a foam-like structure after P-CO_2 injection and the addition of sodium bicarbonate. The P-CO_2 extrudates showed uniform pores relative to the sodium bicarbonate extrudates. This study demonstrated that the floating pellets could be optimized and systematically developed as an extended release dosage form by a continuous process using the HME technology.

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