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BIOAVAILABILITY ENHANCEMENT OF ACYCLOVIR USING HIGH-DENSITY GASTRO-RETENTIVE PELLETS

Nipun Gupta, Vineet Kumar Rai, Tanmay S. Markandeywar and Ghanshyam Das Gupta *

Department of Pharmaceutics, ISF College of Pharmacy, GT Road, Ghal Kalan, Moga - 142001, Punjab, India.

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Correspondence to Author:
Prof. Ghanshyam Das Gupta

Director-cum-Principal, Department of Pharmaceutics, ISF College of Pharmacy (An Autonomous College) GT Road, Ghal Kalan, Moga-142001, Punjab, India.

E-mail: drgdg@rediffmail.com

ABSTRACT: The objective of the present study was to improve the oral bioavailability using high-density gastro-retentive pellets containing solid dispersion of acyclovir. Solid dispersion prepared with polyvinylpyrrolidone (PVP K30) and hydroxypropyl methylcellulose (HPMC) using different ratios 1:1, 1:2, 1:3, 1:4, and 1:5 by solvent evaporation method and characterized for solubility study, dissolution study, FT-IR, DSC, and XRD. High-density gastro-retentive pellets containing solid dispersion of acyclovir was prepared by extrusion/spheronization technique using solid dispersion of acyclovir with PVP K30 (1:3), Barium sulphate as high-density material, microcrystalline cellulose (MCC) as extrusion aid, ethylcellulose (EC) as release retarding polymer and HPMC as swellable gel-forming polymer. The formulation was optimized based on *in-vitro* release profile and characterized for pellets morphology, micro-meritics properties, FT-IR, DSC, XRD, and *in-vivo* study. The optimized formulation F8f showed the release for 12 h with an increase in retention at the absorption site. Release kinetic studies of optimized formulation showed that the data best fit in Higuchi's model. The *in-vivo* studies like pharmacokinetic parameters and X-ray transmission were investigated in Wistar rats. The pharmacokinetic study shows that the bioavailability of high-density gastroretentive pellets containing solid dispersion of acyclovir can be increased as compared to a plain drug, marketed formulation, and solid dispersion. The X-ray analysis further ensures that the optimized formulation is retained at the bottom of the stomach, which is essential to improve the absorption window of acyclovir.

INTRODUCTION: Acyclovir is an antiviral drug used for the management and cure of herpesviruses [HSV-I] & [HSV-II] and varicella-zoster virus. The mechanisms of its antiviral activity include inhibition of viral DNA polymerase, DNA polymerase inhibition, adding into, and abortion of the developing viral DNA chain [1].

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Acyclovir has a half-life of 3 h, and its low oral bioavailability is between 10-30%, and it decreases when the dose increased. Acyclovir slowly consumed from the gastrointestinal tract (GIT) when administered orally. The plasma concentration attains its therapeutic level in 1.5 - 2 h[2].

Solubility is the main problem for certain drugs to formulate a good formulation of the drug for oral administration [3]. Solid dispersion (SD) is a successful strategy to improve the solubility of the drug by changing the drug from crystallite to amorphous form. In this study, solid dispersion can be made through the solvent evaporation method [4, 5].

To prepare delayed and controlled release oral preparations, pellets are being highly investigated. Pellets offer a variety of benefits and exhibit maximum drug absorption and decreased peak plasma fluctuation as they get uniformly distributed in the GIT. Pellets are capable of dose dumping and different types of modified release profiles (pulsed, sustained, or delayed) can be attained by a selection of best polymers and method of preparation [6, 7].

In the present investigation, an effort has been made to design high-density gastro-retentive (GR) pellets to improve retention time of formulation in the upper regions of GIT. Reduced absorption in the upper regions of the GIT is the reason behind the less oral bioavailability from traditional preparations.

Considering its favorable solubility in acidic pH and absorption window in upper regions of the GIT, acyclovir is a good candidate for the GR drug delivery system; as the gastric residence time of GR preparation is extended, little quantity of the drug is continuously released in the absorbable parts of the GIT and thus enhance its bioavailability [8, 9].

To prepare, optimize and characterize high-density gastro-retentive pellets to prolong the release of the drug, and more efficacious delivery of acyclovir, improve patient compliance, fewer side effects, less dosing frequency, and reduce the development of resistance. All the formulations were evaluated for *in-vitro* drug release. Optimized the best formulation and process parameters like micro-meristics properties, DSC, XRD, FT-IR, and in-vivo drug release were also studied.

MATERIALS AND METHODS:

Materials: Acyclovir was purchased from Sigma Aldrich Labs (India), Eudragit RSPO was procured as a gift sample from Evonik Pharma (Mumbai), Polyvinylpyrrolidone K30 and Ethylcellulose were supplied from Central Drug House (New Delhi), Hydroxypropyl methylcellulose was supplied from Sigma Aldrich Labs (India), Microcrystalline cellulose was supplied from DFE Pharma (Cuddalore), Barium sulphate was supplied from LobaChemie (Mumbai), Dimethylsulphoxide was supplied from SDFCL (Mumbai) and all other chemicals used were of analytical grade.

Methods:

Preparation of Solid Dispersion (SD): Solid dispersions were prepared with the help of the solvent evaporation method. The carrier polyvinyl-pyrrolidone (PVP K30) and hydroxypropyl methyl-cellulose (HPMC) were added in the weighed amounts of acyclovir and were mixed properly. This mixture was solubilized in DMSO. A hot air oven at 60 ± 10 °C was maintained to evaporate the solvent. The evaporation was performed until the constant weight was there. Then, solid dispersion formulation was ground with the help of mortar and pestle. The ground powder was sieved and used for study [10].

Optimization of SD: Various process parameters were systematically investigated to determine their effects on the formulation. Based on the solubility profile, the best formulation is obtained. The process parameters included the concentration of drug, carrier, and solvent (DMSO). The different formulations of acyclovir with PVP K30 and HPMC in the ratio of 1:1, 1:2, 1:3, 1:4, and 1:5 (SD1-SD10) respectively were made and optimized. The solubility profile of acyclovir has been found and optimized for the best formulation having the highest solubility than the plain drug.

Characterization of Optimized SD: Optimized SD was characterized for different parameters like solubility profile, percentage yield, DSC, FTIR, XRD, and *in-vitro* drug release [10, 11].

Solubility Study: Solubility studies of different formulations (SD1-SD10) were done in water. An excessive quantity of acyclovir-carrier solid dispersion complex SD1-SD10 was poured into 5 mL of water in separate screw-capped glass vials. All solutions were then shaken on mechanical shaking on a Thermo shaker incubator (LSB-1005RE, Daihan, Korea) operated at 100 rpm and 37 ± 5 °C for 24 h to attain equilibrium.

Each solution was then subjected to centrifugation at 6000 rpm for around 10 min. The supernatant was collected by pipette and filtered with the help of a 0.45 μm syringe filter. 1 mL of filtered solution was further diluted by distilled water and marked a dilution number accordingly. Each dilution was analyzed at 255.42 nm using a UV-Visible spectrophotometer (Shimadzu, Japan).

Percent Yield: Percent yield was calculated to check the efficiency of the method. The percentage yield was performed with the help of the formula.

% Yield = Mass of solid dispersion / Mass of drug + Mass of lipid substances × 100

Dissolution Study of Solid Dispersion: *In-vitro* release studies of acyclovir loaded solid dispersion were performed by using USP I (rotating paddle) dissolution apparatus (Lab India, Mumbai) in 900 mL dissolution medium at 50 rpm at 37 °C, and proper sink conditions were maintained. 0.1N HCl (pH 1.2) was used as a dissolution medium.

Five mL of samples were taken from the middle of the basket with the help of the pipette at preset time intervals and exchanged with fresh dissolution media each time. They were filtered with the help of a syringe filter (5 μm pore), analyzed on UV-Visible spectrophotometer (Shimadzu, Japan) at 255.42 nm wavelength. All measurements were done in triplicate. The graph was made between cumulative percentage drug release versus time.

Preparation of Pellets Containing Solid Dispersion: The whole activity of pelletization was performed by the extrusion-spheronization process. A fixed percentage of SD was added to dry ingredients. These ingredients include micro-crystalline cellulose (Avicel PH101) as a bulking agent, HPMC asa swelling agent, ethylcellulose used as hydrophobic and coating polymer and barium sulphate as high-density material.

Acyclovir loaded SD and other ingredients were shifted to a mortar and a measured amount of deionized water was added whilst the mixture was mixed with the help of a pestle so that a wet mass of required plasticity for extrusion is prepared. Finally, the wet mass was extruded at about 40 rpm in an axial extruder having a die of 0.5 mm diameter circular holes.

The extrudate was spheronized for 2 min, 4 min, and 4 min at 400, 1000 and 1500 rpm on a 120 mm radial plate spheronizer (Cronimach, Gujarat) with the help of a cross-hatch frictional plate of 3 mm × 3 mm pitch and 1.2 mm depth. The prepared pellets were then dried at 37 ± 2 °C for 5 min. in an air-heated oven to constant weight. Dried batches were labeled and stored in an airtight container [12].

Preparation of Coating Solution: Eudragit RSPO and ethylcellulose were used for the coating of pellets containing solid dispersion. The coating polymers were accurately weighed and make different concentrations solution *i.e.* 5%, 7.5% and 10% (w/v). The coating polymer Eudragit RSPO and ethylcellulose were dissolved in isopropyl alcohol (IPA). The solution was homogenized until it became clear. The best formulation was coating with different concentrations of the polymer solution and optimized.

Method for Coating Pellets: Firstly, rinse the pipe of the peristaltic pump with an organic solvent. The solution pipe was filled with a coating solution and ensured that there were no air bubbles and the pipe was attached with a spray gun of accelacota. The door was closed of coating pan and set all the process parameters like; flow rate, temperature, drum speed, and air pressure. Pellets containing solid dispersion were firstly heated and then the preheated pellets were subjected to coating.

Optimization of Pellets:

Optimization of Different Materials Loaded in Pellets: To optimize the concentration of Avicel PH101, HPMC, ethylcellulose, barium sulphate, and solvent *i.e.* deionized water, nine formulations of batch size 10 g, were prepared. Formulations were made with different concentrations of MCC, HPMC, ethylcellulose, barium sulphate, and quantity of water to formulate the optimized SD pellets. These concentrations are optimized on account of the size and shape of the pellets. Then Acyclovir loaded SD pellets were prepared.

Optimization of Process Variables: Optimization of Extruder speed at 30, 35, and 40 rpm along with the optimization of Spheronization speed and time was carried out at 400, 1000, and 1500 rpm and 2, 4, and 4 min respectively, and optimization was done based on shape or the physical appearance of the pellets.

Optimization of Coated Pellets: The coating was done with Eudragit RSPO and ethylcellulose to the pellets containing solid dispersion of acyclovir. Different concentrations (5%, 7.5%, and 10% w/v) of coating solution with Eudragit RSPO and ethylcellulose were made with a mixture of organic solvent (Isopropyl Alcohol), and then it was stirred

until a uniform mixture was formed. The coating of the pellets was done with the help of an accelacota auto-coater and optimized. The formulation was optimized after the coating of the pellets based on the release pattern of the drug. Start the flow of the coating solution at an optimized flow rate. The coating was done in intervals like; spray of atomized coating solution on pellets for 2 min. then drying in hot air for 1 min. likewise, repeat this 3 times. **Table 1** shows the optimization of coating parameters.

TABLE 1: OPTIMIZATION OF COATING PARAMETERS

Formulation code	Concentration (w/v)	Flow rate (mL/min)	Drum speed (rpm)	Temperature (°C)	Air pressure (kg/cm²)
F8a	5% EudragitRSPO	1	25	50 ± 10	2
F8b	7.5% EudragitRSPO	1	25	50 ±10	3
F8c	10% EudragitRSPO	1	25	50 ± 10	3
F8d	5% Ethyl cellulose	1	25	50 ± 10	2
F8e	7.5% Ethyl cellulose	1	25	50 ± 10	5
F8f	10% Ethyl cellulose	1	25	50 ± 10	5

Characterization of Uncoated Pellets and Coated Pellets: Optimized coated pellets and uncoated pellets are evaluated for various parameters pellets morphology, micro-meritics properties, dissolution study, FT-IR, DSC, XRD, and *in-vivo* study.

Dissolution Study of Pellets Containing Solid Dispersion: *In-vitro* release studies of pellets (uncoated and coated) were performed by using USP I (rotating paddle) dissolution apparatus (Lab India, Mumbai) in 900 mL dissolution medium at 50 rpm at 37 °C and proper sink conditions were maintained. 0.1N HCl (pH 1.2) was used as a dissolution medium. Five mL of samples were taken with the help of a pipette at preset time intervals and changed with fresh dissolution media each time. Samples were filtered with the help of a syringe filter (5 µm pore), diluted, and analyzed on a UV-Visible spectrophotometer (Shimadzu, Japan) at 255.42 nm wavelength. All measurements were done in triplicate. The graph was plotted between cumulative percentage drug release and time.

Pellet Size and Sphericity: The size, shape, and area of pellets were done by an optical microscope. Pellet size and shape were measured using an optical DMW2-223 digital microscope (Motic Instruments) equipped with a 1/3'' CCD camera imaging accessory and computer-controlled image analysis software (Motic Images 2000, 1.3 version) at a 10Xobjective lens.

20 pellets were analyzed by microscopic technique, and a variety of parameters like the size of pellets, aspect ratio, roundness, and circularity factor have been used to assess the shape of the pellets. The pellet size was within the 500-1500 µm size range.

Micromeritics Properties of Pellets:

Friability: The Friability test was done on 10 g of the pellets combined with 25 glass beads (3 mm diameter) using a Roche friabilator (Swastika Electric and Scientific works, Ambala Cantt.). The drum was rotated for 4 min at a speed of 25 rpm. The pellets were placed on the sieve with 0.85 mm aperture [13]. The smaller particles were allowed to pass. Percentage friability is calculated using the following formula:

% Friability = Initial weight – Final eight / Initial eight × 100

Angle of Repose: It is used to determine the flow properties of powders, pellets, or granules. It was determined by the funnel method. The angle of repose was checked by pouring the pellets on a conical heap on a level, flat surface and measures the included angle with the horizontal. The angle of repose was calculated by using the following formula:

$$\text{Angle of repose } (\theta) = \text{Tan}^{-1}[h/r]$$

Bulk Density: Pellets were accurately weighed 10gm and were gently poured through a glass funnel into a calibrated 100 mL measuring cylinder. The surface was made smooth carefully with the application of pressure. The volume taken by the sample was noted, and bulk density (g/mL) was calculated and recorded by using the formula given below.

Bulk density = Weight of sample / Bulk volume

Tapped Density: Similar to the bulk density, tapped density was observed by tapping the cylinder 100 times from 3-inch height by using the Bulk density apparatus after pouring the pellets into the measuring cylinder, and the tapped volume was recorded. Finally, the tapped density was recorded by using the formula.

Tapped density = weight of sample / Tapped volume

Hausner's Ratio: The Hausner's ratio is a number that is correlated to the flowability of pellets. The following formula is used to calculate it:

Hausner's ratio = Tapped density / Bulk density

Carr's Index: Carr's index is related to the compressibility of pellets. It was calculated by the following formula:

Carr's index = Bulk volume – Tapped volume / Bulk volume

Fourier Transform Infrared (FT-IR) Spectroscopy: The infrared spectrum of the drug gives information about the groups present in that particular compound. FT-IR spectra were obtained using an FT-IR functional spectrophotometer (Thermo Nicolet-380, USA). FT-IR spectra of acyclovir, PVP K30, physical mixture, solid dispersion, and pellets were recorded to check compatibility, complex formation or any sort of interaction by using FT-IR spectrophotometer (Nicolet omnic software). Samples were placed on a crystal spot (zinc selenide) and the arm was rotated downwards to generate a fine disc of the sample. The scanning range was kept within the range of 4000 to 400 cm^{-1} at a spectral resolution.

Differential Scanning Calorimetry (DSC): It is a technique in which the degree of crystallinity and polymorphic transitions causing energy change is studied. The phase transition of drug, solid dispersion, and pellets was analyzed by differential scanning calorimetry (Netzsch DSC 200F, Maia) in a perforated aluminum sealed pan at a heating rate of 10 ºC/min from 30 -300 ºC using nitrogen as blanket gas.

X-ray Diffraction Analysis (XRD): XRD was carried out to check the impact of processes on the crystallinity behavior of the acyclovir, solid dispersion and pellets. XRD (Bruker D8 Advance, Germany) patterns using XRD Xpert pro with software pan analytical. The voltage applied was 45 kV with a current of 40 mA to monochromatic nickel-filtered copper radiation, and scanning was performed at 2θ range. The sample was triturated before taking each scan.

***In-vivo* studies:**

Bioanalytical Method Development by HPLC For Pharmacokinetic Studies: For plasma studies, 0.3 mL of blood from a rat's eye was collected. Centrifugation was done for around 30 min at 5000 rpm at a fixed temperature of 4 °C. 0.1 mL plasma was taken from the supernatant. Then 0.8 mL acetonitrile was added. 0.1 mL of various concentrations of the drug's solution was added to make 0.1-10 µg/mL concentrations. The sample was brief vortexed for around 5 min, and then centrifugation was done for 10 min at 3000 rpm, and then the supernatant was collected. 1 mL of supernatant was taken out from each was diluted with buffer up to 2 mL. The sample was analyzed at HPLC. The analysis was done using the phosphate buffer and acetonitrile as a mobile phase. Phosphate buffer was made by taking the 13.6 gm of potassium dihydrogen phosphate in 1000 mL water, and pH was adjusted to 2.5 with ortho-phosphoric acid. Phosphate buffer and acetonitrile were used in the ratio of 95:5 (v/v). C18 column (250 × 4.6 mm) was used, and the injection volume was 20 µl which was run at the flow rate of 1 mL/min and analyzed at λ_{max} 254 nm. The first dilution was made in acetonitrile, and the other dilution was made in the mobile phase. Peaks were detected in approximately 30 min.

Experimental Animals: Wistar rats (180-220 gm) were used as an animal model for the present study. All animal experiments were done as per the approved Reg no. ISFCP/IAEC/CPCSEA/Meeting No. 22/2018/ Protocol No.363 by Institutional Animal Ethical Committee (IAEC) formed as per CPCSEA. Wistar rats (180-220 gm) of either sex were procured from the animal house of ISF College of Pharmacy, Moga for pharmacokinetics study of plain Acyclovir, marketed formulation, solid dispersion, and pellets (coated pellets) were administered orally. Wistar rats fasted for 24 h before the experiment, and food was given after 4h post-dosing. The rats were divided into a group of four with six animals each, and each group can be subdivided into three subgroups with two animals

each. Each group was orally administered optimized batch F8f, solid dispersion, marketed formulation and plain acyclovir, an equivalent dose of 40 mg/kg of saline body weight as acyclovir.

Blood samples were taken from the retro-orbital venous plexus of rats at a preset time (t = 1, 2, 4, 6, 8, 12 h). The blood sample was centrifuged for 10 min to separate plasma at 4000 rpm (R-24C, Remi, India) and stored at −20 °C until further analysis.

X-ray Transmission Radiography to Determine the Retention Potential of the Formulation: The behavior of optimized pellets formulation in the rat was done with the help of radiographic imaging techniques. The animals were fasted overnight before the experiment, with free access to water. It includes the utilization of radio-opaque markers like barium sulphate, added in the formulation to check the position of the pellets.

The quantity of the barium sulphate was optimized for no effect on the physical characteristics of the optimized pellets formulation. X-ray images of the stomach of the treated rats were taken at various time points, namely just after administration 2, 4 and 8 h to trace the *in-vivo* movement and behavior of the high-density gastro-retentive pellets in the GIT. X-ray images of the rats in prone positions were captured using L & T Vision 100 (C-arm) X-ray machine, at 64 mAs and 63kV techniques.

RESULTS AND DISCUSSION:

Preparation of Solid Dispersion: The solvent evaporation method was used for the preparation. Accurately weighed the amount of different ratio 1:1, 1:2, 1:3, 1:4, and 1:5 of the drug: carrier polyvinylpyrrolidone (PVP K30) and HPMC and mix them properly then adding the solvent dimethylsulphoxide (DMSO). The drug and carrier can be dissolved to make a dispersion system and the different ratios of solid dispersion (SD1-SD10) were prepared and optimized for the best formulation based on solubility.

Optimization of Solid Dispersion: Table 2 shows that the optimization of different solid dispersion formulations according to its solubility profile and also find the percent yield.

Characterization of Solid Dispersion:

Solubility Study: The different formulations were prepared and optimized. SD3 formulation shows a better solubility than other formulations. The solubility of SD3 was 9.74 ± 1.32 that shows the rise in solubility 7 folds than the drug. The optimization was based on solubility. **Table 2** shows the solubility profile of solid dispersion formulations (SD1-SD10).

Percent Yield: The percentage yield of different formulations (SD1-SD10) can be determined. SD1, SD2 and SD3 show the highest percentage yield than other formulations.

The % age yield of SD formulation was within the range of 88.72%. -84.46%. SD3 formulation was optimized based on solubility, and its % yield was 88.19% which was near to formulation (SD1), having the highest % yield, *i.e.*, 88.72%. **Table 2** shows the % yield of Solid dispersion formulations.

TABLE 2: OPTIMIZATION RATIO OF SOLID DISPERSION FORMULATIONS

Formulation code	Carrier	Ratio	Acyclovir (mg)	Carrier (mg)	Solubility (mg/ml)	%age Yield
SD1		1:1	100	100	5.20 ±1.12	88.72
SD2		1:2	100	200	7.22 ± 1.09	87.95
SD3	PVP K30	1:3	100	300	9.74 ± 1.32	88.19
SD4		1:4	100	400	6.60 ± 1.25	85.86
SD5		1:5	100	500	5.86 ± 1.27	84.57
SD6		1:1	100	100	5.44 ± 1.23	86.40
SD7		1:2	100	200	6.09 ± 1.11	87.22
SD8	HPMC	1:3	100	300	9.06 ± 1.03	86.80
SD9		1:4	100	400	6.27 ± 1.31	84.79
SD10		1:5	100	500	4.53 ± 1.20	84.46

(Data presented as mean ±SD, n=3)

***In-vitro* Drug Release:** It was performed of the optimized solid dispersion (SD3) by using USP I (rotating paddle) dissolution apparatus (Lab India, Mumbai) in 900 mL dissolution medium at 50 rpm at 37 °C, and proper sink conditions were maintained. 0.1N HCl (pH 1.2) was used as a

dissolution medium. Samples were filtered, diluted, and analyzed on a UV-Visible spectrophotometer at 255.42 nm. All measurements were done triplicate. The graph was plotted between cumulative percentage drug release and time. The % drug release of optimized formulation SD3 was found to be (97.90%) in 6 h than the plain drug (95.5%) in 4 h. **Fig. 1** shows the % cumulative release of plain drug compares with solid dispersion.

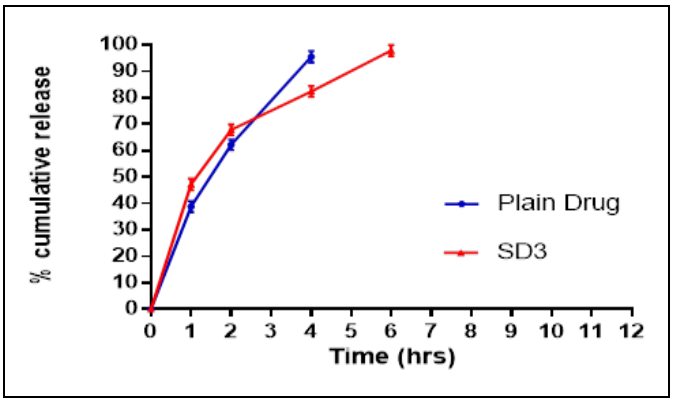

FIG. 1: CUMULATIVE PERCENT RELEASE OF PLAIN DRUG AND SOLID DISPERSION

Preparation of High-density Gastro-retentive Pellets: Pellets can be made by extrusion/spheronization process. For pellets preparation, all the ingredients were weighed and mixed thoroughly. High-density gastro-retentive pellets were made at eight various concentrations of MCC, barium sulphate, hydroxypropyl methylcellulose, and ethyl cellulose. Extrusion/spheronization starts with the extrusion process in which the wet/dough mass is placed into the extruder, where it is continuously changed into cylindrical rods of uniform size and shape. Uniform dispersion and quantity of granulating fluid plays a vital role in the preparation of wet mass as optimum plasticity and cohesiveness directly affect the final production of pellets. After the preparation of extrudates, in the spheronizer, they are rotated or spheronized at higher speed as the friction plate breaks the rod particles into little particles and rounded them to finally form spheres. The pellets containing solid dispersion were characterized based on the release profile then characterized other parameters like pellets shape and size, micromeritics properties, density, and thermal analysis. **Fig. 2** shows the in-process microscopic images of pellets at different time intervals.

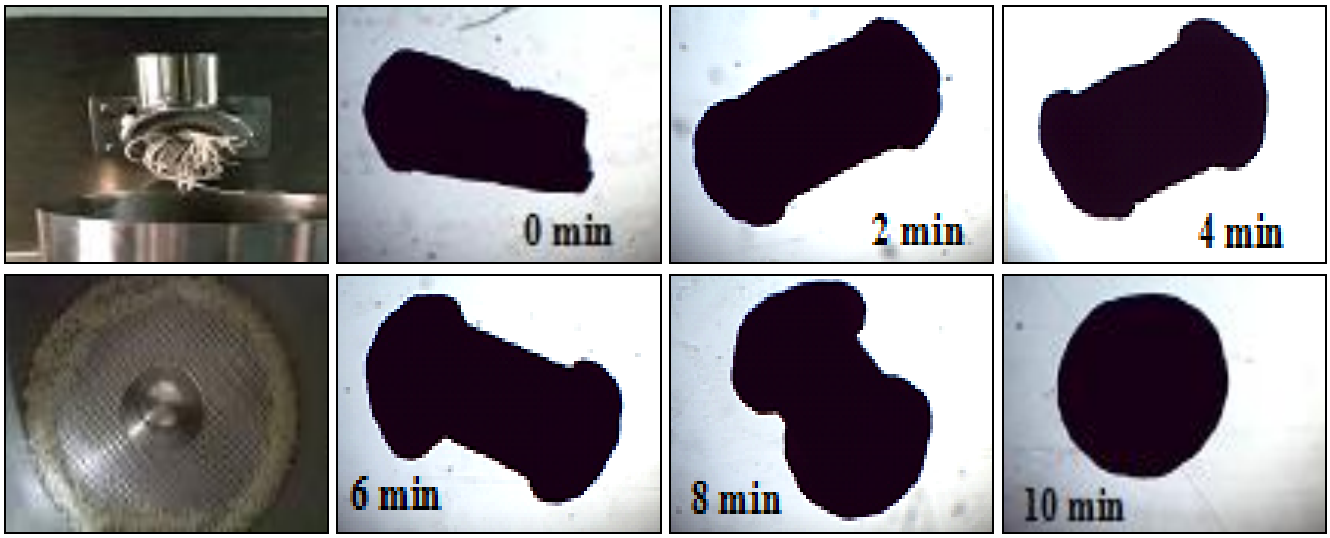

FIG. 2: IN-PROCESS IMAGES OF PELLETS AT DIFFERENT TIME INTERVALS

Optimization of Pellets and Process Variables: The optimization of pellets was done based on the release profile and sphericity of the pellets. In F1 and F2, formulation produces dumbbell shape, F3, F6, F7 and F8 formulation produces spherical pellets while F4 and F5 produce elongated spheroids depending on the amount of excipients utilized and amount of granulating fluid added. The amount of excipients utilized and extrusion / spheronization speed and time are shown in **Table 3** and **Table 4,** respectively.

Preparation of Coating Solution: Eudragit RSPO (pH-dependent) and ethyl cellulose (hydrophilic matrix polymer) were selected based on sustained-release characteristics. Eudragit RSPO provides less pores and channels for effective drug diffusion resulting in low drug release.

The polymers for coating solution (Eudragit RSPO and ethylcellulose) were accurately weighed and dissolve in an organic solvent and make six different concentrations *i.e.*, 5%, 7.5%, and 10% (w/v) to optimize the formulations (F8a-F8f) on behalf of release profile.

TABLE 3: OPTIMIZATION OF DIFFERENT MATERIAL USED IN THE FORMULATION OF PELLETS

Formulation Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Solid dispersion (mg)	1500	1500	1500	1500	1500	1500	1500	1500
Microcrystalline cellulose (mg)	3350	3300	3125	2950	2775	2600	2425	2250
Barium sulphate (mg)	150	200	200	200	200	200	200	200
Ethylcellulose (mg)	-	-	175	350	525	525	525	525
Hydroxypropyl methyl cellulose (mg)	-	-	-	-	-	175	350	525
Distilled water (ml)	4	4	4.5	4.5	4.5	5	5	5

TABLE 4: OPTIMIZATION OF PROCESS VARIABLES

Formulation Code	Extrusion Speed (rpm)	Spheronizing Speed (rpm)	Sheronizing Time (min.)		Shape
F1	30	400	2		Dumbbell shape
		1000	4	4	
F2	30	400	2		Dumbbell shape
		1000	4	4	
F3	30	400	2		Spheroids
		1000	4	4	
F4	35	400	2		Elongated spheroids
		1000	4	4	
F5	35	400	2		Elongated spheroids
		1000	4	4	
F6	40	1000	2		Spheroids
		1500	4	4	
F7	40	1000	2		Spheroids
		1500	4	4	
F8	40	1000	2		Spheroids
		1500	4	4	

Characterization of Pellets:

***In-vitro* Drug Release:** *In-vitro* release studies of formulations, F1 and F2 prepared with varying concentrations of barium sulphate and MCC, in which the release rate of F1 was found to be 98.90 ± 1.91, at the 6^{th} h and for 97.40 ± 2.12 F2 at the end of 6^{th} h. The drug release rate was found to be faster when increasing the concentration of MCC. *In-vitro* release studies of formulations F3, F4 and F5 prepared with ethylcellulose concentrations of 5%, 10% & 15% respectively, in which the release rate of F3 was found to be 96.70 ± 1.98, at the 6^{th} h and for 95.20 ± 2.05 F4 at the end of 6^{th} h and 94.70 ± 2.14 for F5 at the end of 6^{th} h. The release rate was found to be retarded increasing in the concentration of polymer (ethylcellulose). *In-vitro* release studies of formulations F6, F7 and F8 prepared with ethyl cellulose concentration of 15% and with varying HPMC concentrations of 5%, 10% & 15% respectively, in which the release rate of F6 was found to be 96.8 ± 1.98, at the 8^{th} h and for 95.10 ± 1.91 F7 at the end of 8^{th} h and 90.80 ± 2.12 for F8 at the end of 8^{th} h. The release rate was found to be sustained due to an increase in the concentration of ethyl cellulose and HPMC in the case of formulation F8. The results are shown in **Fig. 3**A. But there was not a sufficient sustained release, so it was further coated with a variable amount of Eudragit RSPO and ethyl cellulose. Release rate decrease with the coating. *In-vitro* release studies of formulations F8a, F8b, F8c, F8d, F8e and F8f was found to be 98.75 ± 1.96, 95.64 ± 2.12, 93.4 ± 1.96, 95.5 ± 2.14, 91.6 ± 1.98 and 84.7± 2.09 respectively at the end of 12 h. From the above evaluation parameters, it was concluded that the formulation F8f was having a high percentage of drug release in a controlled manner, so the formulation F8f was selected as the optimized formulation. Hence, the formulation F8f was selected for further study.

The release rate decreases due to an increase in the diffusion pathway. The results are shown in **Fig. 3B,** and **Fig. C** shows the drug release comparison of the uncoated and coated formulation, respectively. **Fig 3D** shows the comparison of release pattern of the plain drug, solid dispersion, uncoated formulation (F8) coated formulation (F8f) in which the coated pellets show the highest release more than 12 h than uncoated, solid dispersion, and plain drug. The F8f formulation follows the Higuchi model shows that the release is in a controlled manner. **Fig. 4** shows the *in-vitro* kinetic release of F8f.

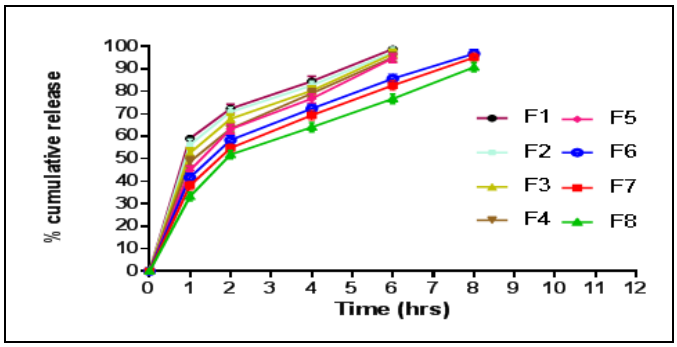

FIG. 3A: CUMULATIVE PERCENT DRUG RELEASE OF UNCOATED

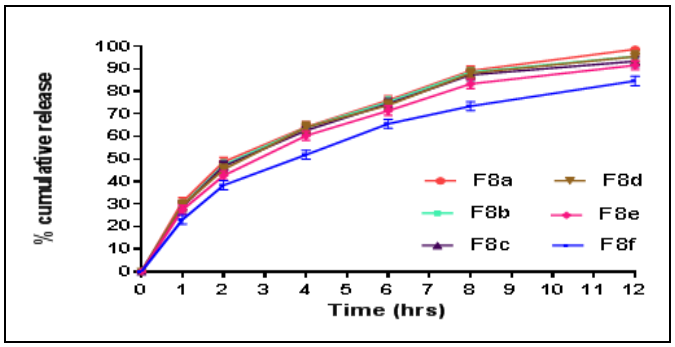

FIG. 3B: CUMULATIVE PERCENT DRUG RELEASE OF COATED

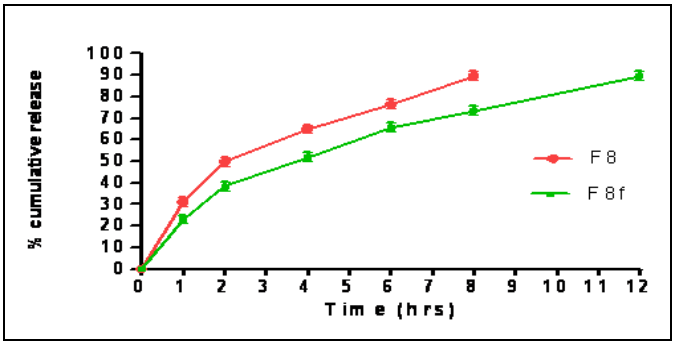

FIG. 3C: COMPARISON BETWEEN DRUG RELEASE OF UNCOATED AND COATED PELLETS

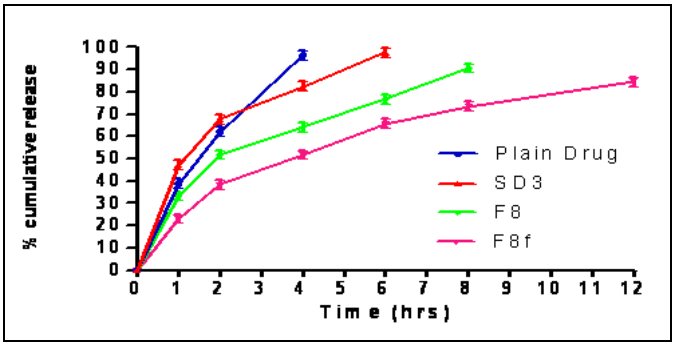

FIG. 3D: RELEASE PATTERN OF PLAIN DRUG, SOLID DISPERSION, UNCOATED AND COATED PELLETS

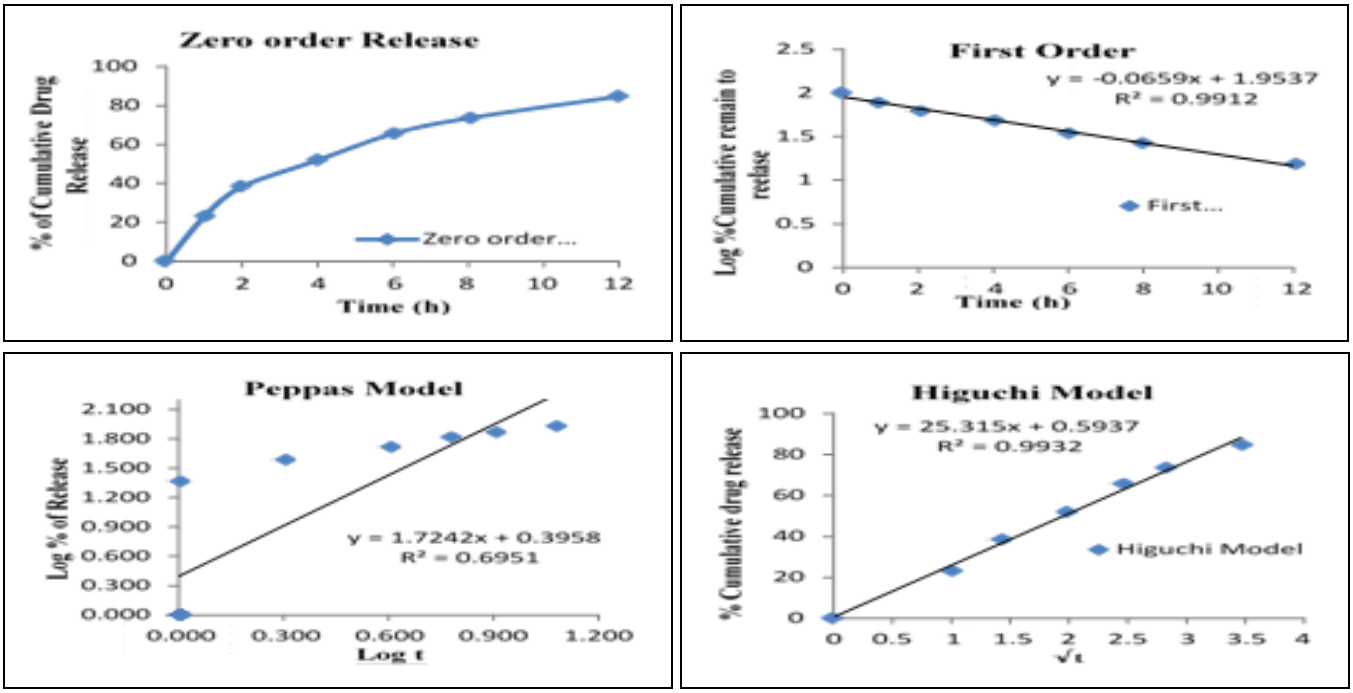

FIG. 4: *IN-VITRO* KINETIC RELEASE OF ACYCLOVIR FROM OPTIMIZED FORMULATION

Pellet Size and Shape Analysis: Particle size analysis revealed that pellet size was observed in the size range of 801 ± 34 µm- 845 ± 41 µm, and all batches showed comparable particle size irrespective of polymer used. The analysis of the image was done to see the effect of the variables on the shape, values of aspect ratio, roundness, and circularity factor. The dumbbell shape, spheroids, and elongated spheroids are the shapes observed. The aspect ratio of F8 was found to be 1.020, which was close to the perfect value 1. Also, F8 showed roundness, and circulatory factor values 1. 008 and 0.990, respectively. The shape of pellets can influence micro-meritics, and thus to check the effect of morphological parameters, flow properties were studied. **Fig. 5A**, **Fig. 5B,** and **Table 5** show the particle morphology of pellets.

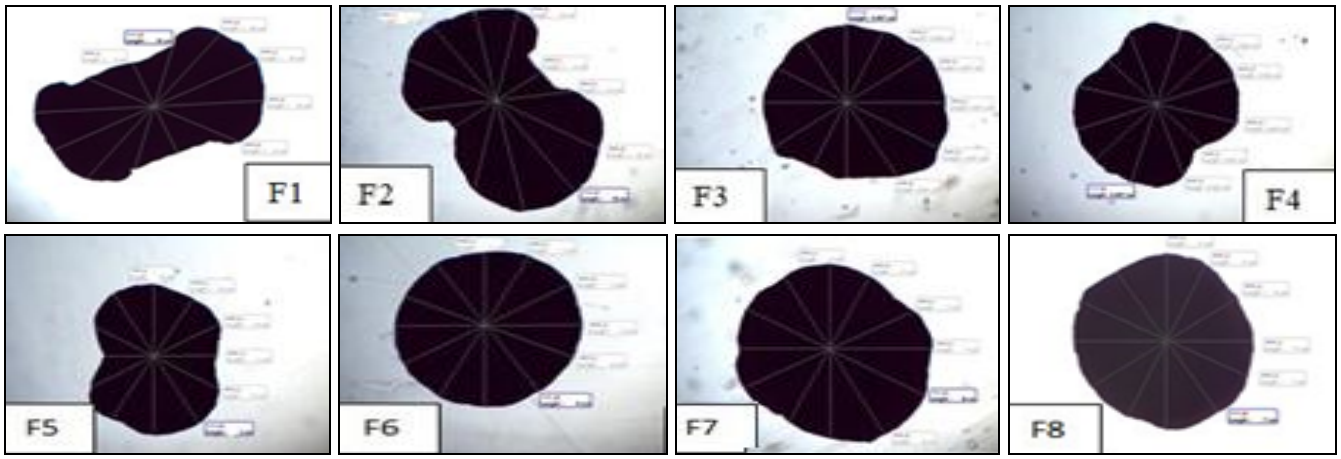

FIG. 5 A: MICROSCOPIC IMAGES OF DIFFERENT UNCOATED PELLETS (F1-F8)

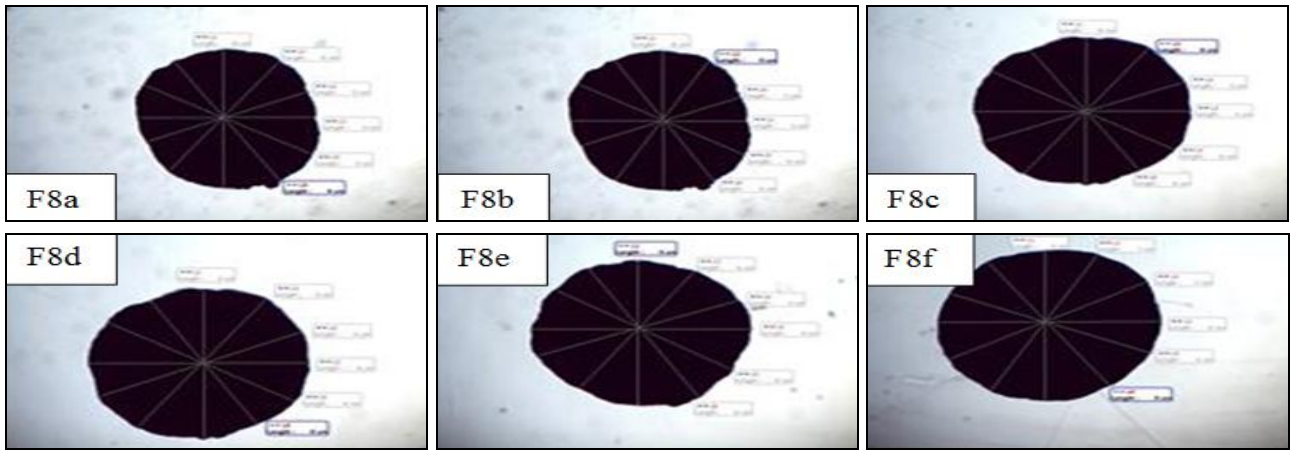

FIG. 5B: MICROSCOPIC IMAGES OF COATED PELLETS (F8A-F8F)

TABLE 5: PARTICLE SHAPE AND SIZE OF DIFFERENT PELLET FORMULATION

Formulation code	Shape	Aspect ratio	Roundness factor	Circularity factor	Size (µm)
F1	Dumbbell	1.317 ± 0.05	1.068 ± 0.04	0.968 ± 0.36	840 ± 40
F2	Dumbbell	1.294 ± 0.12	1.059 ± 0.35	0.971 ± 0.12	845 ± 41
F3	Spheroids	1.016 ± 0.26	1.009 ± 0.29	0.991 ± 0.07	801 ± 34
F4	Elongated spheroids	1.196 ± 0.13	1.021 ± 0.07	0.985 ± 0.18	821 ± 37
F5	Elongated spheroids	1.162 ± 0.09	1.026 ± 0.05	0.981 ± 0.09	809 ± 25
F6	Spheroids	1.035 ± 0.11	1.009 ± 0.08	0.989 ± 0.16	813 ± 44
F7	Spheroids	1.021 ± 0.14	1.010 ± 0.18	0.991 ± 0.14	817 ± 29
F8	Spheroids	1.020 ± 0.26	1.008 ± 0.11	0.990 ± 0.11	815 ± 25
F8a	Spheroids	1.020 ± 0.21	1.008 ± 0.13	0.990 ± 0.12	815 ± 39
F8b	Spheroids	1.020 ± 0.12	1.008 ± 0.10	0.990 ± 0.10	815 ± 42
F8c	Spheroids	1.020 ± 0.10	1.007 ± 0.12	0.991 ± 0.09	816 ± 14
F8d	Spheroids	1.020 ± 0.19	1.007 ± 0.11	0.990 ± 0.14	815 ± 31
F8e	Spheroids	1.019 ± 0.14	1.007 ± 0.08	0.991± 0.11	816 ± 26
F8f	Spheroids	1.019 ± 0.17	1.007± 0.03	0.992 ± 0.10	816 ± 30

(Data presented as mean ±SD, n=3)

Micromeritics Properties of Pellets: The results of the flow properties and friability of various batches of pellet formulations are shown in **Table 6**. The angle of repose (θ) for the pellets was observed in the range of 18.72° ± 1.06 to 25.31° ± 1.16, which showed excellent flow properties of formulations. Similarly, values of Carr's index and Hausner ratio were found in between 11.07 % ± 0.19 to 23.46% ± 0.12 and 1.08 ± 0.09 to 1.28 ± 0.10 respectively. Carr's index and Hausner's ratio are the variables to estimate the flow properties. The friability test was carried out on all formulations to ensure the mechanical strength of pellets. All formulation has possessed good mechanical strength, and friability was observed in the range of 0.18 ± 0.07% to 0.59 ± 0.15%.

TABLE 6: MICROMERITICS PROPERTIES OF PELLETS

Formulation code	Angle of repose(θ)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Hausner's ratio	Carr's index (%)	Friability (%)
F1	25.31±1.16	0.764±0.003	0.821±0.004	1.27±0.09	23.46±0.12	0.59±0.15
F2	22.35±2.15	0.775±0.002	0.829±0.003	1.14±0.17	20.24±0.15	0.50±0.19
F3	19.63±1.21	0.785±0.003	0.832±0.003	1.11±0.49	14.19±0.22	0.24±0.17
F4	24.95±1.18	0.735±0.002	0.823±0.002	1.18±0.20	21.52±0.69	0.42±0.11
F5	18.51±1.13	0.756±0.004	0.820±0.003	1.28±0.10	18.57±0.74	0.39±0.19
F6	18.23±2.09	0.764±0.003	0.829±0.002	1.19±0.23	14.54±0.16	0.29±0.18
F7	18.73±1.21	0.775±0.002	0.845±0.004	1.09±0.17	13.52±0.98	0.26±0.19
F8	18.89±1.10	0.767±0.002	0.843±0.003	1.10±0.11	12.14±0.81	0.21±0.14
F8a	18.87±1.04	0.768±0.003	0.841±0.003	1.10±0.10	12.14±0.5	0.19±0.13
F8b	18.86±1.07	0.765±0.003	0.842±0.004	1.10±0.09	12.11±0.75	0.19±0.10
F8c	18.75±1.02	0.773±0.002	0.851±0.003	1.09±0.11	11.54±0.81	0.18±0.12
F8d	18.85±1.05	0.767±0.003	0.850±0.004	1.10±0.07	11.87±0.45	0.19±0.12
F8e	18.80±1.03	0.770±0.002	0.847±0.002	1.09±0.05	11.42±0.31	0.18±0.10
F8f	18.72±1.06	0.774±0.003	0.853±0.003	1.08±0.09	11.07±0.19	0.18±0.07

(Data presented as mean ±SD, n=3)

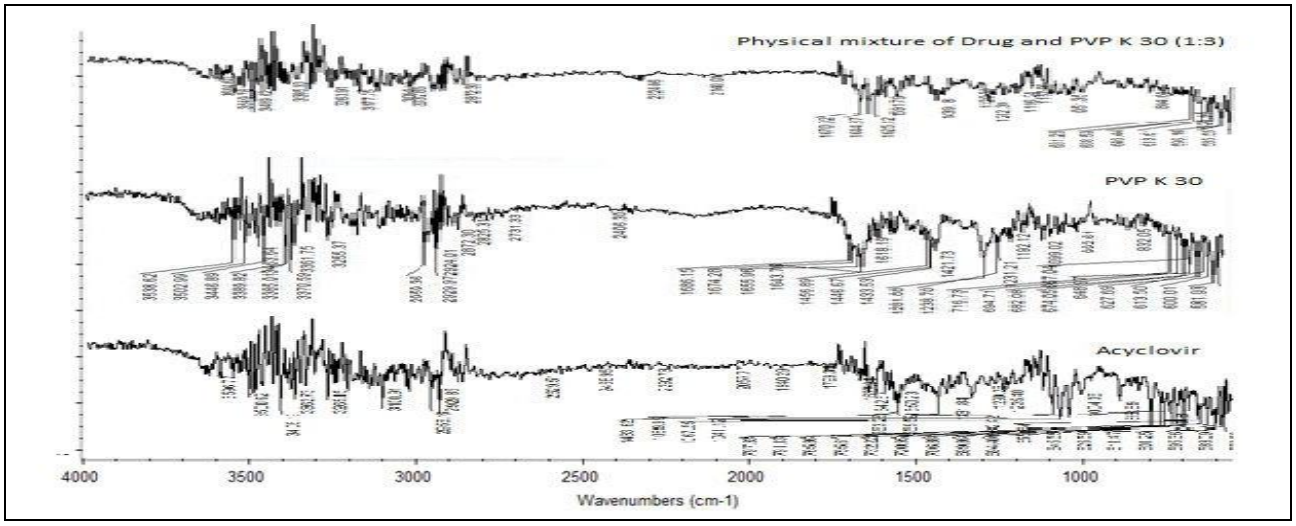

FIG. 6A: IR SPECTRA OF THE DRUG (ACYCLOVIR), PVP K30 AND PHYSICAL MIXTURE OF DRUG AND PVP K30 (1:3)

Fourier Transform Infrared (FT-IR) Spectroscopy: The position of the characteristic peak in the FT-IR spectra of Acyclovir was compared with the standard. The drug shows peaks at 1699.01, 3406.09-3500.62, 1623.01, and 3265.15 cm^{-1} of carbonyl, hydroxyl, amide, and amine group respectively, polymer show a peak of the hydroxyl group at 3385.30 $cm^{-1,}$ and the physical mixture shows peaks of carbonyl, hydroxyl, amide and amine group at 1670.92, 3385.30-3489.62, 1625.12 and 3263.91 respectively. It was observed that there was a small shift in the bond position of functional groups, which interfered there is no interaction between drug and excipient. FT-IR spectra of solid dispersion were shown peaks of carbonyl and hydroxyl groups at 1670.07 and 3322.65-3487.12 $cm^{-1,}$ respectively. The peaks observed in the spectra of solid dispersion containing the drug were near to the spectra of the drug.

The peaks of drug-loaded solid dispersion are of lower intensity than the drug, which means there was no interaction between drug-polymer and shows they are compatible. The IR spectra of pellets show peaks at 1662.34 and 3408.30-3425.26 cm^{-1} of carbonyl and hydroxyl groups. The peaks observed in the spectra of the drug were near to the spectra of pellets containing solid dispersion.

The peaks of pellets loaded solid dispersion are of lower intensity than the drug, which means there was no interaction between the drug-polymer and shows they are compatible. The FT-IR spectra of the drug, polymer, physical mixture, and solid dispersion, blank pellets, and pellets containing solid dispersion were shown in **Fig. 6A** and **Fig. 6B,** respectively.

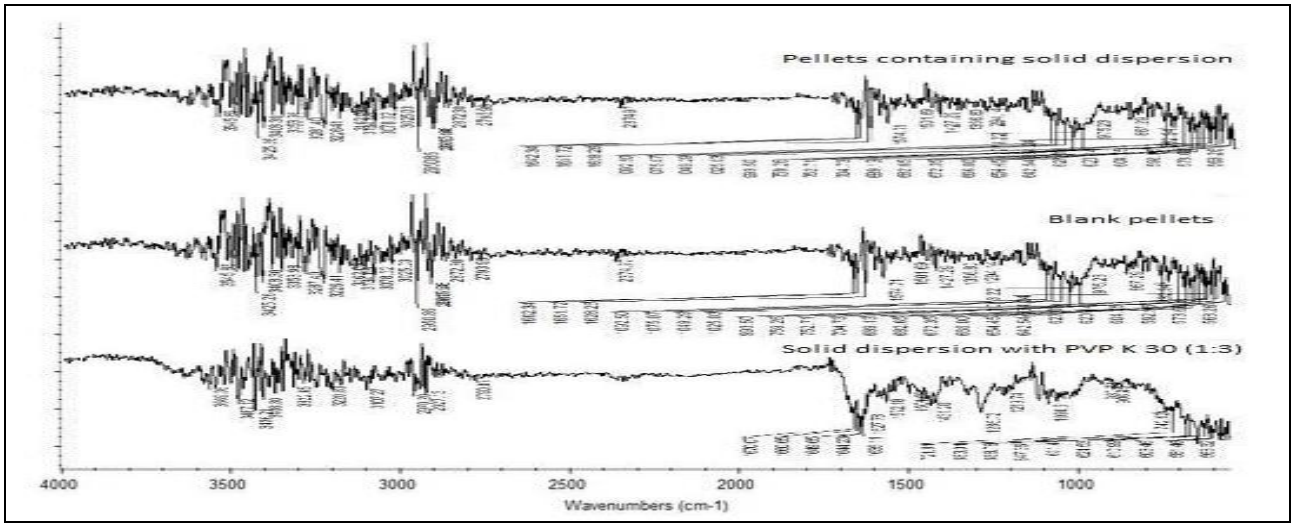

FIG. 6B: IR SPECTRA OF SOLID DISPERSION (SD3), BLANK PELLETS, AND PELLETS CONTAINING SOLID DISPERSION

Differential Scanning Calorimeter (DSC): DSC study of drugs was performed to determine the physical state of the drug. DSC thermogram of Acyclovir shows a sharp endothermic peak at 260.2 °C indicated the crystalline nature of the drug. DSC thermogram of Acyclovir loaded solid dispersion shows a broad endothermic peak at 87.6 °C of polymer and shows no peak of drug indicated that the drug was properly dispersed in the carrier and shows the amorphous nature of the drug.

The DSC thermogram of plain pellets shows no peaks, and pellets containing solid dispersion showed peaks at 124.3 °C and 250.4 °C. The intensity of the drug peak decreased due to the degree of crystallinity may be decreased. The presence of a broad endothermic peak at 250.4 °C shows drug exists in an amorphous state and has no interaction. **Fig. 7** shows the thermogram of drug, solid dispersion, blank pellets, and pellets containing solid dispersion, respectively.

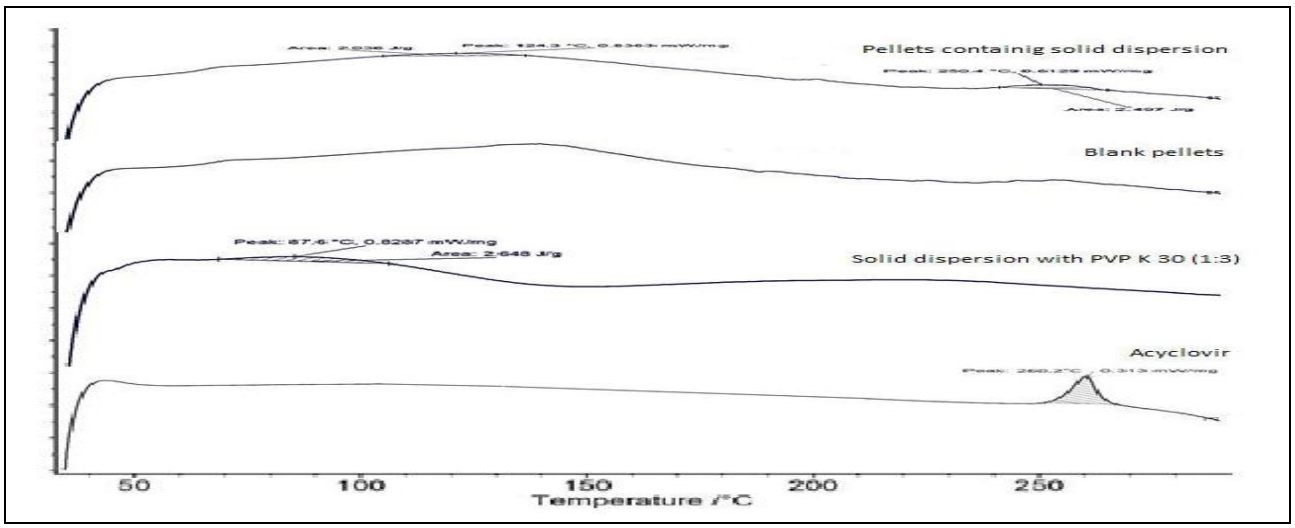

FIG. 7: DSC THERMOGRAM OF ACYCLOVIR, SOLID DISPERSION (SD3), BLANK PELLETS, AND PELLETS CONTAINING SOLID DISPERSION (F8f)

X-Ray Diffraction (XRD): The nature of pure powder, crystalline, or amorphous was confirmed by XRD studies. The sharp peaks indicate that the crystalline nature of the drug. Acyclovir has proved its crystalline nature due to prominent peaks at 2θ = 9.870, 13.000, 14.040, 19.960, 24.140, 25.630, 27.690, 29.810, 32.350, 34.300, 36.220, 37.920 and 38.670. XRD of the drug-loaded solid dispersion did not show the same peaks as plain drug means that the acyclovir endured a conversion from a crystalline state to an amorphous state. The intensities of the acyclovir have been covered with the noise of PVP K30 showing molecular level dispersion of acyclovir in PVP K30, and hence no crystals were found in acyclovir loaded solid dispersion. X-ray diffractogram of blank pellets and pellets containing solid dispersion showed no change, indicating the stability of solid dispersion in the formulation. The XRD pattern of blank pellets and pellets containing solid dispersion shows an additional peak approximately at position 22 (2θ) confirmed the presence of other elements like barium sulphate. **Fig. 8** shows the XRD pattern of drug, solid dispersion, blank pellets, and pellets containing solid dispersion.

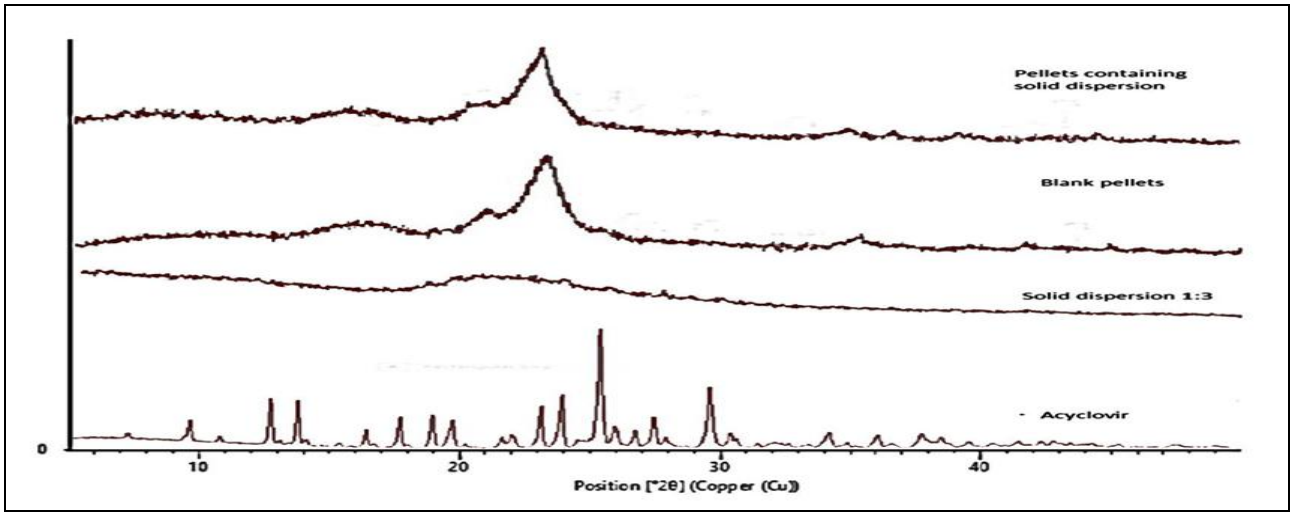

FIG. 8: XRD GRAPH OF ACYCLOVIR, SOLID DISPERSION (SD3), BLANK PELLETS AND PELLETS CONTAINING SOLID DISPERSION (F8f)

***In-vivo* Study:**

Pharmacokinetic Study: A plasma kinetic study was performed to determine the bioavailability and toxicity profile of the prepared formulation.

Results show that the prepared formulation has higher drug availability as compared to the plain drug, marketed formulation, and the solid dispersion, attributed to the delayed release of drug and gastric retention of the formulation at the absorption site also increased. However, the C_{max} of pellets, solid dispersion, marketed formulation, and plain drug was found to be 4.34 µg/mL, 3.61 µg/mL, 2.83 µg/mL, and 2.81 µg/mL, respectively.

However, the t½ and AUC of the prepared formulation are significantly higher than the marketed and the plain formulation, attributed to the controlled drug release behavior of the formulation.

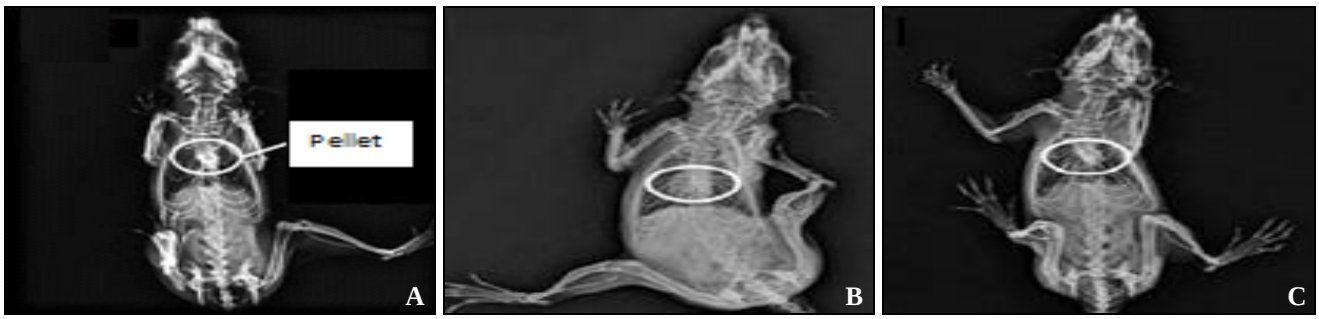

FIG. 9: SHOWS BARIUM SULPHATE CONTAINING GASTRO-RETENTIVE PELLETS (F8F) IN THE STOMACH AFTER ORAL ADMINISTRATION [A] AFTER 2 h [B] AFTER 4 h [C] AFTER 8 h

X-Ray Transmission: Retention efficacy of optimized formulation in the rat was determined using a radiographic imaging technique [14]. **Fig. 9** illustrating X-ray imaging of rat with orally administered high-density gastro-retentive pellets with barium sulphate contrast at a different time interval. Results show that the prepared gastro-retentive formulation was retained in the stomach for a prolonged time. The X-ray analysis further ensures that the prepared formulation is retained at the bottom of the stomach, which is essential to improve the absorption window of acyclovir.

CONCLUSION: In the present investigation, we have designed high-density gastro-retentive (GR) pellets to improve the retention time of formulation in the top regions of GIT. Less absorption in the upper regions of GIT is one of the reasons behind the poor bioavailability of the drug. Considering its favorable solubility in acidic pH and absorption window in upper regions of the GIT, acyclovir is a good candidate for GR drug delivery system; as the gastric residence time of GR formulation is extended, little quantities of the drug are continuously liberated in the parts of the GIT, thereby enhancing its bioavailability.

Also, extended absorption and greater bio-availability of these formulations will help in decreasing the dosing frequency of currently marketed IR formulations. Developed GR formulation might depict a better alternative for prolonged and more efficacious delivery of acyclovir, improve patient compliance, less side effects, less dosing frequency, and reduce the development of resistance. The solid dispersion is made to enhance the solubility of the drug, and the optimization was done based on the solubility profile. Then pellets were made with the help of the extrusion/spheronization method.

The result of the present study revealed that ethylcellulose, HPMC, barium sulphate, and MCC could be used for formulating high-density gastro-retentive pellets of Acyclovir. The efficacy and safety of pellets are expected to offer optimum therapeutic efficacy and improved patient compliance. The prepared pellets were able to maintain a constant plasma concentration for around 12 h and display that rise in the concentration of polymer can cause sustained drug released at 12 h. The C_{max} of the final formulation was greater when compared with plain drug and marketed formulation. The T_{max} of pellets was higher than the plain drug and market formulation. The *in-vitro* drug release of F8f followed Higuchi kinetic model because the value of R is maximum in this (R = 0.9932) and so it can be said that the non-fickian diffusion mechanism was followed. The high-density gastroretentive pellets reside in the stomach more than 8 h that can be checked by the X-Ray imaging of rat at various time intervals after oral dosing. Thus, the preparation of high-density gastro-retentive pellets of solid dispersion containing acyclovir is a promising approach.

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CONFLICTS OF INTEREST: All the authors declare that there is no conflict of interest.

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