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FORMULATION AND IN-VITRO ANTICANCER ACTIVITY OF NILOTINIB IMMEDIATE RELEASE AND IBRUTINIB SUSTAINED RELEASE PELLETS

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ABSTRACT

Background: Blood cancer is a significant contributor to mortality rates worldwide, and its prevalence is projected to rise on a global scale. This trend places considerable strain on healthcare systems and necessitates the expedited development of innovative treatments by pharmaceutical firms to remain competitive. Conventional pellets produce rapid plasma drug levels, but they might cause side effects, decrease effectiveness, and lead to poor therapeutic management. Ibrutinib and Nilotinib are employed to treat leukemia patients. ***Methodology:*** The current research aims to formulate, characterize, and anticancer effect of Nilotinib immediate release (NIR) and Ibrutinib sustained release (ISR) seal sugar-coated pellets. Micrometric properties estimated the characterization of the drug pellets, and surface morphology was estimated using scanning electron microscopy. Drug excipient compatibility studies, stability studies, and *in-vitro* drug release were accessed. ***Result & Discussion:*** The results of pellet formulations FNI-1 to FNI-5 showed that FNI-5 formulations showed 100±6.0 µm size and possessed excellent mechanical strength for giving pellets a good self-life; also, due to the higher drug content up to 99%, FNI-5 was the best suited for pellet formulation and because NIR showed 99.18 ± 2.12 drug release at 2h and ISR 99.03±3.74% up to 12h so that anticancer concentration maintained for prolonged period. The standard dose for cytotoxicity against the THP-1 cell line of Nilotinib was found to be 200 mg, and the maintenance oral dose of Ibrutinib was 140mg, with four times the intake of the drug up to 560 mg. *In an in vitro* study in FNI-5 (final formulation), the dose of Ibrutinib was reduced to 420 mg. **Conclusion:** A synergistic effect of Ibrutinib and nilotinib drugs was observed in the inhibition of cancer cell growth, with an IC50 value of 4.585 µg/mL

INTRODUCTION

The high incidence and mortality rates of blood cancers underscore the need for novel and effective treatments [1]. Leukemia, Lymphoma, Myeloma, Myelodysplastic syndromes (MDSs), and Myeloproliferative neoplasms (MPNs) are forms of blood cancer that can affect the bone marrow, blood cells, lymph nodes, and other lymphatic structures. Leukaemia accounts for 24.7% of subtypes including Acute lymphoblastic

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leukemia (ALL), Acute myeloid leukemia (AML), Chronic lymphocytic leukemia (CLL), Chronic myeloid leukemia (CML), and Juvenile myelomonocytic leukemia (JMML) [2]. Chemotherapy is the most effective cancer treatment. Given the low performance and high toxicity of conventional chemotherapeutics, innovative, effective, and nontoxic cancer treatments are needed [3]. The FDA has authorized Ibrutinib, a very effective, covalent inhibitor of Bruton tyrosine kinase (BTK), a major B-cell receptor signalling pathway kinase, to treat relapsed or resistant MCL. Ibrutinib has a two-hour half-life. The **s**ustained release of this poorly water-soluble drug is to facilitate the dissolution and diffusion of drug molecules into the dissolution molecules [4,5]. Even though Ibrutinib is a sole agent in relapsed/refractory patients, its favourable adverse profile makes it an attractive candidate for investigation in other settings. Combining Ibrutinib with chemo immunotherapy for the first-line therapy of MCL and for the treatment of relapsed/refractory MCL is the subject of ongoing clinical trials [5].

Chronic myelogenous leukemia may be treated with nilotinib resulting in inhibition of ABL, KIT, and PDGFR [6]. Oral administration of drugs through pellets has been a handy and well-recognized delivery route for most therapeutic agents since ancient times. The advantage of the immediate release of drugs is that a wide variety of low-solubility drugs to improve their bioavailability or to attain immediate release [6]. Pellets are geometrically agglomerates made from various starting materials under various processing circumstances [6]. Pellets are oral solid dose forms that deliver drugs to particular gastrointestinal sites. However, pellet Sealing and sugar coating can prolong medication effects [7]. The study formulates pellets and tests nilotinib immediate release and Ibrutinib sustained release pellets on THP-1, a human leukemia monocytic cell line for cytotoxicity.

MATERIALS AND METHOD

Materials

Nilotinib and Ibrutinib were procured as a gift sample from Dr. Reddy's laboratories limited, India, and Sugar Sphere (#30-#35), Hydroxy propyl methyl cellulose (HPMC) 3cps from Colorcon, India, Eudragit RSPO & Eudragit RL PO from Evonik, India, Tri-ethyl citrate from BASF, India, Isopropyl alcohol (IPA) and Dichloromethane (DCM) from Merck chemicals, India. Purified water was produced using a Millipore water filtration system, manufactured by Millipore Corporation, located in Bedford, MA, USA. All the materials used in the experiment were of analytical standard.

Method of preparation of pellets

Nilotinib immediate-release (NIR) pellets and Ibrutinib sustained-release (ISR) pellets executed formulations trials compositions are as mentioned below in **Table 1**.

NIR pellets formulation procedure

Nilotinib immediate-release pellets were prepared by coating the surface of the sugar sphere (size #30-35) with an aqueous core-coat (nilotinib-hydrochloride and HPMC) solution. The fluid bed processer (FBP) (GPCG 1.1) process parameters of the blower fan speed of Wurster GPCG 1.1 pan was set to 60 cfm, inlet temperature (55-60°C), product temperature (35-40°C), and spray rate (5 g/min). Immediate-release pellets of Nilotinib were dried in FBP at a temperature of 40°C and further seal coating was performed. The seal coated Nilotinib immediate release pellets were prepared by coating on Nilotinib coated pellets with aqueous core-coat. The FBP parameters were as blower fan speed (75 cfm), inlet temperature (50-55°C), product temperature (34-42°C) and spray rate (3g/min). The prepared seal coated immediate release pellets of Nilotinib were dried in FBP at 42°C and kept for further analysis**.**

ISR Pellets formulation procedure

The ISR pellets were prepared by coating the surface of the sugar sphere (size #30-35) with aqueous core-coat (Ibrutinib hydrochloride-HPMC) solution and set FBP process parameters such as blower fan speed (63 cfm), inlet temperature (52-58°C), product temperature (36-41°C), and spray rate (7 g/min). The prepared immediate-release pellets of Ibrutinib were dried in FBP at 45°C. Further, Eudragit RSPO & Eudragit RL PO (coat polymers) was dissolved in an organic solvent mixer (isopropyl alcohol and dichloromethane; 80:20) followed by triethyl citrate and stirred for 20 minutes, stirring until it became clear transparent and homogeneous. The ISR pellets were prepared by coating the immediate release pellets of Ibrutinib in FBP at blower fan speed 78 cfm, inlet temperature (30-32°C), product temperature (25-28°C), and spray rate (2.5 g/min).

The prepared ISR pellets were dried FBP at 40°C and kept for further analysis. The coated pellets for immediate-release nilotinib and sustained-release Ibrutinib were prepared using the fluidised bed processor technique in instrument ACG, GPCG1.1, India.

Table 1: Composition of various type of NIR pellets and ISR pellets formulations

Stages		Ingredients	FNI-1 (mg/Cap)	FNI-2 (mg/Cap)	FNI-3 (mg/Cap)	FNI-4 (mg/Cap)	FNI-5 (mg/Cap)
NIR Seal coated pellets	a	**Drug layering-1**					
		Sugar sphere (#30-35 µ)	50.00	50.00	50.00	50.00	50.00
		Nilotinib	200.00	200.00	200.00	200.00	200.00
		HPMC 3cps	30.00	30.00	30.00	30.00	30.00
		Distilled water	q.s.	q.s.	q.s.	q.s.	q.s.
		Drug layer pellets weight (mg)	**280.00**	**280.00**	**280.00**	**280.00**	**280.00**
	b	**Seal coating-1**					
		Drug layer pellets	280.00	280.00	280.00	280.00	280.00
		HPMC-3CPS	13.60	13.60	13.60	13.60	13.60
		Distilled water	q.s.	q.s.	q.s.	q.s.	q.s.
	Total IR pellets weight (mg) = A		**293.60**	**293.60**	**293.60**	**293.60**	**293.60**
ISR seal coated Pellets	c	**Drug layering-2**					
		Sugar sphere (#30-35 µm)	50.00	50.00	50.00	50.00	50.00
		Ibrutinib	420.00	420.00	420.00	420.00	420.00
		HPMC 3cps	21.00	21.00	21.00	21.00	21.00
		Distilled water	q.s.	q.s.	q.s.	q.s.	q.s.
		Drug layer IR pellets Wt. (mg)	**491.00**	**491.00**	**491.00**	**491.00**	**491.00**
	d	**Seal coating-2**					
		Drug layer pellets	491.00	491.00	491.00	491.00	491.00
		HPMC-3CPS	9.82	9.82	9.82	9.82	9.82
		Distilled water	q.s.	q.s.	q.s.	q.s.	q.s.
		Seal coated IR pellets Wt. (mg)	**500.82**	**500.82**	**500.82**	**500.82**	**500.82**
	e	**Polymer coating**					
		Seal coated-2 pellets	500.82	500.82	500.82	500.82	500.82
		Eudragit RS PO	60.62 **(90%)**	61.97 **(92%)**	63.32 **(94%)**	63.99 **(95%)**	65.34 **(97%)**
		Eudragit RL PO	6.74 **(10%)**	5.39 **(8%)**	4.04 **(6%)**	3.37 **(5%)**	2.02 **(3%)**
		Tri ethyl citrate	6.72	6.72	6.72	6.72	6.72
		Isopropyl alcohol (IPA)	q.s.	q.s.	q.s.	q.s.	q.s.
		Dichloro methane (DCM)	q.s.	q.s.	q.s.	q.s.	q.s.
		SR Polymer coated pellets (mg)	**574.90**	**574.90**	**574.90**	**574.90**	**574.90**
	f	**Seal coating-3**					
		SR polymer-coated pellets	574.90	574.90	574.90	574.90	574.90
		HPMC-3CPS	11.50	11.50	11.50	11.50	11.50
		Distilled water	q.s.	q.s.	q.s.	q.s.	q.s.
	Seal coated SR pellets Wt. (mg) = B		**586.40**	**586.40**	**586.40**	**586.40**	**586.40**
Total fill (NIR+ISR pellets) weight (mg) in capsule = (A+B)			**880.00**	**880.00**	**880.00**	**880.00**	**880.00**

Characterization of Pellets

Percent yield of pellets

NIR and ISR pellets were tested by percent yield of pellets for the improvement of the quantity of Eudragit RSPO, Eudragit RLPO, and hydroxypropyl methylcellulose (HPMC), as well as the selection of the optimal formulation, was based on the shape and size of pellets, the percentage of drug release, and micrometric studies. The percentage yield was determined based on polymer and drug weight gains on core spheres of sugar **(Table 2)** by the below formula:

$$\%\ yield = \frac{T_1}{T_2} \times 100$$

T_1 is the practical weight achieved by the final coated pellets, and T_2 is the theoretical total weight of sugar spheres, drugs, and polymers.

Micrometric properties

Pellets were analyzed in triplicate for flow characteristics, including repose angle, bulk, tapped densities, Hausner's ratio, Carr's index, and Friability, to obtain a mean value shown in **Table 2**.

Angle of repose

The flow characteristics could be decided from the angle of repose. Briefly, the sample was allowed to fall gently through a funnel onto a hard surface from a height of 4 cm. The height and diameter of the pile were noted. The angle of repose was reported using the following formula:

$$\text{Angle of repose } (\theta) = \tan^{-1}\left(\frac{h}{r}\right)$$

Where h is the height of the pile, and r is the radius of the pile.

Bulk density

Pellets were accurately weighed to 35 g and gently inserted into a calibrated 100 ml measuring cylinder. The volume was recorded to calculate bulk density (g/ml) using the following formula.

$$Bulk\ density = \frac{Weight\ of\ the\ pellets}{Initial\ volume\ of\ the\ pelletes}$$

Tapped density

Tapped density was observed by tapping the cylinder 500 times using the tapped density apparatus after pouring the pellets into the measuring cylinder. The tapped volume was recorded using the formula.

$$Tapped\ density = \frac{Weight\ of\ the\ pellets}{Volume\ occupied\ by\ pelletes\ after\ tapping}$$

Hausner's ratio

The Hausner's ratio is a number correlated to pellets' flowability. The following formula calculated it:

$$Hausner's\ ratio = \frac{Z^t}{Z^b}$$

Where Z^t is tapped density, and Z^b is bulk density.

Carr's index

The Carr's index is an indication of the compressibility of pellets. The following formula calculated it:

$$Carr's\ index\ (\%) = \frac{Z^t - Z^b}{Z^t} \times 100$$

Z_t is the powder's tapped density, and Z_b is the freely settled bulk density of the powder.

Friability

6.50 gm Pellets were placed in a friabilator (Electrolab, India). Then, the friabilator was subjected to 100 revolutions at 25 rpm. The pellets were collected from the friabilator and again placed on the sieve. The pellets having a smaller diameter than the aperture of the sieve will pass through the sieve, and then the pellets will be reweighed. The friability was determined as the percentage loss of mass of pellets after the test was recorded.

$$Friability\ (\%) = \frac{W_i - W_f}{W_f} \times 100$$

W_i & W_f are the initial and final weights of tablets, respectively.

Surface morphology and pellet size study

Surface morphology and Particle size of optimized pellets were investigated using Scanning electron microscopy (SEM) (Jeol—JSM6100, Japan). NIR and ISR pellets were mounted on aluminum studs as a whole pellet and then sputter coated with gold for approximately 1 min. The electron microscopy pictures were taken at magnifications of 100 µm of X100, X170, X470, and X650, respectively **(Figure 1)**.

Drug-excipients compatibility study

The topic of interest is drug excipients. Compatibility studies were conducted using several analytical techniques, such as Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and differential scanning calorimetry (DSC).

These techniques were used to investigate the interactions between the drug and excipients and evaluate the drug's confirmation, stability, and compatibility within the formulation.

Fourier-transform infrared spectroscopy (FTIR) study

Infrared (IR) spectrums were recorded in the wavelength region of 4500 to 400cm-1. Briefly, the drug alone, polymers alone, and a mixture of drugs and polymers in KBr were compressed into discs by applying a pressure of 7 tons for 5 minutes **(Figure 2)**.

X-ray diffraction (XRD) study

The crystallinity of NIR and ISR pellets was evaluated using an X-ray Diffractometer (Panalytical-Xpert pro, Netherlands). Briefly, Samples were exposed to monochromatic nickel-filtered copper radiation (45 kV, 40 mA) in a wide-angle X-ray diffractometer with 2^{θ} angle **(Figure 3)**.

Differential scanning calorimetry (DSC) study

The degree of crystallinity and polymorphic or thermal transitions involving energy changes throughout the formulation method. The phase transition temperature of NIR pellets and ISR pellets was analyzed by Differential Scanning Calorimetry (SITARAM, Setline DSC+) in perforated aluminum sealed pans at a heating rate of 10°C/min from 30-400°C using nitrogen as blanket gas (10ml/s) to maintain an inert atmosphere **(Figure 4)**.

Percent drug content:

The drug content for the Nilotinib HCl and Ibrutinib loaded pellets was determined by soaking pellets in water for 30 min. The pellets (293.60 mg of NIR and 586.40 mg of ISR pellets) were broken with the spatula in mortar, vortexes for 5 min, centrifuged for 10 min at 2000 rpm, and diluted to 50 ml with PBS 6.8 pH. Then, the drug was estimated by UV spectrophotometer (Shimadzu 1601) at λmax 260 nm and 263 nm for Ibrutinib and Nilotinib HCl, as shown in **Table 3**.

$$Drug\ Content\ (\%) = \frac{A_d}{T_d} \times 100$$

Where, T_d-Theoretical drug content, A_d-Actual drug content

***In-vitro* drug release study:**

Electrolab USP-I (basket) dissolution equipment was used to conduct dissolution experiments. In FNI-5, capsules size 000 were filled with NIR pellets and ISR pellets fitted with USP-I (basket) in the dissolution media. The rotation speed was 100 rpm, and the studies were conducted at 37±0.5°C. 900 ml of HCl solution (pH 1.2), acetate buffer solution (pH 4.5), and PBS (pH 6.8) were selected as the dissolution media. At appropriate time intervals in pH 1.2 solution, 5 min, 15 min, 30 min, 60 min, 120 min, followed by Acetate buffer solution (pH 4.5), 1 hr, 2 hr, 4 hr, 6 hr followed by in PBS (pH 6.8), 1 hr, 4 hr, 8 hr, 12 hr and 16 hr; 5-mL aliquots from the three media's were drawn under replacement of the volume with fresh isothermal medium subsequently. The average cumulative release with standard deviations (Figure 5) was assessed for drug release at 263 nm for Nilotinib and 260 nm for Ibrutinib, respectively, using a UV spectrophotometer (Jeol, JSM6100, Japan).

Kinetics of drug release

To study the release kinetics, the various release models were applied to the dissolution characteristics of different pellet formulations (**Table 4)**.

Zero-order

$$C = (K_{1.}t)$$

Expressed in units of concentration/ time, K_1 is the zero-order release constant, and t is the time in hours.

First-order

$$\text{Log } C = [(\log C_o - Kt) /2.303]$$

Where C is the concentration, C_o is the initial concentration of the drug, K is the first-order rate constant, and t is the time.

Higuchi model

$$Qt = (KH \cdot t^{1/2})$$

Qt is the amount of drug released in time t, K is the kinetic constant, and t is the time in hours.

Korsmeyer's Pappas:

$$(Mt / M_{\infty}) = K \cdot t^{n}$$

Where Mt represents the amount of the released drug at time t, M_{∞} is the total amount (complete dose). The value of n indicates the drug release mechanism related to the geometrical shape of the delivery system.

Cytotoxicity (anticancer study) effect of FNI-5 on THP-1 cell line

MTT Assay was utilized to determine the cytotoxicity of the supplied samples (**Table 5)** against the THP-1 cell line. DMEM media nourished with 10% FBS and 1% antibiotic solution was used to cultivate the cells (10000 cells per well) for 24 hours in 96-well plates at 37°C and 5% CO_2. The next day, cells were treated with formulations ranging from 1 to 1000 g/mL (various concentrations were made in an incomplete medium). Following

a 24-hour incubation period, the cell culture was supplemented with MTT Solution at a final concentration of 250g/mL and then incubated for 2 hours. Upon completion of the procedure, the culture supernatant was disposed of, and the cell layer matrix was allowed to dissolve in 100 l of Dimethyl Sulfoxide (DMSO). Subsequently, the dissolved solution was subjected to spectrophotometric analysis at wavelengths of 540 nm and 660 nm using an Elisa plate reader (iMark, Biorad, USA).

Stability study

Accelerated stability tests for the formulation of FNI-5 pellets were conducted at 40°C and 75% RH for up to six months (180 days) per ICH guidelines (**Table 6).**

RESULTS

As discussed below, the optimization and preparation of Nilotinib immediate release and Ibrutinib sustained release pellets, percent yield, micrometric properties, surface morphology, pellet size, drug-excipient compatibility, percent drug content, in-vitro drug release, kinetics of drug release study, and cytotoxicity effect (anticancer study) were performed.

Optimization and preparation of Nilotinib immediate release and Ibrutinib sustained release pellets

NIR and ISR pellets were prepared using specific particle sizes and the number of sugar spheres with a mesh size (30-35 µm) to reside in the final drug dosage form and kept for further evaluation.

Percent yield

NIR and ISR pellets were tested by percent yield to improve the quantity of Eudragit RSPO, Eudragit RLPO, and Hydroxypropyl methyl cellulose (HPMC) and select the optimal formulation. Table 2 showed 89.25±0.2 to 98.67±0.1% yield, and the highest percent yield of entrapped solid content, 98.67±0.1% on the core sugar sphere, FNI—5, was selected.

Micrometric parameters:

FNI-1 to FNI-5 particle formulations flow properties such as bulk density, tapped density, Hausner's ratio, Carr's index, Angle of repose, and Friability were analyzed. FNI-5 pellets showed bulk and tapered densities of 1.07±0.06 and 1.10±0.04, respectively. Table 2 shows that FNI-5 pellet compositions have excellent flow characteristics, with an angle of repose of 18.66±0.01 and Friability of 0.010±0.02%.

Surface morphology & Pellets Particle size study

The SEM images in Figures 1 (A1), (A2), (B1), and (B2) illustrate pellet morphology. The optimum pellets in the FNI-5 formulation for INR and ISR showed the size 100±6.0 µm. SEM pictures of the ideal formulation showed nearly spherical pellets and were collected before subsequent studies.

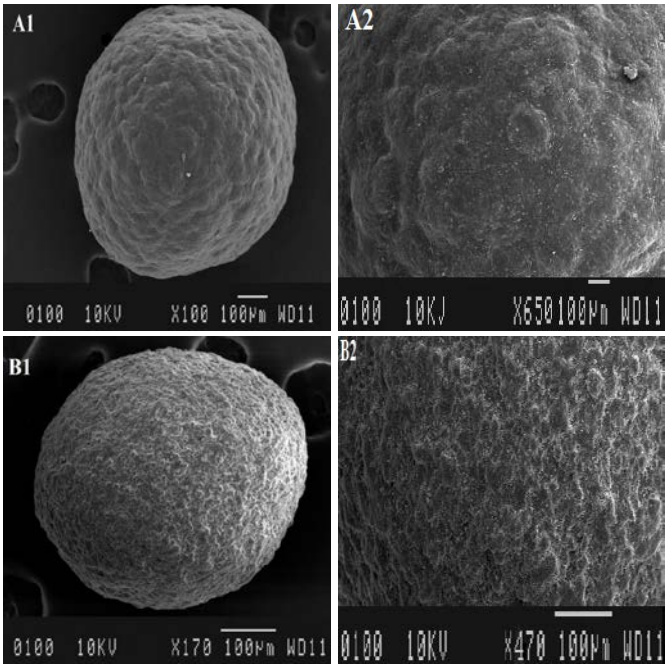

Figure 1: (A1) NIR pellets of FNI-5 formulation, (A2) Magnify the surface of INR pellets of FNI-5 formulation; (B1) ISR pellets of FNI-5 formulation, (B2) Magnify the surface of ISR pellets of FNI-5 formulation.

Drug-excipients compatibility studies

FT-IR study

FT-IR spectroscopy of Nilotinib, Ibrutinib, physical mixture with polymer, and FNI-5 was compared for the drug-polymer compatibility study. Ibrutinib showed characteristics peaks at 3469 cm^{-1} (NH– stretching), 3045 cm^{-1} (Aromatic –CH stretching), 1584,1569 cm^{-1} (Aromatic –C=C- stretching), 2859 cm^{-1} (Aliphatic –CH stretching), 1718 cm^{-1} (C=O stretching), 1637 cm^{-1} (C=N stretching), 1484 and 1457 cm^{-1} (Aromatic –C=C stretching), and 1285 cm^{-1} (C-O-C stretching); Nilotinib at 3255 cm^{-1} (NH– stretching), 3179 cm^{-1} (–OH stretching), 2850 cm^{-1} (Aliphatic –CH stretching), 2032 cm^{-1} (C=O stretching), 1643 cm^{-1} (C=N stretching) in the IR Spectrum of Ibrutinib and Nilotinib. All characteristic peaks of drugs were found in the IR spectrum of FNI-5 and showed no shift and no disappearance of characteristic peaks, indicating no significant interaction between the drug and polymers **(Figure 2).**

Table 2: Percent yield and micrometric properties of various NIR and ISR formulations

Formulation codes	Yield (%)#	Bulk Density# (g/mL)	Tapped Density# (g/mL)	Hausner's ratio#	Carr's Index#	Angle of repose# (Θ) (°)	Friability (%)
FNI-1	89.25±0.2	0.97±0.02	1.07±0.02	1.10±0.03	9.34±0.04	27.78±0.04	0.035±0.02
FNI-2	92.36±0.5	0.98±0.03	1.05±0.01	1.07±0.03	6.67±0.02	28.68±0.02	0.012±0.03
FNI-3	94.67±0.1	0.96±0.02	1.03±0.03	1.07±0.02	6.80±0.03	26.68±0.06	0.025±0.02
FNI-4	95.21±0.6	1.02±0.01	1.09±0.02	1.07±0.01	6.42±0.01	22.66±0.02	0.011±0.04
FNI-5	98.67±0.1	1.07±0.06	1.10±0.04	1.03±0.04	2.73±0.05	18.66±0.01	0.010±0.02

#N=3±S.D

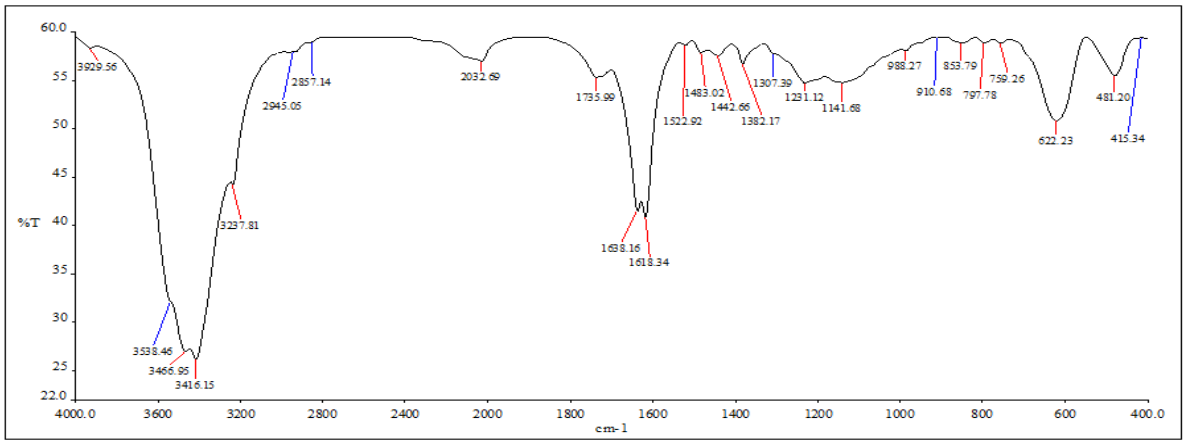

Figure 2 (a): FT-IR spectrum of final formulation FNI-5

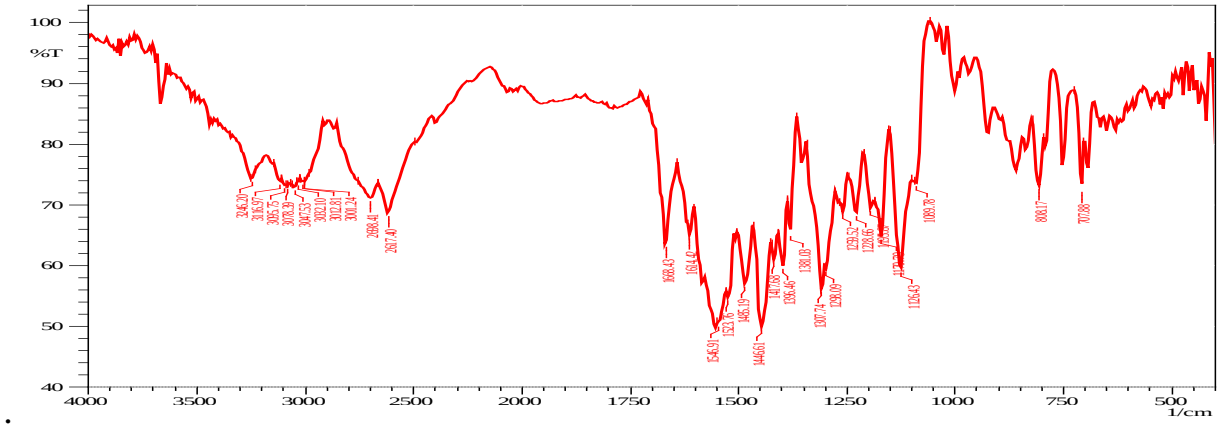

Figure 2 (b): FT-IR spectrum of Nilotinib.

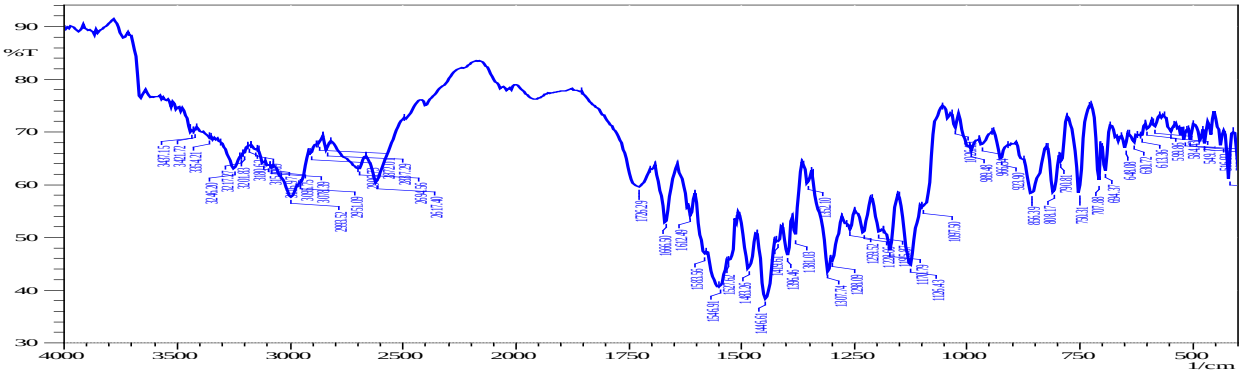

Figure 2 (c): FT-IR spectrum of Physical mixture of Nilotinib-polymer

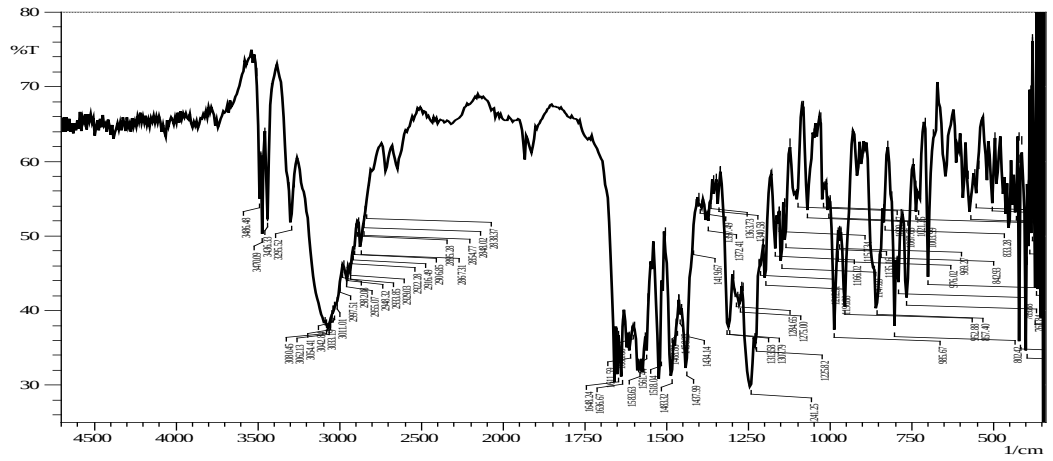

Figure 2 (d): FT-IR spectrum of Ibrutinib.

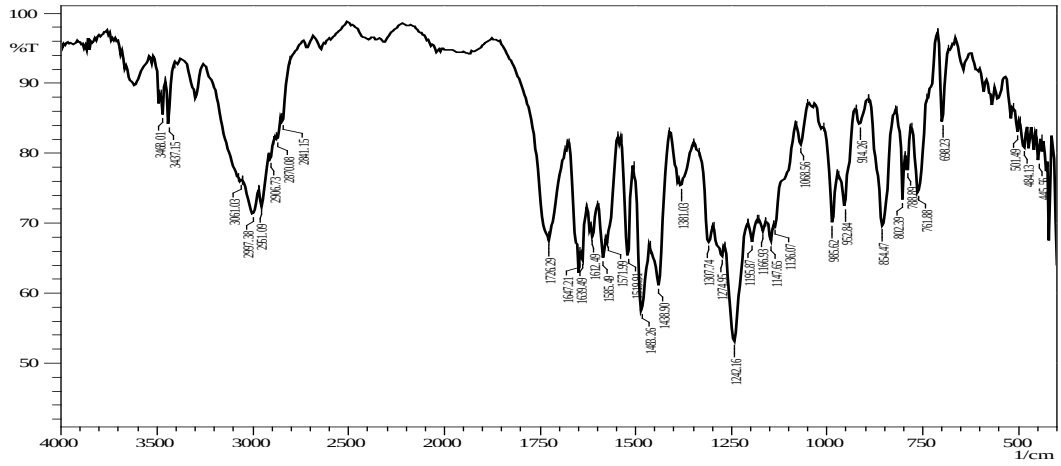

Figure 2 (e): FT-IR spectrum of Physical mixture of Ibrutinib-polymer.

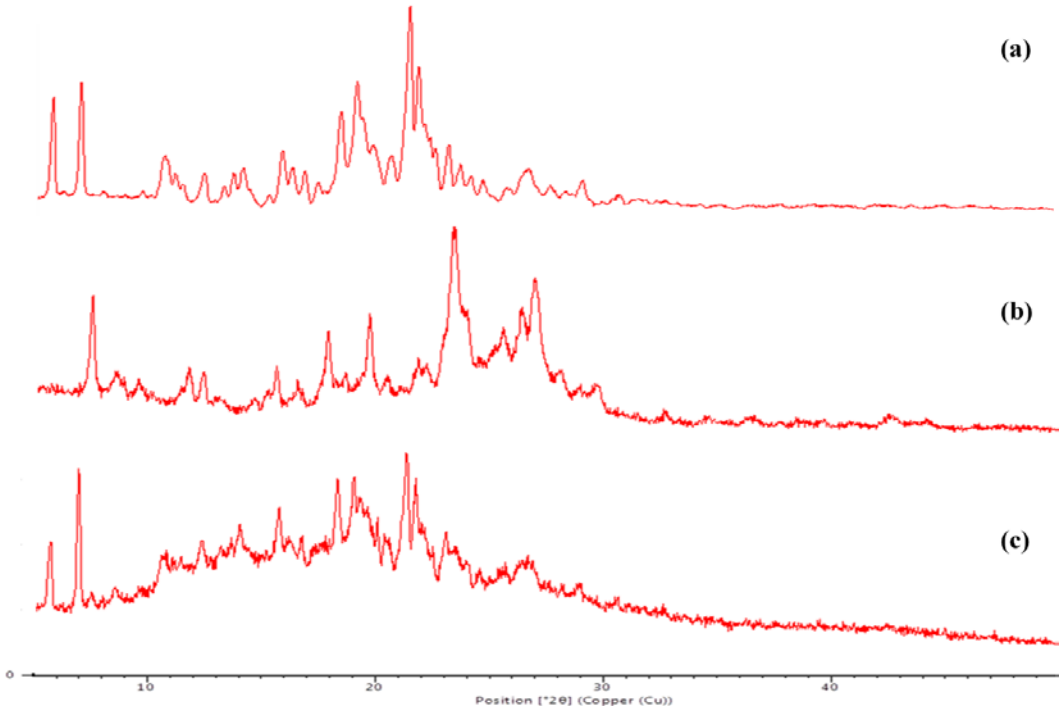

Figure 3: XRD spectrum of (a) Ibrutinib, (b) Nilotinib and (c) FNI-5 formulation

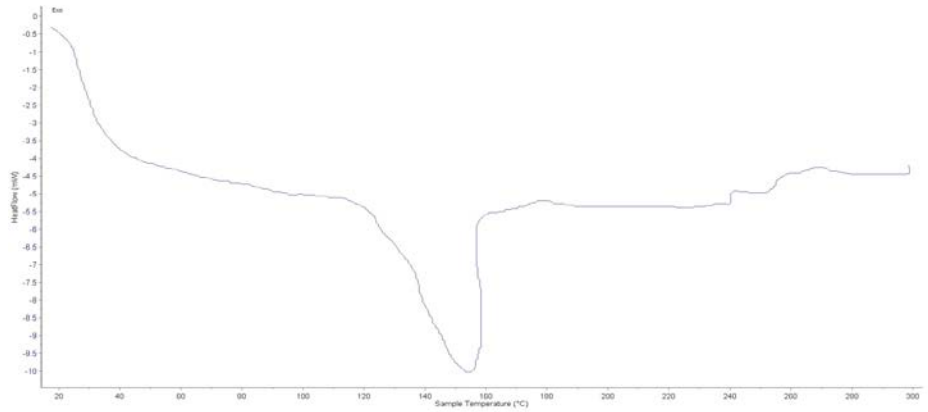

Figure 4 (a): DSC spectrum of Ibrutinib drug

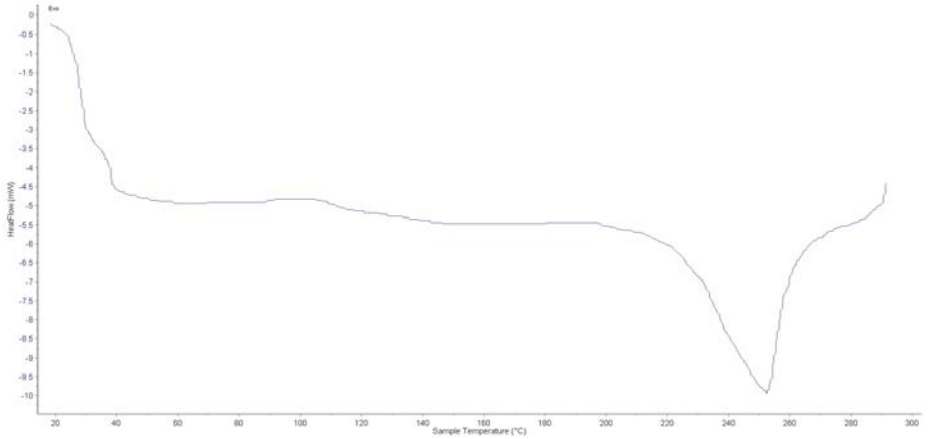

Figure 4 (b): DSC spectrum of Nilotinib

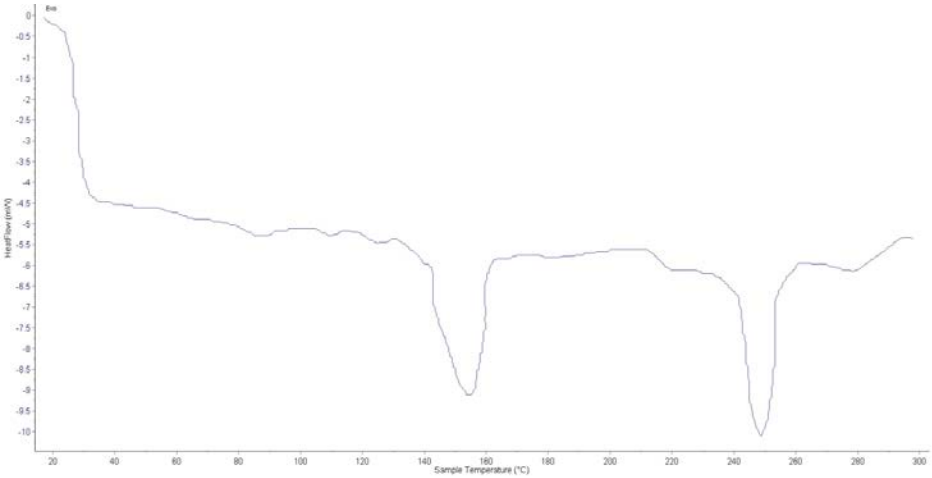

Figure 4 (c): DSC spectrum of FNI-5 formulation

X-ray diffraction (XRD) study:

NIR and ISR pellet crystallinity was measured using an X-ray diffractogram. 2^{θ} value of Pure Ibrutinib showed at 10.6, 11.38, 16.84, 17.44, 19.30, 21.04, 23.74, 25.37 and Nilotinib showed at 5.5, 8.9, 9.9, 12.3, 15.7, 22.9, 27.6, and 36.3. FNI-5 showed no shift or disappearance of characteristic peaks, indicating no significant changes between the drug and Polymers. **Figure 3** depicts XRD patterns for pellets containing NIR and ISR.

Differential scanning calorimetry (DSC) study:

In FNI-5, Ibrutinib and Nilotinib exhibited endothermic peaks at 152.32 °C and 248.85 °C, respectively. In Figure 4, studies showed no significant changes in the endothermic peaks of

drugs, indicating no significant interaction between drug and polymer.

Percent Drug content

The drug content (%) of all formulations was found to be 96.37±0.2 to 99.52±0.6%, but IFN-5 showed the highest drug content, 99.52±0.6%, due to an increase in the concentration of polymers, resulting in a significant increase in drug release.

***In-vitro* percent cumulative drug release study**

In FNI-5 formulations, 3.65% of hydroxypropyl methylcellulose 3cps was used as a binder to bind or entrap the drug Ibrutinib on sugar spheres. **Figure 5** showed that in FNI-5, Nilotinib release 99.18±2.12% was observed for up to 2 hrs, and Ibrutinib release 99.03±3.74% up to 12 hours. So, a ratio of 97:3 of Eudragit RSPO: Eudragit RL PO was optimized based on the observed cumulative percent drug release up to 12 hours.

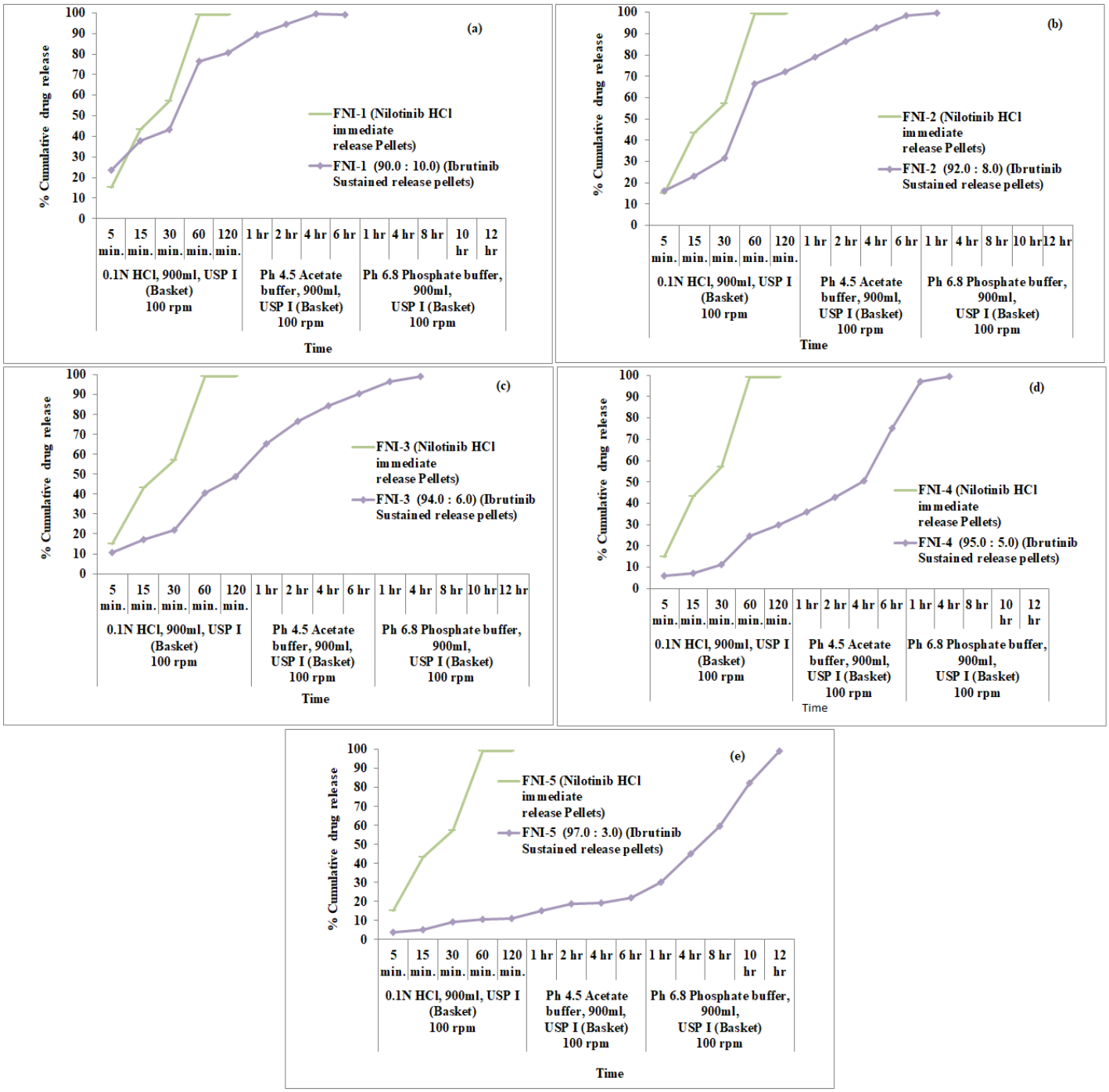

Figure 5: *In-vitro* percent cumulative drug release of Nilotinib and Ibrutinib from different

Table 3: % drug content of various formulations

Formulation codes	Drug Content (%) (mean of 3 ± SD)	
	Nilotinib	Ibrutinib
FNI-1	96.37±0.2	98.26±0.6
FNI-2	97.34±0.3	98.34±0.3
FNI-3	97.21±0.5	96.76±0.4
FNI-4	98.98±0.1	98.69±0.5
FNI-5	99.52±0.6	99.85±0.2

Kinetics of drug release study

Each kinetic model's correlation coefficient (R^2) value was determined to ascertain the drug release process from each formulation. Specifically, in the FNI-5 formulation, Table 4 shows that the R^2 values of the first order and Higuchi are 0.985 and 0.996, respectively. It concluded that drug release depends on the presence of the drug in formation due to NIR and is maintained for a more extended period due to ISR pellets.

Cytotoxicity study or anticancer study on THP-1 Cell line by marketed capsules, capsule, and FNI-5 final formulations:

Figure 6 found that the THP-1 cell line treated with marketed product MC-1 (Nilotinib), MC-2 (Ibrutinib), and Final formulation FNI-5 at 100µg/mL concentrations. MTT assay determined the cytotoxicity of the marketed products MC-1, MC-2, and FNI-5 on the THP-1 cell line (**Table 5)**. The black blotches indicate maximal THP-1 cell line eradication, i.e., blood cancer when treated with 100 mg/mL of the final formulation of FNI-5 and marketed formulations, i.e., MC-1 and MC-2. The standard dose of Nilotinib is 200mg, while the standard oral dose of Ibrutinib is 140 mg, with a maximum daily intake of 560 mg. In the final formulation of FNI-5, the dose of Ibrutinib has been decreased to 420 mg, and it exerts a synergistic effect with Nilotinib in cancer cell killing with an IC50 of 4.585g/mL.

Table 4: Dissolution profile modeling of various NIR & ISR formulation types.

Formulation code	Zero Order		First Order		Higuchi		Korsmeyer peppas	
	R^2	K	R^2	K	R^2	K	R^2	K
FNI-1	0.847	0.409	0.979	0.0042	0.984	0.159	0.825	0.73
FNI-2	0.842	0.415	0.982	0.0045	0.899	0.096	0.819	0.731
FNI-3	0.841	0.422	0.983	0.0048	0.981	0.153	0.814	0.732
FNI-4	0.843	0.431	0.983	0.0053	0.983	0.979	0.811	0.733
FNI-5	0.981	0.456	0.985	0.0072	0.996	0.154	0.906	0.738

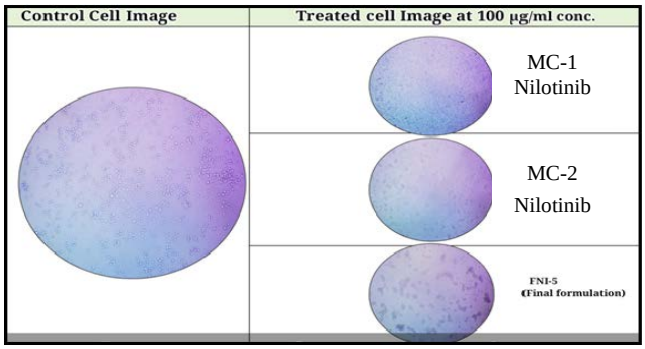

Figure 6: Images of Control THP-1 cell line and treated THP-1 cell line with Ibrutinib API, Nilotinib API, and Final formulation FNI-5 at 100µg/mL.

Stability study

Table 6 shows that nilotinib levels of drug content in NIR pellets and Ibrutinib levels of drug content in ISR pellets were 99.40±0.6% and 99.35±0.2% in FNI-5 at 180 days under 40°±2°C /75±5% RH accelerated conditions. FNI-5 was stable for six months or 180 days after conducting stability testing, and no significant changes were observed in color, odor, or description.

Table 5: IC_{50} value of different sample products along formulation FNI-5

Sample code	Sample name	IC50 value (µg/mL)
Sample-1	Marketed capsule-1 (MC-1)	16.11
Sample-2	Marketed capsule-2 (MC-2)	7.068
Sample-3	FNI-5	4.585

DISCUSSION

Ibrutinib and nilotinib are proven to be the potent anticancer activity [5,6]. The results of the percent drug content of various NIR and ISR formulations showed acceptable physicochemical properties [6]. Since Eudragit is polyanionic and polycationic, it inhibits the rapid release of Ibrutinib in the stomach and intestines. Following this was the approximated higuchi kinetics release of the formulated system, which demonstrated a constant drug release due to the pH-dependent drug release behavior of Eudragit RS and RL. The Eudragit reduced burst release at acidic pH, resulting in effective drug release at higher pH [6, 8]. Distinct ratios of Eudragit Polymer-coated Ibrutinib pellets

exhibited significantly distinct drug release patterns. In addition, according to a drug release study, the uniform and protracted absorption phase coupled with the maintenance of plasma concentration for an extended time following administration of a formulated NIR and ISR capsule reduce the risk of dose-dependent adverse effects and enhance the NIR and ISR effectiveness [9, 10]. The immediate release pellet is to release the drug as soon as it comes into contact with the gastric fluid. NIR pellet dissolves within 120 min, whereas a sustained release oral drug delivery system efficiently retained the maximum drug concentration in a single dose in a desired period of 12 hrs. According to Ahmad et al. (2023) [6], the drug formulated has a controlled-release oral drug delivery system similar to the present study. Pellets adjusted at 3.65% HPMC 3cps, Eudragit RSPO, and Eudragit RLPO in 97:3 ratios, and Carr's index and Hausner's ratios were regular and spherical. A study found that HPMC, Eudragit RSPO, and Eudragit RLPO increase sustained-release pellet shape and size, improving micrometric characteristics. Sustained released pellets of anticancer medications give appropriate plasma concentration with less frequent administration and lessen the side effects of standard-dose forms, including GIT issues [11].

The findings of XRD patterns for pellets containing Nilotinib immediate release and Ibrutinib sustained release showed that the drug-loaded FNI-5 formulation's XRD chart showed no significant changes in peaks. The intensity of Ibrutinib and Nilotinib peaks decreased slightly [12-14], because the encapsulated drug was dispersed in the carrier system at the molecular level, suggesting a uniform coating and affirming the drug's crystallinity in the final formulation FNI-5. The DSC study demonstrated no significant modifications in the degree of crystallinity of drugs, and polymorphic transitions in physical combinations of drugs-polymers and the ultimate formulation of FNI-5 were observed [9]. The reports also showed anticancer activity with an IC50. FNI-5 showed a 99% drug content of nilotinib and Ibrutinib and more effectivity. Nilotinib appreciably enhances the anticancer response of myeloid cancerous cells using MTT assay [6]. Recently, Ibrutinib has been utilized as a novel anticancer drug for a variety of other malignancies, including human blood, ovarian, breast, and lung cancer, as well as gastric carcinoma and glioma [5]. The formulation FNI-5 showed anticancer activities with a maximum percentage of 99% of the drug with stability of six months and IC50 of 4.585g/mL [15-17].

CONCLUSION

NIR and ISR were formulated to evaluate anticancer activity against THP-1 cancer cells. The formulation FNI-5 has maximum drug content (99%) with both immediate and sustained release. Sustained-release pellets release drugs within 12 hours, whereas immediate-release pellets release drug release only by time of 2 hours. Final FNI-5 drug release kinetics were first order and Higuchi. In the final formulation of FNI-5, Nilotinib levels in NIR pellets and Ibrutinib in ISR pellets were 99.40±0.6% and 99.35±0.2% at 180 days under accelerated conditions (40°C ±2°C /75±5% RH). FNI-5 was stable for six months or 180 days after conducting stability testing. FTIR, XRD, and DSC data showed no significant changes in the degree of crystallinity of the drugs or polymorphic transition in the physical combination of drugs and polymers in the final formulation FNI-5. In FNI-5, the Ibrutinib dose was lowered to 420 mg, killing cancer cells synergistically with an IC50 of 4.585g/mL. Finally, nilotinib was immediately released. Ibrutinib sustained-release seal-coated pellets showed potential as a cancer chemotherapeutic agent because of their enhanced drug delivery for a more extended period and consistent favorable release, resulting in optimized absorption and reduced side effects.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Vishal Gupta was involved in data collection, making of the writing-original draft, language, figures, and tables of the manuscript. Jitendra Gupta was in charge of conceptualizing, reviewing, and editing the manuscript.

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