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FORMULATION AND EVALUATION OF BILAYER TABLETS OF TENOFOVIR AND SILYMARIN FOR HEPATITIS INDUCED LIVER CIRRHOSIS

O. Mohammed Fazan, B. Anandha Krishnan and G. Selvi *

Department of Pharmaceutics, C. L. BaidMetha College of Pharmacy, Chennai - 600097, Tamil Nadu, India.

Keywords:

Hepatitis B, Bilayer tablet, Tenofovir, Silymarin, Immediate-release, Sustained-release

Correspondence to Author:
G. Selvi

Associate Professor,
Department of Pharmaceutics,
C. L. BaidMetha College of Pharmacy, Chennai - 600097, Tamil Nadu, India.

E-mail: selviarunkumar@gmail.com

ABSTRACT: Background: Chronic hepatitis B requires effective antiviral therapy combined with hepatoprotective support to manage liver function and prevent disease progression. Bilayer tablets provide a versatile platform to deliver drugs with distinct pattern of the drug release exhibited by a single dosage form. **Objective:** The study aimed to develop a bilayer tablet containing an immediate-release layer of Tenofovir and a sustained-release layer of Silymarin to provide rapid antiviral action with prolonged hepatoprotective effects in a single dosage form. **Methods:** Bilayer tablets were developed by direct compression and optimized through eight trial batches. FTIR confirmed drug–excipient compatibility. Post-compression tests evaluated physical properties and dissolution behavior. Drug-release kinetics were analyzed using zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. Stability was assessed under ICH guidelines. **Results:** The optimized batch (F3: CCS in IR layer + HPMC K100M 10% in SR layer) demonstrated rapid Tenofovir release within 30 minutes and sustained Silymarin release over 12 hours. Kinetic modeling indicated Hixson-Crowell kinetics for Tenofovir (R^2 = 0.9855) and zero-order release with Super Case-II transport for Silymarin (R^2 = 0.9815, n = 1.36), confirming controlled and predictable release. FTIR spectra revealed no significant drug–excipient interactions. Stability studies showed no notable changes in physical, chemical, or dissolution properties during the study period. **Conclusion:** The developed bilayer tablet provides rapid antiviral action and sustained hepatoprotective effects, improving therapeutic outcomes, reducing dosing frequency, and enhancing patient compliance. This formulation is a promising strategy for managing chronic hepatitis B and warrants further *in-vivo* and clinical studies.

INTRODUCTION: The purpose of drug delivery systems is to transport drugs into or throughout the body using various technologies such as oral, injectable, topical, and inhalable routes. Conventional systems often release drugs immediately after administration, leading to fluctuating plasma concentrations and the need for frequent dosing [1].

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To overcome these limitations, advanced systems such as sustained-, controlled-, delayed-, and pulsatile-release systems, have been developed to optimize therapeutic efficacy and improve patient compliance [2, 3, 4].

Among these, bilayer tablets represent a versatile platform that combines two release profiles in a single unit. Typically, one layer provides immediate drug release for rapid onset of action, while the other ensures sustained release for prolonged therapeutic effect. This approach enhances treatment outcomes, lowering the need for frequent dosing, and improving compliance among patients, although challenges remain in

terms of formulation complexity, stability, and regulatory requirements [5, 6]. Viral hepatitis is a major global health concern, with hepatitis B and C accounting for the vast majority of chronic infections and liver-related complications worldwide. Chronic hepatitis B (CHB) is particularly challenging, requiring long-term antiviral therapy to suppress viral replication and prevent progression to cirrhosis or hepatocellular carcinoma. Tenofovir, a potent nucleotide analogue, is a first-line antiviral agent in CHB management, effectively reducing HBV DNA levels and disease progression. However, prolonged therapy necessitates strategies to improve patient compliance.

The combination of Tenofovir, a potent antiviral agent, and Silymarin, a hepatoprotective herbal extract, has been explored as a supportive strategy in the management of chronic hepatitis B. Tenofovir remains a cornerstone of antiviral therapy, effectively suppressing HBV replication and reducing disease progression. Silymarin, a well-known hepatoprotective flavonolignan extract, exhibits antioxidant and anti-inflammatory properties and has been widely studied for liver support. Though it does not possess direct antiviral activity, it reduces liver enzyme levels (ALT, AST) and improves overall liver function without interfering with Tenofovir's efficacy [7, 8].

Combining Tenofovir and Silymarin in a bilayer tablet offers a promising approach for managing CHB by uniting rapid antiviral action with sustained hepatoprotection in a single dosage form.

This dual-release system not only addresses therapeutic challenges but also enhances compliance among patients and treatment outcomes [9]. The objective of the study was to develop and examine a bilayer tablet comprising Tenofovir formulated in an immediate-release layer, combined with a sustained-release layer of Silymarin to address the therapeutic challenges of chronic hepatitis B. The study focused on achieving rapid antiviral activity from Tenofovir and prolonged hepatoprotective support from Silymarin in a single dosage form. Systematic pre-formulation, formulation trials, *in-vitro* dissolution, and kinetic modeling were conducted to optimize the formulation and confirm its performance.

MATERIALS AND METHODS:

Materials and Instruments: Drug substances, excipients, and chemicals required for the formulation of Tenofovir and Silymarin bilayer tablets were kindly provided by SaimirraInnopharm Pvt. Ltd., India. All materials used were of pharmaceutical and analytical grade.

All instruments and equipment required for the formulation and evaluation studies were made available and utilized at the facilities of SaimirraInnopharm Pvt. Ltd. (India).

Preformulation Studies (Characterization of Drug Substances): The samples of Tenofovir and Silymarin were characterized by various parameters to ensure their identity, purity and suitability for formulation.

Physical Appearance: Tenofovir: White, crystalline, hygroscopic powder. Silymarin: Pale yellow, crystalline, slightly hygroscopic powder.

Solubility: Tenofovir: Tenofovir exhibits limited solubility in water, phosphate buffer at pH 6.8, and 0.1N hydrochloric acid, while it is moderately soluble in ethanol and methanol. Silymarin: Silymarin is sparingly soluble in ethanol and methanol, and very slightly soluble in water, phosphate buffer (pH 6.8), and 0.1N HCl[10].

Melting Point Determination: The melting point of Tenofovir was found to be 112-115 °C, and for Silymarin 158–162 °C, which matched the literature values, confirming the identity of the received samples [11].

UV Spectroscopy: The λmax of Tenofovir and Silymarin were determined by scanning standard solutions (10 µg/mL) in the wavelength range of 200–400 nm using methanol. The λ_{max} was found to be 260 nm for Tenofovir and 288 nm for Silymarin, which were used for quantitative analysis.

Calibration Curve and Simultaneous Estimation: Standard solutions of Tenofovir (2–10 µg/mL) and Silymarin (2–10 µg/mL) were prepared in methanol. The absorbances of each solution were measured at both 260 nm and 288 nm to account for spectral overlap in the simultaneous estimation method.

Simultaneous estimation of both drugs in the bilayer tablet was carried out using the simultaneous equations method. Standard curve of Tenofovir and Silymarin are represented in **Fig. 1** and **2**.

TABLE 1: CALIBRATION CURVE OF TENOFOVIR AT 260NM AND 288NM

Calibration curve				
Concentration (µg/ml)	Absorbance (nm) (Tenofovir)	Absorbance (nm) (Silymarin)	Concentration (µg/ml)	Absorbance (nm) (Tenofovir)
	260nm	288nm	260nm	288nm
0	0	0	0	0
2	0.09	0.009	0.06	0.18
4	0.165	0.019	0.125	0.325
6	0.245	0.028	0.17	0.475
8	0.318	0.039	0.235	0.621
10	0.385	0.049	0.29	0.74

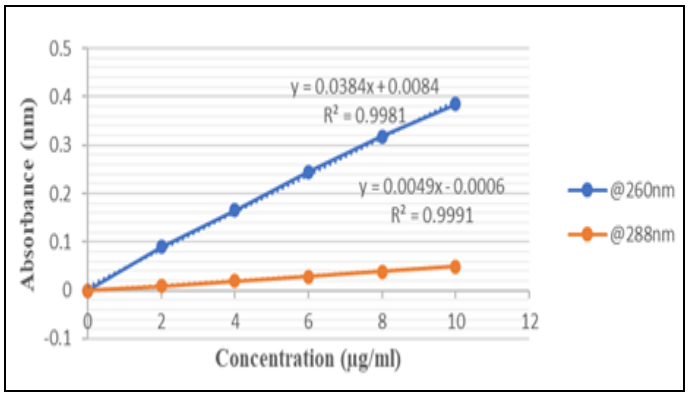

FIG. 1: CALIBRATION CURVE OF TENOFOVIR AT 260NM AND 288NM

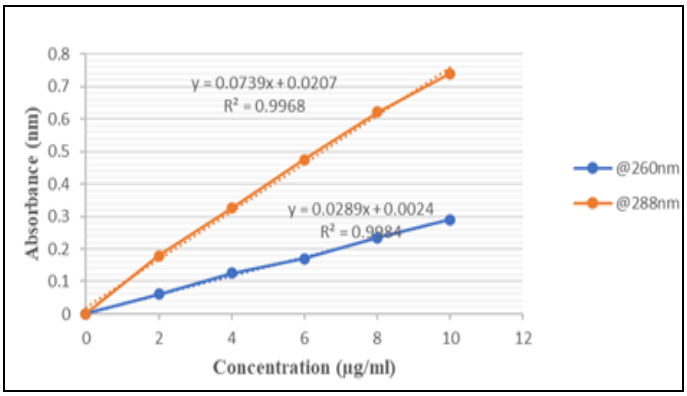

FIG. 2: CALIBRATION CURVE OF SILYMARIN AT 260NM AND 288NM

Simultaneous Equation: Mixture absorbances of Tenofovir and Silymarin were measured at 260 nm (A1) and 288 nm (A2). The amounts of each drug in the bilayer tablet were measured by applying the simultaneous equations method:

$$C_{Tenofovir} = (A_2b_1 - A_1b_2) / (a_1b_2 - a_2b_1)$$

$$C_{Silymarin} = (A_1a_2 - A_2a_1) / (a_1b_2 - a_2b_1)$$

A_1 and A_2 are the absorbance of the mixture at 260 nm and 288 nm respectively. a_1, a_2 = absorptivity of Tenofovir at 259 and 288 nm, b_1, b_2 = absorptivity of Silymarin at 259 and 288 nm

Absorptivity Coefficients Value: The absorptivity coefficients (a) required for simultaneous estimation were calculated from the relationship A=abc, where A is absorbance, b is the path length (1 cm), and c is the concentration of the standard solution.

TABLE 2: ABSORPTIVITY COEFFICIENTS VALUE

Drug	At 260 nm	At 288 nm
Tenofovir	a1=0.0385	a2=0.0049
Silymarin	b1=0.0290	b2=0.0740

These coefficients account for the contribution of each drug at both λmax values and are essential for calculating concentrations in the mixture.

Drug-Excipient Compatibility Study: FTIR spectra of Tenofovir, Silymarin, and their physical mixtures with excipients were recorded using a KBr pellet method in the range of 4000–400 cm^{-1} to evaluate potential drug-excipient interactions [12, 13].

Formulation of Tenofovir and Silymarin Bilayer Tablets: The bilayer tablets containing Tenofovir (immediate release layer) and Silymarin (sustained release layer) were prepared by direct compression method as per the composition given in **Table 3.**

Preparation of Immediate Release (IR) Layer: The IR layer was formulated using Tenofovir as the active pharmaceutical ingredient, with Lactose DCL21 as filler and a superdisintegrant (Crospovidone or Croscarmellose sodium, as per formulation).

All ingredients, except magnesium stearate and Aerosil, were sieved through a #40 mesh and blended by geometric dilution for 10 minutes to ensure uniformity. Aerosil and magnesium stearate (passed through #60 mesh) were then added as glidant and lubricant, respectively, and mixed gently for 2–3 minutes to obtain the IR layer blend.

Preparation of Sustained Release (SR) Layer: The SR layer comprised Silymarin as the active ingredient, with Dicalcium phosphate, Hydroxypropyl cellulose (HPC Klucel-LF), HPMC polymer (K15M/K100M), and MCC 102 as excipients. All ingredients, except magnesium stearate and Aerosil, were passed through a #40 mesh and mixed for 10 minutes. Aerosil and magnesium stearate (sieved through #60 mesh) were added and blended gently for 2–3 minutes to obtain a homogeneous SR layer blend.

Compression of Bilayer Tablets: The tablets were compressed using a Cadmach machine with D-type tooling and a single oblong, round-edged, capsule-shaped plain punch. The compression was performed at a speed of 20 rpm, applying a precompression force of 3 kN and a main compression force of 14 kN. Each tablet weighed 650 mg, with an average thickness of 4.67 mm and a hardness of 8.5 kg/cm².

First Layer Compression (IR Layer): The IR layer blend was loaded into the die cavity and lightly pre-compressed to form a uniform bed.

Second Layer Compression (SR Layer): The SR layer blend was added over the pre-compressed IR layer and final compression was applied at optimized pressure to produce bilayer tablets weighing 650 mg. The different formulations are designated with the formulation codes F1, F2, F3, F4, F5, F6, F7, and F8.

TABLE 3: FORMULATION TABLE OF BILAYER TABLET

Ingredients	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)	F5 (mg)	F6 (mg)	F7 (mg)	F8 (mg)
IR Layer								
Tenofovir	200	200	200	200	200	200	200	200
Lactose Monohydrate	85	85	85	85	85	85	85	85
Disintegrant CCS	9	9	9	9	-	-	-	-
CP	-	-	-	-	9	9	9	9
Magnesium Stearate	3	3	3	3	3	3	3	3
Aerosil	3	3	3	3	3	3	3	3
Total Weight of IR layer	300	300	300	300	300	300	300	300
SR Layer								
Silymarin	140	140	140	140	140	140	140	140
MCC 102	107.5	90	107.5	90	107.5	90	107.5	90
DCP Anhydrous	50	50	50	50	50	50	50	50
HPC Klucel LF	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5
HPMC K15M	35	52.5	-	-	35	52.5	-	-
K100M	-	-	35	52.5	-	-	35	52.5
Magnesium Stearate	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Aerosil	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Total Weight of SR layer	350	350	350	350	350	350	350	350
Total Weight of Tablet	650	650	650	650	650	650	650	650

Pre-Compression Evaluation: Pre-compression evaluation ensures that the powder has suitable flow, compressibility, and uniformity for direct compression. The following tests were performed [14, 15].

Angle of Repose: The angle of repose was determined to evaluate the powder's flow properties. It represents the steepest angle formed between the surface of a powder pile and the horizontal plane, calculated by the formula $\tan \theta = h/r$, where h is the height and r is the radius of the pile.

Bulk Density: Bulk density was measured as the mass of the powder divided by its volume prior to

tapping, reflecting the packing efficiency of the powder blend.

Tapped Density: Tapped density was measured after mechanically tapping the powder to obtain the maximum packing density.

Carr's Compressibility Index and Hausner's Ratio: Carr's index was calculated from the bulk and tapped densities to evaluate the compressibility and flow characteristics of the granules. Hausner's ratio was determined as an indirect measure of inter-particle friction and flowability.

Post-Compression Evaluation [16, 17, 18, 19]:

Tablet Thickness: Tablet thickness was measured with a Vernier caliper to verify consistency across all batches.

Weight Variation: From each batch, twenty tablets were randomly selected and weighed individually. The average weight and standard deviation were then calculated to evaluate weight uniformity.

Hardness: The mechanical strength of the tablets was assessed by measuring the hardness of five tablets from each formulation using a Monsanto hardness tester.

Friability: Twenty tablets were weighed and subjected to friability testing using a Roche friabilator, rotated at 25 rpm for 4 minutes. After the test, the tablets were dedusted and reweighed, and the friability was expressed as the percentage weight loss.

Disintegration Test: The disintegration of the immediate-release (IR) layer was tested using a disintegration apparatus. Tablets were immersed in 0.1 N HCl maintained at 37 ± 0.5°C, and the time required for complete disintegration of the IR layer was recorded.

Drug Content Uniformity: The assay of bilayer tablets was performed using the validated simultaneous estimation method. Twenty tablets were weighed, powdered, and an amount equivalent to the label claim was dissolved, filtered, and suitably diluted. Absorbances were measured at 260 nm and 285 nm, and drug content was calculated using the simultaneous equations. Results were expressed as the percentage of the labelled amount for each drug.

***In-vitro* Dissolution Studies:** Dissolution of the bilayer tablets was carried out using USP Type II apparatus (paddle) at 50 rpm and 37 ± 0.5°C. The IR layer was tested in 0.1N HCl for 2 hours, followed by the SR layer in pH 6.8 phosphate buffer for 10 hours.

Samples were withdrawn at specified intervals, filtered, and analysed at 260 nm and 285 nm. Percentage drug release was calculated using the simultaneous estimation method, and release profiles were compared with pharmacopeial specifications.

Release Kinetics: The *in-vitro* drug release profiles of the bilayer tablet were analyzed layer-wise to understand the release mechanism of each drug. The IR layer Tenofovir released rapidly within 30 minutes. The dissolution data were analysed using first-order, zero-order and Hixson-Crowell kinetic models. The SR layer exhibited controlled release of Silymarin over 12 hours. The dissolution data were fitted to various kinetic models. Zero-order kinetics describes drug release at a constant rate ($Q=K_0t$), while first-order kinetics assumes release proportional to the remaining drug ($\ln (100-Q) = \ln Q_0 - K_1t$). The Higuchi model ($Q = Kt^{1/2}$) represents drug release as a diffusion process, and the Korsmeyer–Peppas model ($M_t / M_\infty = K_Ht^n$) incorporates the release exponent n to indicate the mechanism of release. Here, Q is the percent drug released at time t; Q_0 is the initial drug content; K_0, K_1, K_H are the rate constants, and n characterizes the release mechanism [20, 21].

Stability Studies: The stability of the bilayer tablet, containing Tenofovir (IR) and Silymarin (SR) layers, was evaluated according to ICH Q1A(R2) guidelines over 30 days. Tablets were stored in HDPE containers under room temperature (25°C ± 2°C, 60% RH ± 5%) and accelerated conditions (40°C ± 2°C, 75% RH ± 5%). Samples were withdrawn at 0, 15, and 30 days and assessed for physical appearance, hardness, friability, and drug content of both layers to ensure maintenance of mechanical integrity, assay limits, and release characteristics, confirming the robustness and reliability of the formulation [22].

RESULTS AND DISCUSSION:

Drug-Excipient Compatibility Study: FTIR spectra of pure Tenofovir, pure Silymarin, and the bilayer physical mixture with excipients were recorded separately **Fig. 3, 4** and **5**. The characteristic peaks of Tenofovir (O–H/N–H stretching ~3400–3200 cm^{-1}, C=O stretching ~1700 cm^{-1}, C–N stretching ~1100 cm^{-1}) and Silymarin (O–H stretching ~3400 cm^{-1}, aromatic C=C stretching ~1600 cm^{-1}, C–O–C stretching ~1200–1000 cm^{-1}) were clearly retained in the spectra of the physical mixture without significant shifts or disappearance. This indicates the absence of chemical interaction between drugs and excipients. Thus, FTIR analysis confirmed the compatibility of Tenofovir and Silymarin with the selected excipients, supporting their suitability for bilayer tablet formulation.

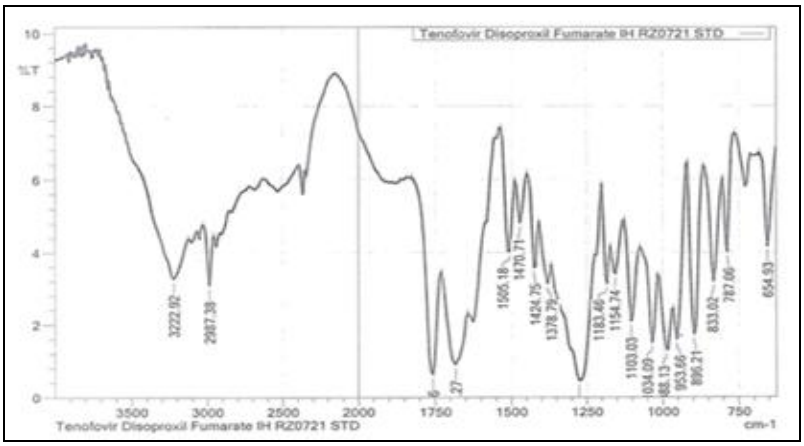

FIG. 3: FTIR SPECTRUM OF PURE TENOFOVIRDISOPROXIL FUMARATE

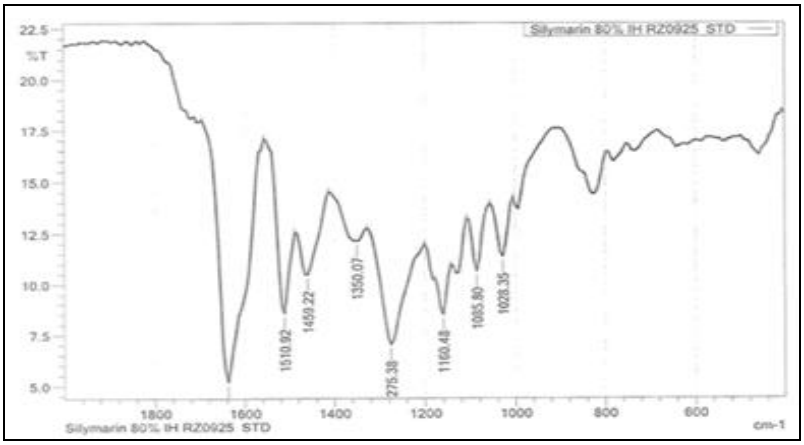

FIG. 4: FTIR SPECTRUM OF PURE SILYMARIN

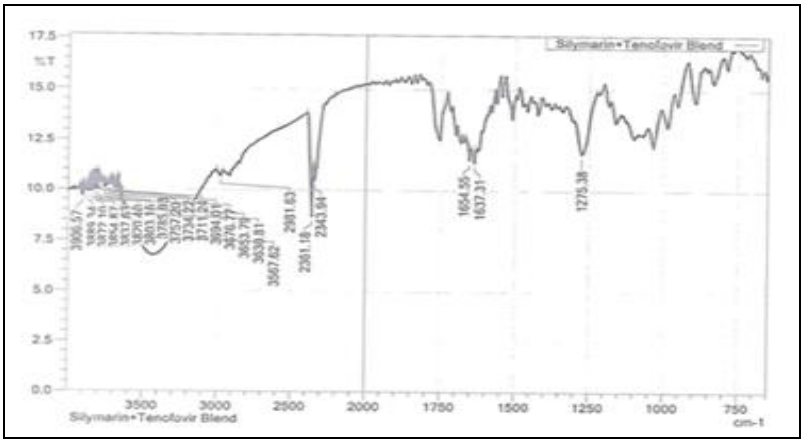

FIG. 5: FTIR SPECTRA OF BOTH TENOFOVIR LAYER AND SILYMARIN LAYER (BLEND)

Pre-Compression Evaluation: All bilayer formulations (F1–F8) showed acceptable pre-compression characteristics, suitable for direct compression. In the IR layer, formulations F1–F4

(containing CCS as superdisintegrant) exhibited good flow, with bulk density 0.328–0.350 g/ml, tapped density 0.372–0.397 g/ml, Hausner's ratio 1.13–1.15, Carr's index 11.82–13.22%, and angle of repose 30°57′–32°71′. Formulations F5–F8 (containing CP as superdisintegrant) also showed fair to good flow, with bulk density 0.328–0.332 g/ml, tapped density 0.382–0.387 g/ml, Hausner's ratio 1.15–1.17, Carr's index 13.08–14.58%, and angle of repose 34°67′–36°87′. In the SR layer, HPMC-based formulations (K15M and K100M at 10% and 15%) also demonstrated acceptable blend properties. Across all batches, bulk density was 0.316–0.341 g/ml, tapped density 0.364–0.400 g/ml, Hausner's ratio 1.14–1.17, Carr's index 12.97–14.75%, and angle of repose 30°65′–36°65′. Most formulations showed good flowability, while a few (F2 and F6) exhibited fair flow, still within acceptable limits for direct compression. All the pre-compression evaluation data of the powder blends are summarized in **Table 4.**

TABLE 4: PRE-COMPRESSION PARAMETERS OF BILAYER TABLET

Formulation code	Bulk density (g/ml)	Tapped density (g/ml)	Hausner's ratio	Carr's index (%)	Angle of repose (ɵ)	Flow property
Layer 1						
F1	0.337	0.384	1.13	12.23	31°66'	Good
F2	0.328	0.378	1.15	13.22	32°71'	Good
F3	0.35	0.397	1.13	11.83	30°57"	Good
F4	0.328	0.372	1.13	11.82	31°43'	Good
F5	0.328	0.384	1.17	14.58	36°87'	Fair
F6	0.33	0.385	1.16	14.28	35°45'	Good
F7	0.331	0.387	1.16	14.47	34°67'	Good
F8	0.332	0.382	1.15	13.08	36°34'	Fair
Layer 2						
F1	0.316	0.364	1.15	13.18	32°54'	Good
F2	0.336	0.393	1.16	14.50	35°78'	Fair
F3	0.321	0.375	1.16	14.40	30°65'	Good
F4	0.332	0.382	1.15	13.08	33°34'	Good
F5	0.336	0.387	1.15	13.17	33°56'	Good
F6	0.331	0.387	1.16	14.47	36°65'	Fair
F7	0.322	0.37	1.14	12.97	31°23'	Good
F8	0.341	0.4	1.17	14.75	32°54'	Good

Post-Compression Evaluation: The prepared bilayer tablets exhibited uniform thickness (4.65–4.66 mm) and complied with pharmacopeial limits for weight variation (650.8–655.7 mg). Hardness values ranged from 8.5–9.2 kg/cm², confirming adequate mechanical strength, while friability values remained well below 1% (0.02–0.06%), ensuring durability during handling and transportation. Disintegration times were within 7.30–8.45 minutes, meeting the requirements for immediate-release dosage forms. Drug content uniformity for both Tenofovir (99.24–102.19%) and Silymarin (99.46–100.61%) was within acceptable pharmacopeial limits, indicating uniform distribution of actives. Overall, both pre-compression and post-compression parameters demonstrated that the prepared bilayer tablets possessed satisfactory flow, compressibility, strength, disintegration, and drug content uniformity. All the post-compression evaluation data of the prepared bilayer tablets are summarized in **Table 5.**

TABLE 5: POST-COMPRESSION PARAMETERS OF BILAYER TABLET

Formulation code	Thickness (mm)	*Weight variation (mg)	Hardness (kg/cm2)	Friability (%)	Disintegration time (mins)	Drug Content	
						Tenofovir	Silymarin
F1	4.65	655.7	8.7	0.02	7.30	99.68	100.61
F2	4.65	651.7	8.5	0.03	7.56	100.33	100.35
F3	4.66	652.2	9.2	0.03	7.40	100.06	100.38
F4	4.65	650.8	8.8	0.05	7.30	99.24	100.46
F5	4.66	652.3	8.7	0.05	8.45	101.09	99.90
F6	4.65	653.2	8,5	0.06	8.35	99.35	100.25
F7	4.66	650.8	8.6	0.05	8.45	101.58	99.46
F8	4.65	652.2	9.1	0.02	8.40	102.19	99.79

***In-vitro* Dissolution Studies:** The *in-vitro* dissolution profiles of all eight bilayer tablet formulations (F1–F8) were evaluated to assess the performance of the Tenofovir IR layer and the Silymarin SR layer. In the IR layer, F1–F4 contained CCS, while F5–F8 contained CP as superdisintegrants. CCS-based formulations exhibited faster disintegration, releasing >50% of Tenofovir within 15 minutes and nearly complete release (>99%) by 30 minutes, whereas CP-based formulations showed slightly slower release. For the SR layer, different grades and concentrations of HPMC were tested. Formulations with HPMC K15M at 10% (F1, F5) released the drug too rapidly, while HPMC K15M at 15% (F2, F6) slowed release but did not achieve complete 12 h release. HPMC K100M at 15% (F4, F8) extended release beyond 15 h, exceeding the target, whereas HPMC K100M at 10% (F3, F7) provided a sustained release close to 12 h with consistent kinetics. Considering both layers, F3 (CCS in IR + HPMC K100M 10% in SR) demonstrated the most desirable biphasic release, with rapid Tenofovir release and sustained Silymarin release for 12 h, and was selected as the optimized formulation. The *in-vitro* dissolution data of all bilayer tablet formulations (F1–F4) are summarized in **Table 6** and F5-F8 are summarized in **Table 7**. The *in-vitro* release profiles of Tenofovir (IR layer) are depicted in **Fig. 6** and **7** and Silymarin (SR layer) are depicted in **Fig. 8** and **9.**

TABLE 6: *IN-VITRO* DISSOLUTION PROFILE OF FORMULATIONS (F1–F4)

Time (hr)	F1		F2		F3		F4	
	%CDR		%CDR		%CDR		%CDR	
	Tenofovir	Silymarin	Tenofovir	Silymarin	Tenofovir	Silymarin	Tenofovir	Silymarin
0	0	0	0	0	0	0	0	0
5 min	12.64	0.14	9.89	0.19	10.44	0.14	10.98	0.02
10 min	32.39	0.61	30.67	0.37	32.39	0.41	31.65	0.26
15 min	52.32	0.98	53.78	0.65	54.32	0.51	51.87	0.38
20 min	77.34	1.10	76.43	0.98	76.34	0.86	75.45	0.51
30 min	99.98	1.87	99.45	1.54	99.57	0.92	98.56	0.67
1 hr	99.23	12.44	100.35	10.34	100.10	9.21	99.94	0.98
2 hr	100.67	23.65	99.56	23.65	100.03	17.55	100.10	5.76
3 hr	99.34	35.54	99.67	35.65	99.90	25.99	99.92	12.76
4 hr	99.65	43.65	99.48	48.43	99.90	34.42	99.95	20.12
5 hr	100.54	55.76	99.89	59.76	99.99	42.23	99.96	27.12
6 hr	99.12	64.98	100.24	65.87	99.96	50.48	99.93	34.31
7 hr	99.76	76.54	99.76	72.43	99.89	59.27	99.89	41.98
8 hr	99.43	85.89	99.41	79.54	99.45	67.65	100.13	49.98
9 hr	100.76	95.76	99.12	85.78	99.89	76.12	99.23	57.12
10 hr	99.89	100.54	99.65	91.76	99.86	84.38	99.36	65.34
11 hr	-	-	100.65	96.98	99.84	93.06	99.87	73.21
12 hr	-	-	99.65	100.89	100.54	100.82	100.34	79.99
13 hr	-	-	-	-	-	-	100.08	87.65
14 hr	-	-	-	-	-	-	99.98	96.54
15 hr	-	-	-	-	-	-	100.28	100.97

TABLE 7: *IN-VITRO* DISSOLUTION PROFILE OF FORMULATIONS (F5–F8)

Time (hr)	F5		F6		F7		F8	
	%CDR		%CDR		%CDR		%CDR	
	Tenofovir	Silymarin	Tenofovir	Silymarin	Tenofovir	Silymarin	Tenofovir	Silymarin
0	0	0	0	0	0	0	0	0
5 min	10.32	0.18	8.99	0.12	9.89	0.15	10.72	0.03
10 min	28.65	0.56	27.57	0.55	29.72	0.39	27.99	0.19
15 min	48.66	0.95	49.15	0.76	46.83	0.55	45.67	0.4
20 min	74.23	1.29	75.87	1.01	72.62	0.8	74.29	0.55
30 min	91.98	1.65	92.65	1.65	91.73	0.96	92.61	0.71
1 hr	99.66	11.98	99.87	12.65	100.42	10.01	99.56	0.89
2 hr	99.78	25.76	99.48	24.98	99.63	18.23	99.45	6.23
3 hr	99.51	36.75	99.83	35.43	99.73	26.54	99.74	13.76
4 hr	100.44	45.23	100.74	47.99	99.81	34.98	99.83	21.54

5 hr	99.82	54.87	99.34	60.02	99.09	43.73	99.25	26.98
6 hr	99.38	65.98	99.75	66.87	99.87	51.56	99.78	35.32
7 hr	99.41	76.34	99.39	73.1	100.02	60.33	100.87	44.45
8 hr	99.95	85.99	99.83	78.98	99.87	68.32	99.08	50.32
9 hr	99.35	96.52	99.27	86.09	99.11	76.78	99.45	56.74
10 hr	99.89	100.22	99.32	90.65	99.79	85.21	99.57	62.87
11 hr	-	-	99.38	95.23	100.33	94.87	99.43	72.56
12 hr	-	-	100.68	100.81	99.94	100.1	99.87	78.88
13 hr	-	-	-	-	-	-	99.52	88.43
14 hr	-	-	-	-	-	-	100.67	97.44
15 hr	-	-	-	-	-	-	100.08	100.08

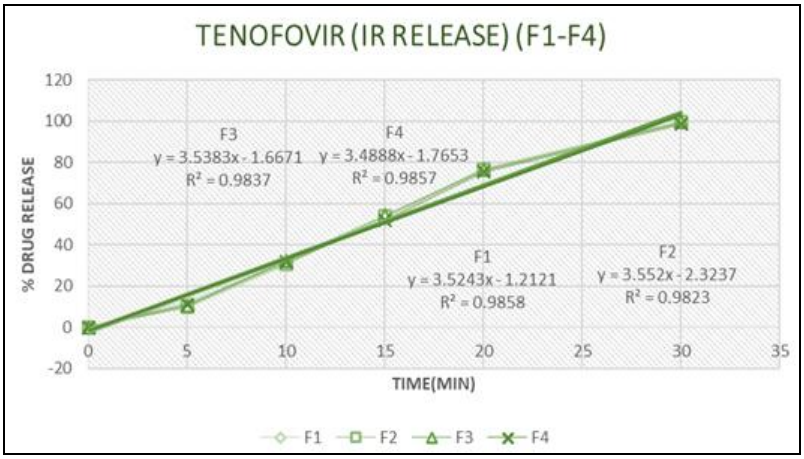

FIG. 6: *IN-VITRO* DRUG RELEASE PROFILE OF TENOFOVIR FROM F1 TO F4

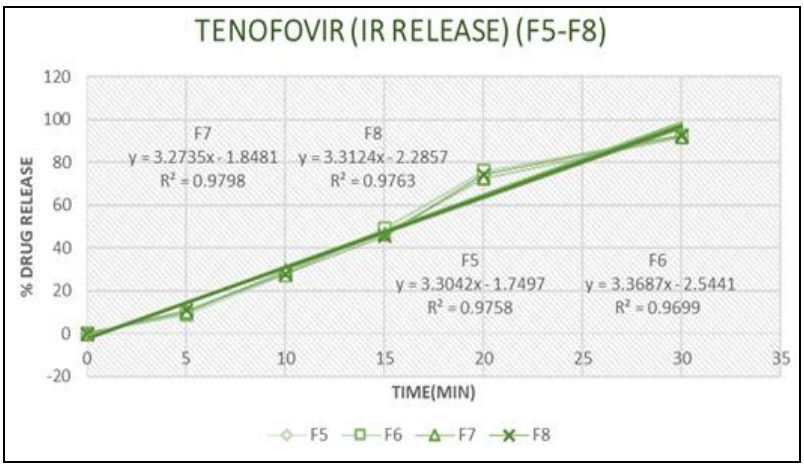

FIG. 7: *IN-VITRO* DRUG RELEASE PROFILE OF TENOFOVIR FROM F5 TO F8

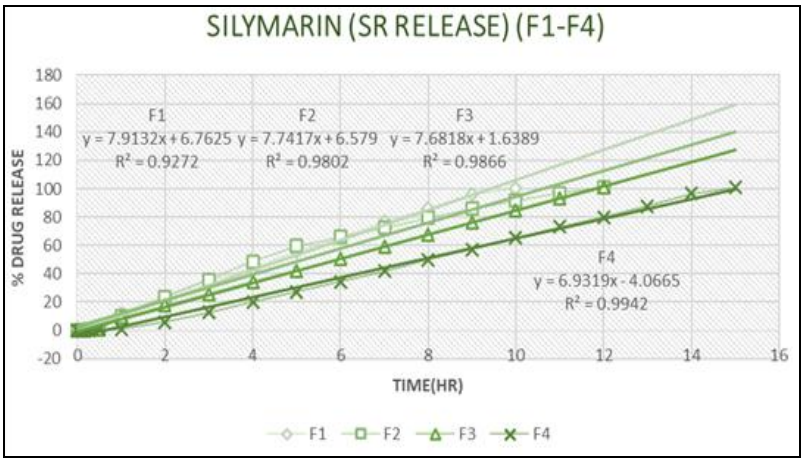

FIG. 8: *IN-VITRO* DRUG RELEASE PROFILE OF SILYMARIN FROM F1 TO F4

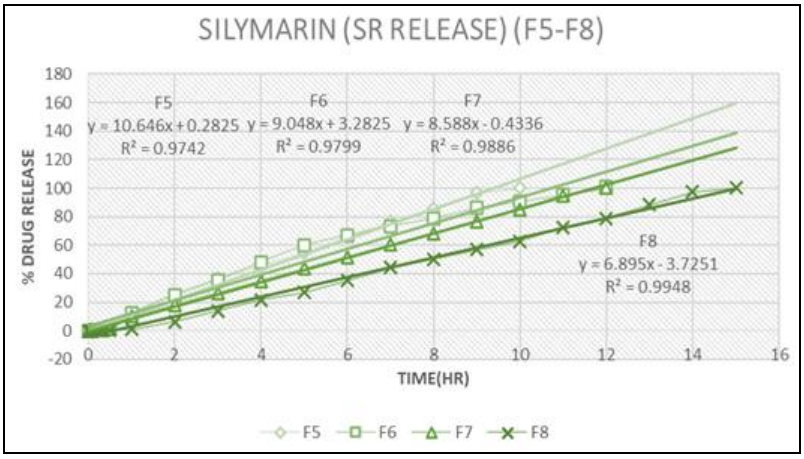

FIG. 9: *IN-VITRO* DRUG RELEASE PROFILE OF SILYMARIN FROM F5 TO F8

***In-vitro* Kinetic Studies:** The *in-vitro* drug release profiles of the bilayer tablet were analyzed layer-wise to elucidate the release kinetics of Tenofovir (IR layer) and Silymarin (SR layer). The Tenofovir IR layer followed the Hixson–Crowell model (R^2 = 0.9855), indicating surface area–controlled drug release due to rapid disintegration. The Zero-order model (R^2 = 0.9837) also showed good fit, while the First-order model (R^2 = 0.7929) showed poor correlation, confirming a surface area–dependent release mechanism. The fitting results about immediate release layer were listed in **Table 8** and corresponding model-dependent plots are presented in **Fig. 10, 11** and **12.** The SR layer of Silymarin demonstrated controlled release over 12 hours. Among the kinetic models tested, zero-order kinetics showed an excellent correlation (R^2 = 0.9992), whereas first-order kinetics displayed a poor fit (R^2 = 0.3039). The Higuchi model (R^2 = 0.9507) indicated a diffusion-based component, and the Korsmeyer-Peppas model (R^2 = 0.9815) with an n value of 1.36 suggested a non-Fickian (anomalous) release mechanism involving a combination of diffusion and matrix erosion. The fitting results about sustained release layer were listed in **Table 9**. Overall, these results demonstrate that the optimized F3 bilayer tablet successfully achieves rapid release of Tenofovir from the IR layer and sustained release of Silymarin from the SR layer, highlighting the effectiveness of the formulation design. All the R^2 values for the kinetic models are summarized in **Table 10** and corresponding model-dependent plots are presented in **Fig. 13, 14**, **15** and **16.**

TABLE 8: *IN-VITRO* DRUG RELEASE MODEL FOR TENOFOVIR (IMMEDIATE RELEASE)

Time (hours)	%CDR	% Cumulative drug remaining	Log of % Cumulative drug remaining
0	0	100	2
0.0833	10.44	89.56	1.95211
0.1667	32.39	67.61	1.83001
0.25	54.32	45.68	1.65972
0.3333	76.34	23.66	1.37401
0.5	99.57	0.43	-0.36653

TABLE 9: *IN-VITRO* DRUG RELEASE MODEL FOR SILYMARIN (SUSTAINED RELEASE)

Time (hours)	Log time	Square root of time	%CDR	Log of %CDR	% Cumulative drug remaining	Log of % Cumulative drug remaining
0	∞	0	0	0	0	0
0.0833	-1.07935	0.288617	0.14	-0.85387	99.86	1.999392
0.1667	-0.77806	0.408289	0.41	-0.38722	99.59	1.998216
0.25	-0.60206	0.5	0.51	-0.29243	99.49	1.997779
0.3333	-0.47716	0.577321	0.86	-0.0655	99.14	1.996249
0.5	-0.30103	0.707107	0.92	-0.03621	99.08	1.995986
1	0	1	9.21	0.96426	90.79	1.958038
2	0.30103	1.414214	17.55	1.244277	82.45	1.916191
3	0.477121	1.732051	25.99	1.414806	74.01	1.86929
4	0.60206	2	34.42	1.536811	65.58	1.816771
5	0.69897	2.236068	42.23	1.625621	57.77	1.761702

6	0.778151	2.44949	50.48	1.703119	49.52	1.694781
7	0.845098	2.645751	59.27	1.772835	40.73	1.609914
8	0.90309	2.828427	67.65	1.830268	32.35	1.509874
9	0.954243	3	76.12	1.881499	23.88	1.378034
10	1	3.162278	84.38	1.92624	15.62	1.193681
11	1.041393	3.316625	93.06	1.968763	6.94	0.841359
12	1.079181	3.464102	99.99	1.999957	0.01	-2

TABLE 10: KINETIC MODELS WITH R^2 VALUE

Formulation	Kinetic Model					
	Zero order "R^2"	First order "R^2"	Higuchi model "R^2"	Korsmeyer-Peppas model		Hixson-Crowell model "R^2"
				"R^2"	Slope	
Immediate Release Tenofovir	0.9837	0.7929	-	-	-	0.9855
Sustained Release Silymarin	0.9992	0.3039	0.9507	0.9815	1.3608	-

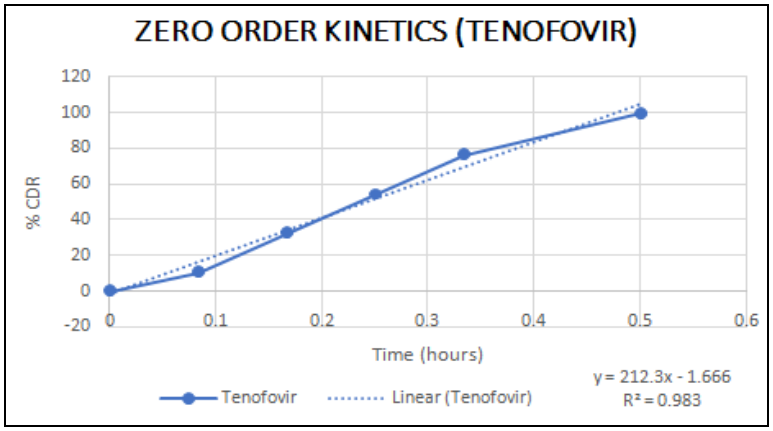

FIG. 10: ZERO ORDER RELEASE KINETICS FOR TENOFOVIR

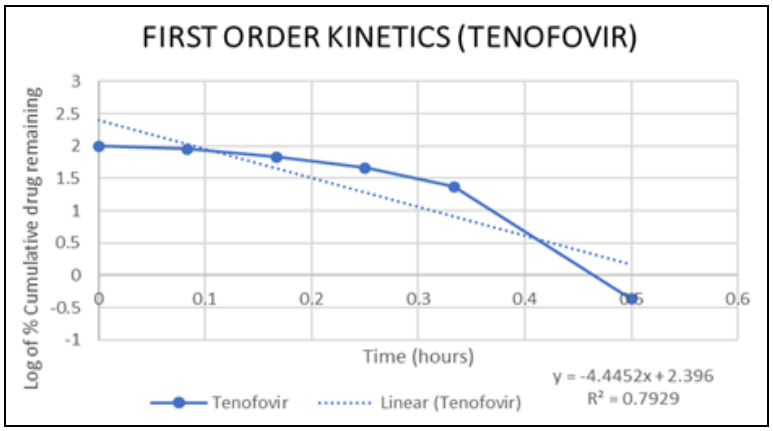

FIG. 11: FIRST ORDER RELEASE KINETICS FOR TENOFOVIR

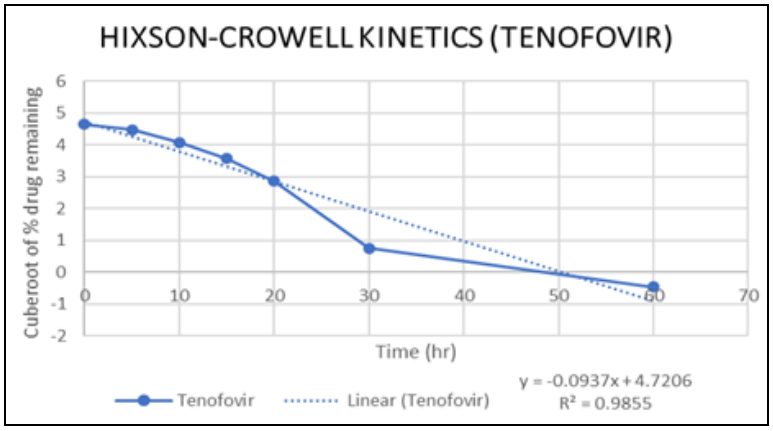

FIG. 12: HIXSON-CROWELL KINETICS FOR TENOFOVIR

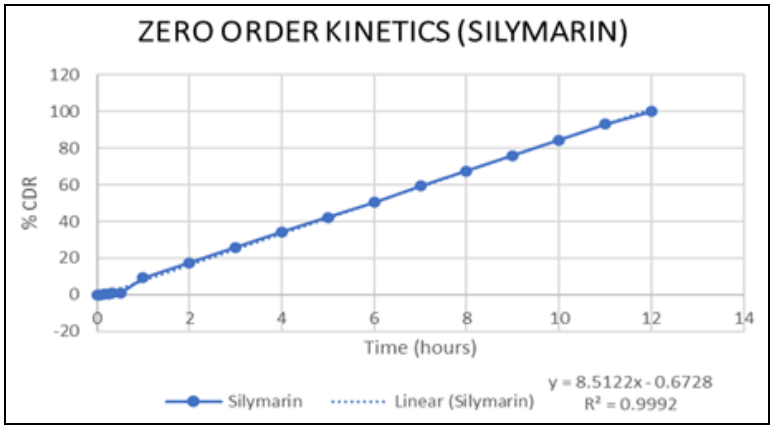

FIG. 13: ZERO ORDER RELEASE KINETICS FOR SILYMARIN

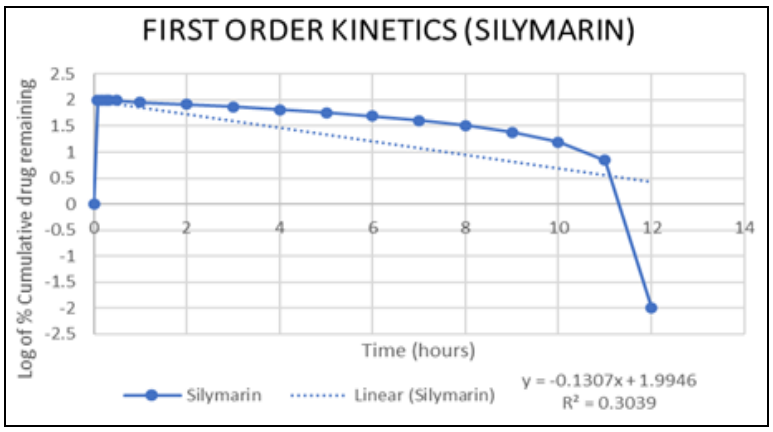

FIG. 14: FIRST ORDER RELEASE KINETICS FOR SILYMARIN

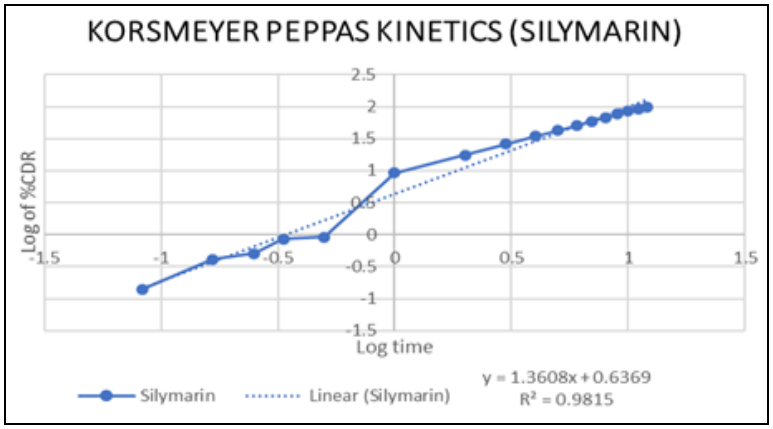

FIG. 15: KORSMEYER-PEPPAS RELEASE KINETICS FOR SILYMARIN

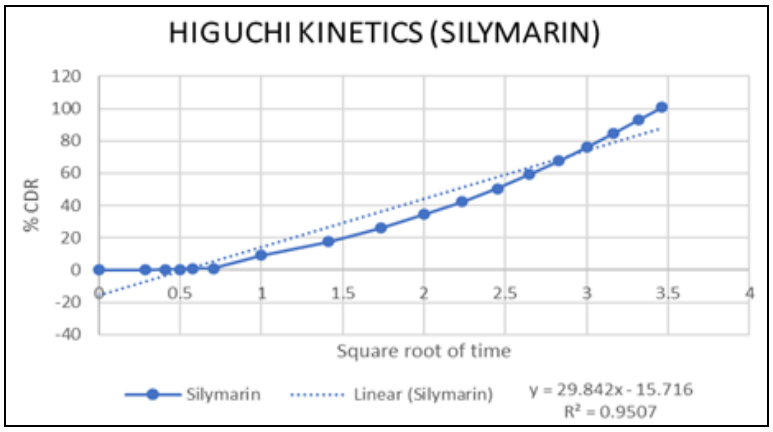

FIG. 16: HIGUCHI RELEASE KINETICS FOR SILYMARIN

Stability Studies: Stability studies were carried out for the optimized bilayer tablet formulation (F3) in accordance with ICH guidelines to assess the effect of storage conditions on product quality. The results obtained **Table 11** indicate that all evaluated parameters remained within acceptable limits under both storage conditions, with no significant changes observed in physical appearance, mechanical strength, drug content and invitro drug release. The optimized bilayer tablet formulation (F3) was found to be stable under both room temperature and accelerated conditions, demonstrating that the product maintains its quality attributes during the study period. This confirms the suitability of the formulation for further development and potential scale-up.

TABLE 11: STABILITY REPORT OF BILAYER TABLET

Stability condition	Hardness (kg/cm2)			Friability (%)			Drug content (%)						Drug release (%)					
	Initial	15 days	30 days	Initial	15 days	30 days	Initial		15 days		30 days		Initial		15 days		30 days	
							Ten	Sil	Ten	Sil	Ten	Sil	Ten	Sil	Ten	Sil	Ten	Sil
Room temperature	8.5±0.3	8.6±0.3	8.6±0.3	0.02	0.02	0.03	99.61	100.3	99.66	99.64	99.56	99.84	100.8	100.2	100.7	100.6	100.1	100.6
40°C ± 2°C, 75% ± 5% RH	8.5±0.2	8.5±0.5	8.5±0.5	0.03	0.03	0.03	99.30	100.2	99.61	100.2	99.51	100.4	99.98	100.1	100.4	100.7	100.5	100.3

CONCLUSION: The present study successfully developed a bilayer tablet of Tenofovir (Immediate Release) and Silymarin (Sustained Release) using direct compression. The formulation strategy addresses the therapeutic needs of hepatitis-induced liver cirrhosis by combining rapid antiviral action with prolonged hepatoprotective effects in a single dosage form. Among eight trial formulations, F3 (CCS in IR layer + HPMC K100M 10% in SR layer) was optimized, achieving the desired biphasic release profile — immediate Tenofovir release within 30 minutes and sustained Silymarin release over 12 hours. Kinetic modeling confirmed that Tenofovir release followed Hixson-Crowell kinetics, while Silymarin release followed zero-order kinetics with Super Case-II transport, ensuring predictable and controlled drug release. Stability studies under ICH conditions further validated the robustness of the optimized formulation, with no significant variations in physical, chemical, or release parameters. Overall, this bilayer tablet system enhances therapeutic efficacy, reduces dosing frequency, and improves patient compliance, representing a promising platform for integrated antiviral and hepatoprotective therapy. Further *in-vivo*, pharmacokinetic, and clinical evaluations are warranted to establish its clinical applicability.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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