Design, Prepare and In Vitro Evaluation of Bilayer Tablet Containing Clavulanic Acid as Immediate Release Layer and Amoxicillin as Sustained Release Layer

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ABSTRACT :
The present work aimed to design, prepare and evaluate bilayer tablets containing clavulanic acid as an immediate-release (IR) layer and amoxicillin as a sustained-release (SR) layer. The IR layer was formulated by direct compression using clavulanate with microcrystalline cellulose and Super disintegrates to ensure rapid disintegration, while the SR layer of amoxicillin was prepared by direct compression method using hydroxypropyl methylcellulose (HPMC) as the hydrophilic matrix former. Bilayer tablets were compressed on a double-layer rotary press and evaluated for pre- and post-compression quality parameters. All formulations complied with pharmacopeial requirements for weight variation (799–801 mg), hardness (5.5–6.8 kg cm⁻²) and friability (<0.3 %), demonstrating adequate mechanical strength and low risk of layer separation. Disintegration time of the IR layer was within 12–27 min, ensuring prompt availability of clavulanic acid. Assay of the active ingredients showed amoxicillin 73.7–85.7 % and clavulanic acid 78.8–86.9 %, with the optimized formulations achieving >80 % of label claim for both drugs. In-vitro dissolution testing revealed a rapid initial release of the IR layer followed by controlled release of amoxicillin from the SR matrix. After 8 h, all batches released more than 92 % of amoxicillin, while maintaining a moderate release (≈20–35 % at 2 h) during the early phase, consistent with sustained-release performance. The release data fitted best to the Higuchi model, indicating diffusion-controlled drug release through the hydrated HPMC matrix. This study demonstrates the feasibility of a single-tablet bilayer system capable of providing the desired immediate release of clavulanic acid and sustained release of amoxicillin, potentially improving therapeutic efficacy and patient compliance in the management of infections requiring combined β-lactam/β-lactamase inhibitor therapy.
Keywords: Clavulanic acid, Amoxicillin, FTIR studies, Direct compression method, polymers, In vitro drug release studies.

I. INTRODUCTION

The development of novel drug delivery systems has gained significant attention in recent years to overcome the limitations of conventional dosage forms, enhance pharmacological response, and improve patient compliance.[1]A bilayer tablet combining immediate release clavulanic acid and sustained release amoxicillin offers an effective strategy for synchronized pharmacological action: rapid β-lactamase inhibition followed by extended antibacterial activity.[2] Such a dosage form may reduce dosing frequency, improve bioavailability, enhance therapeutic outcomes, and potentially reduce antibiotic resistance associated with subtherapeutic exposure.[3]Amoxicillin, a broad-spectrum β-lactam antibiotic belonging to the penicillin class, is widely used in the treatment of bacterial infections caused by susceptible gram-positive and gram-negative organisms.[4] The objective of the present study is to design, formulate, and evaluate a bilayer tablet comprising an immediate release clavulanic acid layer and a sustained release amoxicillin layer. The formulation was optimized using suitable polymers, excipients, and manufacturing conditions to achieve desired drug release profiles.[5] The prepared bilayer tablets were subjected to physicochemical characterization and in-vitro dissolution studies to assess their performance and suitability as an improved oral antibiotic delivery system.

II. EXPERIMENTAL WORK

MATERIALS

Clavulanic Acid and Amoxicillin were procured from Hetero Labs, HYD. HPMC E15, Ethyl cellulose, Sodium starch glycolate and Croscaramellosewere obtained from Synpharma Research Labs, Hyderabad. Other chemicalsand the reagents used were of analytical grade.

METHODOLOGY

Identification of Clavulanic Acid and Amoxicillin by FT-IR

IR spectroscopy was used to determine the molecular interaction between polymer and drugs. All physical mixtures and drugs sample were mixed with dried KBR in ratio 1:1. Then small fraction of mixtures was compressed on automatic IR Press at pressure 10 tones to form transparent pellet. Then the IR spectrum of pellets was taken on FTIR spectrophotometer. [6,20]

FORMULATION DEVELOPMENT[7,8,9]

Preparation of bilayer tablets:

Step 1: Weigh all the ingredients in required quantity.
Step 2: Transfer all ingredients into a mortar, triturate for 10minutes until to get fine powder and sieve the material. (#60)
Step 3: then transfer the material into blender for proper distribution of drug in blend for 10minutes.
Step 4: then addition of lubricant, mix well.
Step 5: Perform the micromeritic properties (Precompression studies).
Step 6: Compression.

Table-1: Composition of Immediate release tablets

S.NO.	INGREDIENTS	F1 (mg)	F 2 (mg)	F 3 (mg)	F 4 (mg)	F 5 (mg)	F 6 (mg)	F 7 (mg)	F 8 (mg)
1	Clavulanic Acid	125	125	125	125	125	125	125	125
5	Croscaramellose Sodium	5	10	15	20	-	-	-	-
6	Sodium starch glycolate	-	-	-	-	5	10	15	20
8	Microcrystalline Cellulose	15	10	5	-	15	10	5	-
9	Talc	2	2	2		-	2	2	2
	Magnesium stearate	3	3	3	3	3	3	3	3
10	Total weight	150	150	150	150	150	150	150	150

Table-2: Composition of Sustained release tablets

S.NO.	INGREDIENTS	F1 (mg)	F 2 (mg)	F 3 (mg)	F 4 (mg)	F 5 (mg)	F 6 (mg)	F 7 (mg)	F 8 (mg)
1	Amoxicillin	500	500	500	500	500	500	500	500
5	HPMC E15	25	50	75	100	-	-	-	-
6	Ethyl cellulose	-	-	-	-	25	50	75	100
8	Microcrystalline Cellulose	120	95	70	45	10	10	10	10
9	Talc	2	2	2	2	2	2	2	2
	Magnesium stearate	3	3	3	3	3	3	3	3
10	Total wt	650	650	650	650	650	650	650	650

SR layer (Amoxicillin) — blend

- Weigh ingredients for SR layer
- In a suitable blender, add MCC and polymers mix 1–2 min to distribute polymer.
- Add amoxicillin slowly and blend for 5–10 min (low speed) to obtain uniform blend.
- Add Lubricant and glidant blend 1–2 min to improve flow.
- Sift final blend to break agglomerates if any.
- Take samples for precompression tests (flow, bulk/tapped density, LOD, blend uniformity assay).

IR layer (Clavulanic acid) — blend

- Weigh IR ingredients.
- Add clavulanic acid and mix gently 5–8 min to ensure homogeneous distribution
- Add MCC and super disintegrates and blend 1–2 min.
- Add magnesium stearate (lubrication) and blend for final 1–2 min (short mixing to avoid over-lubrication).
- Take samples for precompression tests.

Bilayer compression (Direct compression)

- Compression order: Compress the SR layer first (bottom) then deposit IR powder and compress second (top). This reduces risk of crushing the SR matrix and gives a stronger interface.
- Set tablet press parameters: punch/die size matching target weight and thickness; pre-compression and final compression forces tuned during development. Monitor tablet hardness, weight, and layer integrity during runs.
- For each tablet: dose SR powder into die → pre-compress (low force) to form lower layer → add IR powder on top of pre-compressed SR → final compression at target force.
- Collect sample tablets every 5–10 minutes for in-process QC (weight, thickness, hardness). Monitor for lamination, capping, and delamination.

EVALUATION PARAMETERS

Weight Variation: Twenty tablets were selected randomly and weighed individually. Average weight was calculated and compared the individual tablet weight to the average weight.[10]

Thickness and diameter: Thickness and diameter of tablets were accurately measured by using digital Vernier calliper for desired uniformity in size and shape. [11]

Hardness: Tablet requires certain amount of strength or hardness which was measured by Monsanto hardness tester. Ten tablets were randomly picked from each formulation and was subjected for relative hardness and the value were expressed in Kg/cm ^{2}. [12]

Friability: The tablets were subjected to the test of friability with initial weight (Wi) almost equivalent to 6.5g of the tablets. The tablets were allowed to fall on it from a height of 6 inches while the friabilator drum was rotated at 25 rpm for 4 minutes.[13] The final weight (Wf) of the tablets after subjecting to friability was noted and the friability was calculated according to the formula

Friability (%) =(initial weight–final weight)/initial weight*100

Disintegration test: Six tablets were selected randomly from each batch for the disintegration test (Electrolab ED-2L). Disintegration test was performed in simulated gastric fluid using Electrolab Disintegration tester (USP). Disintegration time (DT) was measured for immediate release layer tablets and also for bilayer tablets. [14]

Drug Content:20 tablets were accurately weighed and powdered. Then powder equivalent to 10 mg of Repaglinide was shaken vigorously with 50 ml of 0.1 M hydrochloric acid for 10 min and added sufficient 0.1M hydrochloric acid to produce 100 ml and filtered. Each ml of filtrate was suitably diluted to 10 ml distilled water. The absorbance of resulting solution was measured at maximum 241 nm. From the absorbance the drug content of the tablets was calculated. [15]

In-vitro dissolution Study In vitro drug release study was performed using type II (paddle) apparatus (Electro lab TDT- 08L plus, Dissolution tester USP Mumbai, India) at 50 rpm in 900 ml simulated gastric fluid 1.2 pH for 1hr. and after 6.8 phosphate buffer for 7 hrs. Temperature was maintained at 37 ± 0.5oC. The 5 ml sample was withdrawn at predetermined time intervals and replaced with same fresh dissolution media to maintain sink condition. The withdrawn samples were filtered through membrane filter 0.45µm, suitably diluted and analyzed by using UV spectrophotometer (UV Lab India 3000+) at λmax 247 nm. [16]

Kinetics of In-vitro drug release:[17] To study the release kinetics of In-vitro drug release data of above selected batches were applied to kinetic models such as zero order, first order, Higuchi and Korsmeyer- Peppas.

Zero order
Where K0 is the zero-order rate constant expressed in units of concentration/time and t is the time in hrs.
First order Where C0 is the initial concentration of drug, K is the first order constant, and t is the time in hrs.

Higuchi equation

$$\%R=Kt^{0.5}$$

This model is applicable to systems with drug dispersed in uniform swellable polymer matrix as in case of matrix tablets with water soluble drug.

Korsmeyer-Peppas equation :

$$\%R=Kt^{n}$$

This model is widely used, when the release phenomenon could be involved.

Stability Studies and Storage Condition:[18-19]Store packaged samples at ICH conditions (e.g., 40°C/75% RH for accelerated; 25°C/60% RH long term). At predefined intervals (1, 2, 3, months for accelerated; test assay, dissolution, hardness, moisture, appearance, layer adhesion. Acceptance: Meet release spec at each time point. Monitor degradation products of clavulanic acid especially.

III. RESULTS AND DISCUSSION

Fourier transformation infra-Red (FTIR) analysis :
Infra-red spectroscopy analysis was performed by Fourier Transformation Infrared Spectrophotometer Alpha Brooker FTIR (Tokyo, Japan). The instrument was calibrated by using polystyrene film.

Fourier Transformation Infra-Red (FTIR) analysis of Clavulanic Acid :

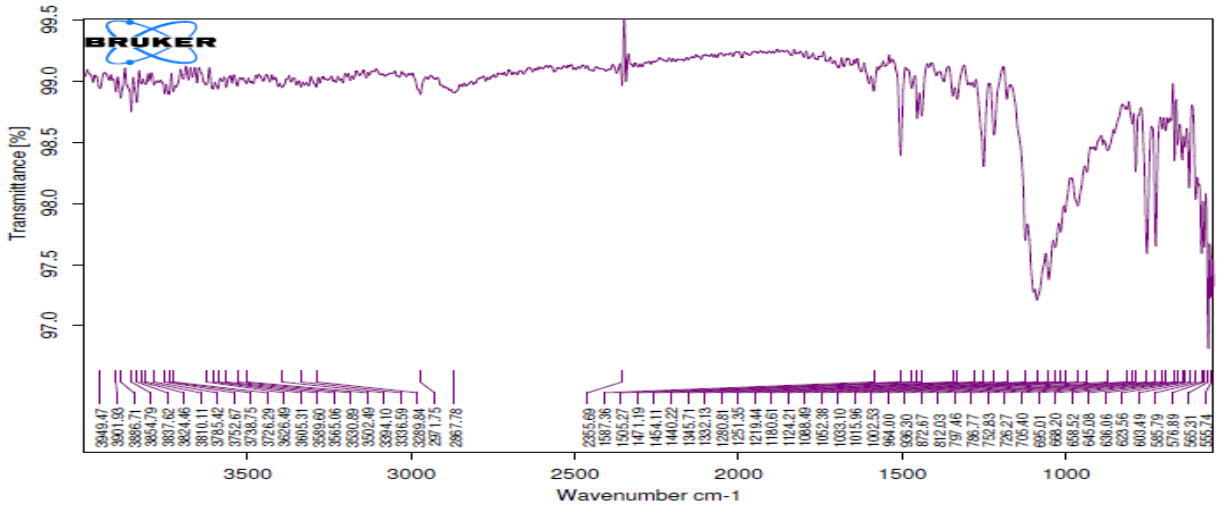

Fig-1: FTIR Studies of Clavulanic Acid

Fourier Transformation Infra-Red (FTIR) analysis of Amoxicillin :

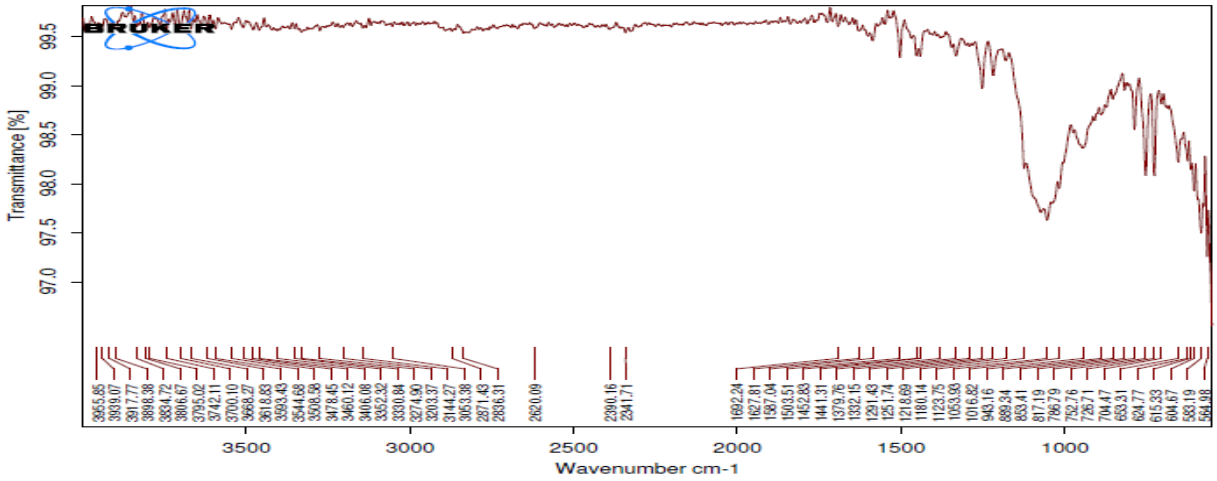

Fig-2:FT-IR graph for Amoxicillin

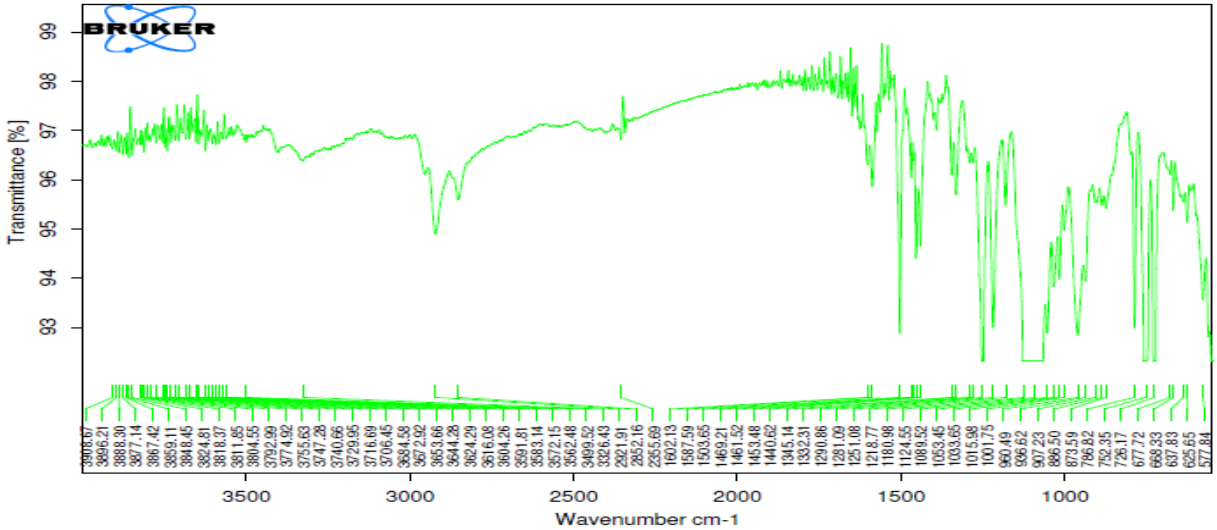

Fig-3:FT-IR graph for Balayer optimized formulation

The IR spectrum of Clavulanic Acid and Amoxicillin Drug Excipients mixture was shown. In the present study, it has been observed that there is no chemical interaction between Clavulanic Acid and Amoxicillin and the polymers used. From the figure it was observed that there were no changes in these main peaks in IR spectra of mixture of drug and polymers, which show there were no physical interactions because of some bond formation between drug and polymers. This further confirms the integrity of pure drug and compatibility of them with excipients.

EVALUATION OF PREPARED BILAYERED TABLETS FOR POST COMPRESSION PARAMETERS:

Table-3: Evaluation of post compression parameters for Bilayered tablets

Parameter	F1	F2	F3	F4	F5	F6	F7	F8
Weight variation	800	799	801	800	799	800	799	800
Thickness (mm)	3.9	3.5	3.8	3.1	4.1	3.6	3.9	4.2
Hardness (kg/cm^2)	5.9	5.6	6.2	5.5	6.3	6.8	5.9	6.1
Friability (%)	0.15	0.26	0.19	0.24	0.23	0.19	0.17	0.15
Disintegration time (min)	19	22	25	27	23	19	18	12
Assay of Amoxicillin	73.69	75.81	79.58	80.12	82.36	83.69	78.85	85.69

Assay of Clavulanic Acid	79.86	78.82	80.12	82.36	82.34	85.96	79.45	86.93

Weight variation: Individual tablet weights ranged from 799 to 801 mg, which is well within the ±5% limit specified in the pharmacopeia for tablets of this size. This confirms uniform die fill and good flow of both immediate- and sustained-release granules during compression.

Thickness and hardness: Tablet thickness varied between 3.1 and 4.2 mm, reflecting minor differences in powder bed packing and compression force. Hardness values were consistently within 5.5–6.8 kg cm^{-2}, indicating adequate mechanical strength for handling and packaging without risk of breakage or chipping. The absence of capping or lamination further supports good inter-layer adhesion.

Friability: All formulations exhibited friability well below the 1 % USP limit (0.15–0.26 %), confirming satisfactory resistance to abrasion and suitability for routine handling.

Disintegration time: The disintegration time of the immediate-release layer ranged from 12 to 27 min. Most batches disintegrated within the 30 min pharmacopeial limit for uncoated tablets. F8 showed the shortest disintegration (12 min), likely due to a higher proportion of superdisintegrant or optimal moisture content, while F4 exhibited the longest time (27 min), suggesting that compression force or binder content could have retarded water penetration.

Assay of active ingredients: Amoxicillin content ranged from 73.69 % (F1) to 85.69 % (F8), while clavulanic acid content was 78.82 % (F2) to 86.93 % (F8). Although all batches contained both actives, early formulations (F1–F3) gave lower amoxicillin assay values (<80 %), indicating either incomplete mixing of the sustained-release granules or possible degradation of amoxicillin during processing. Progressive optimization of granulation and compression conditions in later batches appears to have improved uniformity, with F5–F8 achieving both actives above 80 % of label claim.

In vitro Dissolution studies

Table-4: *In-Vitro* dissolution studies of all formulations

Time	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	12.69	22.58	27.89	29.67	16.39	17.63	16.98	17.89
2	33.58	33.69	35.69	36.49	37.48	38.46	36.89	35.16
3	42.58	42.50	43.37	45.27	46.38	47.98	45.12	46.19
4	55.69	55.89	56.91	55.69	56.29	55.38	56.37	55.82
5	67.49	69.72	67.89	68.12	63.97	65.29	63.18	65.24
6	75.48	76.89	77.82	78.49	79.68	72.22	73.65	75.69
7	80.12	80.25	82.16	83.26	85.39	83.19	84.59	82.12
8	92.36	92.38	94.35	95.67	96.37	93.46	94.56	98.69

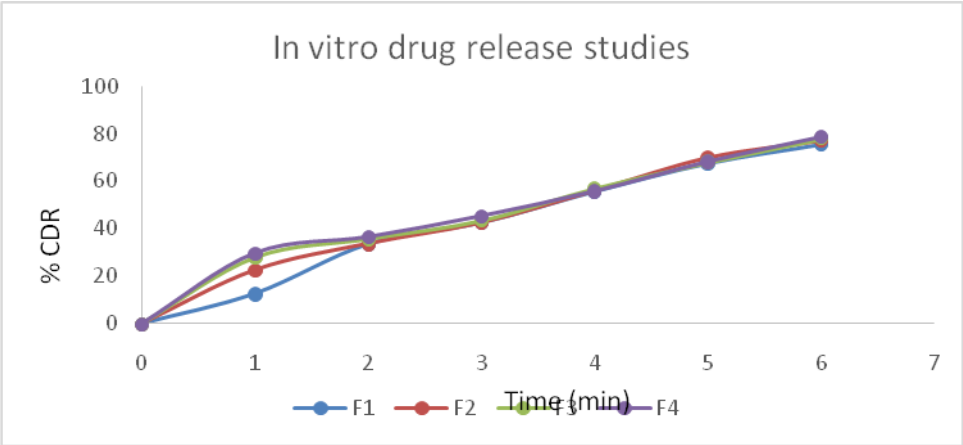

Fig-4: Drug release studies of F1-F4 formulations

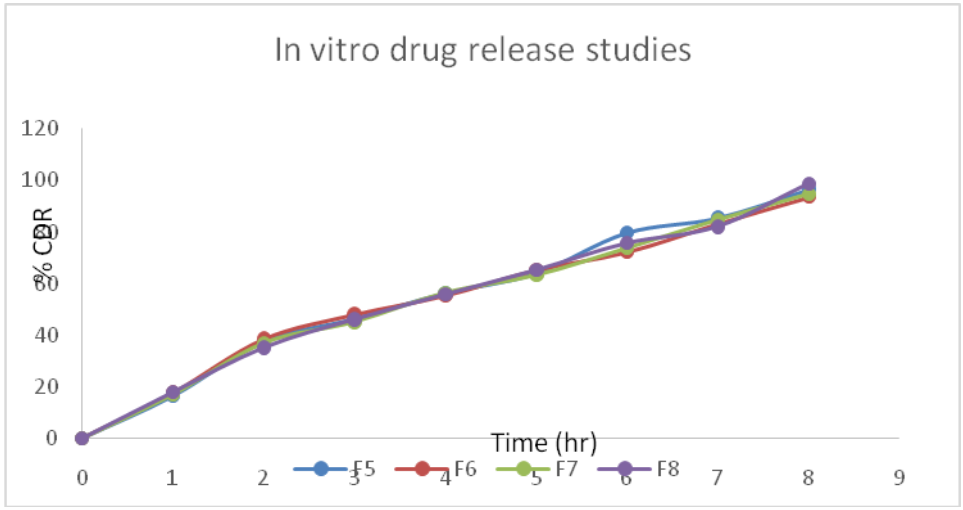

Fig-5: Drug release studies of F5-F8 formulations

All eight bilayer tablet formulations (F1–F8) exhibited a progressive increase in cumulative drug release over the 8-hour dissolution study. The release was initially rapid during the first 2 h, followed by a more gradual increase and achieving >90 % drug release by 8 h in every formulation. This pattern is consistent with the intended design: an immediate release of clavulanic acid combined with a sustained-release amoxicillin layer.

All formulations demonstrated satisfactory sustained-release behavior with complete drug release within the targeted 8-hour period. Minor variations in early-phase release can be tuned by adjusting polymer grade or concentration. Among the tested batches, F8 provided the most desirable profile, combining a modest initial burst with nearly complete release by 8 h, making it the most promising candidate for a bilayer tablet delivering clavulanic acid as an immediate-release layer and amoxicillin as a sustained-release layer.

Drug release kinetic profile
Zero order kinetics

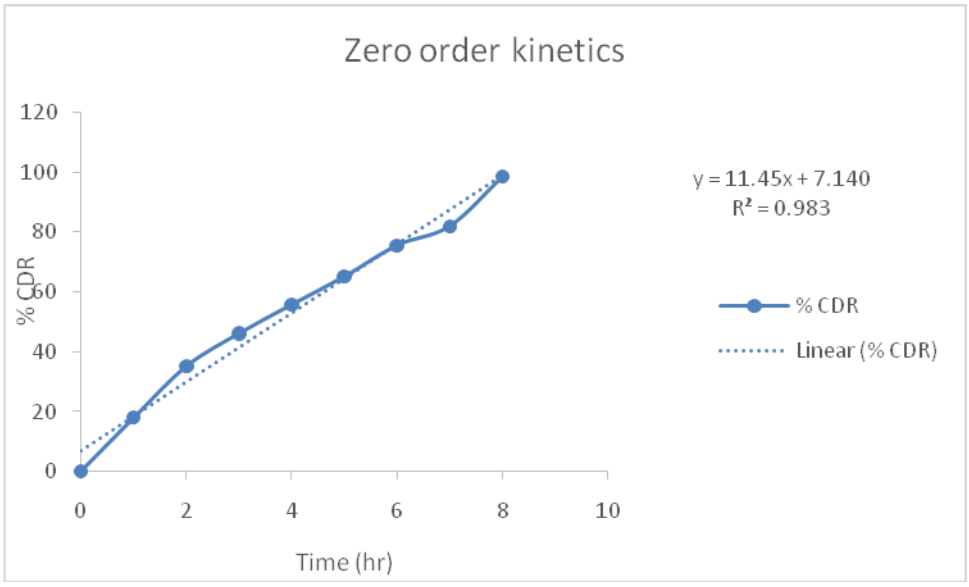

Fig-6: Drug release kinetics of zero order kinetics
First order kinetics

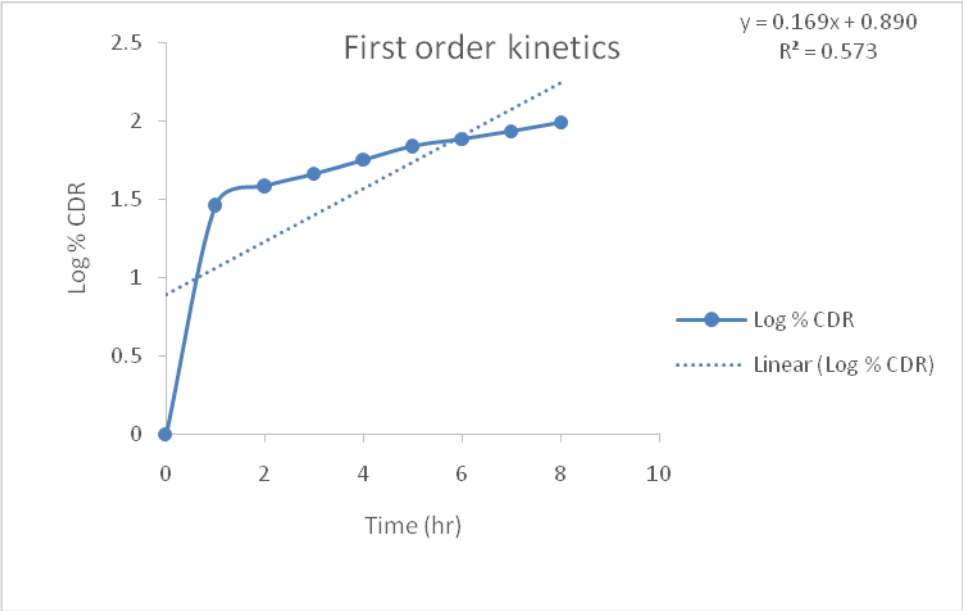

Fig-7: Drug release kinetics of First order kinetics

Higuchi model

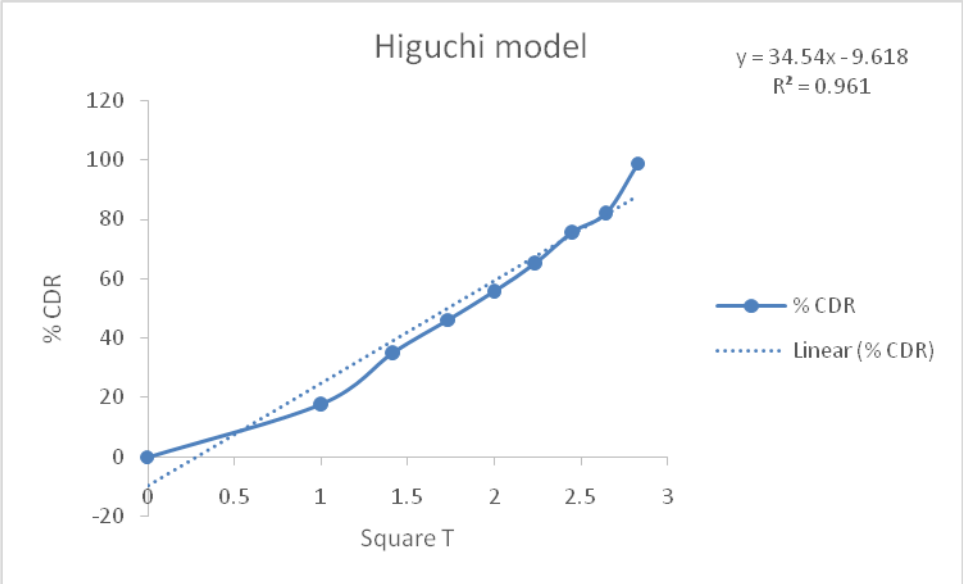

Fig-8: Drug release kinetics of Higuchi plot

Korsmeyer peppas

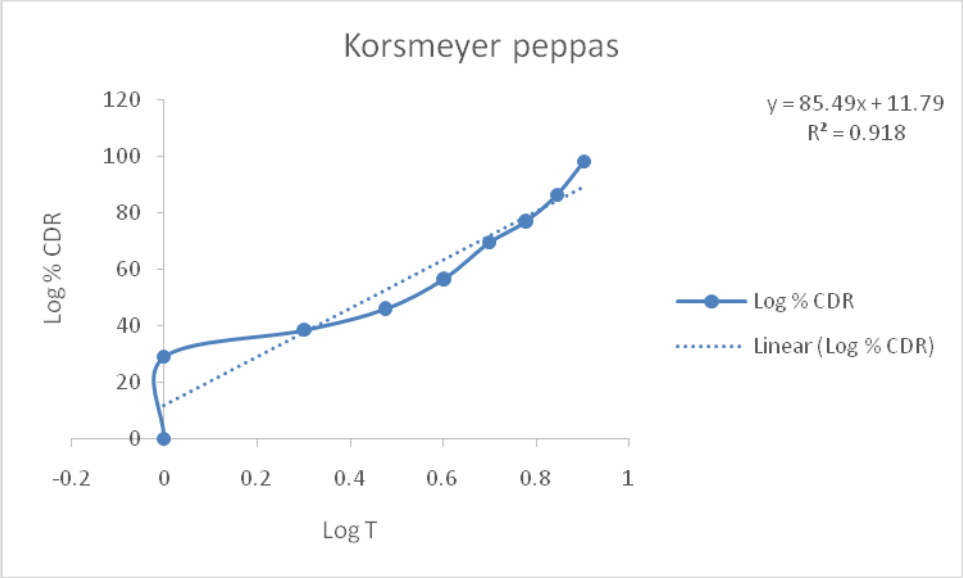

Fig-9: Drug release kinetics of Korsmeyer peppas

The drug release from the tablets were explained by the using mathematical model equations such as zero order, first order, Higuchi`s Korsmeyer-Peppas equation. Based on the regression values it was concluded that the optimized formulation F8 followed zero order release where the regression value was found to be 0.983. It was also found that the drug was released by diffusion as the regression in Higuchi's plot was 0.961.

Stability Study

There was no significant change in physical and chemical properties of the tablets of formulation F-8 after 90 days. Parameters quantified at various time intervals were shown.

Table-5: Stability study for optimized formulation

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-8	**25^{0}C/60%RH**	98.69	97.68	96.58	95.81	**Not less than**
F-8	**30^{0}C/75% RH**	98.69	97.52	96.16	95.82	**Not less than**
F-8	**40^{0}C/75% RH**	98.69	97.16	96.32	95.60	**Not less than**

CONCLUSION

Bilayer tablets combining clavulanic acid (IR) and amoxicillin (SR) were successfully formulated and met all quality control requirements.The immediate-release layer ensured prompt delivery of the β-lactamase inhibitor, while the sustained-release layer provided a controlled release of amoxicillin over 8 h, potentially maintaining therapeutic plasma levels and improving patient compliance.This bilayer approach offers a promising strategy for the co-delivery of β-lactam antibiotics and β-lactamase inhibitors, reducing dosing frequency and enhancing the overall effectiveness of antimicrobial therapy.

REFERENCES

1. Benet LZ, Kroetz DL, Sheiner LB. Pharmacokinetics: The Dynamics of Drug Absorption, Distribution, and Elimination. In: Goodman & Gilman's The Pharmacological Basis of Therapeutics. 13th ed. McGraw-Hill; 2018.

2. Munjal M, Kaur G, Garg R. Formulation and Evaluation of Bilayer Tablets: A Review. Int J Appl Pharm Sci Res. 2020;5(2):24-32.
3. Bussemer T, Bodmeier R. Formulation and Evaluation of Sustained Release Matrix Tablets. Eur J Pharm Biopharm. 2003;56(2):261-270.
4. Rani S, Gupta GD. Design and Evaluation of Bilayer Tablet for Immediate and Sustained Release of Combination Antibiotics. Int J Pharm Sci Res. 2018;9(9):3687-369.
5. Nanjwade BK, Adichwal SA, Manvi FV. Bilayer Tablets for Combination Therapy. J Pharm Res. 2011;4(6):1819-1823.
6. Patel DM, Prajapati BG. Formulation and In-Vitro Evaluation of Sustained Release Amoxicillin Tablets. Int J Pharm Sci Rev Res. 2010;4(2):123-130.
7. Sampath Kumar K P, Bhowmik D, Chiranjib, Chadira M and Tripathi K K, "Innovations in sustained release drug delivery system and its market opportunities', J Chem Pharm Res, 2010, 2(1), 349-360.
8. Shaikh T. K., Gadhave M.V., Jadhav S.L., Gaikwad D.D., "Different techniques of bi-layer tablet: a review", International Journal of Universal Pharmacy and Life Sciences, 2012, 2(2), 450-460.
9. Shangraw R, Mitrevej A, Shah M. A new era of tablet disintegrants. Pharm Technol 1980; 4(10): 49–57.
10. Sharma A, Jain A, Purohit A, Jatav R and Sheorey RV, "Formulation and evaluation of aceclofenac fast dissolving tablets", Int J Pharm & Life Sci, 2011, 2 (4), 681-686.
11. Shiyani B, Gattani S, Surana S, "Formulation and evaluation of bi-layer tablet of Metoclopramide hydrochloride and Ibuprofen", AAPS Pharm Sci Tech, 2008, 9(3), 818-827.
12. Sowmya, C.Suryaprakash, Reddy, S.G.Tabasum, V.Varma, "An overview Bilayer Tablets", International Journal of Pharmacy Technology, 2012, 4(2), 2143-2156.
13. Su vakanta dash et. al., kinetic modeling on drug release from controlled drug delivery systems, Acta Poloniae Pharmaceutica ñ Drug Research, Vol. 67 No. 3 pp. 217ñ223, 2010.
14. Kulkarni A and Bhatia M. Development and evaluation of bilayer floating tablets of Amoxycillin and lovastatin for biphasic release profile. Iran J Pharm Res. 2009;8(1):15-25.
15. Mohideen S and Satyanarayana T. Development and evaluation of two layered tablet of glimepiride and metformin hydrochloride for the treatment of hyperglycemia. Inter JBioPharam. 2011;2(1):31-35.
16. Yadav A and Jain DK. Formulation development and in vitro characterization of player and floating
17. Bioadhesive tablets of propranolol hydrochloride. Asia J Phar Life Scie.2011;1(1):2.
18. Dey S and Chatto PS. Formulation and evaluation of fixed dose combination of bilayer gastro retentive matrix tablet containing atorvastatin as fast release and Amoxycillin as sustained release. BiomedRes Inter. 2014;10(1155):396106
19. Bhargav Bhongiri*, Vadivelan Ramachandran, Bharathi Bathula, Nagarani Racha, Development and characterisation of fenofibrate-loaded nanoparticles, International Journal of Advanced Research In Medical & Pharmaceutical Sciences, 2021, Volume.6, Issue.4.
20. Bhargav Bhongiri*, Vadivelan Ramachandran, and Raman Suresh Kumar. "Preformulation Studies of S-Equol." Journal of Pharmaceutical Negative Results, 2022, 1020-1029.IF-0.654.