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Enhancement of Antibacterial Activity of Clarithromycin using Piperine and its Formulation Development

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Clarithromycin, Piperine, Gastroretentive Floating tablets, Design Expert 11 software.

Abstract

The current study focuses on increasing antibacterial action of clarithromycin and its formulation. Although various clarithromycin tablets are available in the market, but its bioavailability is around 45% and dose given is 250-500mg twice daily. We propose to make use of Piperine for the first time in the prepared formulations to enhance its bio-efficacy and reduce dose. The literature has described Piperine as a potent bio-efficacy booster. Preformulation studies of pure drug Clarithromycin was done by determining its melting point, FTIR, DSC and λmax determination, Standard Calibration curve, etc. which confirmed drug. The compatibility of the drug with the excipients was then assessed using DCS, XRD, and SEM, which revealed that the drug is compatible with every excipient. Zones of inhibition against Staphylococcus aureus and Escherichia coli were measured after mixing CL and piperine in various ratios (1:0.5, 1:1, 1:1.5, 1:2, 1:3, and 1:4). Initially MIC of standard drug was evaluated against Staphylococcus aureus and Escherichia coli. Out of all the ratios, the 1:2 ratio displayed the greatest activity, following which a persistent zone of inhibition was seen. The optimized ratio, 1:2, was employed to develop the formulation. Gastro retentive effervescent floating tablets of Clarithromycin using different concentrations of HPMC K15, Polyethylene Oxide and Sodium Bicarbonate were taken. Formulation was optimized by using Design Expert 11 software, total 9 batches F1- F9 were formulated using two independent variables PEO (X1) and HPMC K15 (X2) whereas Floating Lag Time (Y1) and % Drug Release after 24 hr (Y2) were selected as dependent variable. Out of all these formulations F7 was found to be the optimized formulation. All the formulations were evaluated by various parameters and the results obtained were within the limits. Optimized formulation was also studied for release kinetics.

1. Introduction

Infectious diseases are the leading cause of global morbidity and mortality. *Staphylococcus aureus* (S. aureus) and *Escherichia coli* (E. coli) are a major cause of various humans and animal's infections. A few illnesses that people get in the community and in hospitals are seriously caused by E. coli and S. aureus. One of the most prevalent nosocomial organisms that causes enterocolitis and urinary tract infections (UTIs) is E. coli. Additionally, S. aureus is an etiological infection agent that contributes significantly to morbidity and mortality. [1]

A substance that is antimicrobial either kills or prevents the growth of germs. A chemical component or physical agent could be the microbial agent. These substances prevent harmful organisms like bacteria, fungus, parasites, viruses, and others from growing and reproducing. Antimicrobial prophylaxis and antimicrobial chemotherapy are the terms used to describe the use of antimicrobial medications to treat and prevent infections, respectively.

Minimum inhibitory concentration (MIC) is the lowest concentration of a chemical which prevents visible growth of a bacterium. MIC results are crucial in diagnostic labs to confirm microbiological resistance to an antimicrobial agent and to track the action of new antimicrobials. Clinicians utilise MIC scores to determine an appropriate antibiotic dose and which medications to provide to patients with particular infections. This is crucial because populations of bacteria that have been

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exposed to a medicine insufficiently or to a broad-spectrum antibiotic may develop resistance to it. Therefore, MIC scores aid in improving outcomes for patients and preventing evolution of drug-resistant microbial strains. There are various methods to determine the MIC such as agar dilution method, broth dilution, micro-dilution. Agar dilution and broth dilution are the most commonly used techniques to determine the minimal inhibitory concentration (MIC) of antimicrobial agents [2].

Clarithromycin (CLA) is a macrolide antibiotic with broad spectrum of activity. It is bacterial protein synthesis inhibitor. It is administered to treat skin and soft tissue infections, as well as respiratory tract infections. It lowers CYP3A4 enzyme activity, which gradually inhibits the metabolism of both the medicine being co-administered and the patient. The gastrointestinal tract (GI) quickly and completely absorbs CLA. An oral dosage of CLA has a 55% bioavailability in the systemic circulation due to first-pass metabolism. A dose taken orally is recovered as the parent chemical in around 22% of cases, with the remaining 18% of cases being recovered as the parent compound in urine and 4% in faeces. As the drug is effective when the plasma fluctuations are minimized, sustained release dosage form of CLA is desirable. The short biological half life of drug (~3–5 h) also favors development of sustained release formulation.

Gastro retentive drug delivery (GRDDS) [17-22] is one of the site specific drug delivery for the delivery of the drugs at stomach. Oral drug delivery is widely used in pharmaceutical field to treat the diseases. Some drugs are absorbed at specific site only; these require release at that specific site. Gastro retentive formulation shows various advantages like enhanced bioavailability, reduced frequency of dosing by sustaining drug deliver, achieved targeted therapy for local ailments in the upper GIT, improved selectivity in receptor activation and minimized adverse activity at the colon, etc. As early as 1962, the idea of a floating drug delivery system was discussed in the literature. Its bulk density is lower than that of gastric fluids, therefore it floats in the stomach for a long time without slowing down the rate at which the stomach empties. The medicine is slowly released from the system at the desired rate while floating on the gastric contents. As a result, the GRT is raised and the oscillations in plasma drug concentration are better managed. FDDS CLA is stable in gastric acidic medium and has a narrow absorption window in gastrointestinal tract. Hence increasing the gastric retention time of the CLA in the stomach may improve its bioavailability and therapeutic efficacy [3].

Piperine (1-Piperoyl piperidine) is a significant alkaloid found in the Piperaceae family plants Piper nigrum Linn. and Piper longum Linn. and it has a variety of therapeutic uses. It has been demonstrated to have bioavailability-improving effects with a variety of structurally and therapeutically varied medications. Piperine enhances the bioavailability of different drugs in allopathic system of medicine like Indomethacin, phenytoin, ofloxacin, Oxytetracycline, cefotoxime sodium & cyclosporine A. Bio-efficacy enhancing property of Piperine may be attributed by increasing absorption, blood supply to the GIT and enzymes like gamma-glutamyl transpeptidase, Leucine amino peptidase and Glycyl-glycine dipeptidase activity due to the changes in enzyme kinetic, which participate in active and passive transport of nutrients to the intestinal cells. Additionally, by interacting with nearby lipids and hydrophobic protein regions due to its apolar nature, which may reduce the tendency of membrane lipids to act as stearic constraints to enzyme proteins and thereby modify enzyme conformation, bioefficacy is increased by decreasing HCl secretion, which prevents the breakdown of some drugs. [7-11]

having an advantages like sufficient structure to form a cohesive gel barrier, maintaining an overall specific gravity lower than that of gastric contents and drug dissolve slowly enough to serve as a drug reservoir.

Clarithromycin is highly soluble in acid and its bioavailability is about 45%, so high dose is required with twice dosing. Therefore, increasing the dose form's stomach residence time in the bacterium's ecological niche is a sensible strategy to increase the antibiotic's therapeutic efficiency. Because of the large levels of clarithromycin in the stomach, the infection will be effectively treated locally. This necessitates the creation of clarithromycin dosage formulations that are gastro-retentive.

2. Experimental Methods

CLA is obtained as gift sample from Sun Pharma, Mumbai and piperine is obtained as gift sample from Synthyte,

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Kochi, Kerla. All the required chemicals were procured from S.D. Fine Chemicals, Mumbai and Loba Chemicals, Mumbai.

1.Preparation of physical mixture of clarithromycin with piperine by Kneading method.

The mixture of CL and Piperine was prepared in 1:0.5, 1:1, 1:1.5, 1:2, 1:3 and 1:4 molar ratios by Kneading method [30-32].

- **Kneading method**

CL with Piperine in different molar ratios (1:0.5, 1:1, 1:1.5, 1:2, 1:3 and 1:4) was taken in mortar and 10ml of a solvent blend of DMSO is added while triturating to get slurry like consistency. Then thick slurry was kneaded for 45 min. and dried at 40^0 C for 24 hrs, pulverized and finally passed through sieve no.40. Then prepared mixture were kept in desicators over the fused calcium chloride

2. Antibacterial activity testing [32-52]

a) Sample Preparations

Solutions of drug-piperine mixture in different molar ratios (1:0.5, 1:1, 1:1.5, 1:2, 1:3 and 1:4) were prepared in Dimethyl Sufoxide (DMSO). Further Concentrations of prepared solutions were prepared using water. Clarithromycin (10mg/ml) was used as a positive control and DMSO as a negative control.

b) Antibacterial Assay

The modified agar well diffusion method was used to test the antibacterial activity of various ratios. Nutrient agar plates were seeded with 0.2 ml of a 24 hour broth culture of S. aureus. For one hour, the plates were dried. Four evenly spaced wells were bored into each plate using a sterile 8 mm borer, and then 0.5 ml of solutions in various ratios of mixture and clarithromycin were added there at random. For 24 hours, the plates were incubated at 370C. The diameter of the inhibitory zones (measured in mm) was used to assess the antibacterial activity. Three times the experiments were conducted.

c) Determination of minimum inhibitory concentrations (MIC)

3. The lowest concentration of a substance needed to inhibit microbial growth is known as the MIC. The CL-Piperine mixture was dissolved in DMSO to a concentration of 100 mg/ml in order to calculate the respective minimum inhibitory concentration values. After that, water was added to these solutions to create various concentrations. Then, 0.5 l of each of these solutions was added to each cup separately. There were three duplicates of each test run.

4. Formulation Development [53-77]

1. Preparation of powder blend for Clarithromycin Floating tablets

Clarithromycin Floating tablets were prepared by Direct Compression method. All the ingredients were sifted through 40# sieve, mixed thoroughly in mortar using pestle. Finally, glidant and lubricant were added with proper mixing.

Table 1. Formulation of Clarithromycin Floating Tablets

Batch	Drug (mg)	Piperine (mg)	MCC (%)	PEO (%)	HPMC (%)	Sod. Bicarbonate (%)	Mg. Sterate (%)	Talc (%)
F1	50	100	33	30	-	6	0.5	0.5
F2	50	100	31	30	-	8	0.5	0.5
F3	50	100	31	30	-	10	0.5	0.5

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F4	50	100	21	40	-	8	0.5	0.5
F5	50	100	11	50	-	8	0.5	0.5
F6	50	100	31	23	7	8	0.5	0.5
F7	50	100	31	25	5	8	0.5	0.5
F8	50	100	31	27	3	8	0.5	0.5
F9	50	100	31	20	10	8	0.5	0.5

3. Result and Discussions

1. Preformulation study

a. Organoleptic Properties

Table 2. Organoleptic Properties of CL

Parameters	**Properties**
Colour	White colour powder
Odour	Odourless

b. Melting Point Determination

Melting point of CL was determined by Capillary Method. It was found to be 222^0C. The observed melting point of drugs was confirmed with the standard melting point of that drug.

c. Solubility determination

It is soluble in DMSO, acetone, slightly soluble in methanol, ethanol, and acetonitrile, and practically insoluble in water.

d. Determination of λ max

The maximum absorption wavelength of CL was found at 210 nm. Therefore 210 nm was recorded as λmax of CL. The observed λmax of drug was found to be similar as given in literature. Hence the drug was considered to be pure. The UV spectrum of CL was showed in Figure 1.

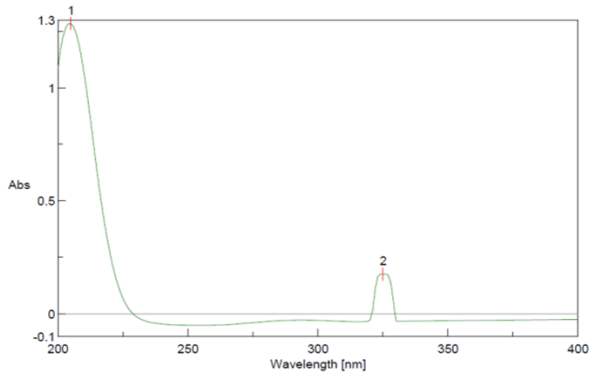

Figure 1. UV spectra of Clarithromycin

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e. Drug confirmation by FT-IR

The IR spectrum of CL (Figure 2) and Piperine (Figure 3) showed similar characteristics peaks to that of reported spectra of CL and Piperine respectively. From FTIR study the sample of CL and Piperine was identified. The observed peak and their functional groups were given in Table 3 and 4 respectively.

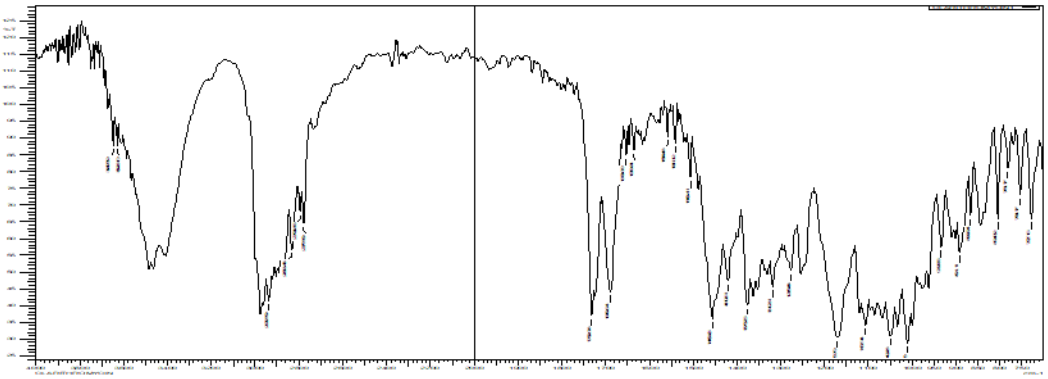

Figure 2. IR spectra of Clarithromycin

Table 3. Functional groups and observed peak value of Clarithromycin

S. No	Peaks cm^{-1}		
	Absorbance assignment	**Reported**	**Observed**
1	-C=O stretching vibration from ketone group	1690 - 1685	1687
2	-O-C=O stretching vibration in the lactone ring	1740 - 1729	1732
3	-O-ether functional bands	1055 - 1049	1051
4	Alkane Stretching	3600 - 3417	3640
5	-O-ether functional bands	1108	1107
6	Alkyl- CH_3 substitution bands	2948 - 2935	2939

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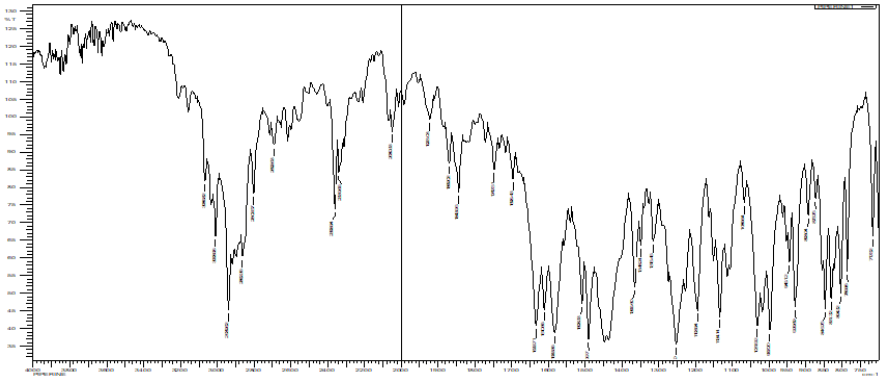

Figure 3. IR spectrum of Piperine

Table 4. Functional groups and observed peak value of Piperine

S. No	Peaks cm^{-1}		
	Absorbance assignment	**Reported**	**Observed**
1	Aromatic C- H Stretching	3000 - 2900	2998
2	Aromatic C = C (diene)	1635- 1608	1632
3	- CO- N-	1639- 1649	1635
4	Asymmetrical Stretching =C-O-C	1250 - 1190	1238
5	-CO- Stretching	930	932
6	-CH2- Bending	1450-1430	1442

Standard calibration curve

The standard calibration curve of CL was plotted between the absorbance v/s concentrations. The λmax of CL in 0.1 N HCl pH 1.2 was determined at 210 nm. The standard calibration curve of CL was plotted between 10 -50 µg/mL as shown in Figure 4. (0.1 N HCl pH 1.2).

Table 5. Standard calibration curve data of 0.1 N HCl pH 1.2

Sr. No.	Concentration (µg/mL)	Absorbance 0.1 N HCl pH 1.2
1	10	0.1015
2	20	0.3010
s3	30	0.4050
4	40	0.5490
5	50	0.6820

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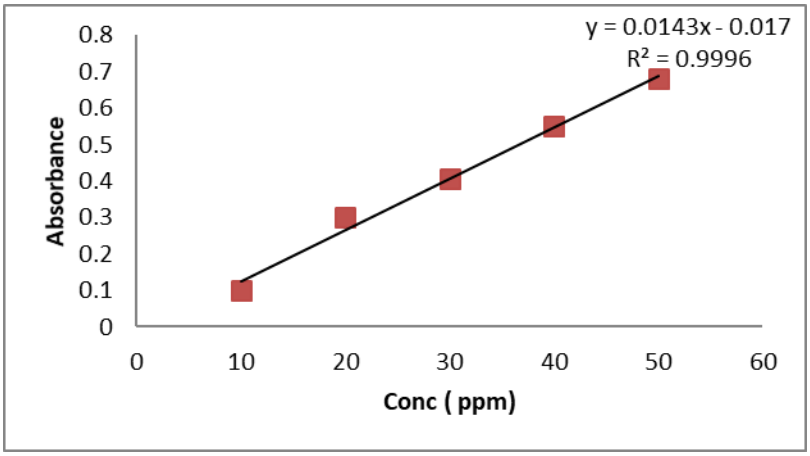

Figure 4. Standard calibration curve of 0.1 N HCl pH 1.2

3. Characterization of Drug – Piperine Complex

1. FTIR studies

FTIR is a highly sensitive method of analysis. The FTIR spectrum of drug and Piperine showed no significant shift or reduction in intensity of peaks of CL it was shown that the characteristics peaks of CL was present in the combination spectra of Piperine, so indicated the compatibility of the drug and Piperine which was shown in Figure 5.

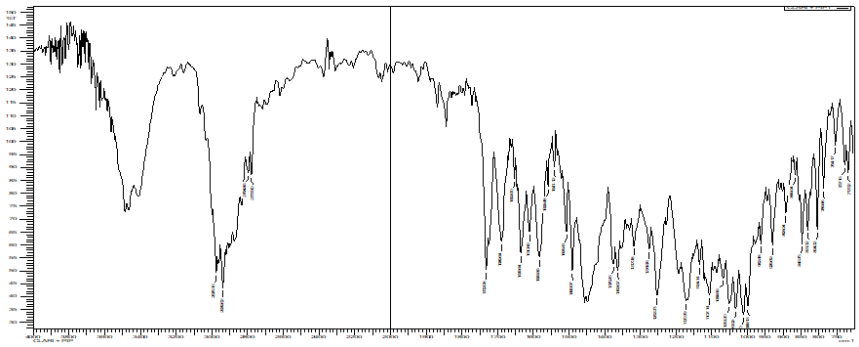

Figure 5. FTIR spectra of CL – Piperine (Complex)

Table 6. Functional groups and observed peak value of CL – Piperine Complex

S. No	Peaks cm^{-1}		
	Absorbance assignment	**Reported**	**Observed**
1	-C=O stretching vibration from ketone group	1690 - 1685	1687
2	-O-C=O stretching vibration in the lactone ring	1740 - 1729	1732

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3	- CO- N-	1639- 1649	1640
4	-CO- Stretching	930	933
5	-O-ether functional bands	1108	1107
6	Alkyl- CH_3 substitution bands	2948 - 2935	2939

2. Differential Scanning Calorimetry (DSC)

CL - Piperine complex (Figure 6) was analysed by Differential Scanning Calorimetry which shows the confirmation of the formulation of complexes. CL exhibit a characteristic endothermic peak at 226.40^0C and Piperine showed peak at 127.74^0C. The DSC spectrum of CL with Piperine formulated by kneading method has shown the peaks at 129.04^0C indicating that drug melting endotherm had shifted from original 226.40^0C to $129.04\ ^0$C endotherm as sharp peak which can be attributed to the complex formation between CL – Piperine.

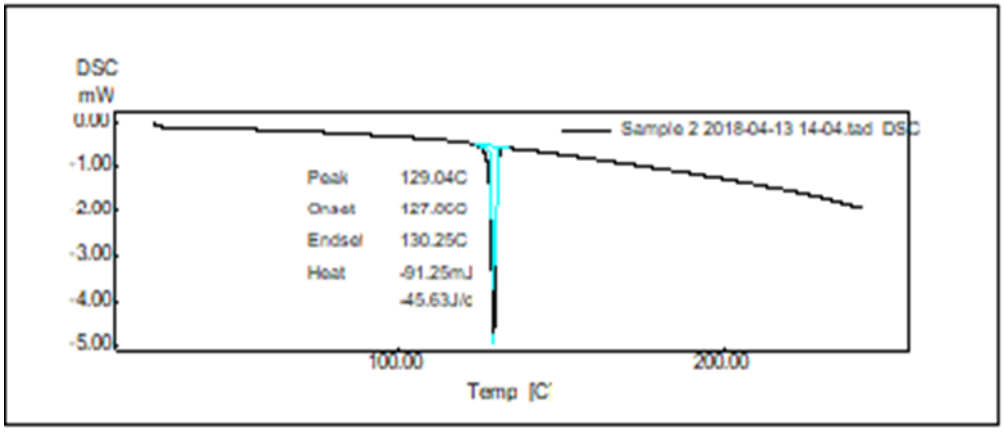

Figure 6. DSC thermogram of CL - Piperine complex

3. X-ray Powder Diffraction Studies

The X-ray diffractogram of CL confirms its crystalline nature, as evidenced from the number of sharp and intense peaks situated between 10^0 and 30^0(2θ) (Figure 7). This data indicated that the drug is in the crystalline and stable form. However, the diffraction pattern of CL Piperine complex represents similar intense peak especially those situated between10^0 and 30^0 (2θ). These diffraction patterns suggest that there is a no change in crystallinity of drug due to Kneading (Figure 7). Thus, Piperine does not inhibits the crystallization of drug and remain CL into Crystalline form, during the preparation of the complex.

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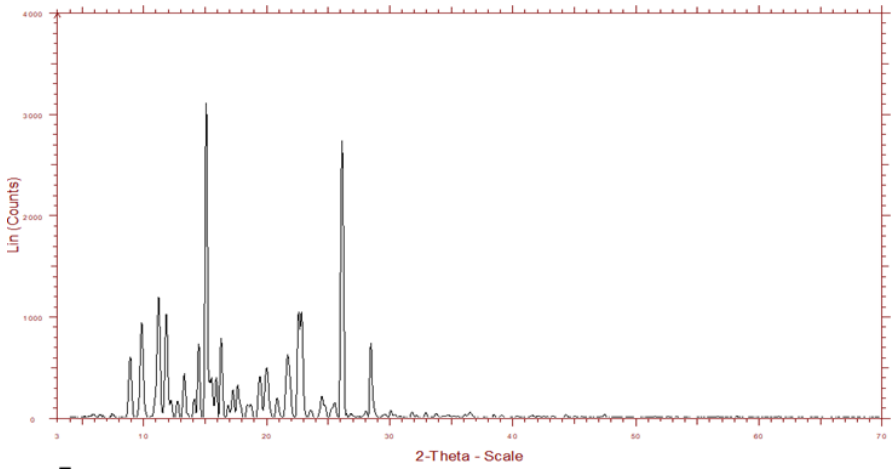

Figure 7. XRD of CL

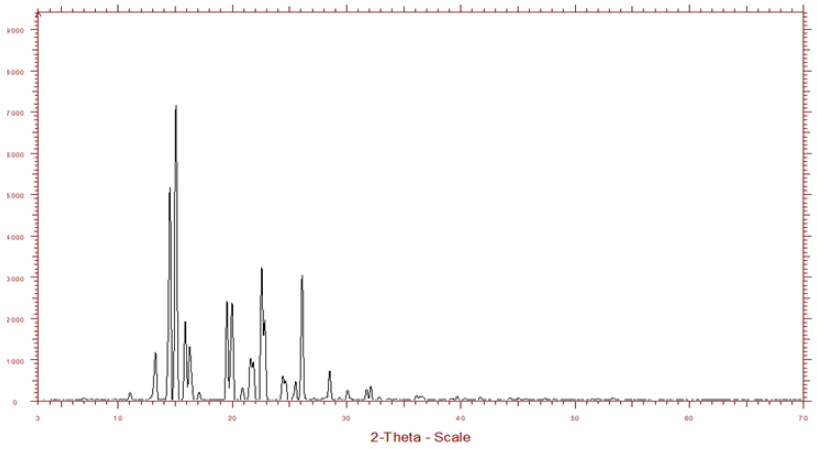

Figure 8. XRD of CL – Piperine Complex

4. Scanning Electron Microscopy (SEM) Studies

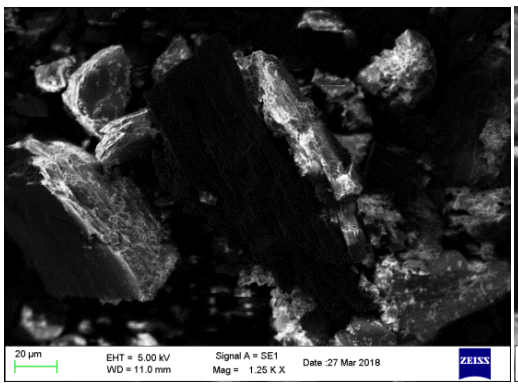

Figure 9. SEM image of pure Clarithromycin

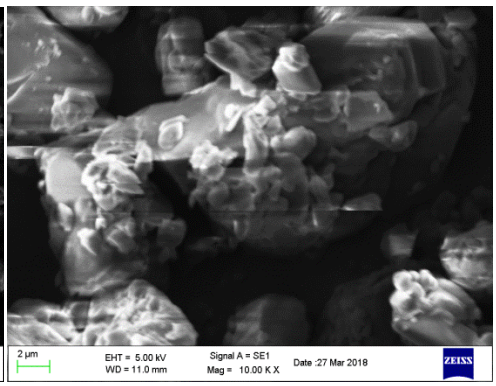

Figure 10. SEM image of pure Piperine

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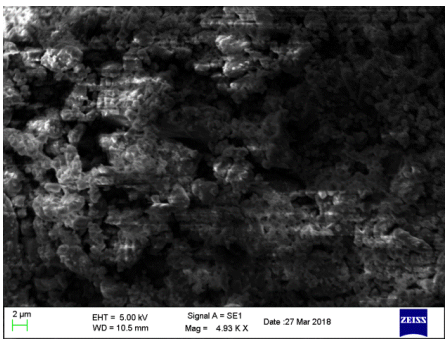

Figure 11. SEM image of CL- Piperine Complex

From the above images it was concluded that, SEM image of pure CL i.e., fig.9 showed rough and irregular surface and SEM image of pure Piperine i.e., in fig.10 showed globular form. The surface morphology of Complex indicates that CL interacted with Piperine and homogeneously dispersed. SEM pictures images suggested that the individual surface properties of CL and Piperine were lost during kneading and the formation of effective systems. These findings demonstrated that the drug was thoroughly mixed in the Pipeine with a negligible loss of little crystallinity.

4. Antimicrobial Activity of Drug – Piperine Complex

1. MIC Determination

The MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganisms. The MIC of Drug, Piperine and Complex in both culture i.e *S aureus* and *E.coli* found as shown in table 7.

Table 7. MIC of Drug, Piperine and Complex against S. aureus and E. coli

Bacteria	Clarithromycin Conc.(µ/ml)	Piperine Conc.(µ/ml)	1:1 Complex Conc.(µ/ml)
S.aureus	**0.25**	**10**	**0.05**
E.coli	**0.2**	**12**	**0.05**

(a) **(b)**

Figure 12. MIC of Clarithromycin at 0.2 µ/ml (a) *S. aureus* and (b) *E.Coli*

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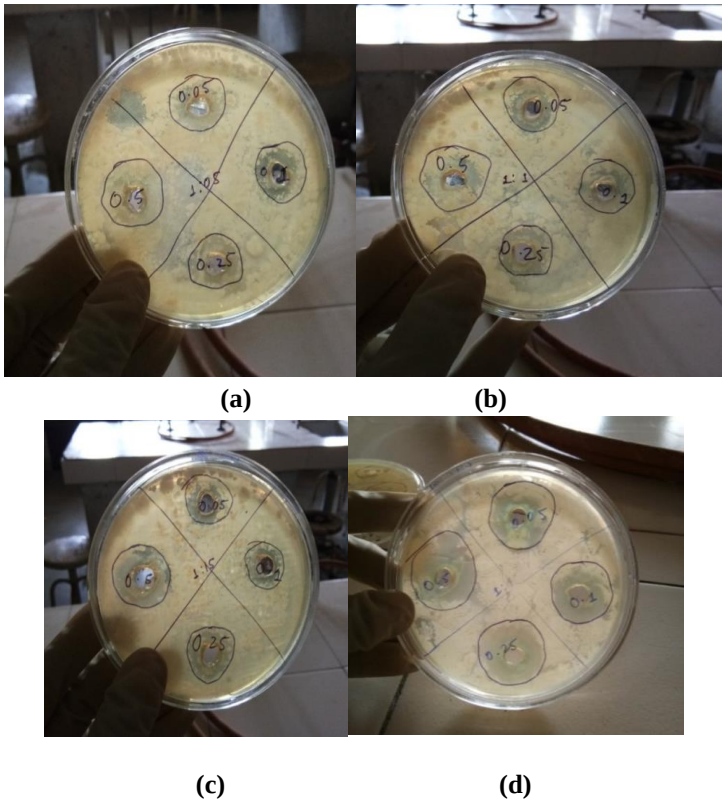
From the results above it was observed that the Drug – Piperine Complex showed maximum zone of inhibition as compared to standard drug clarithromycin, whereas Piperine showed least antimicrobial activity.

2. **Antimicrobial activity of different ratios of complex against *Staphylococcus aureus.***

Zone of inhibition of different conc. of Drug Piperine complex 1:05, 1:1, 1:1.5 and 1:2 against *Staphylococcus aureus* are shown in fig.a, fig b, fig c and in fig.d respectively.



(a) **(b)**

(c) **(d)**

Figure 13. Zone of inhibition of different conc. of Drug Piperine complex (a)1:05, (b)1:1, (c)1:1.5 and (d)1:2 against *S. aureus*.

Table 8. Zone of Inhibition (mm) on *S aureus*

Complex	**0.05 (µg/ml)**	**0.1(µg/ml)**	**0.25(µg/ml)**	**0.5(µg/ml)**
Clarithromycin	-	-	12	22
01:00.5	-	8	16	23
01:01	5	12	17	23
01:01.5	5	13	17	24

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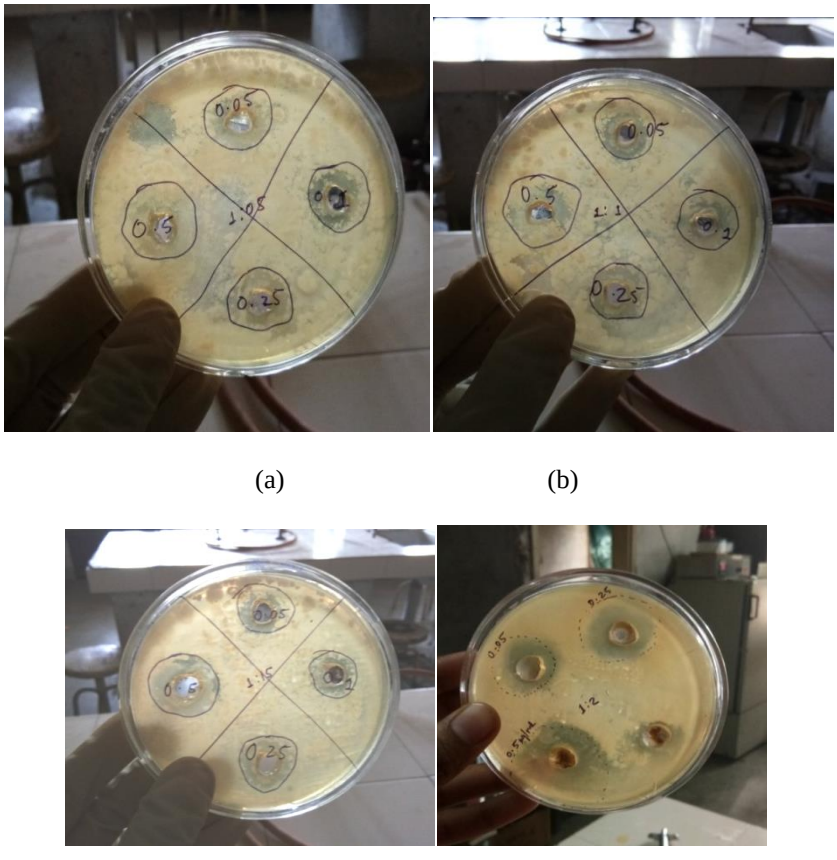
01:02	**25.6**	**27**	**29.6**	**30**
01:03	26	27	29.5	30.1
01:04	26	27.2	29.7	30
Piperine	-	-	-	-

From the results above it was observed that the 1:2 Drug – Piperine Complex showed maximum zone of inhibition against S. aureus as compared to other ratios, after which constant results were observed. Thus, 1:2 ratio was selected for formulation.

3. **Antimicrobial activity of different ratios of complex against *Escherchia Coli***

Zone of inhibition of different conc. of Drug Piperine complex 1:05, 1:1, 1:1.5 and 1:2 against *Escherichia Coli* are shown in fig.a, fig b, fig c and in fig.d respectively.



(a) (b)

(c) **(d)**

Figure 14. Zone of inhibition of different conc. of Drug Piperine complex (a) 1:05, (b)1:1, (c)1:1.5 and (d)1:2 against *E.coli*.

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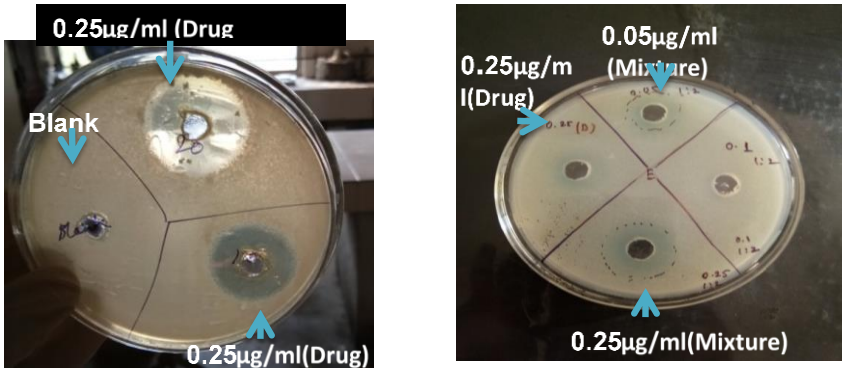
Table 9. Zone of Inhibition (mm) on *E. coli*

Complex	0.05 (µg/ml)	0.1(µg/ml)	0.25(µg/ml)	0.5(µg/ml)
Clarithromycin	-	-	10	20
1: 0.5	-	06	14	20.6
1: 1	07	11	15	21
1: 1.5	7.8	13	17	22.4
1: 2	**20.1**	**25**	**26.6**	**28**
1: 3	22.4	25.2	27.5	28.5
1: 4	22.8	25.9	27.7	28.7
Piperine	-	-	-	-

From the results above it was observed that the 1:2 Drug – Piperine Complex showed maximum zone of inhibition against E. coli as compared to other ratios, after which constant results were observed. Thus, 1:2 ratio was selected for formulation.



(a) (b)

Figure 15. Final Comperative plates of Standard Drug and 1:2 Complex of Drug- Piperine (a) *S. aureus* (b) *E. coli*

This final plate shows that 0.25µg/ml conc of both Standard Drug and Drug – Piperine Complex gives drastic change in Zone of inhibition. Also, when 0.05 µg/ml conc of Drug – Piperine Complex and 0.25 µg/ml conc of Drug was tested, it showed increased Zone of inhibition in Drug - Piperine Complex than Standard drug. Hence it can be concluded that Piperine enhances the antimicrobial activity of Clarithromycin, thus can be used as bioenhancer.

5. Formulation Development

The two independent variables such as PEO (X_1) and HPMC K15 (X_2) were selected on the basis of the preliminary studies carried out before the experimental

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design is being implemented whereas Floating Lag Time (Y1) and % Drug Release after 24 hr (Y2) were selected as dependent variable. Experimental design was generated by Design-Expert® 11 software.

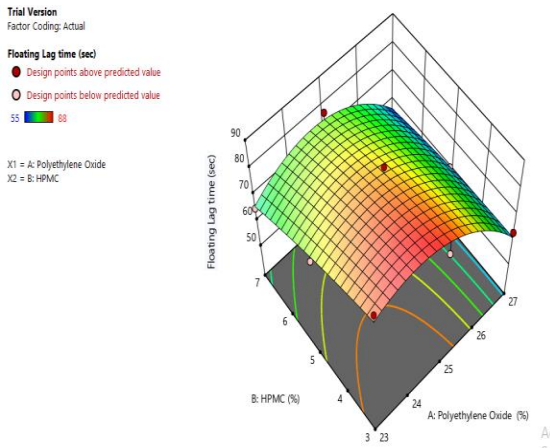

Figure 15. 3D plot of Floating Lag Time

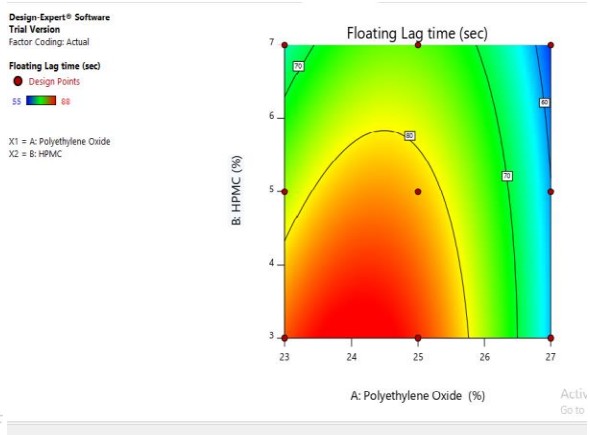

Figure 16. Response surface Plot of Floating Lag Time

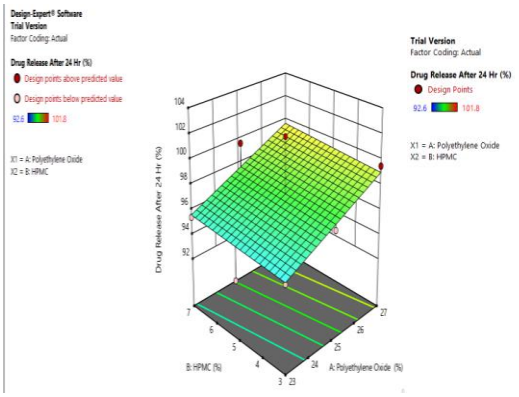

Figure 17. :3D plot of % Drug Release in 24 hrs

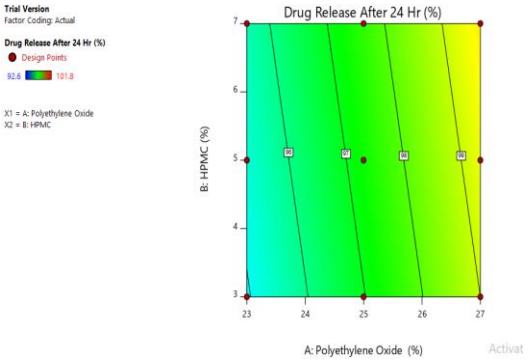

Figure 18 Response surface Plot of % Drug Release in 24 hrs.

Table 10. Optimization of Floating formulation

Constraint			
Name	**Goal**	**Lower limit**	**Upper Limit**
Polyethylene Oxide (PEO)	Optimum	23	27
HPMC K 15	Optimum	3	7
Floating Lag Time	Minimum	55	88
In-vitro %drug release after 24 hr	Maximum	92.56	99.68

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6. Evaluation of pre-compression parameters of floating tablet:

Table 11. Micromeritic properties of CL- Piperine powder mix of floating matrix tablets (FMT). (Mean ± SD, n = 3)

Formulations	Angle of repose (˚)	Bulk Density(g/cm3)	Tapped Density (g/cm3)	Carr's Index (%)	Hausner's Ratio
F1	25.49±0.72	0.72±0.01	0.84±0.02	14.28±0.42	1.16±0.01
F2	26.24±0.71	0.60±0.01	0.72±0.05	16.66±0.67	1.20±0.04
F3	29.05±0.73	0.54±0.04	0.66±0.03	18.18±0.56	1.22±0.10
F4	26.97±0.81	0.62±0.06	0.75±0.04	17.33±0.67	1.20±0.02
F5	29.25±0.11	0.53±0.02	0.61±0.02	13.11±0.59	1.15±0.11
F6	33.65±0.17	0.56±0.05	0.66±0.03	10.00±0.37	1.17±0.09
F7	26.56±0.32	0.61±0.05	0.71±0.04	16.39±0.28	1.16±0.11
F8	27.33±0.12	0.70±0.06	0.82±0.05	14.63±0.14	1.17±0.01
F9	26.43±0.16	0.57±0.04	0.69±0.02	17.39±0.43	1.21±0.03

7. Evaluation of post compression parameters of CL - Piperine:

Table 12. Results of physical parameters, Drug Content, Swelling Index of CL floating matrix tablets (FMT). (Mean ± SD, n = 3)

Batch	Hardness (kg/cm)	Friability (%)	Thickness	Assay	Swelling Index after 8 hr (%)
F1	4.5±0.388	0.55±0.15	4.50±0.210	102±0.60	90.80
F2	5.2±0.055	0.18±0.19	4.45±0.07	99.55±0.83	100.75
F3	5.0±0.035	0.25±0.10	4.65±0.06	93.55±0.20	106.26
F4	5.5±0.135	0.29±0.25	4.35±0.05	101.67±0.67	96.55
F5	4.5±0.025	0.45±0.18	4.55±0.03	94.80±0.75	107.62
F6	4.9±0.050	0.51±0.23	4.50±0.02	101.56±o.77	115.60
F7	5.5±0.157	0.60±0.15	4.48±0.01	96.65±0.15	102.51
F8	5.3±0.267	0.45±0.35	4.53±0.050	99.95±0.15	114.77
F9	4.8±0.165	0.53±0.51	4.50±0.02	100.50±0.56	115.65

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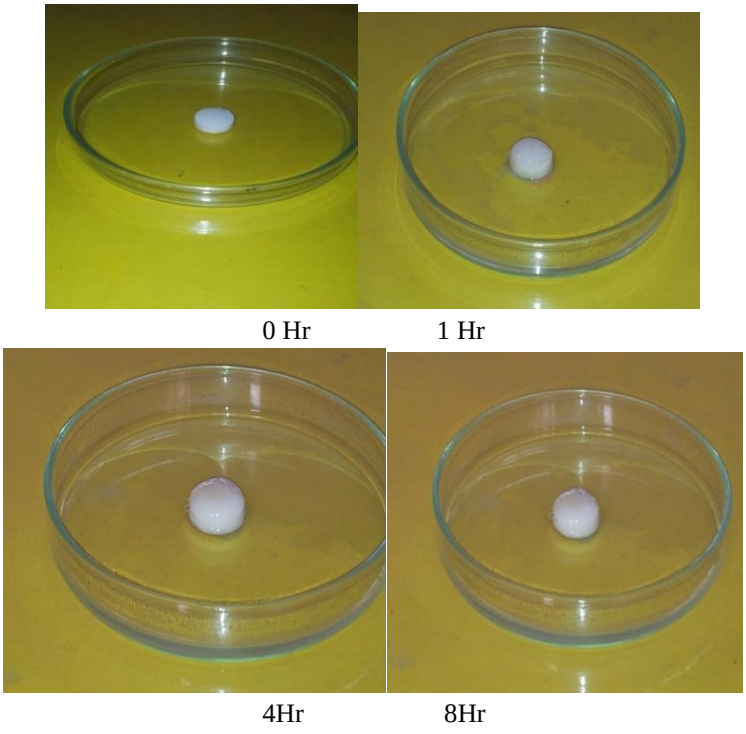

Figure 19. Pictures of F7 optimized tablet during swelling index studies at different time intervals

Table 13. Floating lag time and Total floating time of CL (FMT).

Batch	Floating lag time(sec)	Floating time(hr.)
F1	150	7
F2	30	20
F3	8	10
F4	60	20
F5	55	20
F6	50	20
F7	40	24
F8	60	21
F9	72	20

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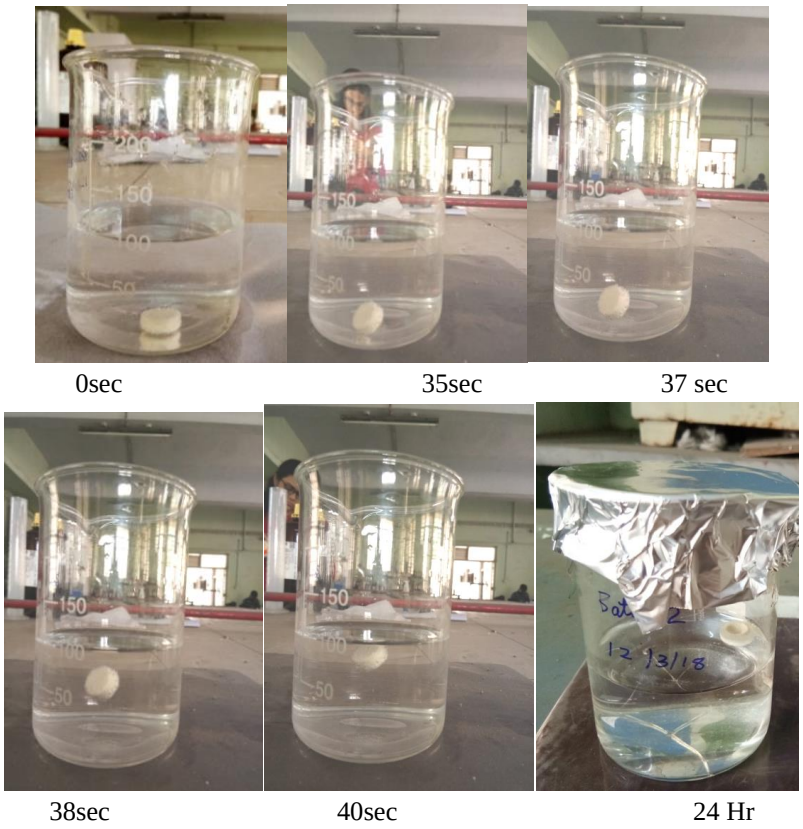

Figure 20. Photographs taken during *in vitro* buoyancy study of formula F7 in 100 ml 0.1 N HCl at different time intervals

Sodium Bicarbonate was used in three different conc. i.e 6%, 8% and 10% out of which 6% and 10% formulations did not show proper floating during *in vitro* buoyancy study. Hence from the above observations, 8% was the optimized conc. as it showed the desired floating lag time and floating time and the other two batches (F1 and F3) were discarded from Drug release studies.

- **Results of dissolution studies:**

The *in-vitro* drug release studies of all tablets from each batch, Marketed preperation along with Plain drug tablet with same excipients without piperine was also carried out in 0.1N HCl by USP XXIV model and the values are shown in table 17. The plot of percent drug release v/s time (min) was plotted and depicted in figure 21.

Table 17: Results of dissolution studies of Clarithromycin floating matrix tablets. (Mean ± SD, n = 3)

Time (Hr.)	F2	F4	F5	F6	**F7**	F8	Plain drug tablet	Marketed tablet
0	0	0	0	0	**0**	0	0	0
0.5	19.85	23.92	14.51	12.77	**18.62**	16.02	12.6	24.92
1	26.76	34.68	16.4	29.37	**30.09**	30.63	28.25	31.68

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2	31.58	43.69	29.77	38.71	**47.36**	39.99	36.72	43.69
4	47.22	46.12	43.71	46.88	**54.52**	49.71	48.38	46.12
6	58.36	51.36	56.62	63.75	**65.17**	56.4	58.18	49.36
8	67.61	56.29	68.5	74.76	**72.8**	67.74	66.29	56.29
10	79.78	68.14	83.16	83.51	**83.97**	84.06	76.97	65.01
12	89.72	95.97	88.31	91.58	**87.17**	91.31	83.59	96.97
20	97.46	96.47	94.62	95.1	**96.29**	94.7	97.84	98.47
24	98.35	98.63	95.24	97.4	**99.98**	97.35	98.24	100.6

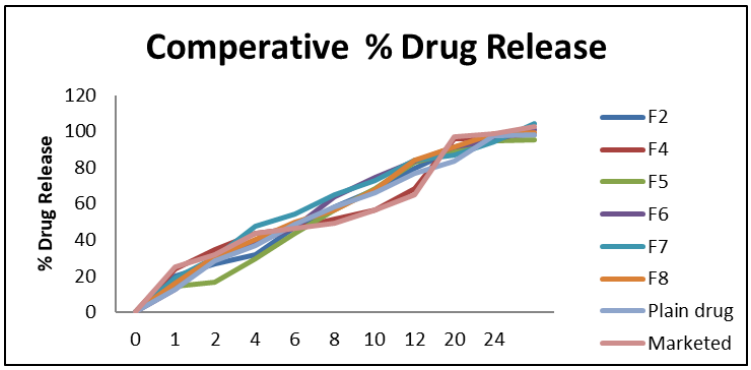
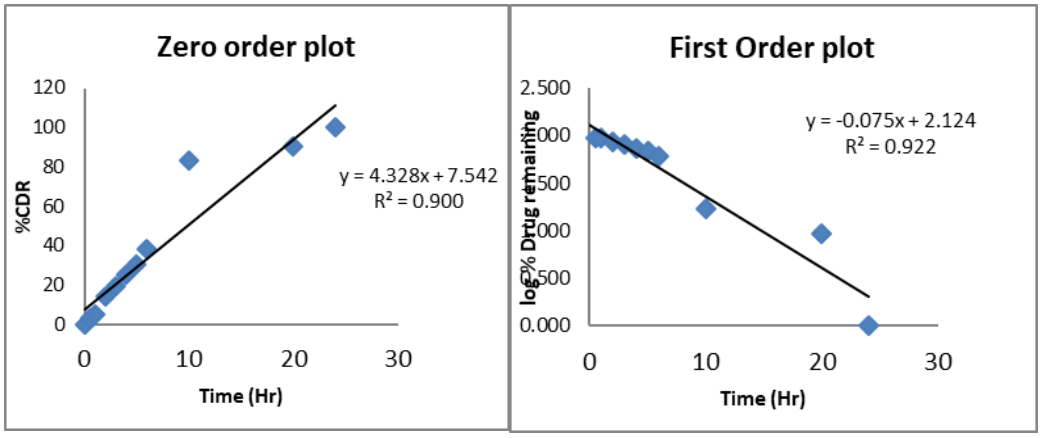


Figure 21. Release profiles of Clarithromycin from floating matrix tablets

The batch F7 gives sustained release of drug for 24 hrs. The release rate constant was calculated from the slope of the appropriate plots, and the regression coefficient (R^2) and release exponent (n) was calculated. It was found that the *in vitro* drug release of F7 sustained release tablet was best explained by Kosmeyer peppas model, plots showed the linearity (R^2 = 0.9710) of optimized batch F7.

- **Drug Release Kinetics**



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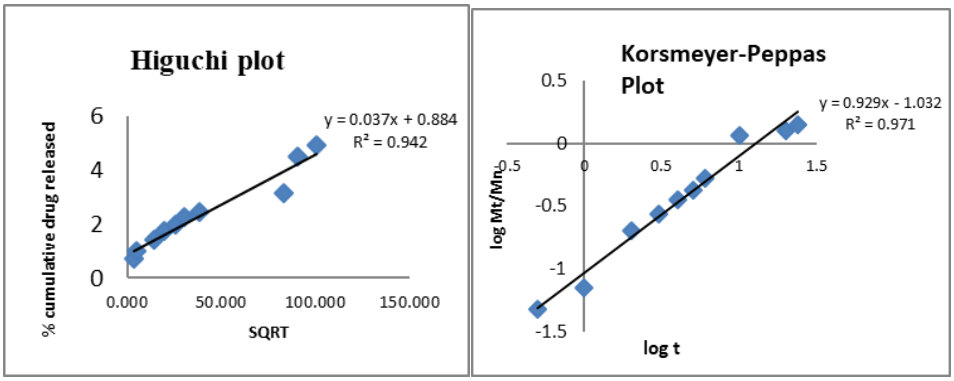

Figure 22. Zero order plot, First order plot, Higuchi plotand Kosmeyer-peppas plot for drug release.

Table 18. Correlation coefficient (R^2) values of optimized batch.

	REGRESSION COEFFICIENT			
Formulation Code	**Zero order plots**	**First order plots**	**Higuchi plots**	**Korsemeyer-peppas plots**
	(R^2)	**(R^2)**	**(R^2)**	**(R^2)**
L9	0.900	0.9220	0.9420	0.9710

Prime objective of development CL Floating formulation was to sustain drug release over 24 hours for reduction of dosing frequency and to improve the patient compliance. Initial trial batches were prepared using 23, 25, 27% PEO and 3, 5, 7% HPMC K15M and based on those result further experimental factorial batches were prepared.

Based on various evaluation parameters like hardness, thickness, friability, drug content, weight variation, release kinetics, factorial batch F7 with 25% PEO and 5% HPMC K15M and was considered to be optimized batch for further study.

7.9. Comparative study of prepared tablet with the marketed formulations and standard antibiotic

The comparative antimicrobial activity was performed on the basis of zone of inhibition of prepared formulation F7, marketed formulation and standard antibiotic clarithromycin. From the results obtained as below it was observed that prepared tablet F7 showed more zone of inhibition than Standard drug and marketed formulation.

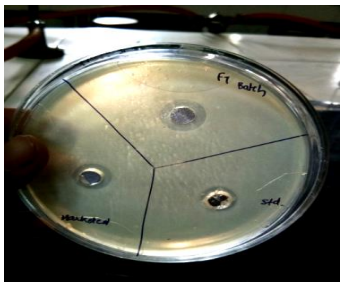

Figure 30. Comparative study of antimicrobial activity of prepared formulations with the marketed formulation and standard antibiotic.

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8. Stability Study

Stability testing on optimized batches of Compression coated tablets as per ICH guidelines was carried out. After checking the physical parameters, no significant change was found in all the batches before and after stability study. The results obtained as shown in Table 19

Table 19. Evaluation parameters of stability batch F7

Evaluation parameters	Before stability Storage	After 3 month storage
Hardness (kg/cm^2)	4.2	4.2
Friability (%)	0.43±0.015	0.45±0.018
Weight variation (mg)	351.1±1.56	350.9±1.53
Drug content (%)	101.39±0.23	99.04±0.22
Floating Lag Time (sec)	62	60
Floating Time (hr)	24	24
% Drug release after 24 hr	99.03±0.19	98.98 ± 0.17

The data are presented as mean value ± S.D. (n = 3)

4. Conclusion

The present research work deals with enhancement of antimicrobial activity of clarithromycin by using *Piperine* for the first time in the formulations so as to enhance its bioefficacy and reduce dose. Piperine and Clarithromycin were kneaded in different ratios but the one which shows maximum zone of inhibition at minimum concentration was selected for further studies so as to achieve maximum antimicrobial activity at minimum concentration of drug and piperine. After optimization of CL- Piperine ratio, gastroretentive floating tablets were formulated by direct compression method using Design Expert 11 software. For formulation optimisation, several quantities of sodium bicarbonate were utilised as a gas generator and PEO and HPMC K15 as a polymer. It was observed that the drug release profile was delayed by increasing the concentration of polymers (HPMC K15MCR and PEO). Additionally raising the sodium bicarbonate concentration decreased the floating lag time. However, the total floating time of the formulations was unaffected by the amount of sodium bicarbonate. The sodium bicarbonate concentrations had no discernible impact on the medication release profile. Therefore, it can be inferred from the current work that hypromellose (HPMC K15MCR) and PEO (Polyyox WSR303) in the right concentrations can be employed to create sustained release floating tablets of clarithromycin by adding the right

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concentration of sodium bicarbonate for gas formation. Such system can remain buoyant for 24 hours along with the sustained drug release for the same duration.

The optimized formulation was found to follow Kosmeyer-peppas plot, which showed its sustained release pattern following non fickian release (n=0.5517). Further research can be made to study on Helicobacter Pylorus, and also on different antimicrobial drugs.

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