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STRATEGIC DEVELOPMENT AND CHARACTERIZATION OF CLARITHROMYCIN-LOADED CUBOSOMAL GEL FOR TARGETED THERAPY OF ACNE VULGARIS

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ABSTRACT

Topical therapy offers a targeted approach to drug delivery, enhancing local concentrations while minimizing systemic side effects. This study focuses on the development and characterization of a Clarithromycin-loaded cubosomal gel, leveraging cubosomes as innovative vesicular nanocarriers to improve drug penetration and retention for acne management. The gel was formulated using a top-down approach with Glyceryl monooleate (GMO) as the lipid phase, Poloxamer 407 as a stabilizer, and distilled water as the aqueous phase. The optimal formulation, F5, demonstrated an entrapment efficiency of 85.229±0.12%, an average particle size of 150.6 nm, and a drug content uniformity of 96.88±0.41%. Zeta potential analysis indicated a stable surface charge of -23.7 mV. *In vitro* diffusion studies revealed a maximum drug release of 82.53±0.13%. The cubosomal formulation was then incorporated into a 1% w/w Carbopol 934 gel, which exhibited a homogenous appearance, a pH of 6.28±0.14, viscosity ranging from 3055 cps at 5 rpm to 1025 cps at 50 rpm, spreadability of 19.16±0.11 g.cm/sec, and an extrudability % decrease of 98.33±0.23%, a drug content of 90.50±0.15% and a cumulative drug release of 78.568±0.18% over 8 hours, following zero-order (R^2=0.9919) and Higuchi diffusion kinetics (R^2=0.8926). Stability studies at 25±2°C and 60±5% RH confirmed the gel's robustness and sustained efficacy. The Clarithromycin-loaded cubosomal gel presents a promising strategy for managing *Acne vulgaris*, with enhanced drug stability and controlled release, making it a valuable approach for effective treatment.

KEYWORD:- Clarithromycin, cubosomes, top-down approach, *Acne vulgaris*, gel.

INTRODUCTION

Transformative advances in pharmaceutical sciences have revolutionized drug delivery, enabling more innovative and effective methods of administering medications.[1] Novel Drug Delivery Systems (NDDS) have emerged as a result of improved understanding of pharmacokinetics and pharmacodynamics, offering a more strategic and rational approach to drug formulation and delivery.[2] Numerous NDDS technologies have been developed, utilizing various routes of administration to facilitate controlled and targeted drug delivery.[3]

Targeted drug delivery refers to a technique that delivers medication in such a way that maximizes its concentration in the desired tissues while minimizing its distribution to other areas of the body.[4] These are stable colloidal dispersions featuring a three-dimensional bicontinuous lipid bilayer structure, forming a continuous cubic symmetry with two interwoven but non-overlapping aqueous nanochannels, resembling a honeycomb-like liquid crystal pattern; their size generally ranges from 100 to 500 nm.[5-6] Additionally, they are thermodynamically stable, biodegradable, and biocompatible nanocarriers that can be stored for long periods without phase separation and are preferred due to their solvent-free, environmentally friendly manufacturing process.[7]

Acne vulgaris is a common skin disorder involving excess oil production, bacterial growth (*P. acnes*, *S. aureus*), and inflammation.[8] Topical agents are widely used as first-line or supportive treatments due to their ability to deliver high drug concentrations to the target site while minimizing systemic side effects. The effectiveness of these therapies depends on skin penetration and retention at the target site.[9] As a result, topical delivery is preferred for anti-acne medications.[10]

Clarithromycin is a broad-spectrum macrolide antibiotic used to treat severe acne and various skin infections, and is available as a 1% w/w topical gel.[11] Oral use may lead to liver, kidney, or heart complications, making topical delivery a safer and more targeted option that avoids gastrointestinal irritation and the first-pass effect.[12] However, traditional topical formulations like creams and gels mostly act on the skin's surface and may cause irritation, limiting their effectiveness. To overcome these challenges, there is a growing need for advanced

delivery systems that enhance skin retention and improve penetration, ultimately boosting Clarithromycin's therapeutic impact.[13]

Despite the wide availability of acne treatments, none provide a lasting cure, and patient adherence remains low, emphasizing the need for more efficient solutions.[14-15] Recognizing these limitations, this study aims to develop a Clarithromycin-loaded cubosomal gel to offer deeper skin penetration, prolonged retention, and better patient compliance.[16]

MATERIALS AND METHODS

Materials

Clarithromycin was supplied from Yarrow Chem products, Mumbai. All other excipients and solvents used are of analytical pharmaceutical grades.

METHODS

1. Preformulation studies[12,17]

i. Organoleptic characteristics

The physical properties of the drug, such as its state, colour, and smell, were described using specific terms. These characteristics help in identifying the drug.

ii. Determination of melting point

The melting point of Clarithromycin was measured using the capillary method. A small amount of the drug was placed in a capillary tube, which was then inserted into a melting point apparatus. The temperature at which the drug melts is recorded as its melting point.

2. Spectroscopic studies[18]

i. Determination of Absorbance Maximum (λ_{max}) of Clarithromycin

A stock solution (1000 µg/ml) was made by dissolving 100 mg Clarithromycin in methanol and phosphate buffer pH 7.4. From this, 10 ml was diluted to 100 ml with buffer to get 100 µg/ml. A 10 µg/ml solution was prepared and scanned between 200–400 nm using a UV spectrophotometer. The wavelength showing the highest absorbance (λ_{max}) was recorded.

ii. Standard calibration curve

Solutions of Clarithromycin were prepared at concentrations of 2, 4, 6, 8, and 10 µg/ml using phosphate buffer pH 7.4. Absorbance of each solution was measured, and a calibration curve was plotted with concentration on the X-axis and absorbance on the Y-axis.

3. Drug-excipient compatibility studies[11]

FTIR spectroscopy was used to check compatibility between Clarithromycin and excipients. About 10 mg of drug and formulation were directly placed in the FTIR instrument, and spectra were recorded between 4000 - 400 cm^{-1}.

Development of clarithromycin-loaded cubosomes[17,19]

Cubosomes were commonly prepared using the top-down approach, which involves the dispersion of bulk cubic phase structures into smaller, stable particles through high-energy processing. The steps involved in this method are detailed below.

Step 1: Melting of components

Glyceryl monooleate (GMO) and Poloxamer 407, in varying concentrations as per the formulation, were melted together at 70°C using a water bath.

Step 2: Incorporation of drug

Clarithromycin (1%) was added to the molten lipid mixture and stirred thoroughly to ensure uniform drug distribution.

Step 3: Formation of cubosomal dispersion

The clear lipid-drug solution was added dropwise to distilled water (also maintained at 70°C) with continuous stirring at 1000 rpm to obtain a homogeneous dispersion.

Step 4: Cooling and Sonication

The dispersion was allowed to cool to room temperature and then ultrasonicated using a probe sonicator for 10 minutes to obtain a stable cubosomal formulation.

Table No. 1: Formulation chart of Cubosomes.

Formulation code	GMO (%)	Poloxamer 407 (%)	Clarithromycin (%)	Purified Water (ml)
F1	4.8	0.2	1	100
F2	4.6	0.4	1	100
F3	4.4	0.6	1	100
F4	4.2	0.8	1	100
F5	4.0	1.0	1	100
F6	3.8	1.2	1	100
F7	3.6	1.4	1	100
F8	3.4	1.6	1	100

Characterization of cubosomes containing clarithromycin

1. Entrapment efficiency % (EE %)[20]

Various cubosomal formulations were subjected to centrifugation using a cooling centrifuge at 16,000 rpm for 1 hour at 4 °C to separate the cubosomal nanovesicles from the unentrapped drug. The supernatant containing the free drug was filtered, adequately diluted, and analysed for drug concentration using a UV spectrophotometer at a predetermined λ_{max}.

The entrapment efficiency percentage (EE%) was calculated using the following formula:

$$EE\ \% = \frac{Total\ amount\ of\ drug - free\ drug\ in\ supernatant}{Total\ amount\ of\ drug} X100$$

2. Drug content[21]

To determine the total drug content, 1 mL of the formulation was disrupted using sufficient quantity of methanol followed by dilution using buffer and the solution was analysed spectrophotometrically at λ_{max}. Methanol was chosen as a suitable solvent for disrupting the prepared vesicles.

3. Zeta potential[22]

The zeta potential of clarithromycin-loaded cubosomes was measured using a Zetasizer Nano (Malvern Instruments Ltd., Worcestershire, UK), a laser-based device that analyses particle movement and surface charge. To measure it, 0.5 ml of the cubosome sample was diluted with deionized water to make a total of 30 ml. All measurements were carried out at room temperature (25°C) and repeated three times for accuracy.

4. Particle Size and Polydispersity index[23]

The average particle size (PS) and polydispersity index (PDI) of all formulations were measured using a Zetasizer (Malvern Instruments). The samples were properly diluted with distilled water and analysed at 25 °C using a 90° angle to ensure accurate light scattering.

5. *In-vitro* drug diffusion study[11]

In-vitro drug release was evaluated using a Franz diffusion cell. A cellophane membrane, pre-soaked overnight in phosphate buffer pH 7.4, was placed between the donor and receptor compartments. The donor compartment held a cubosome formulation equivalent to 10 mg of drug, while the receptor compartment was filled with phosphate buffer. The system was maintained at 37 ± 0.5°C and stirred at 50 rpm. At specific time intervals, 1 ml samples were withdrawn from the receptor compartment and replaced with fresh buffer to maintain sink conditions. Drug concentration was determined using a UV spectrophotometer.

Development of gel containing cubosomes

Based on the optimal entrapment efficiency and drug content observed in the cubosomes, they were incorporated into a gel formulation using Carbopol 934 as the gelling agent. Carbopol 934 (1% w/w) was first moistened with water and left to stand for 2 hours. It was then dispersed by continuous stirring at 600 rpm using a magnetic stirrer for 1 hour. The optimized cubosomal dispersion, containing the equivalent of 1% w/w drug, was added to the gel base and mixed thoroughly. Propylene glycol was included as a penetration enhancer, glycerol was added to adjust viscosity, and methyl paraben was used as a preservative. Finally, triethanolamine was added dropwise to neutralize the pH of the gel.[24]

Table No. 2: Formulation chart of clarithromycin loaded cubosomal gel.

Ingredients	Quantity
Clarithromycin loaded cubosomes	Eq. to 1% w/w
Carbopol 934	1% w/w
Propylene glycol	3ml
Glycerol	0.25ml
Methyl paraben	0.075mg
Triethanolamine	q.s
Distilled water	q.s. to 100ml

Characterization of cubosomal gel

1. Physical appearance[25]

Prepared gel was inspected visually for appearance, clarity, homogeneity and grittiness.

2. Ph[26]

The pH of the gel was measured using a digital pH meter. For this, 1 gram of the gel was mixed with 25 ml of distilled water, and the pH value was recorded.

3. Viscosity[27]

The viscosity of the gels was measured using a Brookfield Viscometer. The gel samples were tested at speeds of 5, 10, 20, 30, and 50 rpm using spindle number 64. The dial readings were noted at each speed, and the test was repeated in triplicate.

4. Spreadability study[28,29]

To test the spreadability of the gel, about 1 gram of gel was placed between two glass slides (each 14 X 5cm). A weight of 1000 grams was placed on the top slide for 1 minute to let the gel spread. After removing the weight, the diameter of the spread area was measured.

$$S = \frac{M\ X\ L}{T}$$

Where,
S is the spreadability
M is the weight placed on the top slide
L is the length of the glass slide
T is the time the weight was applied.

5. Extrudability[30]

Extrudability is used to check how easily the gel comes out of the tube when pressure is applied. The gel was

filled into a collapsible tube, sealed, and its initial weight was recorded. A weight of 100 g was then placed on the tube to apply pressure, causing the gel to extrude. The amount of gel extruded was collected and weighed.

The percentage of gel released was calculated. This test is important, as proper extrudability ensures the accurate and convenient delivery of the gel during use.

$$\%\ decrease\ of\ weight(extrude) = \frac{Total\ wt\ in\ tube - wt.of\ gel\ extruded}{Total\ wt\ in\ tube} X\ 100$$

6. Drug content[31]

To determine the drug content, 500 mg of cubosomal gel was accurately weighed and dissolved in 100 ml of methanol in a volumetric flask. The mixture was stirred for 2 hours using a mechanical stirrer to ensure complete dissolution of Clarithromycin. The solution was then filtered through Whatman filter paper and analysed using a UV spectrophotometer.

7. *In-vitro* drug diffusion study[32]

The diffusion study was done using a Franz diffusion cell with a 50 ml receptor compartment. A synthetic cellophane membrane was placed between the donor and receptor compartment. 1 gram of cubosomal gel was placed on the membrane (donor side), and the receptor compartment was filled with phosphate buffer pH 7.4. The setup was kept on a magnetic stirrer at 50 rpm, and the temperature was maintained at 37 ± 0.5°C using a water jacket. Samples were collected at 1regular intervals and the same amount of fresh buffer was added back. The collected samples were analysed using a spectrophotometer.

8. *In-vitro* drug release kinetics[33]

To understand how the drug is released from the cubosomal gel and the mechanism behind it, the *in-vitro* drug release data was analysed using different kinetic models. These included **Zero-order model** (cumulative % drug remaining vs. time), **First-order model** (log % drug remaining vs. time), **Higuchi model** (cumulative % drug released vs. square root of time), **Korsmeyer-Peppas model** (log % drug release vs. log time).

From the graphs plotted for each model, the rate constant (K) and regression coefficient (R^2) were calculated using regression analysis to determine which model best fits the release profile.

The equations used for each model are:

Zero-order: $Q_t = Q_0 + k_0 t$

First-order: $logQ_t = logQ_0 - \left(\frac{k_1 t}{2.303}\right)$

Higuchi model: $Q_t = k_H t^{1/2}$

Korsmeyer-Peppas model: $\frac{M_t}{M_\infty} = kt^n$

Where,

Q_t = amount of drug released at time, t

Q_0 = initial drug amount (usually zero)

k_0, k_1, k_H, k = rate constants for each model

$\frac{M_t}{M_\infty}$ = fraction of drug released at time, t

n = exponent indicating the type of drug release mechanism

t = time

9. Stability studies[34]

Accelerated stability studies were carried out for the gel formulation in accordance with ICH guidelines. The samples were stored at 25 ± 2°C and 60% ± 5% relative humidity (RH), and evaluated at intervals of 0, 30, 60, and 90 days. At each time point, the gel was assessed for physical appearance, pH, drug content, and drug release to monitor any changes over time.

10. Comparison with marketed formulation[11]

Clarithromycin-loaded cubosomal gel was compared with a marketed 1% Clarithromycin gel (**Crixan Gel 1% w/w**) by conducting ***in-vitro* drug release studies** to evaluate performance differences.

RESULTS AND DISCUSSIONS

1. Pre-formulation study of the drug

Table No. 03: Pre-formulation study observations of pure drug.

Properties		Reported	Observed
Organoleptic properties	Colour	White	White
	Nature	White crystalline powder	White crystalline powder
	Odour	Odourless	Odourless
Melting point		217-220°C	218°C

From above examination it was found that all the observed values were within the reported literature limits indicating that the drug is pure.

2. Spectroscopic studies

1. Determination of absorption maxima (λ_{max})

The UV spectrum of Clarithromycin, recorded between 200-400 nm using a phosphate buffer at pH 7.4, exhibited a prominent absorbance maximum (λ_{max}) at 203.5 nm, consistent with the standard peaks. The corresponding peak is illustrated in Fig. No.01.

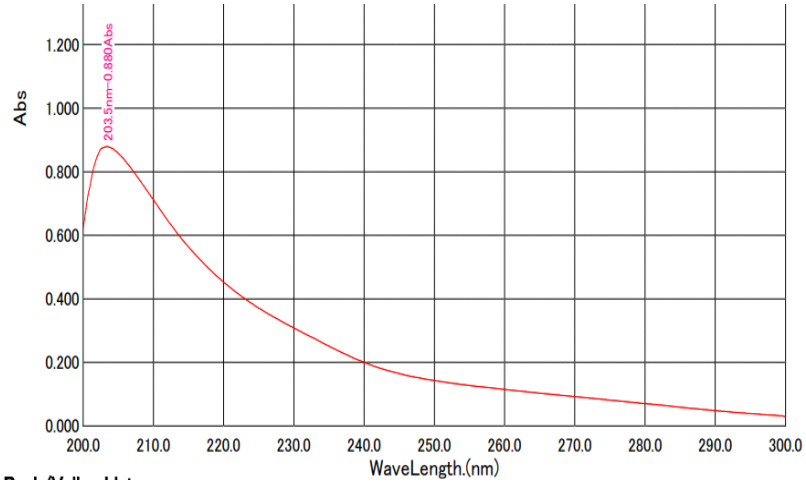

Fig. No. 01: λ_{max} of Clarithromycin in pH 7.4 phosphate buffer.

2. Calibration curve for clarithromycin

Table No. 04: Calibration data of Clarithromycin in phosphate buffer of pH 7.4.

Concentration (µg/ml)	Absorbance*
0	0
2	0.188±0.002
4	0.357±0.004
6	0.527±0.001
8	0.703±0.001
10	0.856±0.005

*Data expressed as mean ± SD, n=3

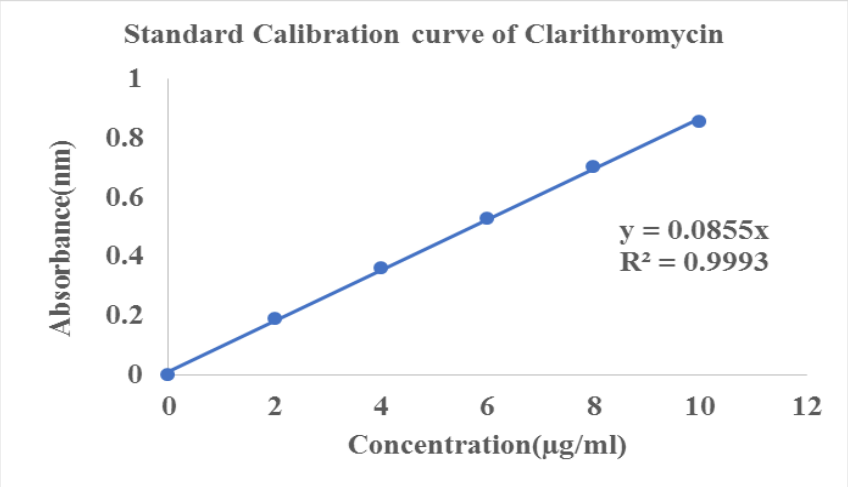

Fig No. 02: Calibration curve of Clarithromycin in phosphate buffer pH 7.4.

The calibration curve of Clarithromycin, along with the slope, intercept, and regression coefficient, was determined and is presented in Fig No.02. The absorbance values exhibited linearity and complied with Beer's Law over the concentration range of 2–10 µg/ml, with an R^2 value of 0.9993.

3. Drug-polymer compatibility studies

FTIR analysis was successfully conducted for Clarithromycin, Glyceryl monooleate, Poloxamer 407 and its formulation.

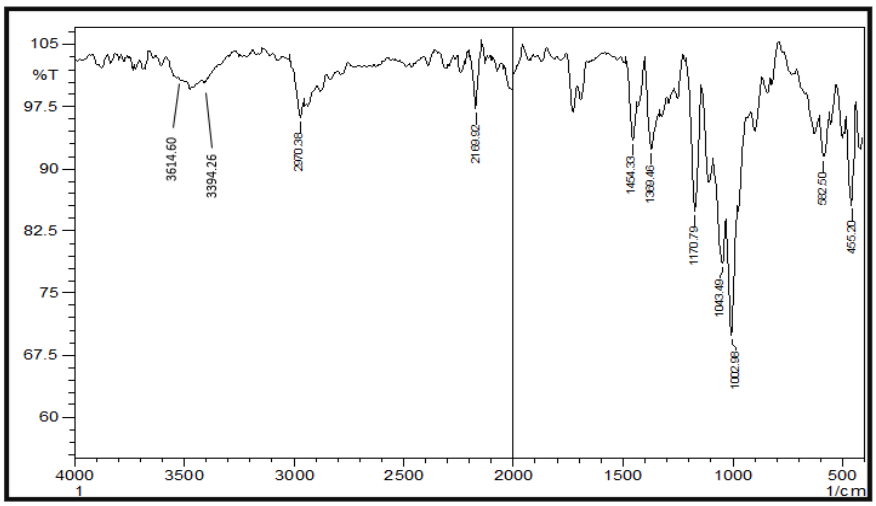

Fig. No. 03: FTIR spectrum of Clarithromycin.

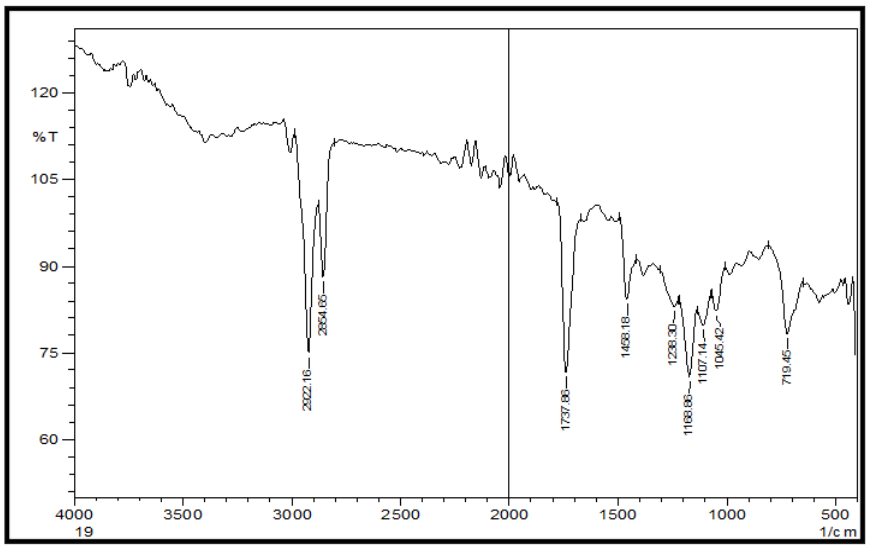

Fig. No. 04: FTIR spectrum of Glyceryl monooleate.

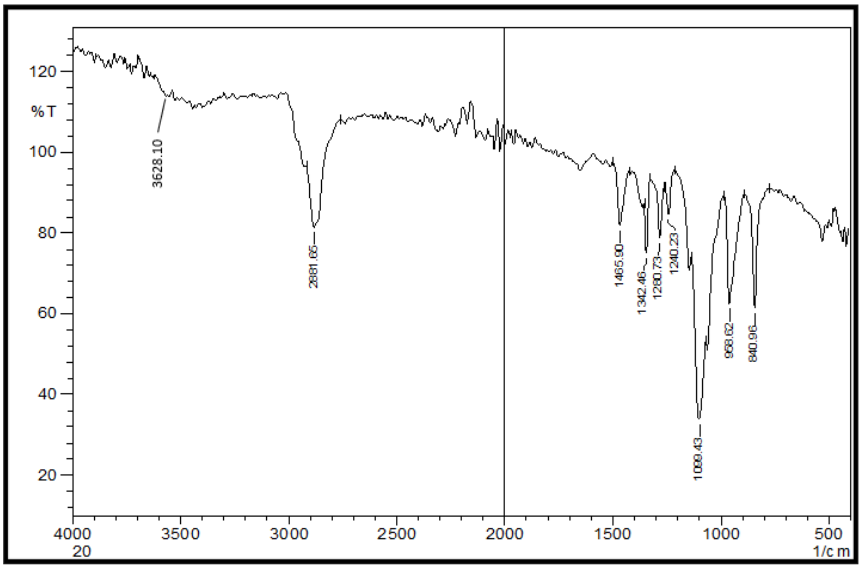

Fig. No. 05: FTIR spectrum of Poloxamer 407.

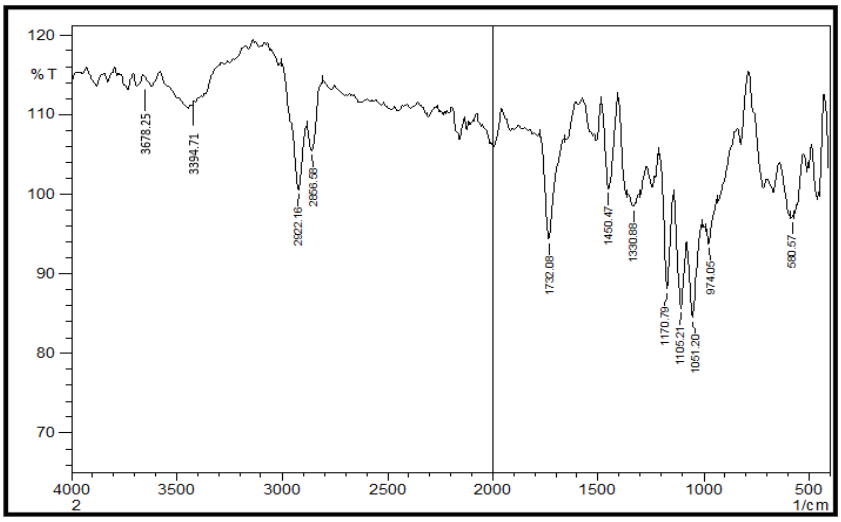

Fig. No. 06: FTIR spectrum of Cubosomal formulation.

Table No. 05: Comparison of FTIR spectra of Pure drug and excipients.

Components	Functional group	Reported frequency(cm^{-1})	Observed frequency(cm^{-1})
Pure drug Clarithromycin	C=O Stretching	1680-1740	1724.36
	N-H Stretching	3300-3400	3394.26
	C-H Stretching	2850-3000	2970.38
	C-O Stretching	1000-1200	1170.79
	O-H Stretching	3500-3600	3614.60
	$-OCH_3$ bending	1450-1500	1454.33
Glyceryl monooleate	C-H Stretching	2850-3000	2922.16
	C=O Stretching	1680-1740	1737.86
	C-O Stretching	1000-1300	1168.86
	C-C Stretching	800-1000	989.48
	$-CH_3$ bending	1370-1400	1379.10
Poloxamer 407	C-H Stretching	2850-3000	2881.65
	C-O Stretching	1000-1300	1099.43
	C-C Stretching	800-1000	958.62
Cubosome formulation	C=O Stretching	1680-1740	1732.08
	C-H Stretching	2850-3000	2922.16
	C-O Stretching	1000-1300	1170.79
	C-C Stretching	800-1000	974.05
	N-H Stretching	3300-3400	3394.71
	O-H Stretching	3500-3600	3678.25
	$-OCH_3$ bending	1450-1500	1450.47

The compatibility between the drug and polymers was checked using the FT-IR peak matching method. Specific functional group peaks were compared, and results are shown in Table No.05.

- Clarithromycin showed peaks at 1724.36 cm^{-1} (C=O), 3394.26 cm^{-1} (N-H), 2970.38 cm^{-1} (C-H), 1170.79 cm^{-1} (C-O), 3614.60 cm^{-1} (O-H), and 1454.33 cm^{-1} ($-OCH_3$).
- Glyceryl monooleate showed peaks at 2922.16 cm^{-1} (C-H), 1737.86 cm^{-1} (C=O), 1168.86 cm^{-1} (C-O), 989.48 cm^{-1} (C-C), and 1379.10 cm^{-1} ($-CH_3$).
- Poloxamer 407 showed peaks at 2881.65 cm^{-1} (C-H), 1099.43 cm^{-1} (C-O), and 958.62 cm^{-1} (C-C).
- Cubosome formulation showed similar peaks, confirming no major shifts.

The FT-IR spectra of the F5 formulation showed all the important peaks of the drug and polymers with slight shifts, indicating good compatibility without any chemical changes.

Preparation of Clarithromycin loaded cubosomes by Top-down Approach

Clarithromycin loaded cubosomes was successfully prepared using the top-down approach. Prepared cubosomal dispersion was white in colour as shown in Figure No.08.

Fig. No. 07: Preparation of Clarithromycin loaded Cubosomes using Magnetic stirrer and Probe sonicator.

Fig. No. 08: Prepared batches of cubosome formulation.

Characterization of cubosomes containing clarithromycin

1. Entrapment Efficiency % (EE %)

Table No. 06: Entrapment Efficiency % (EE%) of Clarithromycin loaded cubosomes.

Formulation	%EE*
F1	68.833±0.04
F2	72.087 ±0.06
F3	75.422±0.02
F4	82.929±0.06
F5	85.229±0.12
F6	78.092±0.11
F7	73.018±0.03
F8	65.572±0.05

*Data expressed as mean ± SD, n=3

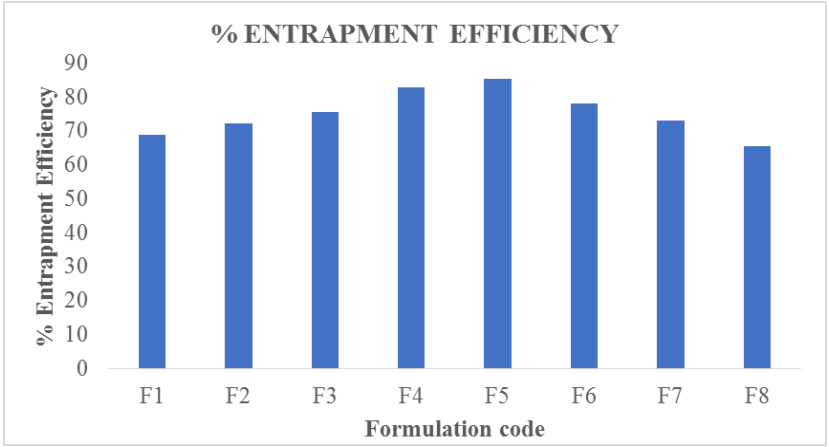

Fig. No. 09: Entrapment Efficiency % of Clarithromycin loaded Cubosomes (F1-F8).

The entrapment efficiency (%EE) of formulations F1 to F8 ranged from 65.57% to 85.23% (Table No.06). Maximum entrapment was observed near a 4:1 ratio of glyceryl monooleate (GMO) to poloxamer 407. %EE increased from F1 to F5, peaking at F5 (85.23%), suggesting that higher poloxamer 407 concentrations enhance cubosome stabilization. However, beyond F5, %EE declined (F6–F8), indicating that excessive poloxamer 407 may disrupt cubosome structure and lower drug entrapment.

2. Average particle Size and PDI

Table No.07: Particle size and PDI of Clarithromycin loaded cubosome formulation F5.

Formulation	Particle size(nm)	PDI
F5	150.6	0.458

The average particle size of cubosomes (F5) was 150.6 nm (Table No.07), influenced by glyceryl monooleate (GMO) and Poloxamer 407 concentrations. Higher GMO levels increased particle size due to greater viscosity, while increased Poloxamer 407 reduced particle size by enhancing stabilization. The PDI was 0.458, indicating a relatively uniform size distribution, likely due to effective emulsification by Poloxamer 407.

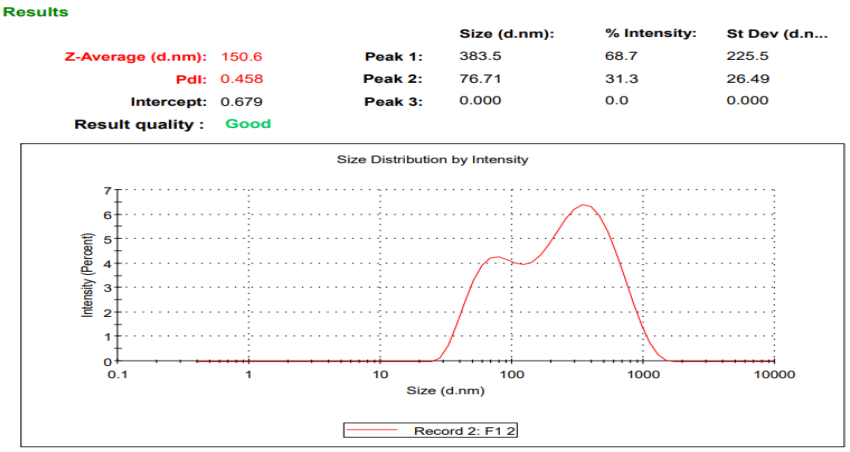

Fig. No. 10: Particle size of F5 Clarithromycin loaded cubosomes.

3. Zeta potential

Table No. 08: Zeta potential of Clarithromycin loaded cubosome formulation F5.

Formulation	Zeta potential
F5	-23.7

Zeta potential is a key physico-chemical parameter influencing the stability of cubosomes by affecting particle interactions. The F5 cubosome formulation showed a zeta potential of -23.7 mV (Fig No.11), indicating good stability. The negative charge helps prevent aggregation, suggesting that the formulation will remain well-dispersed and stable over time.

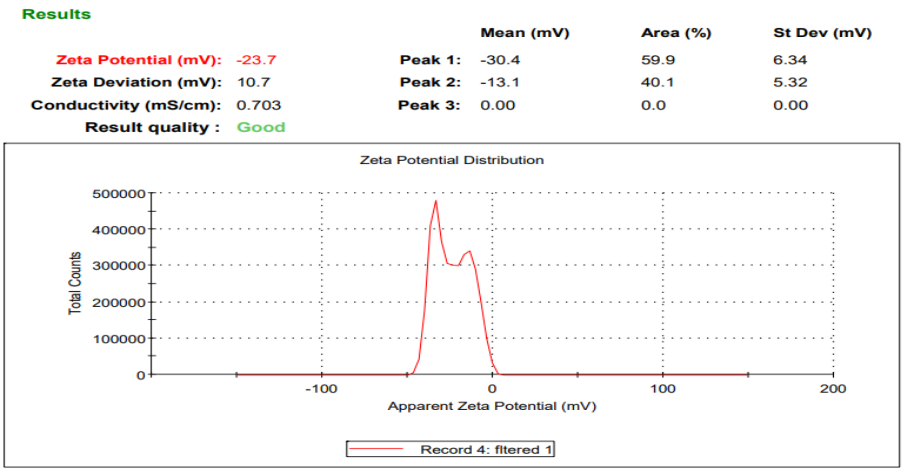

Fig. No. 11: Zeta potential of F5 Clarithromycin loaded cubosomes.

4. Drug content

Table No. 09: Drug Content of Clarithromycin loaded cubosomes.

Formulation	Drug Content (%) *
F1	86.851±0.12
F2	89.640±0.50
F3	91.160±0.31
F4	93.657±0.14
F5	96.888±0.41
F6	88.220±0.15
F7	87.214±0.42
F8	85.823±0.12

*Data expressed as mean ± SD, n=3

The drug content of the developed cubosomes ranged from 85.823±0.12% to 96.888±0.41%. The optimal formulation, F5, exhibited the highest drug content of 96.888±0.41%, which aligns with the PDI and particle size results. The detailed study results are presented in Table No.09.

5. *In –vitro* drug diffusion studies from cubosomes

Table No. 10: *In –vitro* drug diffusion studies from Clarithromycin loaded cubosomes.

Time	% Cumulative Drug Release							
	F1	F2	F3	F4	F5	F6	F7	F8
0 min	0	0	0	0	0	0	0	0
15 min	4.42 ± 0.19	5.22 ± 0.10	5.40 ± 0.30	5.83 ± 0.16	6.44 ± 0.16	4.51 ± 0.18	4.44 ± 0.10	4.14 ± 0.02
30 min	7.89 ± 0.10	8.79 ± 0.23	9.34 ± 0.02	9.93 ± 0.21	10.61 ±0.04	9.87 ± 0.18	7.87 ± 0.05	8.29 ± 0.12
45 min	12.62 ± 0.20	13.76 ±0.07	13.40 ±0.15	14.07 ±0.23	15.25 ±0.13	11.12 ±0.12	11.26 ±0.16	12.54 ±0.03
1 hr	16.86 ± 0.12	19.53 ±0.02	17.45 ±0.30	18.29 ±0.06	19.33 ±0.16	17.17 ±0.07	15.07 ±0.14	16.09 ±0.04
2 hr	23.64 ± 0.04	26.20 ±0.03	21.83 ±0.05	27.04 ±0.14	28.46 ±0.18	18.86 ±0.17	20.63 ±0.04	20.62 ±0.12
3 hr	30.57 ± 0.02	32.74 ±0.13	35.89 ±0.02	34.75 ±0.05	34.38 ±0.16	24.67 ±0.18	29.08 ±0.07	28.23 ±0.01
4 hr	38.08 ± 0.02	40.32 ±0.24	39.92 ±0.13	44.28 ±0.16	42.76 ±0.11	33.56 ±0.17	37.18 ±0.16	39.12 ±0.16
5 hr	47.89 ± 0.04	49.34 ±0.12	44.07 ±0.02	54.01 ±0.06	53.21 ±0.12	43.17 ±0.13	48.31 ±0.80	47.25 ±0.01
6 hr	57.91 ± 0.14	59.12 ±0.013	56.67 ±0.011	61.01 ±0.04	62.30 ±0.18	51.41 ±0.08	57.48 ±0.18	56.84 ±0.08
7 hr	67.76 ± 0.20	61.12 ±0.01	68.80 ±0.23	69.96 ±0.19	75.86 ±0.08	62.09 ±0.17	65.08 ±0.01	64.32 ±0.11
8 hr	72.18 ± 0.12	73.60 ±0.32	75.51 ±0.14	79.17 ±0.17	82.53 ±0.13	78.82 ±0.18	73.31 ±0.27	69.86 ±0.19

*Data expressed as mean ± SD, n=3

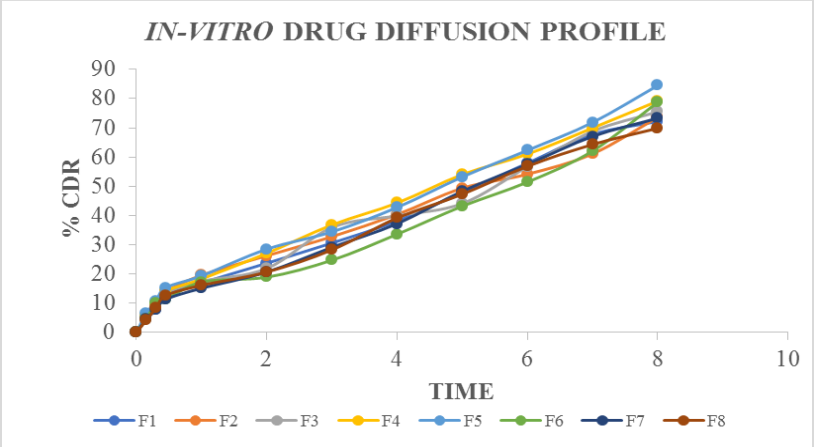

Fig No. 12: *In –vitro* drug diffusion studies from Clarithromycin loaded cubosomes (F1-F8).

The % cumulative drug release over 8 hours for all formulations (F1-F8) is shown in Fig No.12. The drug-polymer composition significantly affects the *in-vitro* drug release rate from cubosomes. All formulations exhibited a biphasic release profile, with an initial rapid release phase for the first 2 hours, followed by a controlled release over the remaining 6 hours. The rapid initial release is likely due to the unentrapped drug present on the surface of the cubosomes or in the solution. Variations in the release profiles may be attributed to factors such as vesicle size, drug entrapment, lamellarity, and membrane fluidity. Formulation F5 demonstrated the highest release, with 82.53±0.13% released after 8 hours.

Characterization of developed cubosomal gel of clarithromycin

Based on the % entrapment efficiency, drug content, and *in-vitro* diffusion studies, formulation F5 showed the best results. Therefore, it was incorporated into a Carbopol gel for further evaluation.

1. Physical examination

Table No. 11: Physical examination of Clarithromycin loaded Cubosomal gel.

Formulation	Colour	Clarity	Homogeneity	Grittiness
Clarithromycin loaded Cubosomal gel	White	Clear	Good	Absent

The prepared gel was white in colour. Its clarity was checked by visual inspection on both black and white backgrounds, and it was graded as clear. The gel was also evaluated for physical appearance and found to be homogeneous, without any gritty particles or aggregates.

Fig. No. 13: Prepared Clarithromycin loaded Cubosomal gel.

2. Determination of pH, spreadability, extrudability and drug content uniformity of Cubosomal gel of Clarithromycin

Table No. 12: Determination of pH, spreadability, extrudability and drug content uniformity of Cubosomal gel of Clarithromycin.

Formulation	pH*	Spreadability* g.cm/sec	Extrudability % decrease*	Drug Content*
F5	6.28±0.14	19.16±0.11	98.33±0.23	90.501±0.15

*Data expressed as mean ± SD, n=3

The pH of the Clarithromycin-loaded cubosome gel was found to be 6.28±0.14 (Table No. 12), which is similar to the skin's pH and causes no irritation, making the gel compatible with the skin.

The spreadability of the Clarithromycin 1% gel was tested, and its spreadability factor was 19.16 ± 0.11 g.cm/sec, indicating good spreadability. This ensures easy application and helps the gel cover a larger area of the skin, allowing for better drug delivery.

The gel also showed excellent extrudability, which is important for ease of use. The extrudability was 98.33±0.23% when 100 g of weight was applied to the tube, confirming a good balance of the gel's properties and tube compatibility.

The drug content of the Clarithromycin 1% gel was 90.501 ± 0.15%, indicating that the gel is suitable for pharmacological action.

3. Viscosity

Table No. 13: Viscosity data of Cubosomal gel of Clarithromycin.

RPM	Viscosity (cps)
5	3055
10	2869
20	2025
30	1694
50	1025

The viscosity of the Clarithromycin-loaded cubosomal gel was measured using a Brookfield viscometer. The gel was tested at shear rates ranging from 5 to 50 rpm using spindle number 64. The results showed that the gel behaves as a non-Newtonian pseudoplastic, with its viscosity decreasing from 3055 centipoise (cps) at 5 rpm to 1025 cps at 50 rpm, indicating a shear-thinning effect.

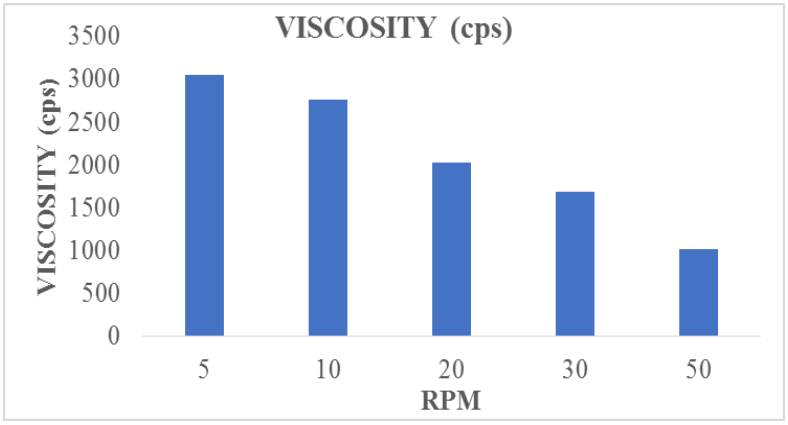

Fig. No. 14: Graphical representation of Viscosity of gel against rpm.

6. *In-vitro* drug diffusion studies

The *in-vitro* drug diffusion study of the F5 Clarithromycin-loaded cubosomal gel was performed using a Franz diffusion cell. It showed a cumulative drug release of 79.568±0.18% after 8 hours. The release pattern is shown in Fig No.15.

Table No. 14: *In vitro* diffusion study of Clarithromycin loaded cubosomal gel.

Time	% CDR *
0 min	0
15 min	4.175±0.02
30 min	8.196±0.12
45 min	11.972±0.03
1 hr	17.389±0.17
2 hr	24.292±0.11
3 hr	32.227±0.14
4 hr	39.012±0.15
5 hr	49.836±0.11
6 hr	58.025±0.12
7 hr	72.194±0.14
8 hr	79.568±0.18

*Data expressed as mean ± SD, n=3

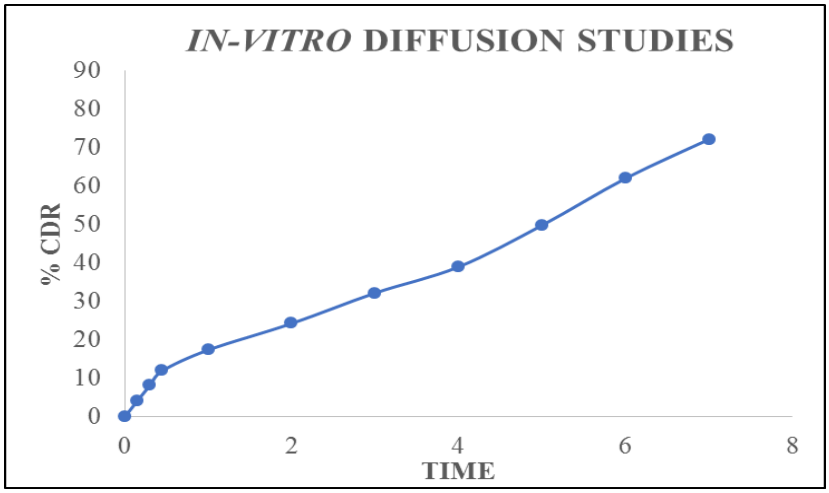

Fig No. 15: *In vitro* release profile of Clarithromycin loaded cubosomal gel.

4. *In-vitro* drug release kinetics

Table No. 15: Drug release kinetics of Clarithromycin loaded cubosomal gel.

Time	%CDR	% Drug remaining	Square root of time	Log % cumulative drug remaining	Log time	Log cumulative %drug released
0 min	0	100	0	2	0	0
15 min	4.175	95.825	0.387298335	1.981478828	-0.82391	0.62065648
30 min	8.196	91.804	0.547722558	1.962861604	-0.52288	0.91360195
45 min	11.972	88.028	0.670820393	1.944620835	-0.34679	1.078166708
1 hr	17.389	82.611	1	1.917037879	0	1.240274607
2 hr	17.389	82.611	1.414213562	1.917037879	0.30103	1.240274607
3 hr	32.227	67.773	1.732050808	1.83105671	0.47712	1.508219879
4 hr	39.012	60.988	2	1.785244392	0.60206	1.591198216
5 hr	49.836	50.164	2.236067977	1.700392159	0.69897	1.697543177
6 hr	60.025	39.975	2.449489743	1.601788472	0.77815	1.778332169
7 hr	70.194	29.806	2.645751311	1.474303697	0.8451	1.846299991
8 hr	80.568	19.432	2.828427125	1.288517502	0.90309	1.906162583

Zero order release kinetics

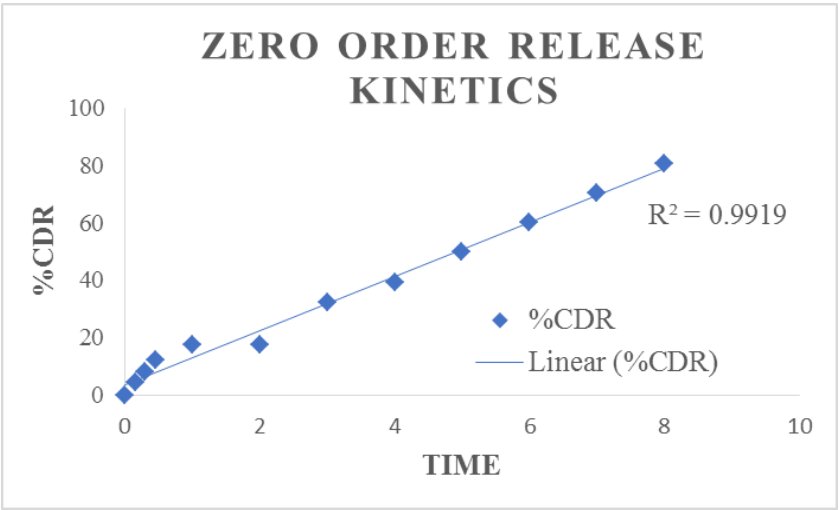

Fig. No. 16: Plot of Percentage CDR v/s Time (Zero order kinetics).

First order release kinetics

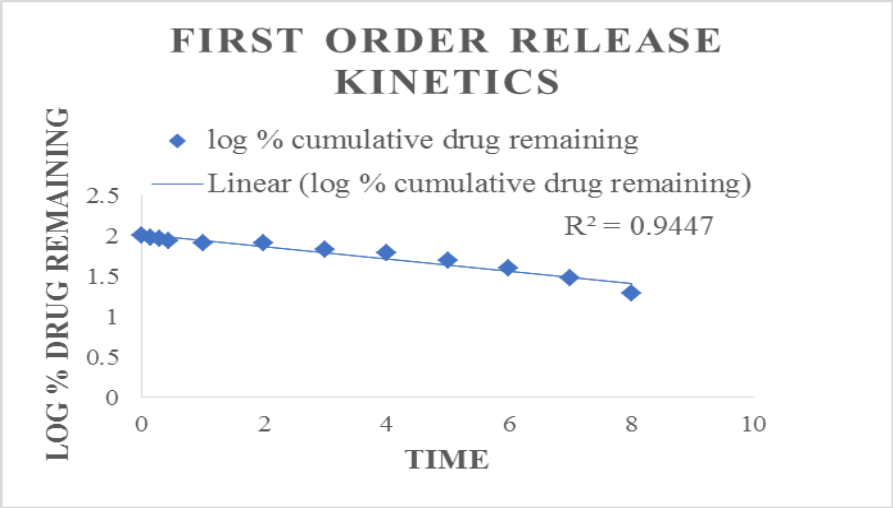

Fig. No. 17: Plot of Log % CDR remaining v/s Time (First order kinetics).

Higuchi Model

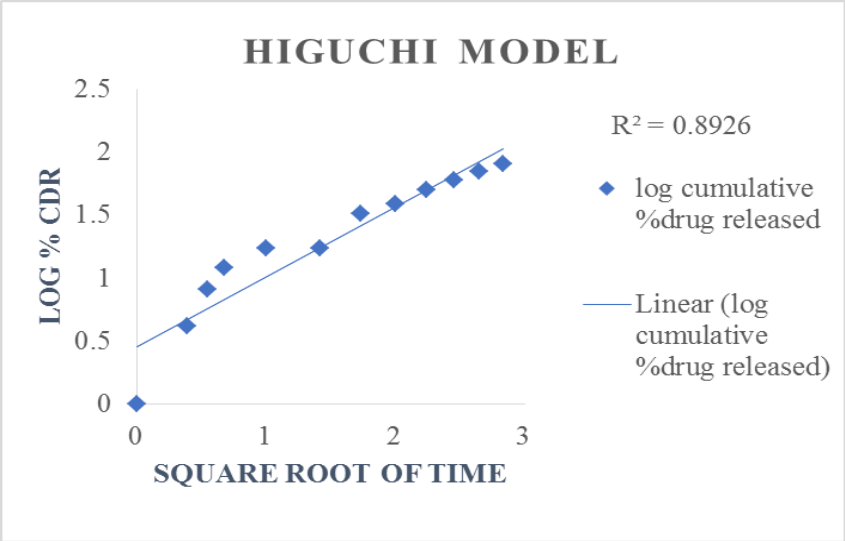

Fig. No. 18: Plot of Log %CDR v/s √ t (Higuchi model).

Korsmeyer Peppas Model

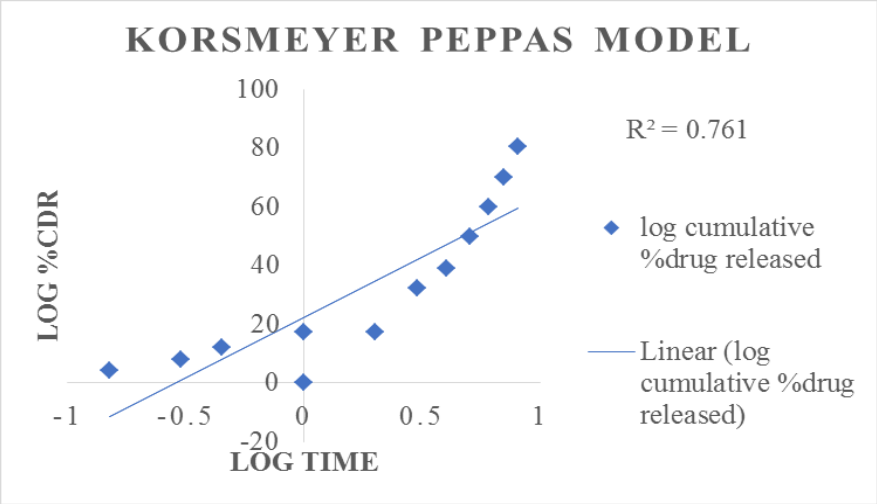

Fig. No. 19: Plot of Log %CDR v/s Log Time (Korsmeyer Peppas Model).

Table No. 16: Regression values of kinetic release study of Clarithromycin loaded cubosomal gel.

Kinetic modelling	R^2
Zero order kinetics	0.9919
First order kinetics	0.9447
Higuchi model	0.8926
Korsmeyer peppas model	0.7610

To determine the drug release mechanism from the F5 Clarithromycin-loaded 1% gel, *in-vitro* release data were fitted into various models including Zero-order, First-order, Higuchi, and Korsmeyer-Peppas. Regression analysis was performed using MS-EXCEL, and release constants were calculated from the slopes of the respective plots. The results showed that the drug release followed Zero-order kinetics, with a high regression coefficient (R^2 = 0.9919), as shown in Fig. 16 and Table 16. The Higuchi model (R^2 = 0.8926) also supported a diffusion-controlled release mechanism.

5. Comparative study with marketed formulation

Table No. 17: Comparative study of cubosomal gel with marketed formulation.

Time (mins)	%CDR of cubosomal gel F5 *	%CDR of Crixan gel 1%w/w Marketed formulation *
0 min	0	0
15 min	5.513±0.15	8.076±0.16
30 min	9.340±0.04	29.941±0.17
45 min	14.756±0.03	41.013±0.16
1 hr	19.673±0.23	52.458±0.12
2 hr	26.926±0.12	67.803±0.26
3 hr	33.278±0.17	82.324±0.12
4 hr	41.037±0.16	91.617±0.18
5 hr	49.678±0.17	-
6 hr	62.388±0.16	-
7 hr	73.883±0.24	-
8 hr	80.192±0.14	-

*Data expressed as mean ± SD, n=3

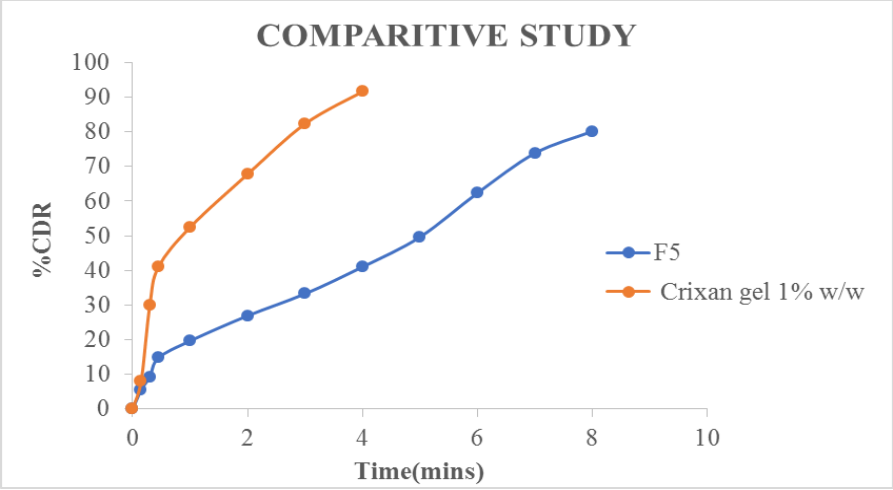

Fig. No. 20: *In -vitro* release pattern of cubosomal gel (F5) and marketed formulation.

Based on the drug release comparison studies, it was observed that the marketed product exhibited a rapid release, reaching 91.617% in 4 hrs whereas F5 Clarithromycin loaded cubosomal gel showed gradual release, achieving 80.192% at the end of 8hrs.

6. Stability Studies as per ICH guidelines

Table No. 18: Evaluation of F5 cubosomal gel for stability study.

Evaluation parameters	Time(days) Accelerated condition 25±2°C at (60±5% RH)			
	0 day	30 days	60 days	90 days
Colour	White	White	White	White

pH	6.28±0.04	6.27±0.02	6.22±0.01	6.24±0.02
Drug content (%) *	90.501±0.12	88.785±0.20	86.816±0.17	86.051±0.14
In vitro diffusion in 8 hr *	78.512±0.04	77.194±0.13	77.986±0.11	76.008±0.08

*Data expressed as mean ± SD, n=3

The stability study for the F5 Clarithromycin 1% gel was conducted under accelerated conditions (25±2°C and 60±5% RH) for 3 months (at 30, 60, and 90 days) following ICH guidelines. The results confirmed that the formulation remained stable over the study period, with no significant changes observed in appearance, pH, drug content, or *in-vitro* drug release after 90 days.

CONCLUSION

- A Clarithromycin-loaded cubosomal gel was successfully developed using Glyceryl monooleate (GMO) and Poloxamer 407.
- Eight cubosome formulations were prepared and evaluated for entrapment efficiency, particle size, zeta potential, drug content, and *in-vitro* drug release.
- Formulation F5 showed the best results and was incorporated into a Carbopol gel, improving its physical properties, pH, viscosity, and uniformity.
- The 1% Clarithromycin gel exhibited a controlled and prolonged drug release over 8 hours, following zero-order kinetics supported by the Higuchi model.
- *In-vitro* diffusion studies showed that the cubosomal gel provided a slower and more sustained release compared to a marketed product.
- Stability studies confirmed that the formulation remained stable with no significant changes in its properties, highlighting its potential as an effective drug delivery system for Clarithromycin.

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