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Formulation and Evaluation of Rifampicin Cubosomes

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ABSTRACT

The present study was focused on formulating and evaluating Rifampicin containing Cubosomes formulation for in vitro studies. Cubosomal formulations were prepared by using top down method and were evaluated for in vitro characteristics, stability studies. Cubosomal formulation displayed highest entrapment efficiency with desired particle size. SEM analyses showed that Cubosomal formulation was spherical in shape. Cubosomes containing Pluronic-F 127 percentage of drug release after 8 h as compared to other formulations. F-7 formulation was found to be stable at the end of the study on storage condition. The present study suggested that cubosomes formulations provide sustained and prolonged delivery of drug with enhance bioavailability.

Keywords: Cubosomes, Rifampicin, bioavailability, Top down method, in vitro drug release studies.

I. INTRODUCTION

Cubosomes are discrete, sub-micron, Nano-structured particles of bicontinuous cubic liquid crystalline phase. Cubosomes possess the same microstructure as the parent cubic phase but have larger specific surface area and their dispersions have much lower viscosity in comparison to the bulk cubic phase. [1] Cubosomes have honeycombed (cavernous) structures whose size range from 10–500 nm in diameter. They appear like dots, which are slightly spherical in structure.[2] Rifampicin (RIF) is a major component in fixed dose combination therapy for the treatment of tuberculosis. Rifampicin (RIF) was selected as a pharmaceutical model based on the fact that it belongs to the four first-line drugs in TB chemotherapy and the most widely investigated.[3] Especially, RIF has not only a broad antibacterial spectrum for gram-positive and -negative bacteria and protozoa, but also antibacterial activity for MDR-TB.1 In contrast to other antibiotics, RIF binds directly to an enzyme involved in RNA polymerization, namely DNAdependent RNA polymerase; the same enzyme, present in a variety of eukaryotic species, is completely resistant to the actions of RIF.[4] Drug-loaded microspheres (MS) have received extensive attention for the treatment of tuberculous osteomyelitis as they can penetrate well into bone tissue and have been extensively used for the treatment of other types of bacterial osteomyelitis, that is, osteoarthritis, spondylitis, spondylodisciitis, and arthritis.[5]

II. MATERIALS

Rifampicin was obtained from Alkem Pvt Mumbai, Pluronic acid F-127 and ethanol procured from SD fine chemicals Mumbai. Other chemicals and the reagents used were of analytical grade.

III. METHODOLOGY

Fourier Transform Infrared Spectroscopy:

Fourier transform IR spectra were obtained on Shimadzu FT-IR spectrometer. Samples were prepared in KBr disks (2mg sample in 200mg KBr). The scanning range was 450-4000 cm^{-1} and the resolution was 4 cm^{-1}.[6]

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Formulation Development
Top-Down Method

Table-1: Formulation Development

Ingredients	F1(1:1)	F2(1:2)	F3(1:3)	F4(1:4)	F5(1:5)	F6(1:6)	F7(1:7)	F8(1:8)
Rifampicin	10	10	10	10	10	10	10	10
Fluronic F-127	10	20	30	40	50	60	70	80
Ethanol	10	10	10	10	10	10	10	10
Water	10	20	30	40	10	20	30	40

Method of Preparation:
Top-Down Method

Rifampicin was weighed into a glass vial and heated at 40°C until free flowing. Aqueous solution containing different concentrations of Pluronic F-127, prepared from a 2 wt% stock solution of F-127, were added to the vial containing Rifampicin. Subsequently, this mixture was homogenized by ultra-sonication (Hielscher, Teltow,Germany)at 40°C, amplitude 80%,pulse cycle 1 for 30min until a milky dispersion was formed. To study the effect of ethanol on the Cubosome formation, different amounts of ethanol were added to the F-127 aqueous solution. In order to examine the influence of the initial temperature on the cubosomes, they were also prepared at 60 and 70°C. By this method the formulations of various formulation ratios were determined. [7]

Evaluation of Cubosomes:
Size and Size Distribution:

Size and size distribution studies were done for cubosomes prepared by Thin Film hydration. The cubosomes suspension (100 mg) was hydrated in a small glass test tube using 10 ml of pH 7.4 phosphate buffer solution. The dispersion was observed under optical microscope at 40X magnification. Size and size distribution of 200–300cubosomes were noted using calibrated stage and ocular micrometres (Elico Instruments, Hyderabad).[8]

Entrapment Efficiency:

To 0.2 g of cubosomes, weighed in a glass tube, 10 ml phosphate buffer pH 7.4 was added. The aqueous suspension was then sonicated. Cubosomes containing Rifampicin were separated from untrapped drug by centrifugation at 9000rpm for 45 min at 4◦C. The supernatant was recovered and assayed spectrophotometrically using UV-spectrophotometer. [9]

The encapsulation percentage of drug (EP) was calculated by the following equation:

$EP = [(C_t - C_r)/ C_t] * 100$

Where,
Ct, concentration of total Rifampicin,
Cr, concentration of free Rifampicin.

Vesicle Physical Analysis:

The shape, surface characteristics, and size of the cubosomes were observed by scanning electron microscopy. [10]

Zeta Potential

The zeta potential of a particle represents the overall charge of the particle and stability of the formulation. Zeta potential measurement was carried out using Zeta sizer Nano-ZS90, Malvern Instrument Ltd., UK by differential light scattering (DLS) technique. Nanoparticle samples redispersed in Milli-Q water. All measurements were carried out in triplicates at 25 °C.[11]

In Vitro Drug Release Study:

In vitro release studies were carried out using unjacketed vertical Franz diffusion cells with a diffusional surface area of 6.154 cm^2 and 20 mL of receptor cell volume. Prior to the study, the dialysis membrane was soaked in phosphate buffer pH 7.4 Formulation equivalent to 5mg of Rifampicin was placed in the donor compartment. The receptor compartment consisting of PB pH 7.4 was maintained at 37±2°C under constant stirring upto 24 hrs. The donor chamber and the sampling port were covered with lid to prevent evaporation during the study. Aliquots of 5 mL were withdrawn periodically at different time intervals (5, 10, 15, 30, 1, 2, 3, 4, 5, 6, 7 hrs) and replaced with equal volume to maintain constant receptor phase volume. At the end of the study, the samples were suitably diluted and the amount of drug was determined spectrophotometrically.[12]

Kinetics of Drug Release:[13]

To study kinetices data obtained from invitro release were plotted in various kinetic models.

- **Zero-Order Equation:**

% R = Kt

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This model represents an ideal release profile in order to achieve the pharmacological prolonged action. This is applicable to dosage forms like transdermal systems, coated forms, osmotic systems, as well as matrix tablets with low soluble drugs.

- **First Order Equation:**

Log % unreleased = Kt / 2.303

This model is applicable to study hydrolysis kinetics and to study the release profiles of pharmaceutical dosage forms such as those containing water soluble drugs in porous matrices.

- **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:

The formulations stored in glass vials covered with aluminium foil were kept at room temperature and in refrigerator (4◦C) for a period of 90 days, samples were withdrawn and hydrated with phosphate-buffered saline (pH 7.4) and observed for any sign of drug crystallization under optical microscope. Furthermore, the samples were also evaluated for particle size and percent retention of Rifampicin.[14]

IV. RESULTS AND DISCUSSION

Drug - Excipient Compatibility Studies (FT-IR):

The compatibility between the drug and the selected lipid and other excipients was evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the drug-lipid mixture, which confirmed the absence of any chemical interaction between the drug, lipid and other chemicals.

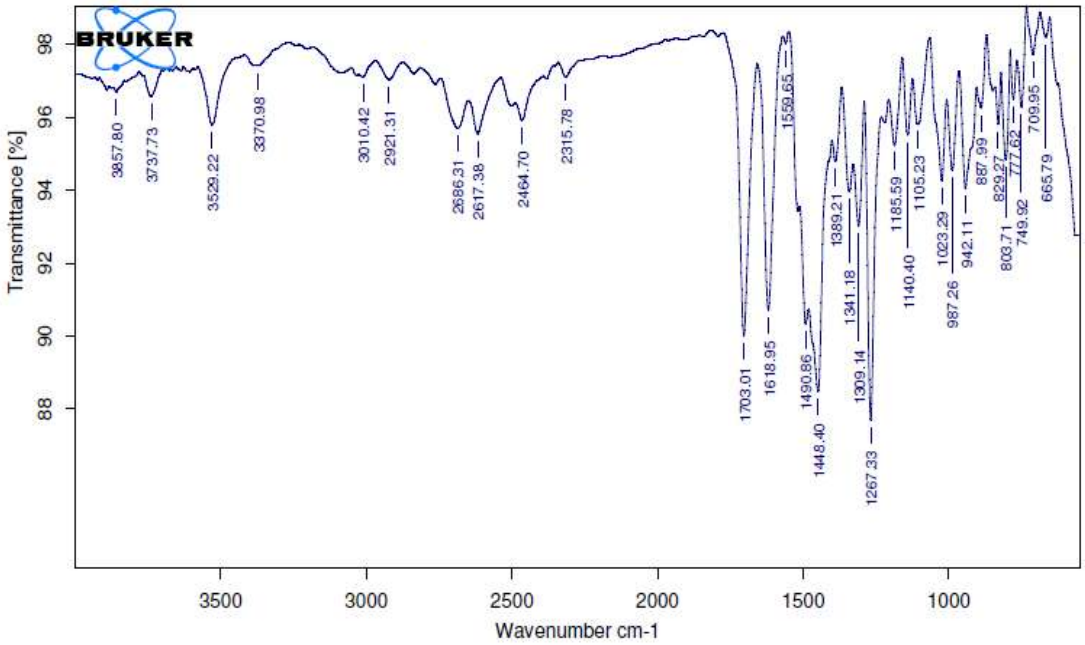

Fig-1: FT-IR Sample for Rifampicin

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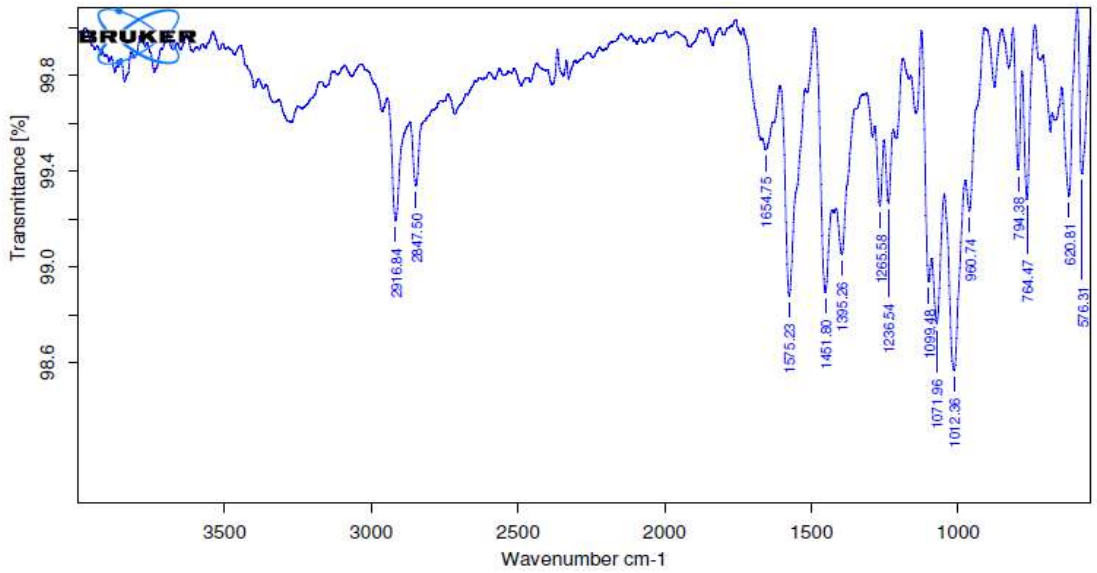

Fig-2: FT-IR Sample for Optimized Formulation

EVALUATION PARAMETERS:

Entrapment Efficiency:

Separation of unentrapped drug from Cubosomes was done by exhaustive dialysis method. A measured quantity of Cubosomes was placed in a dialysis tube to which osmotic cellulose membrane was attached securely on one side and the dialysis tube was suspended in 100 ml of phosphate buffer pH 7.4 which was stirred continuously using magnetic stirrer. Through the osmotic cellulose membrane the unentrapped drug was separated into the medium.Foreveryonehourthewholemediumwasrepl acedwithsamequantityof freshmedium and continued for about 9 to 12hrs till the absorbance of collected medium reaches a constant reading indicating complete separation of unentrapped drug. The Cubosomes in the dialysis tube was further lysed with propane–1–ol and the entrapped drug was estimated with the help of double beam UV spectrophotometer at 275nm. The entrapment efficiency was measured in % with the help of following equation,

$$\%\text{Entrapment efficiency} = \frac{\text{Amount of drug entrapped}}{\text{Total amount of drug added}} \ 100$$

Table-2: Drug entrapment Efficiency of all Formulation

F. No.	Drug Entrapment Efficiency
F1	65.68
F2	68.23
F3	64.25
F4	65.22
F5	68.92
F6	68.35
F7	69.83
F8	67.86

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Determination of Vesicle Morphology and Size

The morphological characteristics of formulated Cubosomes were carried by using Scanning electron microscopy (SEM). A small drop of Cubosomes was placed between two rivets fixed on a gold plated copper sample holder. The whole system was slushed under vacuum in liquid nitrogen. The sample was heated to -85^{0}C for 30 min to sublime the surface moisture. Finally the sample was coated with gold and allowed the SEM to capture the images at a temperature of - 120^{0}c and voltage of 5kV.Scanning electron microscopy is the direct method to measure cubosomes, physical characterization of cubosomes with the former method being used for morphological examination.

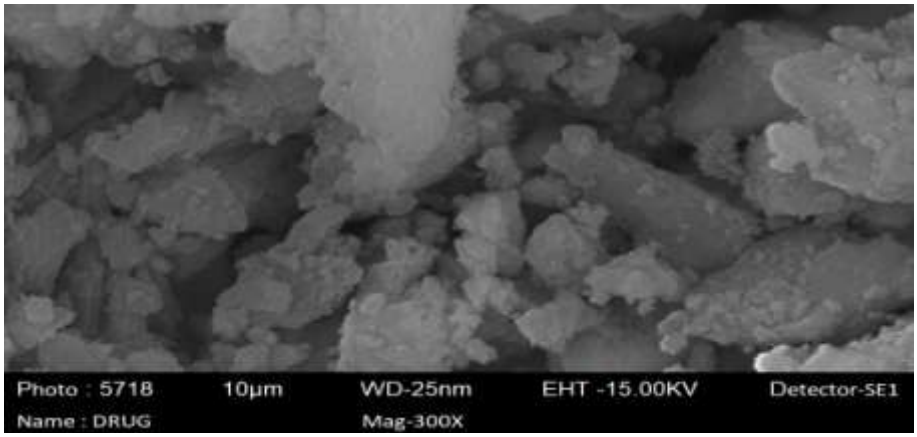

Fig-3: SEM Analysis of Optimized Formulation

Zeta Potential:

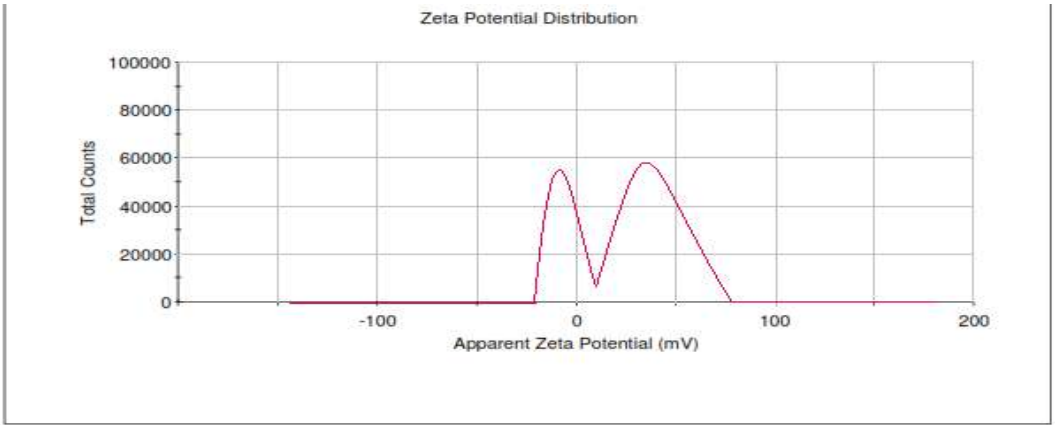

Fig-4: Zeta Potential of Optimized Formulation

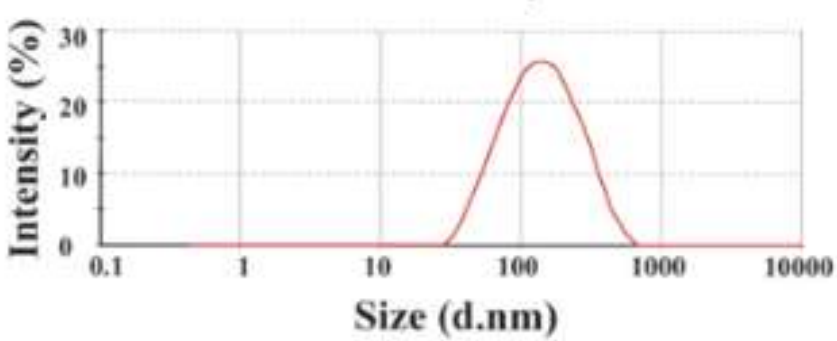

Fig-5: Particle Size of Optimized Formulation

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Table-3: Evaluation Studies of Particle Size and Zeta Potential Cubosomes

F. No.	Particle Size (nm)	Zeta Potential
F1	100.23	-26
F2	125.42	-32
F3	119.65	-28
F4	126.48	-30
F5	135.49	-23
F6	146.98	-27
F7	135.48	-30
F8	140.22	-33

In Vitro Drug Release Studies:

The release of drug from Cubosomes was investigated using Franz diffusion cell method. All the formulations were separately placed in a dialysis membrane of 5cm length with closed ends which was washed and soaked in phosphate buffer pH 7.4 for about 15min. The membrane was suspended in a beaker containing 500ml of phosphate buffer pH 7.4 as diffusion medium maintained at a temperature of 37 ± 0.5^0 C and stirred continuously by means of magnetic stirrer at a constant speed. At a regular time interval of one hour 5ml of diffusion medium was withdrawn periodically for about 8hrs and immediately replaced with same amount of fresh diffusion medium to maintain sink condition. The collected samples were measured spectrophoto metrically at 275 nm.

Table-4: Cumulative Percentage Drug Release from Various Formulations of Cubosomes

Time	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	11.45	12.58	13.85	14.59	15.12	14.50	19.89	17.82
2	25.93	27.98	29.86	27.86	25.89	27.93	28.99	24.87
3	30.59	32.82	35.47	36.98	35.90	36.55	39.82	35.44
4	43.59	46.75	48.93	47.44	48.90	45.22	49.87	48.21
5	55.82	56.98	57.80	56.82	55.42	56.98	59.89	55.40
6	69.68	68.90	67.88	62.66	63.89	62.04	70.25	71.52
7	79.89	78.89	77.81	76.59	75.40	74.96	80.42	80.21
8	89.82	90.25	92.67	90.25	92.68	93.61	95.99	94.66

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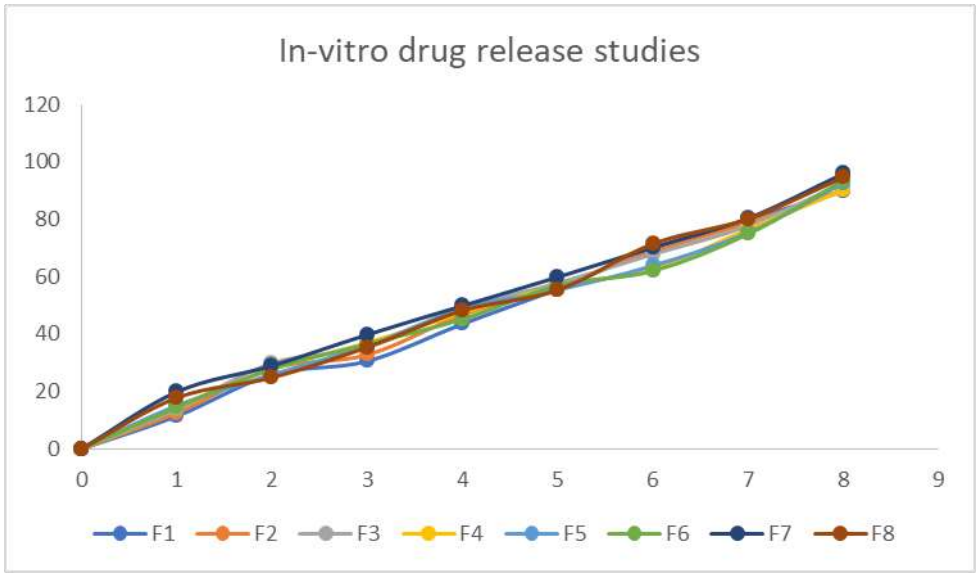

Fig-6: In Vitro Drug Release for (F1- F8) Formulations

Drug Release Kinetics

Thekinetic of drug release for formulation F7 was calculated and plotted. The formulation F7 follows first order release kinetics and the drug release mechanism was found to be non-Fickian anomalous diffusion.

In-Vitro Dissolution Study and Kinetic Modelling of Drug Release:

The results obtaining in vitro release studies were plotted in different model

1. Zero order rate kinetics
2. First Order rate Kinetics
3. Higuchi's models
4. KorsmeyerPeppas

Table-5: Drug Release Kinetics of Formulation F7

TIME	%CDR	SQARE T	LOG T	LOG%CDR	ARA	LOG%ARA
0	0	0	0	0	0	0
1	19.89	1	0	1.298634783	80.11	1.903686732
2	28.99	1.414213562	0.301029996	1.462248215	71.01	1.851319513
3	39.82	1.732050808	0.477121255	1.600101256	60.18	1.779452183
4	49.87	2	0.602059991	1.697839368	50.13	1.700097705
5	59.89	2.236067977	0.698970004	1.777354313	40.11	1.603252662
6	70.25	2.449489743	0.77815125	1.846646329	29.75	1.47348697
7	80.42	2.645751311	0.84509804	1.905364069	19.58	1.291812687
8	95.99	2.828427125	0.903089987	1.982225992	4.01	0.603144373

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Zero Order Kinetics

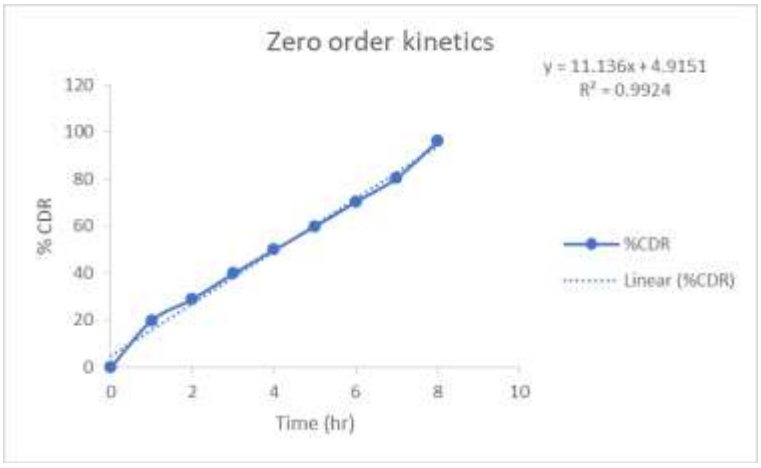

Fig-7: Zero Order Kinetics of Optimized Formulation

First Order Kinetics

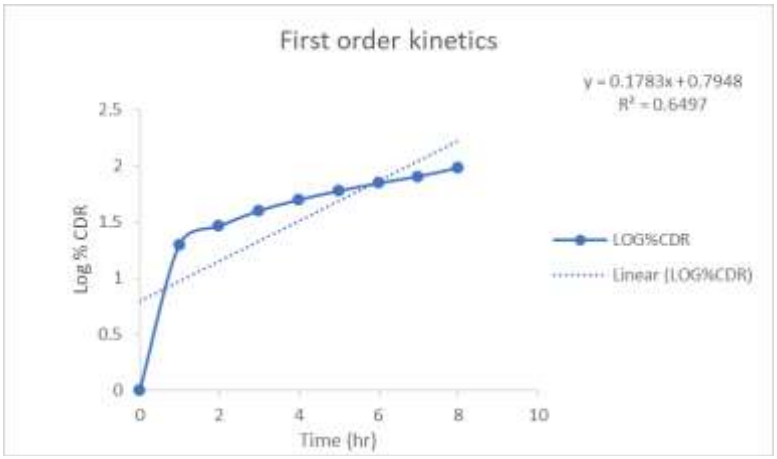

Fig-8:First Order Kinetic of Optimized Formulation

Higuchi Model

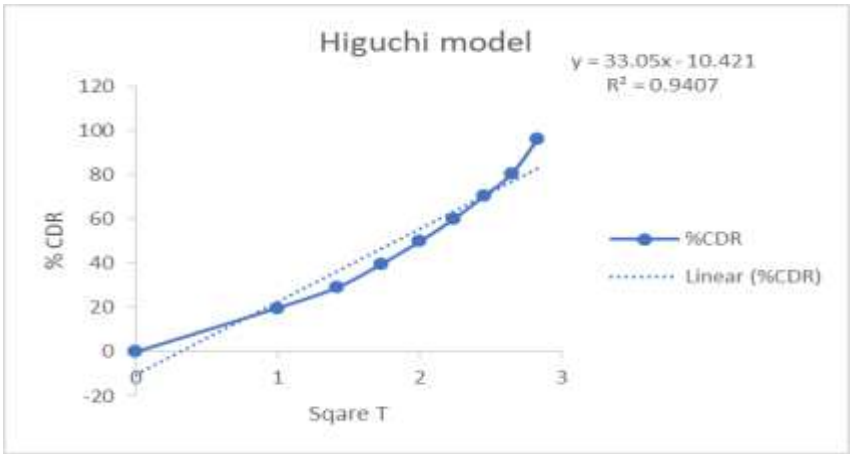

Fig-9: Higuchi Model of Optimized Formulation

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Krosmayer Peppas

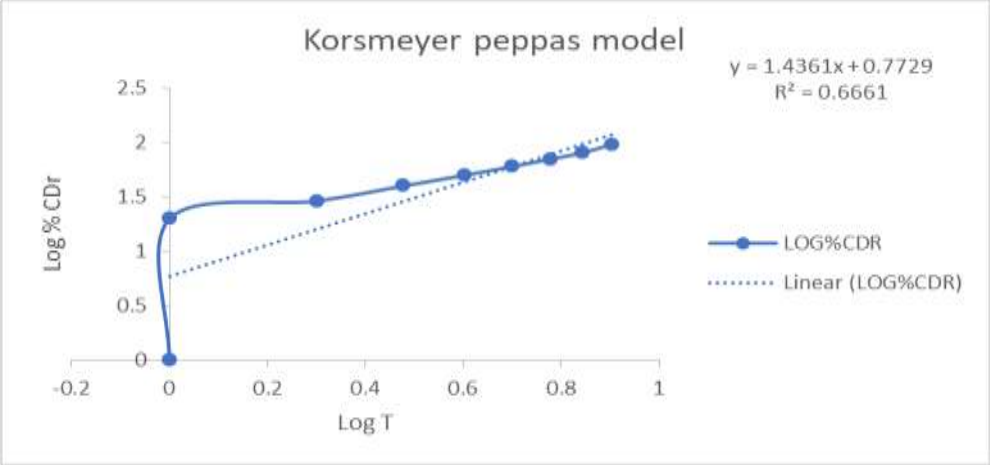

Fig-10: Krosmayer Peppas of Optimized Formulation

The values of in vitro release were attempted to fit into various mathematical models. Plots of zero order, first order, Higuchi matrix, Peppas were respectively

Stability Studies

There was no significant change in physical and chemical properties of the cubosomes of formulation F -7 for 3 months. Parameters quantified at various time intervals were shown

Table-6: Results of Stability Studies of Optimized Formulation F-7

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-7	25^0C/60%RH % Release	95.99	94.86	93.95	92.68	Not less than 85 %
F-7	30^0C/75% RH % Release	95.99	94.80	93.78	92.63	Not less than 85 %
F-7	40^0C/75% RH % Release	95.99	94.12	93.65	92.50	Not less than 85 %

V. CONCLUSION

Rifampicin cubosomes stabilized with F127 were prepared by the Top down (TD) method with ethanol as the liquid precursor and removed using rotary evaporator, and the top-down (TD) method using ultrasonication. This study aimed Rifampicin cubosomes were prepared by top up method. Our results suggest that Cubosome formulation is an ideal candidate for many Rifampicin cubosomes required in various applications. In vitro study revealed that cubosomes formulations F7(1:7) containing 70 mg F 127 shown better releases than other dispersion. In conclusion cubosomes are promising control release vehicle for the effective drug delivery of Rifampicin.

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