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Research Article

FORMULATION AND CHARACTERIZATION OF SOLID LIPID NANOPARTICLES OF RIFAMPICIN

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ABSTRACT

The purpose of this work was to develop prolonged release solid lipid nanoparticles (SLNs) of Rifampicin (RIF) for oral drug delivery and to improve the bioavailability of RIF. SLNs were designed by using cetyl palmitate (CP) and as lipid core materials and polaxamer 188 as stabilizer. SLNs were prepared by o/w microemulsion technique and characterized by particle size analysis, Fourier transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC), drug entrapment efficiency, scanning electron microscopy (SEM), Zeta potential, *in vitro* evaluation studies, storage stability, membrane permeation study. At highest speed the resultant SLNs were smaller in size and their size increased with increase in lipid concentration. Nanoparticles prepared using 5.0% lipid, 1.5% Poloxamer 188 and 9,500 rpm shows smaller particle size, better drug entrapment, excellent surface characteristic and controlled drug release. Release from RIF-SLNs was studied using a dialysis bag method. The results of *in-vitro* release study shows that the dialysis membrane after 12 and after 24 h the release pattern of drug slightly increases. The surface characters were found to be smooth with all the lipid carriers. Short term stability studies indicated no significant change, when stored between 2-8°C for one month.

Keywords: Solid lipid nanoparticles, Rifampicin, Microemulsion Technique, Ultra stirrer, Dialysis membrane's

1. INTRODUCTION

Solid lipid nanoparticles (SLNs) recently emerged as a novel approach to parenteral drug delivery systems. Solid lipid nanoparticles combine the advantages of lipid emulsion systems and polymeric nanoparticle systems. Utilizing biological lipids is theorized to minimize carrier cytotoxicity, and the solid state of the lipid is theorized to permit more controlled drug release due to increased mass transfer resistance. SLNs are also useful as drug carriers to treat neoplasm [1].

Mycobacterial organisms cause tuberculosis, *Mycobacterium avium* complex (MAC) disease, and leprosy. Tuberculosis (TB) is second only to HIV/AIDS as the greatest killer worldwide due to a single infectious agent. In 2011, 8.7 million people fell ill with TB and 1.4 million died from TB. Over 95% of TB deaths occur in low- and middle-income countries, and it is among the top three causes of death for women aged 15 to 44. In 2010, there were about 10 million orphan children as a result of TB deaths among parents. TB is a leading killer of people living with HIV causing one quarter of all deaths. Multi-drug resistant TB (MDR-TB) is present in virtually all countries surveyed [2].

The rifamycins (rifampin, rifabutin, rifapentine) are a group of structurally similar, complex macro cyclic antibiotics produced by *Amycolatopsis mediterranei* rifampin (RIFADIN; RIMACTANE) is a semi synthetic derivative of one of these rifamycin B. Rifampin (RIF) inhibits DNA-dependent RNA polymerase of mycobacteria and other microorganisms by forming a stable drug-enzyme complex, leading to suppression of initiation of chain formation in RNA synthesis Rifampicin possess protein binding of 89% and shorter half-life 1-5 hrs which gets well absorbed from gastrointestinal tract with bioavailability varied from 90-95 [3, 4].

The present study is aimed at the development and characterizations of SLNs of RIF. SLNs prepared by the Microemulsion technique yield SLNs with good drug loading capacity and are possible to alter the therapeutic index and the duration of activity of drugs. The process of formation of these SLNs is simple with high reproducibility and safe as biodegradable lipid carriers cetyl palmitate (CP), the surfactants is used like polaxamer 188 which produced chemically stabled system.

2. MATERIAL AND METHODS

Rifampicin was obtained as a gift sample from Novartis-Sandoz, Pvt. Ltd, Maharashtra. Cetyl palmitate was procured from Loba Chemicals, Pvt. Ltd, Mumbai. Polaxamer-188 was obtained from S.D. Fine Chem Ltd., Mumbai. Dialysis membrane was purchased from Himedia laboratories Pvt. Ltd., Mumbai.

2.1. Formulation Design

2.1.1. Procedure for preparation of SLN'S by Microemulsion Technique

SLNs were prepared from o/w microemulsion technique containing lipid Cetyl palmitate carrier, polaxamer 188 as surfactant. Cetyl palmitate was added drop wise maintained at 70°C into ice cold water (2-3°C) with continuous stirring (IKA-Ultra Turrax T25 USA) to form SLNs. The composition of Formulation was shown in Table 1.

2.2. Characterization of SLNs

2.2.1. Compatibility studies of drug and polymers [5]

Compatibility studies of drug and polymers were done by FTIR spectrophotometer (Thermo Nicolate, Japan). The infrared spectra of the drug and polymers were run individually. Then it was investigated for any possible interaction between polymer and drug. I.R spectral data are shown in Spectra and tabulated in Table 2.

Table 1: Formulation Design of RIF loaded CP SLNs

Formulation Codes	Lipid %W/V	Drug (mg)	Conc. of Surfactant W/V	Speed (rpm)
Lipid concentration (%)				
FCL-01	2.5%	10mg	1.5%	9,500
FCL-02	5.0%	10mg	1.5%	9,500
FCL-03	7.5%	10mg	1.5%	9,500
Surfactant concentration (%)				
FCP-04	5.0 %	10mg	2 %	9,500
FCP-05	5.0 %	10mg	1.5%	9,500
FCP-06	5.0 %	10mg	1 %	9,500
Speed of ultra-stirrer (rpm)				
FCS-07	5.0 %	10mg	1.5 %	7,500
FCS-08	5.0 %	10mg	1.5 %	11,500

***FCL**- Formulation of Cetyl palmitate with changing lipid conc., **FCP**- Formulation of Cetyl palmitate with changing Poloxamer conc., **FCS**- Formulation of Cetyl palmitate with changing speed*

2.2.2. Differential scanning calorimetry (DSC) [6]

Studies are also a qualitative identification of substance in the pure form and in combination. DSC was carried by the action of Argon purging with 80ml/min, where it was hermetically sealed with Aluminum Pans, from this sample of 40µl was used. The program is run at 25-250°C. The onset, end set and the peaks are recorded for individual pure drug, lecithin and in formulations. The spectra's are shown in fig. 2. The peaks values are tabulated in Table 3.

2.2.3. Particle Size Analysis [7]

The nanotrac measurement technique is that of dynamic light scattering. The velocity distribution of a sample particle suspended in a dispersing medium is known function of particle size. Light from a laser diode is coupled to sample through an optical power splitter/probe assembly. Light scattered from each particles is Doppler-shifted by the particle motion (Brownian motion). The Doppler-shifted scattered light is mixed with coherent un-shifted light in a silicon photo-detector and down converted to the audio range. The detector output signal is then amplified, filtered, digitized and mathematically analyzed by the microtrac windows software. Clean the cell with Mineral Water. Check the background (BVG should not cross than 0.1) then Add Mineral Water and Set Zero. 5 ml Sample was inserted into sample cell with a pipette. And Allow 10 cycles RUN to measure size.

2.2.4. Scanning Electron Microscopy

Surface morphology of the specimens will be determined by using a scanning electron microscope (SEM), Model JSM 840A, JEOL, Japan. The samples are dried thoroughly in vaccum desicator before mounting on brass specimen studies, using double sided adhesive tape. Gold-palladium alloy of 120 Å was coated on the sample using sputter coating unit (Model E5 100 Polaron U.K.)

in Argon at ambient of 8-10 Pascal with plasma voltage about 20MA. The sputtering was done for nearly 5 minutes to obtain uniform coating on the sample to enable good quality SEM images. The SEM was operated at low accelerating voltage of about 15KV with load current of about 80MA.The condenser lens position was maintained between 4.4-5.1. The objective lens aperture has a diameter of 240 microns and the working distance WD=39mm [8].

2.2.5. Entrapment Efficiency

Entrapment efficiency of RIF-SLNs was determined by centrifugation of samples at 10,000 rpm for 10 min. The amount of free drug was determined in the clear supernatant by UV spectrophotometer (Thermo-spectronic Genesys-6. USA) at 479.5 nm using supernatant of non-loaded nanoparticles on basic correction. The entrapment efficiency (EE %) could be achieved by the following equation [9].

$$EE\ (\%) = \frac{W\ \textit{initial drug} - W\ \textit{free drug}}{W\ \textit{initial drug}} \times 100$$

2.2.6. Short term stability study

The short term stability of the SLNs was studied below 2-8°C. The SLNs were evaluated at 2-8°C for a period of 1month. The samples were observed for particle size analysis, To, Entrapment efficiency and *In vitro* release studies [10].

2.2.7. Zeta potential

The particle charge is one of the factors that determine the physical stability of emulsions and suspensions. The higher is the electrostatic repulsion between the particles the higher is the physical stability. Typically the particle charge is quantified as the so called zeta potential, which is measured by using Zeta meter (Malvern Instruments Ltd., UK), about 1 ml of SLN was dispersed in 1 ml of distilled water by sonication and it was subjected to Zeta potential analyzer [11].

2.2.8. In-Vitro Release Study

In vitro release studies were performed using dialysis bag diffusion technique. Dialysis membrane (molecular weight - 12,000 Da) was soaked in double distilled water for 12 h before use for experiment. RIF nanosuspension equivalent to 1 mg of RIF were placed in the dialysis bag containing 50 mL of dissolution medium at 37±0.5°C with continuous magnetic stirring at 200 rpm. At fixed time intervals, the samples were withdrawn; same dissolution medium was replaced by fresh medium to maintain constant volume. Sink conditions were maintained for release studies. Samples were analyzed by UV visible spectroscopy at 479.5nm. The results are calculated and tabulated in table 5 [12].

2.3. Data Analysis

The Colloidal systems were reported to follow the zero order release rate by the diffusion mechanism for the release of the drug.

To analyze the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted in to Zero order, First order, Higuchi matrix and Krosmeyer and Peppas model. Comparing the r-values obtained, the best-fit model was selected.

2.3.1. Zero order kinetics

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly, assuming that the area does not change and no equilibrium conditions are obtained can be represented by the following equation:

$$Q_t = Q_o + K_o t$$

Where Q_t = amount of drug dissolved in time t, Q_o= initial amount of drug in the solution and K_o= zero order release constant.

2.3.2. First order kinetics

To study the first order release rate kinetics the release rate data were fitted to the following equation.

$$\text{Log } Q_t = \log Q_o + K_1 t / 2.303$$

Where Q_t is the amount of drug released in time t, Q_o is the initial amount of drug in the solution and K_1 is the first order release constant.

2.3.3. Higuchi model

Higuchi developed several theoretical models to study the release of water-soluble and low soluble drugs incorporated in semisolids and or solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media, the equation is:

$$Q_t = K_H . t^{1/2}$$

Where Q_t = Amount of drug released in time t, K_H = Higuchi dissolution constant.

2.3.4. Krosmeyer and Peppas release model

To study this model the release rate data are fitted to the following equation:

$$M_t / M_\infty = K.t^n$$

Where M_t / M_∞ is the fraction of drug release, K is the release constant, t is the release time and n is the Diffusion exponent for the drug release that is dependent on the shape of the matrix dosage form.

2.3.5. Hixson- Crowell model

To study the Hixson-Crowell model the release rate data are fitted to the following equation:

$$W_o^{1/3} - W_t^{1/3} = Kst$$

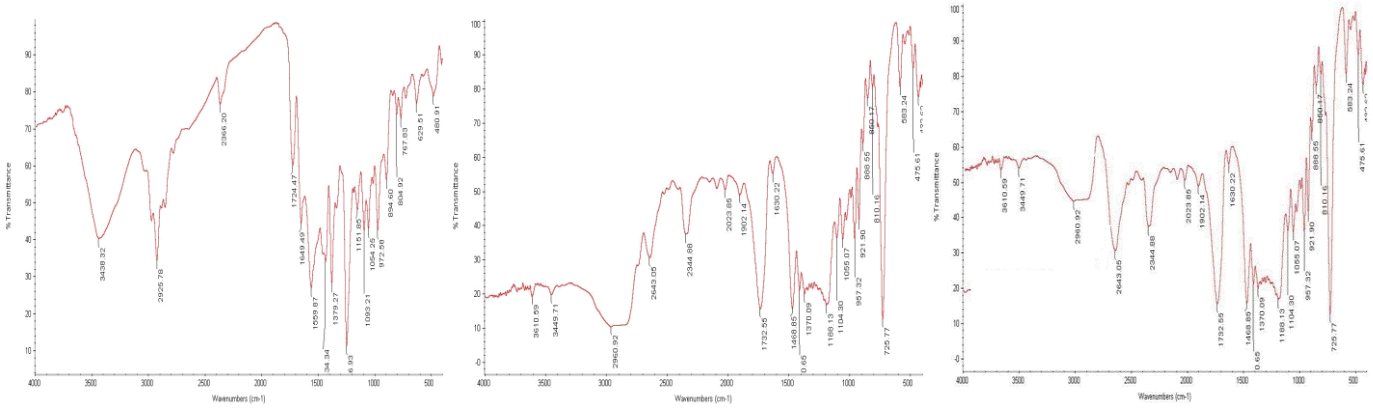
Where W_o is the amount of drug in the dosage form, W_t is the remaining amount of drug in the pharmaceutical dosage form, Ks is a constant incorporating the surface-volume relationship [13].

3. RESULTS & DISCUSSION

3.1. Compatibility

From the FTIR spectral analysis it was found that spectrum Rifampicin with lipids Cetyl palmitate showed all characteristic peaks in combination with no significant changes as shown in Fig.1 & Table 2.

DSC studies indicate that, thermal peak of Rifampicin in the pure form was identical with physical mixtures; this indicates that there was no interaction as shown in fig. 2 & table 3.

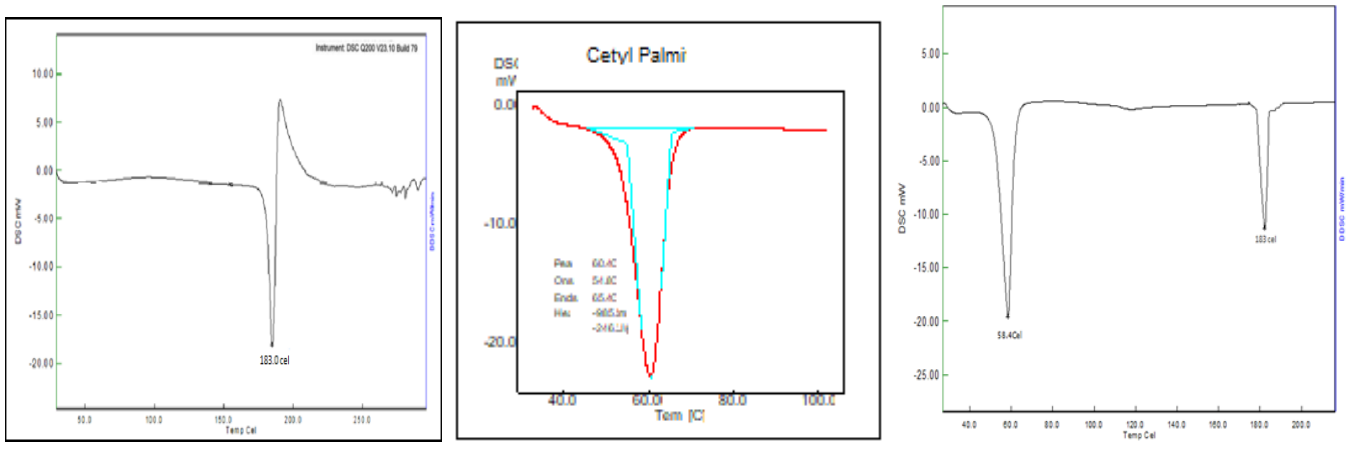


(a) (b) (c)

Fig. 1: FTIR spectra of (a) Rifampicin (b) Cetyl Palmitate (c) Rifampicin & Cetyl Palmitate

Table 2: Data obtained from Compatibility study of drug polymer and formulations by FTIR spectroscopy

Polymer/Drug	Important IR Spectral peaks of different groups, wave length in cm^{-1}			
	OH Stretch	CH Stretch(Aliphatic)	C=0 Stretch	C-O-CStretch
Rifampin	3438.32	2925.78	1724.47	1151.85
Cetyl palmitate	3449.71	2960.92	1732.55	1188.13
Rifampin;Cetylpalmitate	3449.21	2960.92	1732.07	1246.87



(a) (b) (c)

Fig. 2: DSC spectra of (a) Rifampicin (b) Cetyl Palmitate (c) Rifampicin & Cetyl Palmitate

Table 3: Data obtained from Compatibility study of drug and polymer by Differential scanning calorimetry

Drug/Polymer/ Formulation	Peaks	
Pure Rifampin	--------	183°C (Rifampin)
Rifampin & Cetylpalmitate	58.6°C (Cetyl palmitate)	182.5°C (Rifampin)

Table 4: Mean Particle Size & % EE of SLNs of RIF by using Cetyl Palmitate as a lipid carrier and Poloxamer 188 as Surfactants

Formulation code	FCL-01	FCL-02	FCL-03	FCP-04	FCP-05	FCP-06	FCS-07	FCS-08
Particle size (nm)	394	506	431	394	316	306	372	365
% EE	61.60%	69.72%	74.38%	75.48%	80.98%	72.75%	54.84%	50.48%

3.2. Formulation of RIF loaded SLNs

RIF loaded SLNs were successfully prepared by using o/w microemulsion technique. The SLNs were obtained immediately when dispersing the warm microemulsion into cold water with the aid of a homogenizer. The cold water facilitated rapid lipid crystallization and prevented lipid aggregation. Different lipid carriers were used like cetyl palmitate along with mixtures of surfactants.

3.3. Evaluation of SLNs

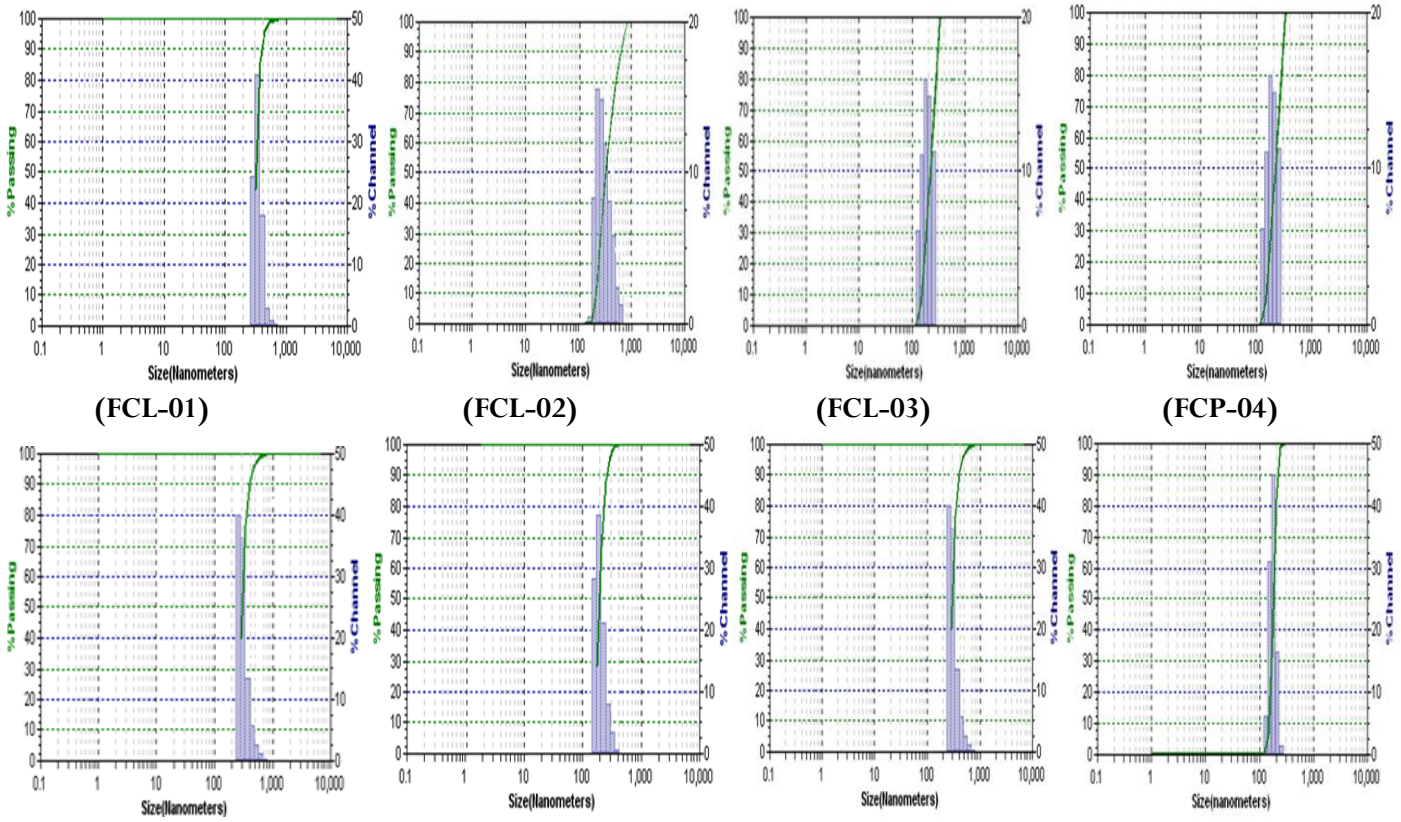
3.3.1. Particle size

The particle sizes of all formulations were listed in Table 4 and the size distribution graphs were showed in Graph 4. Particles size of nanoparticles of Rifampicin was found to be as shown by Cetyl palmitate such as FC-1 to FC-8 showed particle size ranging from 93 nm to 394nm.

The particle size analysis reveals that the size reduction was with varying speeds and size increment with varying lipid carrier concentrations.

3.3.2. Influence of speed (rpm) on SLN

Decreasing of the particle size with the increasing of stirring rate can be explained by the intensification of the micro mixing (*i.e.* mixing on the molecular level) between the multi-phases. High micro mixing efficiency enhanced the mass transfer and the rate of diffusion between the multiphase, which induced high homogenous super saturation in short time and thus rapid nucleation to produce smaller drug particles. Hence, higher stirring rate favored the formation of the smaller and more uniform drug particles.



(FCL-01) **(FCL-02)** **(FCL-03)** **(FCP-04)**

(FCP-05) **(FCP-06)** **(FCS-07)** **(FCS-08)**

Fig. 3: Particle Size Range of RIF SLNs of different formulations

3.3.3. Influence of lipid concentration on SLN

As the lipid concentration was increased, more particles were aggregated resulting in a decreased yield. The yield was <50% at lipid concentration of 2.5% and dropped to the 5% range when the lipid concentration exceeded 10%. When the concentration of the lipid exceeded 1.0% with a fixed concentration of Surfactants, there were insufficient surfactants available to coat the surface of all the lipid droplets, resulting in particle aggregation and an increase in particle size.

3.3.4. Entrapment Efficiency

The formulations prepared with cetyl palmitate (50% to 81%) respectively as shown in table 4. The entrapment efficiencies of the RIF loaded SLNs was in the order of FC. The higher entrapment efficiency with Rifampicin is attributed to the high hydrophobicity due to the long chain fatty acids attached to the triglyceride resulting in increased accommodation of lipophilic drugs.

3.3.5. In-vitro Dissolution Studies

In-vitro drug release data from the SLNs were carried out for 12hrs and graphically represented as % CDR v/s time profile. The Cumulative Percent drug released after 12hrs FC-1 to FC-8 was 73.84to 89.13 % respectively. Interestingly, the particle size had no influence on the *in vitro* release of RIF. The release of a drug from the SLN can be influenced by nature of the lipid matrix and its concentration. Since the surfactant concentration was optimized at 1 % in the present investigation, the drug release profile was affected by other parameters such as lipid nature, solubility of the drug in lipid and partition coefficient. In such a model, the drug enriched core is surrounded by a practically drug-free lipid shell. Due to the increased diffusion distance and hindering effects by the surrounding solid lipid shell, the drug has a controlled release profile. The cetyl palmitate had shown slow release which can be attributed to the hydrophobic long chain fatty acids of the triglyceride that retain lipophilic drugs.

Table 5: Comparative *In-vitro* Drug Release Study of Cetyl palmitate Nanoparticles of RIF

Time/formulation Code	% Cumulative drug release (CDR)					
	2h	4h	6h	8h	10h	12h
FCL-01	35.6209	49.0956	58.5947	66.7704	74.9026	82.4875
FCL-02	32.3304	46.6382	54.0156	63.7443	70.193	78.7689
FCL-03	29.826	42.4735	51.1356	59.2434	67.9686	73.8487
FCP-04	38.9739	54.2573	63.3652	71.5947	78.7486	83.1568
FCP-05	34.1782	57.993	68.7339	75.0034	82.6587	89.1345
FCP-06	36.1683	51.8989	60.5408	69.7304	76.1634	83.5965
FCS-07	37.8596	53.2547	62.5287	71.8764	78.5692	81.1269
FCS-08	40.2587	55.3139	65.5269	74.6325	79.4583	83.4785

Table 6: Kinetic data of various models for release study (Cetyl Palmitate)

Formulation	Zero order		First order		Higuchi		Peppas			Best fitting
Code	R	K	R	k	R	k	R	k	N	model
FCL-01	0.7221	4.9371	0.7450	-0.039	0.8426	20.14	0.9425	0.3636	0.3252	Peppas
FCL-02	0.7692	4.9762	0.8134	-0.037	0.9010	22.40	0.9400	0.4001	0.3769	Peppas
FCL-03	0.8197	4.8801	0.8691	-0.036	0.8952	0.9633	0.9442	0.4404	0.3825	Peppas
FCP-04	0.7305	5.1459	0.7667	-0.043	0.8649	20.99	0.9203	0.3603	0.2627	Peppas
FCP-05	0.7017	5.1559	0.7315	-0.044	0.9033	21.26	0.8206	0.3411	0.3699	Peppas
FCP-06	0.8786	6.6492	0.9744	-0.073	0.9693	25.64	0.9893	0.5031	0.2832	Peppas
FGS-07	0.8851	6.4000	0.9890	-0.068	0.9497	24.69	0.9988	0.4533	0.2620	Peppas
FGS-08	0.8727	6.5380	0.9929	-0.072	0.9957	25.38	0.9995	0.4369	0.2867	Peppas

3.3.6. Kinetic Study

The release study was further investigated for the kinetic studies. Various kinetic models were applied and their values were noted. Almost all formulations (with different lipid carrier) were found to follow the Peppas model. From the n values obtained it can be said that the diffusion followed non-Fickian mechanism.

3.4. Optimization of Results

On the basis of low particle size, high entrapment efficiency and controlled of release profile, We selected each best formulation as optimized from different type of lipids formulation (FCP-05) the selected optimized formulation were subjected for further evaluation.

Table 7: Data for Optimize formulation Table

Formulation Codes	Lipid % (W/V)	Surfactant Conc. (W/V)	Speed (rpm)	Particle size	% E.E
FCP-05	5.0 %	1.5 %	9,500	316 nm	80.98%

3.4.1. Scanning Electron Microscopy

The smooth surface of SLNs is because of presence of polaxamer188. The surface morphology of the SLNs had not been altered by the type of lipid carrier, concentration of lipid carrier and speed.

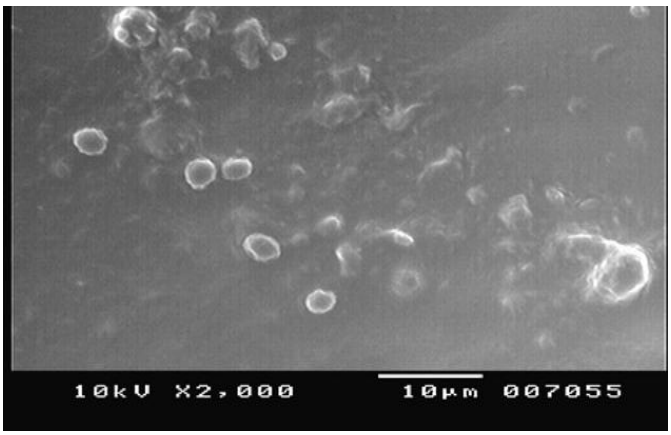


Fig. 4: SEM of optimized batch of RIF SLNs

3.4.2. Zeta Potential

In general, particle aggregation is less likely to occur for charged particles (high Zeta potential) due to electric repulsion. Lower zeta potential facilitates aggregation. The Zeta potential of both lipid based Nano formulations was found to be -25.2, which will not allow aggregation.

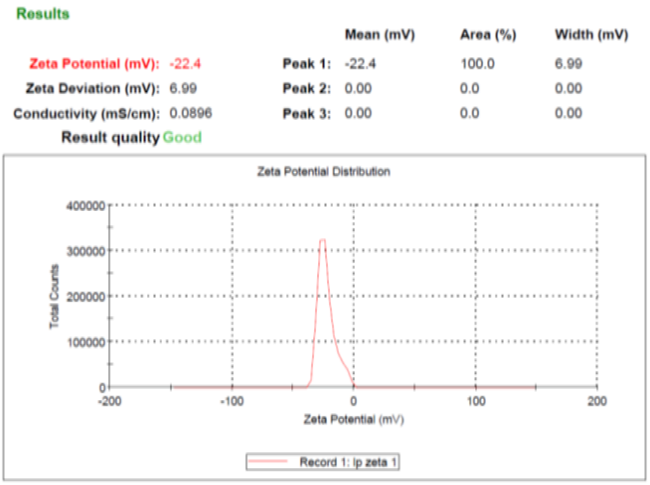


Fig. 5: Zeta potential of optimized batch of RIF SLNs

3.4.3. Short term Stability Studies

The selected formulations (one from each set) was stored at 2-8°C for a month. There was no significant change of particle size, entrapment efficiency and *In-vitro* drug release. The reason for good stability was use of emulsifying agents appears to produce mixed surfactant films at the interface having high surfactant coverage as well as sufficient viscosity to promote stability.

Table 8: Short term stability studies of SLNs after 1 month of storage at 2-8°C

Code	Particle size (before storage)	particle size (after storage)	EE (before storage)	EE (after storage)	% CDR (before storage)	% CDR (after storage)
FCP-05	316 nm	320 nm	80.98%	82.12 %	38.255	39.22

4. CONCLUSION

RIF loaded SLNs can be successfully formulated from Microemulsion Technique to enhance the efficacy of drug by reducing the side effects from the dose. The optimized SLNs formulations of RIF showed particle sizes in the range of 316 nm-320 nm; with good surface characters. Increment in particle size was observed with increased concentrations of lipid carriers. Smaller size was obtained with increasing stirring speed. Interestingly, the particle size had no influence on the *in vitro* drug release was observed. Cetyl Palmitate (CP) had shown controlled release and maximum entrapment efficiency which can be attributed to the hydrophobic long chain fatty acids of the triglyceride that retain lipophilic drugs and also increased accommodation of lipophilic drugs. The study reveals the influence of formulation parameters

(concentration of surfactant, concentration of lipid carrier) and process parameter (stirring speed) on the entrapment efficiency and *in vitro* release of lipophilic drug.

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