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# FORMULATION AND IN-VITRO EVALUATION OF RIFAMPICIN NIOSOMAL DRUG DELIVERY SYSTEM

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**Abstract:**

*The present study focused on the formulation, characterization, and in-vitro evaluation of rifampicin-loaded niosomes as a novel drug delivery system aimed at improving solubility, stability, and sustained release of the drug. Preformulation studies revealed that rifampicin is a reddish-brown crystalline powder, tasteless, odorless, with a melting point of 183–188 °C, and is poorly soluble in water (~2 mg/mL) but freely soluble in organic solvents such as methanol, ethanol, and chloroform. Drug–excipient compatibility assessed by FT-IR indicated no chemical interactions. Niosomes were prepared using non-ionic surfactants and cholesterol, with optimization based on entrapment efficiency, particle size, and zeta potential. The prepared formulations exhibited entrapment efficiencies ranging from 76.35–83.59 %, with the optimized formulation (F7) showing 83.59 % EE. SEM analysis confirmed nearly spherical vesicles with rough surfaces, while dynamic light scattering revealed a mean particle size of 168 nm and a zeta potential of –25 mV, indicating good physical stability. In-vitro release studies conducted using the Franz diffusion cell demonstrated sustained release of rifampicin up to 8 hours, with the optimized formulation achieving 97.55 % cumulative drug release. Release kinetics fitted best to the zero-order model, suggesting a constant release rate, and the Higuchi model indicated a diffusion-controlled mechanism. Accelerated stability studies over three months at 40 ± 2 °C and 75 ± 5 % RH confirmed the stability of the formulation with negligible drug degradation. Overall, the study concluded that rifampicin niosomes provide a promising approach for enhanced solubility, sustained release, and stability, potentially improving therapeutic efficacy and patient compliance.*

***Keywords:*** *Niosomes, FTIR Studies, In vitro drug release studies*

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## INTRODUCTION:

Niosomes, which are non-ionic surfactant-based vesicular drug delivery systems, have emerged as a promising alternative to conventional carriers such as liposomes. Niosomes are composed of non-ionic surfactants and cholesterol, forming closed bilayer structures capable of encapsulating both hydrophilic and lipophilic drugs.[1] The vesicular nature of niosomes allows for controlled and sustained release of the encapsulated drug, leading to prolonged plasma residence time and reduced dosing frequency. Furthermore, niosomal formulations can protect rifampicin from enzymatic degradation and improve its penetration into infected tissues, thereby increasing therapeutic efficacy[2].Rifampicin is a first-line antitubercular drug widely used in the treatment of TB due to its potent bactericidal activity against Mycobacterium tuberculosis.[3] It acts by inhibiting DNA-dependent RNA polymerase, thereby suppressing bacterial RNA synthesis.[4] In vitro evaluation of rifampicin-loaded niosomes plays a crucial role in assessing their physicochemical properties and performance.[5]The present research work is aimed at the formulation and in vitro evaluation of a rifampicin niosomal drug delivery system. The study seeks to develop a stable and efficient niosomal formulation that can overcome the limitations associated with conventional rifampicin therapy and offer an improved approach for the management of tuberculosis.[6]

## MATERIALS

Rifampicin was procured from Hetero Labs, Hyderabad. Cholesterol and Span 80was obtained from Synpharma Research Labs, Hyderabad. Other chemicalsand the reagents used were of analytical grade.

## METHODOLOGY

### Fourier Transform Infrared Spectroscopy (FTIR) study

FTIR is a useful technique to check and confirm any interaction that may occur between excipients and drug. The FTIR spectra of drug, excipients, briefly, solid sample (1 mg) along with 100 mg dried potassium bromide was compressed into a disc. The sample were place onto NaCl or KBr aperture plate and sandwiched it under another aperture plate, such that no gas bubbles were trapped. The sample allowed formation of a thin liquid membrane between the two aperture plates. Thereafter, sample was scanned for absorbance over the range from 4000 to 400 (cm-1) wave numbers. The obtained spectrum was then compared with standard group frequencies of Rifampicin.[7]

### Preparation of Niosomes

Rifampicin niosomes were prepared using thin film-hydration method. Accurately weighed quantities of the surfactant (Span 80 and Tween 80) and cholesterol in different Ratios in around-bottom flask. Afterwards, Rifampicin dissolved in 10 ml of chloroform: methanol mixture (2:1) was added to the lipid solution. The organic solvents were removed under vacuum in a rotary evaporator at 40◦ C for 20min to form a thin film on the wall of the flask, and kept in a desiccator under vacuum for 2 h to ensure total removal of trace solvents. After removal of the last trace of organic solvents, hydration of the surfactant film was carried out using 10mL of distilled water at 55°C. The resulting niosomal suspension was mechanically shaken for 1 h using a horizontal mechanical shaking water bath at 55 ◦ C. Then, the vesicle suspension was sonicated in 3 cycles' of1min "on" and 1min "off" leading to the formation of multi lamellar niosomes. The niosomal suspension was left to mature overnight at 4 ◦ C and stored at refrigerator temperature for further studies.[8]

**Table-1: Composition of Niosomal Rifampicin (F1 to F8)**

| S. No. | Ingredients (mg) | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Rifampicin | 450 | 450 | 450 | 450 | 450 | 450 | 450 | 450 |
| 2 | Cholesterol: Span80 | 1:1 | 1:2 | 1:3 | 1:4 | 1:5 | 1:6 | 1:7 | 1:8 |
| 5 | Methanol | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 6 | Chloroform | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

**Fig-1: Rotary evaporator**

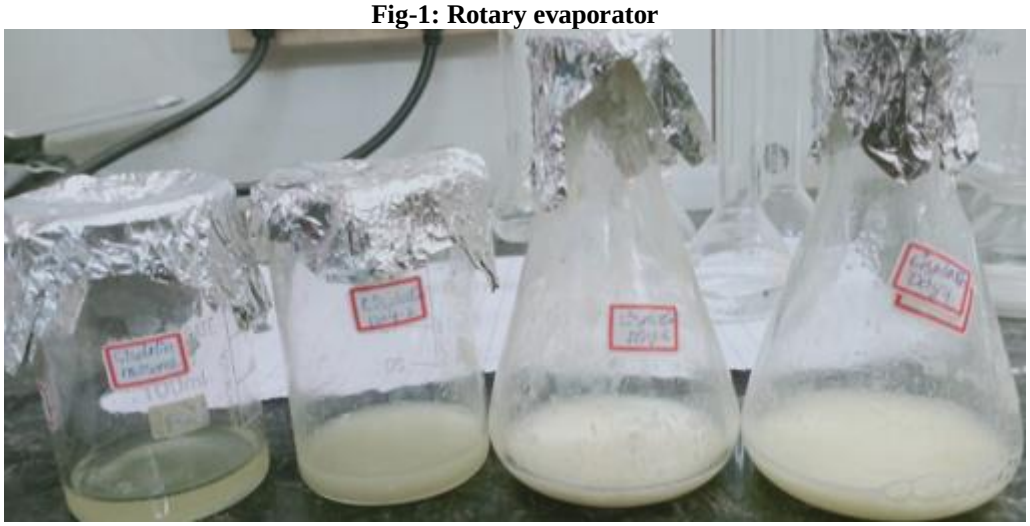

**Fig-2: Niosomes**

**Evaluation of Niosomes**

**Particle size**

Size and size distribution studies were done for niosomes prepared from Niosomes hydration. The Niosomes (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. Similarly, size was noted for niosomes formed spontaneously from Niosomes after hydration without agitation in a cavity slide.

**Zeta-potential**

The sample was diluted with distilled water (1:100 (V/V)) and zeta potential was determined using Malvern zetasizer (Nano ZS, Malvern Instruments, United Kingdom). Measurement was based on the electrophoretic mobility of the particles, which was converted to the zeta potential by inbuilt software based on the Helmholtz-Smoluchowski equation. [10]

**SEM Analysis**

The shape, surface characteristics, and size of the niosomes were observed by scanning electron microscopy. Once again, 0.2 g of the Niosomes in a glass tube was diluted with 10 ml of pH 7.4 phosphate buffer. The niosomes were mounted on an aluminium stub using double-sided adhesive carbon tape. Then the vesicles were sputter-coated with gold palladium (Au/Pd) using a vacuum evaporator (Edwards) and examined using a scanning electron microscope (Hitachi 3700N, Germany) equipped with a digital camera, at 10 kV accelerating voltage.[11]

**Entrapment efficiency**

Place a known volume (e.g., 1 mL) of the niosomal suspension into ultracentrifuge tubes. Centrifuge at ~15,000–20,000 rpm for 30–60 min at 4 °C. Collect the clear supernatant—this contains the unentrapped rifampicin. The supernatant was recovered and assayed spectrophotometrically using UV spectrophotometer (UV-1800 Shimadzu, Japan), at 335 nm[12]

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.

***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 up to 8 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 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 at 335 nm.[13]

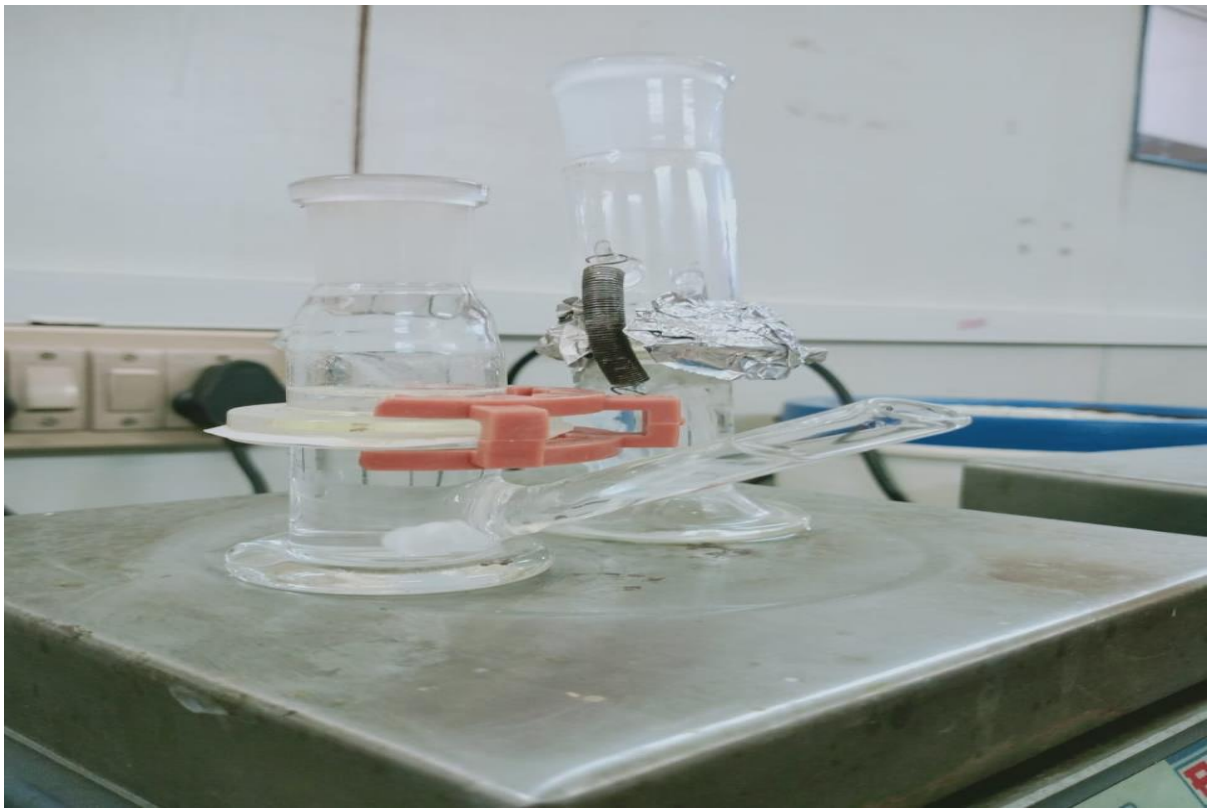

**Fig-3: Franz diffusion cell**

**Kinetic modelling of drug release[14]**

All the 8 formulation of prepared Rifampicin niosomes were subjected to in vitro release studies these studies were carried out using dissolution apparatus.

The results obtaining in vitro release studies were plotted in different model of data treatment as follows:

1. Cumulative percent drug released vs. time (zero order rate kinetics)
2. Log cumulative percent drug retained vs. time (First Order rate Kinetics)
3. Cumulative percent drug released vs. square root of time (Higuchi's Classical Diffusion Equation)
4. Log of cumulative % release Vs log time (Peppas Exponential Equation)

**Stability Studies[15]**

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. At definite time intervals (10, 20, and 30 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.

## RESULTS AND DISCUSSION:

**Drug - excipient compatibility studies (FT-IR)**

Using the FTIR peak matching approach, the compatibility of the medicine with the chosen polymer and other excipients was assessed. The drug-Excipients mixture showed no peaks that appeared or vanished, indicating that there was no chemical interaction between the medication, lipids and other molecules.

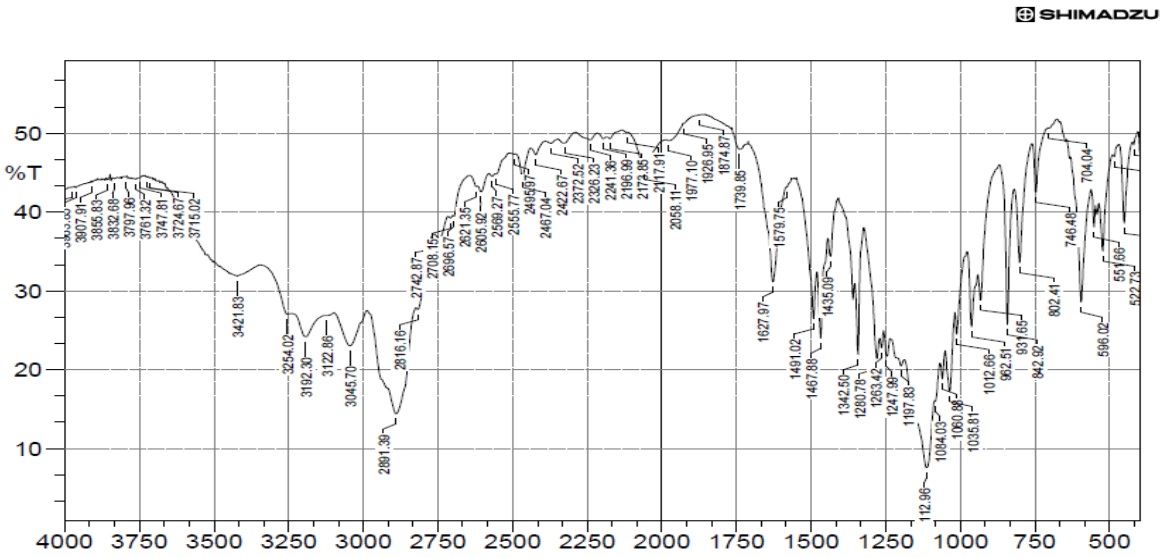


**Fig-4: FT-IR Sample for Pure drug**

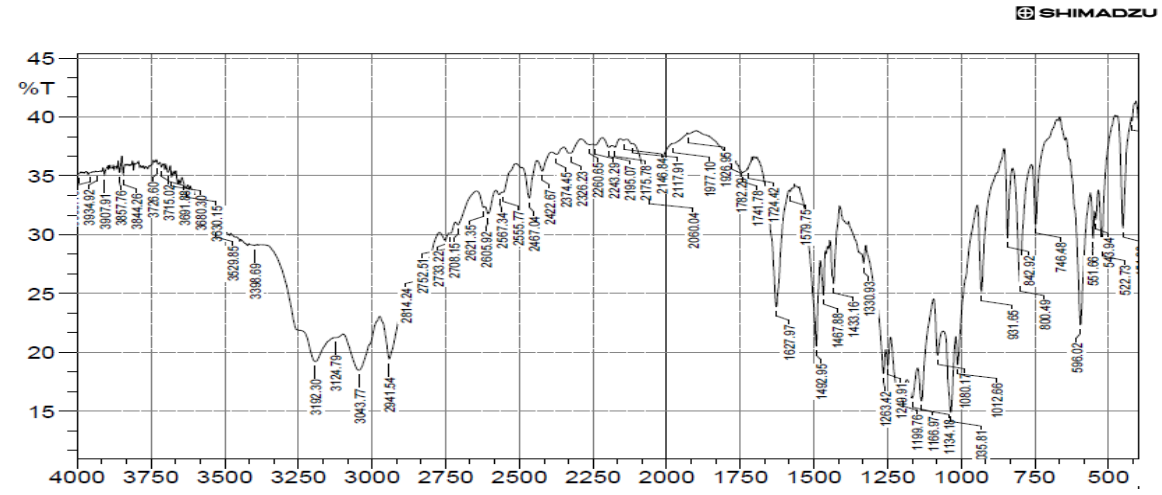


**Fig-5: FT-IR Sample for Optimized formulation**

**EVALUATION PARAMETERS:**

**Entrapment Efficiency:**

**Table-2: Drug entrapment efficiency of all formulation**

| F.no | Drug entrapment efficiency |
|---|---|
| F1 | 79.68 |
| F2 | 80.13 |
| F3 | 76.35 |
| F4 | 81.39 |
| F5 | 77.81 |
| F6 | 79.36 |
| F7 | 83.59 |
| F8 | 82.30 |

The formulate niosomes formulation's entrapment efficiency was in the range of 76.35-83.59 %. The % EE of the prepared optimized formulation was found to be 83.59%.

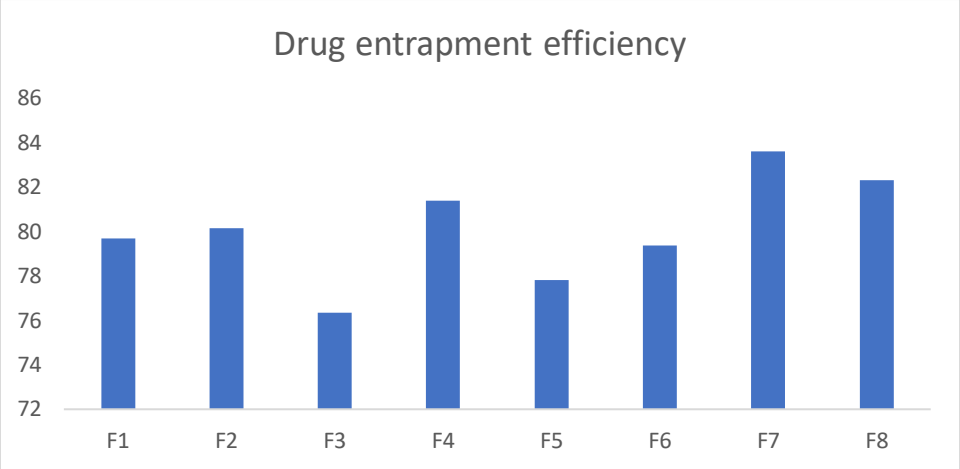


**Fig-6: Drug entrapment efficiency of all formulation**

**Determination of Vesicle morphology and Size**

The morphology of the prepared diverse types of nanoparticles was found to be virtually spherical in shape and have a rough surface, as illustrated in SEM photomicrographs of the nanoparticles.

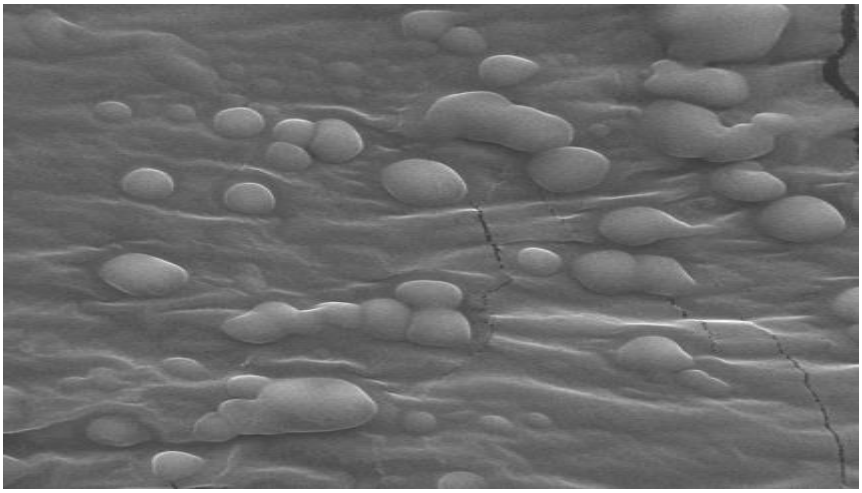

**Fig-7: SEM analysis of Optimized niosomes**

**Evaluation Studies of particle size and Zeta potential Niosomes**

**Particle size**

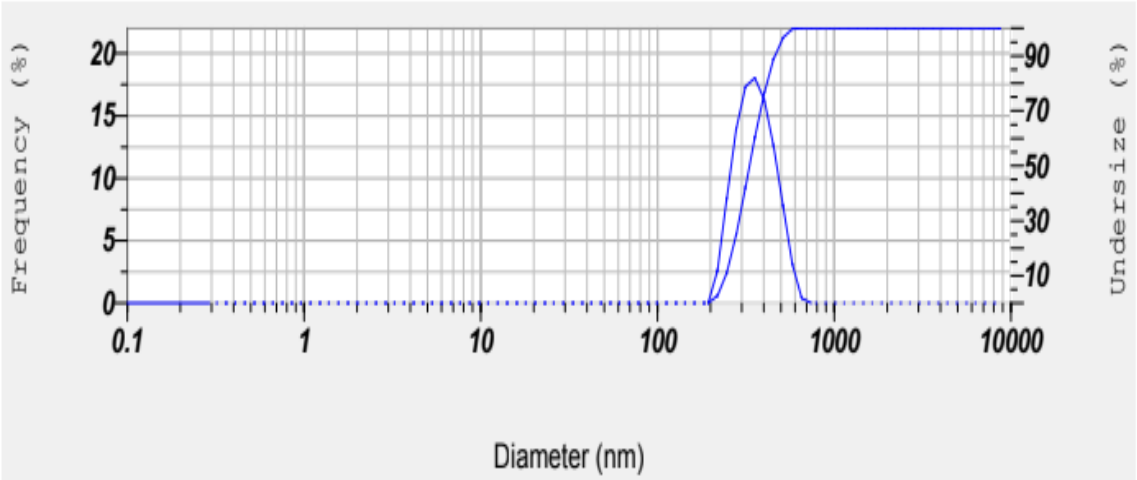


**Fig-8: Particle size analysis of Optimized niosomes**

**Zeta potential**

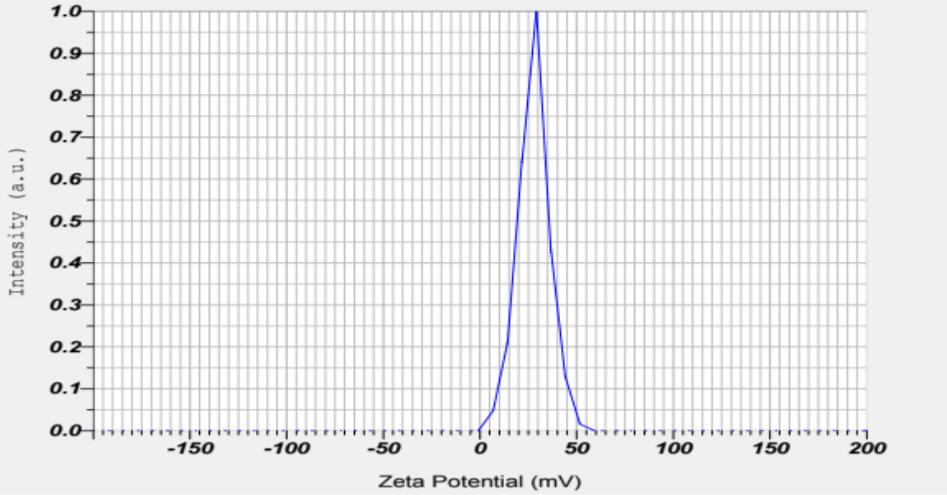


**Fig-9: Zeta potential analysis of Optimized niosome**

The ZP or change in the surface of colloidal particles in niosomeswas studied to determine the charge on the particles to avoid agglomeration. Figure indicates the ZP of the optimized formulation as -25 mV.

**Table-3: Evaluation Studies of particle size and Zeta potential Niosomes**

| F. No | Particle size (nm) | Zeta potential |
|---|---|---|
| F1 | 153 | -27 |
| F2 | 149 | -28 |
| F3 | 168 | -29 |
| F4 | 169 | -26 |
| F5 | 155 | -21 |
| F6 | 173 | -28 |
| F7 | 168 | -25 |
| F7 | 170 | -27 |

The mean particle size of optimized niosomes was found to be 168 nm.

***In vitro* drug release studies:**

**Table-4: *In vitro* drug release profiles of Rifampicin niosomes (F1-F6)**

| Time (hr) | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 16.98 | 17.63 | 16.67 | 15.68 | 17.54 | 15.96 | 18.56 | 16.39 |
| 2 | 28.13 | 27.46 | 28.64 | 29.64 | 25.19 | 27.14 | 26.93 | 25.47 |
| 3 | 35.17 | 36.89 | 38.98 | 30.25 | 39.83 | 34.67 | 42.46 | 36.25 |
| 4 | 47.89 | 48.24 | 49.82 | 45.58 | 50.10 | 49.14 | 51.37 | 45.69 |
| 5 | 58.10 | 59.68 | 60.16 | 59.45 | 62.14 | 60.20 | 61.64 | 57.48 |
| 6 | 69.76 | 70.25 | 72.34 | 67.45 | 71.25 | 72.36 | 73.35 | 68.21 |
| 7 | 81.53 | 82.34 | 83.15 | 78.69 | 83.15 | 83.15 | 85.19 | 83.16 |
| 8 | 93.67 | 94.57 | 92.68 | 89.55 | 94.63 | 95.84 | 97.55 | 92.20 |

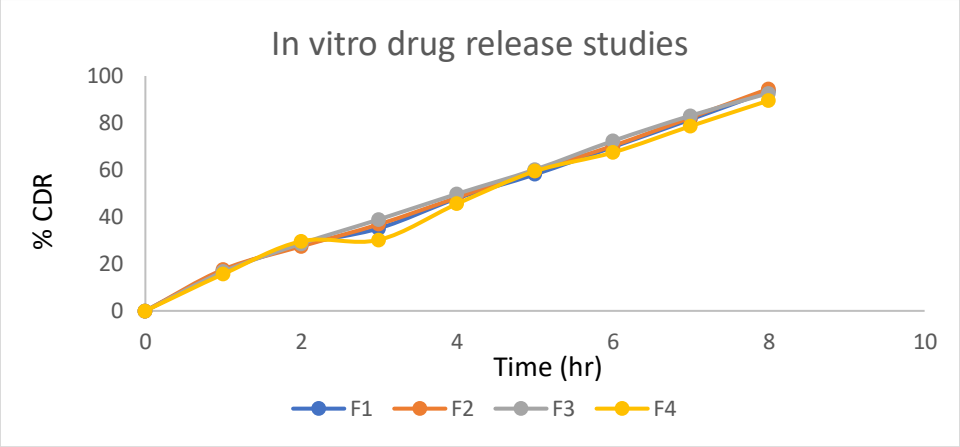


**Fig-10: Drug release of (F1-F4) formulations**

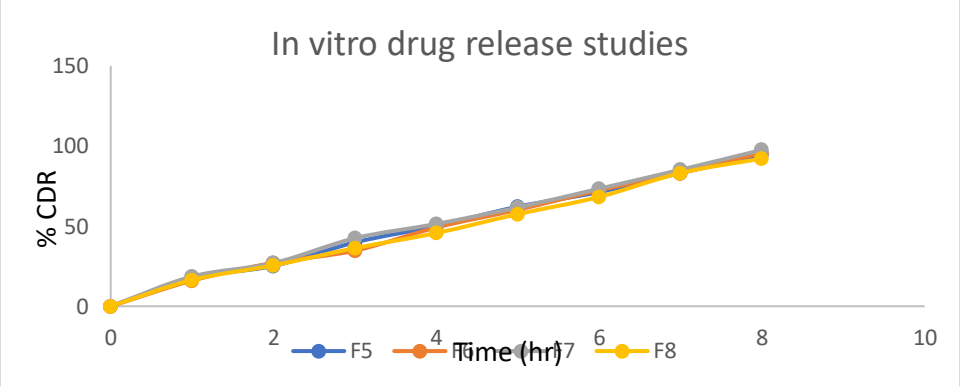


**Fig-11: Drug release of (F5-F8) formulations**

The drug release studies of all formulations of Rifampicin were conducted by means of Franz diffusion cell apparatus for a time period of 8 hrs. From the drug release studies as depicted in Figure, the results showed that 4th formulation showed maximum drug release rate of 97.55% within 8 hrs.

**Drug release kinetics**
**Zero order kinetics**

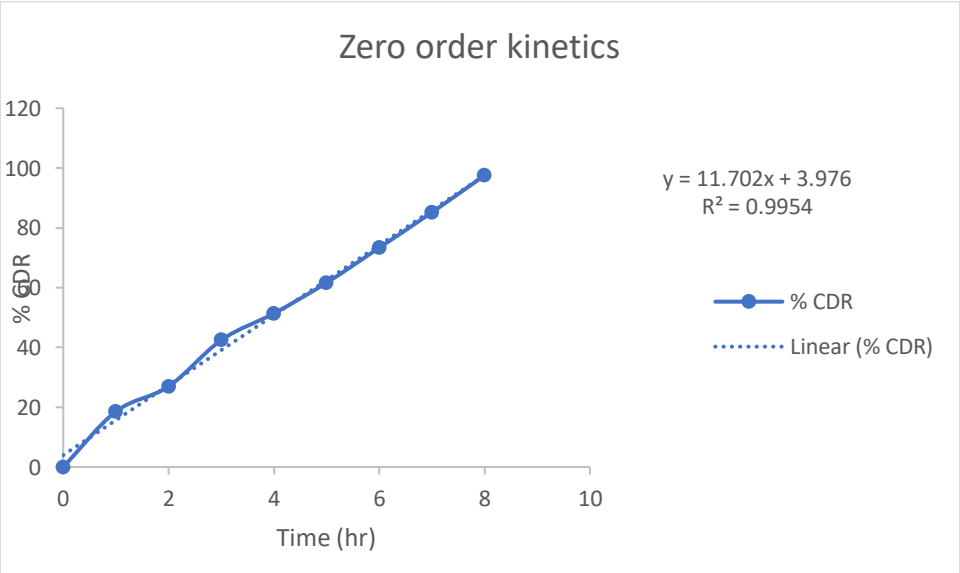


**Fig-12: Zero order kinetics of optimized formulation**

**First order kinetics**

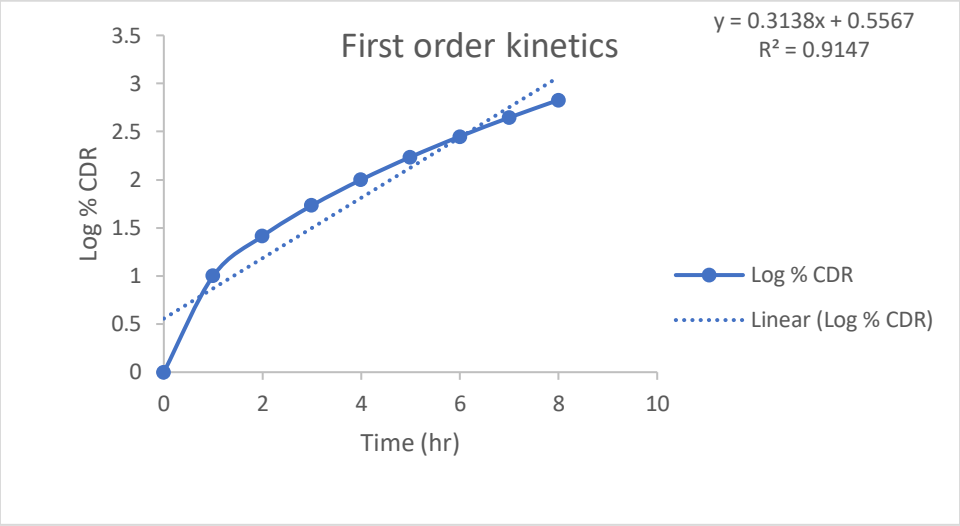


**Fig-13: First order kinetics of optimized formulation**

**Higuchi model**

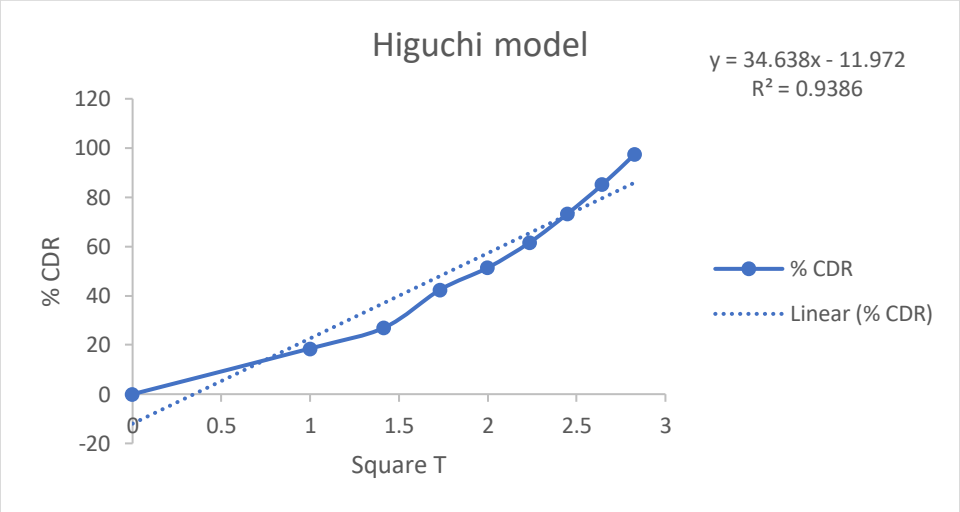


**Fig-14: Higuchi model of optimized formulation**

**Korsmeyer peppas**

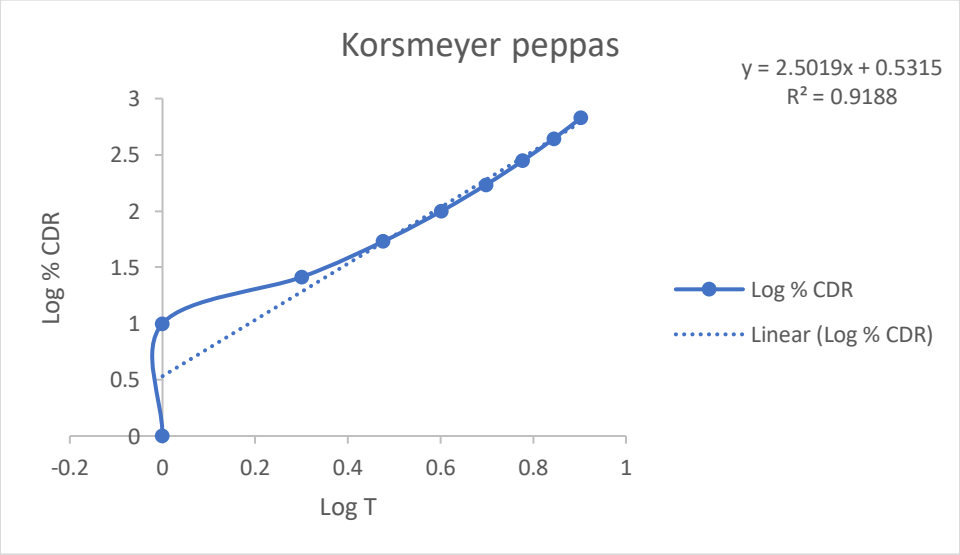


**Fig-15: Korsmeyer peppas of optimized formulation**

The values of in vitro release were attempted to fit into various mathematical models. Plots of zero order, first order, Higuchi matrix and Peppas. Regression values are higher with Zero order release kinetics. Therefore, all the Rifampicin niosomes follows Zero order release kinetics.

**Stability studies:**

Optimized formulations F7 was selected for accelerated stability studies as per ICH guidelines. The Niosomes were observed for drug release for a period of three months.

**Table-5: Stability studies of optimized formulations at 40 ± 2 $^0$C and 75 ± 5% RH for 3 months**

| Formulation Code | Initial | 1st Month | 2nd Month | 3rd Month | Limits as per Specifications |
|---|---|---|---|---|---|
| F-7 | 97.55 | 96.46 | 95.37 | 94.80 | Not less than 85 % |
| F-7 | 97.55 | 96.35 | 95.23 | 94.67 | Not less than 85 % |
| F-7 | 97.55 | 96.19 | 95.10 | 94.55 | Not less than 85 % |

## CONCLUSION:

Rifampicin-loaded niosomes were successfully formulated and characterized.The optimized batch (F7) exhibited high entrapment efficiency (83.59 %), nanoscale particle size (~168 nm), good surface charge (–25 mV), and nearly complete sustained drug release (97.55 % over 8 h).Drug–excipient compatibility was confirmed by FT-IR and the formulation remained physically and chemically stable for at least three months under accelerated conditions.Overall, these findings demonstrate that niosomal encapsulation is an effective strategy to enhance the controlled delivery and stability of rifampicin, potentially improving its therapeutic performance and patient compliance.

## REFERENCES:


1. Baillie, A. J., Florence, A. T., Hume, L. R., Muirhead, G. T., & Rogerson, A. (1985). The preparation and properties of niosomes—Non-ionic surfactant vesicles. Journal of Pharmacy and Pharmacology, 37(12), 863–868.
2. Uchegbu, I. F., & Vyas, S. P. (1998). Non-ionic surfactant based vesicles (niosomes) in drug delivery. International Journal of Pharmaceutics, 172(1–2), 33–70.
3. Kaur, I. P., & Kanwar, M. (2002). Ocular preparations: The formulation approach. Drug Development and Industrial Pharmacy, 28(5), 473–493.
4. Torchilin, V. P. (2005). Recent advances with liposomes as pharmaceutical carriers. Nature Reviews Drug Discovery, 4(2), 145–160.
5. Rang, H. P., Dale, M. M., Ritter, J. M., & Flower, R. J. (2019). Rang and Dale's Pharmacology (9th ed.). Elsevier, London.
6. Goodman, L. S., Brunton, L. L., Hilal-Dandan, R., &Knollmann, B. C. (2018). Goodman & Gilman's The Pharmacological Basis of Therapeutics (13th ed.). McGraw-Hill Education, New York.
7. Pandey, R., Sharma, A., Zahoor, A., Sharma, S., Khuller, G. K., & Prasad, B. (2003). Poly (DL-lactide-co-glycolide) nanoparticle-based inhalable sustained drug delivery system for experimental tuberculosis. Journal of Antimicrobial Chemotherapy, 52(6), 981–986.
8. Sharma, A., & Sharma, U. S. (1997). Liposomes in drug delivery: Progress and limitations. International Journal of Pharmaceutics, 154(2), 123–140.
9. Senior, J., Crawley, J. C. W., &Gregoriadis, G. (1985). Tissue distribution of liposomes exhibiting long half-lives in the circulation after intravenous injection. *Biochimica et Biophysica Acta (BBA)-General Subjects, 839*(1), 1-8.
10. Finkelstein, M., &Weissmann, G. (1979). The introduction of enzymes into cells by means of liposomes. *Journal of Lipid Research, 20*(3), 289-303.
11. Grit, M., de Smidt, J. H., Struijke, A., &Crommelin, D. J. (1993). Hydrolysis of phosphatidylcholine in aqueous liposome dispersions. *International Journal of Pharmaceutics, 90*(2), 131-140.
12. van Winden, E. C. (2003). Freeze-drying of liposomes: theory and practice. *Methods in Enzymology, 367*, 99-110.
13. Crowe, J. H., Crowe, L. M., Carpenter, J. F., &AurellWistrom, C. (1988). Stabilization of dry phospholipid bilayers and proteins by sugars. *Biochemical Journal, 242*(1), 1-10.
14. Ozer, A. Y., Talsma, H., &Crommelin, D. J. (1988). The stability of liposomes upon nebulization. *International Journal of Pharmaceutics, 43*(1-2), 131-134.
15. Grit, M., Underberg, W. J., & Crommelin, D. J. (1991). Hydrolysis of saturated soybean

phosphatidylcholine in aqueous liposome dispersions. *Journal of Pharmaceutical Sciences*, *80*(5), 434-436.