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Original Research Article

FORMULATION AND EVALUATION OF SOLID LIPID NANOPARTICLES OF RIFAXIMIN

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ABSTRACT
The present study focuses on the formulation and evaluation of Rifaximin-loaded solid lipid nanoparticles (SLNs) to enhance its solubility, stability, and sustained-release performance. Rifaximin, a poorly water-soluble antibiotic, presents limited bioavailability, making it a suitable candidate for nanoparticulate drug delivery. SLNs were prepared using the ionotropic gelation method, and five formulations (F1–F5) were developed by varying polymer and crosslinker concentrations. The nanoparticles were evaluated for particle size, zeta potential, entrapment efficiency, drug content, *in vitro* drug release, kinetic modeling, and stability. Among all formulations, F3 demonstrated the most promising physicochemical properties with a particle size of 65.45 ± 0.15 nm, entrapment efficiency of 78.85 ± 0.41%, and high drug content of 99.12 ± 0.47%. Zeta potential analysis revealed a value of −38.12 mV, indicating excellent colloidal stability. *In vitro* release studies showed a sustained release pattern, reaching 98.12% drug release at 12 hours. Kinetic modeling indicated that the release followed Zero-order kinetics (R^2 = 0.983), confirming a consistent and controlled release mechanism. Stability studies conducted over three months showed minimal changes in entrapment efficiency and drug content, establishing the stability of the optimized formulation. The study demonstrates that rifaximin-loaded SLNs, particularly formulation F3, possess favorable characteristics for improved drug delivery, offering a promising approach to enhance Rifaximin's therapeutic efficiency and patient compliance.

Keywords: Rifaximin, Solid lipid nanoparticles, Ionotropic gelation, Entrapment efficiency, Sustained drug release, Zeta potential, Nanotechnology, Controlled release formulation.

INTRODUCTION

Rifaximin is a semi-synthetic, non-systemic oral antibiotic derived from rifamycin, widely used for traveler's diarrhea, hepatic encephalopathy, irritable bowel syndrome, and small intestinal bacterial overgrowth due to its broad-spectrum antimicrobial activity against enteric pathogens (Scarpignato *et al.*, 2005). Its poor aqueous solubility, minimal permeability, and extremely low systemic absorption classify it as a BCS Class IV drug, resulting in variable bioavailability and reduced therapeutic effectiveness (Patel *et al.*, 2021). In addition, rifaximin demonstrates food-dependent dissolution and limited absorption across the gastrointestinal tract, necessitating frequent dosing to maintain therapeutic levels (Jiang *et al.*, 2017). These pharmaceutical and biopharmaceutical limitations highlight the need for an optimized drug delivery system capable of enhancing solubility, dissolution, and sustained availability.

Solid lipid nanoparticles (SLNs) have emerged as promising nanocarriers composed of physiological lipids solid at room and body temperature, stabilized by surfactants to create a nanosized delivery matrix (Mehnert *et al.*, 2012). SLNs offer multiple advantages including improved solubility, enhanced stability, protection of drugs from degradation, controlled release, and excellent biocompatibility (Mukherjee *et al.*, 2009). Their lipidic structure facilitates lymphatic uptake and improved permeation, providing superior oral bioavailability for poorly soluble drugs (Üner *et al.*, 2021). Additionally, SLNs have practical benefits such as low production cost, scalability, and regulatory acceptability compared to polymeric nanoparticles (Pardeike *et al.*, 2009).

Incorporation of rifaximin into SLNs is expected to overcome its poor solubility and permeability by enhancing dissolution, prolonging gastrointestinal residence, and enabling controlled drug release. Recent studies demonstrate that lipid-based nanoparticles significantly enhance the delivery and antimicrobial efficacy of poorly soluble antibiotics (Pandey *et al.*, 2021; Rodenak-Kladniew *et al.*, 2020). However, limited literature exists specifically on rifaximin-loaded SLNs, presenting a substantial gap in research.

Therefore, the present study aims to formulate, optimize, and evaluate solid lipid nanoparticles of rifaximin to improve its solubility, sustain drug release, enhance intestinal absorption, and ultimately increase therapeutic performance.

MATERIALS AND METHODS

Materials

The formulation development of rifaximin-loaded nanoparticles was carried out using high-quality analytical-grade materials. Rifaximin was procured from Bioplus Life Sciences Pvt. Ltd., Bangalore. Various solvents and reagents, including methanol, ethanol, and chloroform, were obtained from Qualigens Fine Chemicals, Mumbai. Potassium bromide was sourced from Thomas Baker, Mumbai, while buffer salts such as disodium hydrogen phosphate, dipotassium hydrogen orthophosphate, sodium chloride, hydrochloride, and sodium hydroxide were purchased from S. D. Fine Chem. Ltd., Mumbai. Additionally, potassium dihydrogen phosphate was supplied by Loba Chemie Pvt. Ltd., Mumbai. All chemicals used were of appropriate purity suitable for nanoparticle formulation and analytical studies.

Methods

Preparation of solid lipid nanoparticles (SLNs) of Rifaximin

Solid lipid nanoparticles of rifaximin were prepared using the ionotropic gelation method as described by Muller *et al.* and co-researchers in their work on lipid-based nanoparticle systems (Müller *et al.*, 2007; Müller *et al.*, 1997). Initially, a 1% w/v chitosan stock solution was prepared by dissolving chitosan in 1% v/v acetic acid at room temperature. Rifaximin (10 mg) was then incorporated into the chitosan solution to obtain a homogeneous drug–polymer mixture. Separately, a 1% sodium tripolyphosphate (TPP) solution was prepared using distilled water. The TPP solution was added dropwise to the chitosan–drug mixture under continuous magnetic stirring to allow ionic crosslinking and nanoparticle formation. Stirring was continued for 30 minutes, after which the dispersion was filtered and washed with distilled water to remove unbound materials. The resulting nanoparticles were air-dried for 24 hours and subsequently oven-dried at 40 °C for six hours to obtain dry rifaximin-loaded SLNs.

Table 1: Formulations of Solid Lipid Nanoparticles

Sr. No	Formulation Code	Rifaximin (mg)	Chitosan (mg)	STPP (mg)
1.	F1	10	250	500
2.	F2	10	250	750
3.	F3	10	250	1000
4.	F4	10	500	500
5.	F5	10	500	750
6.	F6	10	500	1000

Coating of chitosan nanoparticles

Nanoparticles were coated with Eudragil S-100 (ES) using solvent evaporation method. Nanoparticles (10mg) were dispersed in 10 mL of coating solution prepared by dissolving 500 mg of ES-100 in ethanol: acetone (2:1) to give 5:1 (coat: core ratio). This organic phase was then poured in 70 mL of light liquid paraffin containing 1% wt/vol Span 80. The system was maintained under agitation speed of 1000 rpm at room temperature for 3 hours to allow for the evaporation of solvent. Finally, the coated nanoparticles were filtered, washed with n-hexane, and dried in desiccators.

Evaluation of Nanoparticles

Particle Size and Zeta Potential

The particle size and zeta potential of the prepared nanoparticles were determined using photon correlation spectroscopy with a Malvern Zetasizer, as described by Joshi *et al.* (2008). This technique provides accurate measurement of hydrodynamic diameter and surface charge, which are critical for evaluating nanoparticle stability and dispersibility.

Entrapment Efficiency

Entrapment efficiency of rifaximin-loaded nanoparticles was determined using the dialysis method as reported by Lin *et al.* (2007). The nanoparticle formulations were placed inside dialysis bags and immersed in 50 ml of phosphate-buffered saline (PBS, pH 7.4) for 24 hours to allow unentrapped (free) drug to diffuse out. The dialysate was then analyzed spectrophotometrically at 272 nm against PBS blank under identical conditions. The concentration of free drug was calculated from the standard calibration curve of rifaximin prepared in the same medium. Entrapment efficiency was expressed as the ratio of drug associated with nanoparticles to the total drug initially used.

Total Drug Content

The total drug content was quantified following the method adapted from Lacerda *et al.* (2011). An aliquot (1 ml) of the nanoparticle suspension was diluted with 10 ml of PBS (pH 7.4) and ethanol mixture to ensure complete dissolution of the drug. The absorbance was measured at 433 nm using a UV–visible spectrophotometer, with placebo nanoparticles serving as the blank. The total rifaximin content was calculated based on the standard calibration curve.

***In vitro* drug release**

In vitro release of rifaximin from the nanoparticles was evaluated using the USP XXII paddle-type dissolution apparatus, following the theoretical principles described by Higuchi *et al*. (1963). A weighed amount of formulation (100 mg) was dispersed on the surface of 900 ml dissolution medium (PBS, pH 7.4) maintained at 37 ± 0.2°C and stirred at 100 rpm. Samples were collected at predetermined intervals and immediately replaced with fresh medium to maintain sink conditions. Each withdrawn sample was diluted to 10 ml with PBS and analyzed spectrophotometrically to determine rifaximin concentration based on the standard curve. The cumulative drug release was calculated to evaluate release kinetics.

Several kinetic models have been proposed to describe the release characteristics of a drug from matrix. The following three equations are commonly used, because of their simplicity and applicability. Equation 1, the zero-order model equation (Plotted as cumulative percentage of drug released vs time); Equation 2, Higuchi's square-root equation (Plotted as cumulative percentage of drug released vs square root of time); and Equation 3, the Korsemeyer-Peppas equation (Plotted as Log cumulative percentage of drug released vs Log time).

Stability studies

To ensure the physicochemical stability and integrity of the drug-loaded formulation, a short-term stability study was conducted under two different temperature conditions: refrigeration temperature (4.0 ± 0.2℃) and ambient room temperature (25–28 ± 2℃) over a period of 3 month. The formulation was stored in chemically inert, airtight borosilicate glass containers to prevent any potential interaction between the formulation and the container material. Borosilicate glass is known for its high thermal resistance and chemical stability, which makes it suitable for pharmaceutical stability testing. At regular time intervals during the 3-week study period (initial, 1st, 2nd, and 3rd month), the samples were withdrawn and evaluated for important parameters. The drug content was quantitatively determined by UV spectrophotometric analysis to assess any loss in potency or chemical degradation over time. Observations from the stability study provided essential insights into the physical and chemical stability of the formulation, ensuring that the drug maintains its efficacy and consistency under different storage conditions throughout its shelf life.

RESULTS AND DISCUSSION

The prepared rifaximin-loaded nanoparticles exhibited suitable physicochemical characteristics, indicating successful formulation using the selected method and excipients. Particle size analysis revealed that all formulations were within the nanometric range (65–110 nm), demonstrating efficient nanoparticle formation. Among all batches, formulation F3 showed the smallest particle size (65.45 ± 0.15 nm), which can be attributed to optimum polymer–crosslinker concentration and effective ionic gelation. Smaller particle size generally facilitates enhanced drug diffusion, improved cellular uptake, and higher bioavailability, thereby making F3 the most promising formulation.

Entrapment efficiency (EE%) ranged from 65.58% to 78.85%. Formulation F3 again demonstrated the highest value (78.85 ± 0.41%), indicating that optimal chitosan-to-TPP ratio favors the encapsulation of rifaximin. Higher entrapment suggests better drug loading capacity, reduced drug wastage, and improved therapeutic potential of the nanoparticles. Drug content among all batches was consistently high (95.58–99.12%), reflecting uniform drug distribution and minimal loss during processing.

Zeta potential analysis of the optimized formulation F3 showed a negative charge (−38.12 mV), confirming good colloidal stability. Nanoparticles with zeta potential values beyond ±30 mV are generally considered physically stable due to strong electrostatic repulsion that prevents aggregation. Hence, the stability of F3 indicates suitability for long-term storage and enhanced dispersion properties.

In vitro drug release studies demonstrated a controlled and sustained release pattern over 12 hours. The optimized batch (F3) showed an initial slow release (3.12% at 1 hour), followed by a gradual increase, reaching 98.12% at 12 hours. This biphasic release initial diffusion followed by a sustained phase can be attributed to surface-bound drug release in the early phase and matrix diffusion during the later phase. Such sustained release behavior is desirable for maintaining prolonged therapeutic levels of rifaximin, potentially reducing dosing frequency.

Kinetic modeling revealed that the drug release from F3 followed Zero-order kinetics ($R^2 = 0.983$), suggesting that release occurred at a constant rate independent of drug concentration. This is an ideal release profile for sustained-release formulations, indicating predictable and uniform delivery of rifaximin.

Stability studies conducted at two temperature conditions (4°C and 25–28°C) over three months indicated no significant changes in entrapment efficiency or total drug content. Although a slight decline was observed over time, the values remained within acceptable limits, confirming that the optimized formulation is physically and chemically stable under both refrigerated and room-temperature conditions. This stability is essential for ensuring long shelf-life and effectiveness during storage and transportation.

The findings indicate that formulation F3 possesses optimal particle size, high entrapment efficiency, excellent stability, and a desirable sustained release profile. These characteristics collectively demonstrate the potential of the developed rifaximin-loaded nanoparticles as an efficient delivery system for enhancing therapeutic performance and improving patient compliance.

Table 2: Result for particle size, entrapment efficiency and drug content of drug loaded nanoparticles

Formulation Code	Particle size (nm)	Entrapment Efficiency (%)	Drug Content (%)
F1	110.25±0.25	65.58±0.15	97.07±0.69
F2	95.65±0.32	72.23±0.19	98.85±0.58
F3	65.45±0.15	78.85±0.41	99.12±0.47
F4	88.32±0.22	69.95±0.47	96.65±0.41
F5	98.85±0.29	70.23±0.55	95.58±0.52

Table 3: Particle size and entrapment efficiency of optimized nanoparticles

Formulation Code	Particle size (nm)	Entrapment Efficiency	Zeta potential
F3	65.45±0.15	78.85±0.41	-38.12

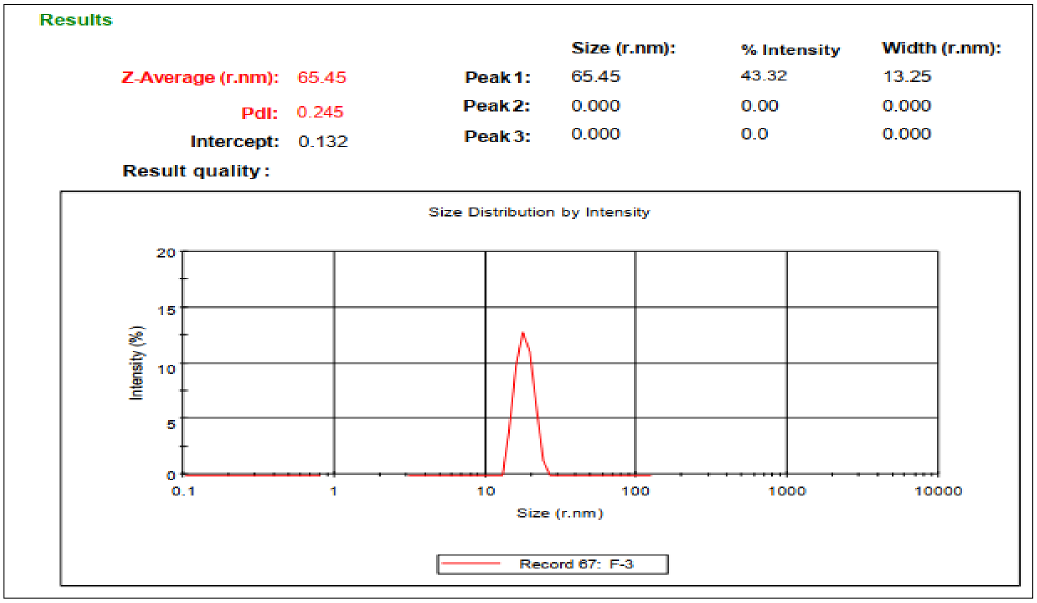

Figure 1: Particle size of Optimized nanoparticles formulation F3

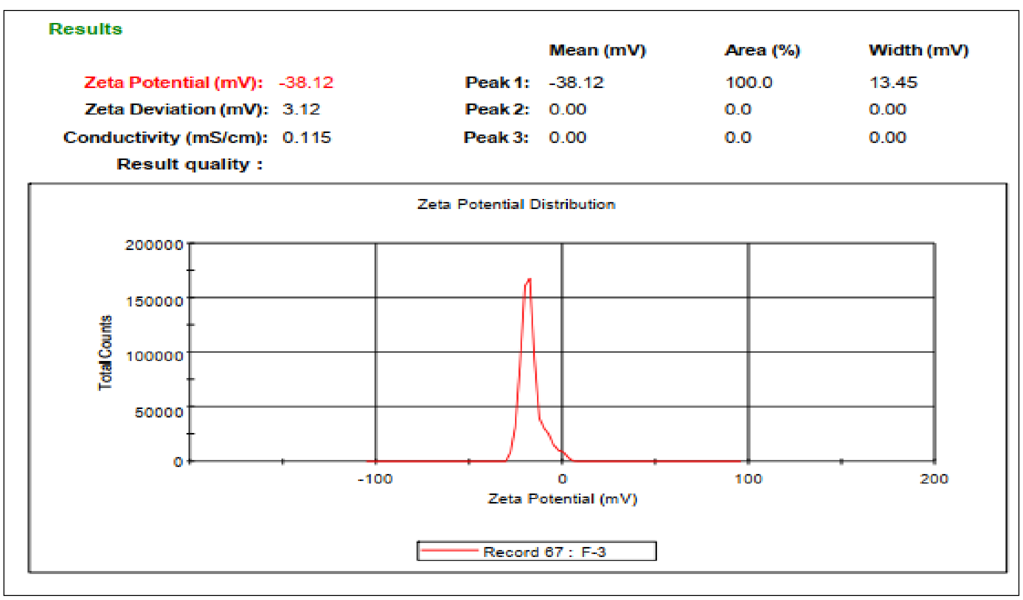

Figure 2: Zeta potential of Optimized nanoparticles formulation F3

Table 4: Cumulative % drug release

S. No.	Time (hrs)	% Cumulative Drug Release
1	1	3.12
2	2	8.98
3	3	14.65
4	4	20.32
5	5	34.45
6	6	41.32
7	7	58.98
8	8	65.32
9	9	78.85
10	10	88.98
11	12	98.12

Table 5: Regression analysis data of optimized formulation F3

Batch	Zero Order	First Order
	R^2	R^2
F3	0.983	0.814

Table 6: Stability of optimized formulation

Characteristic	Time (Month)					
	1 Month		2 Month		3 Month	
Temp.	4.0 ± 0. 2℃	25-28 ± 2℃	4.0 ± 0. 2℃	25-28 ± 2℃	4.0 ± 0. 2℃	25-28 ± 2℃
Entrapment Efficiency (%)	78.25	80.25	76.65	78.85	73.32	75.65
Total Drug Content (%)	99.12	98.75	99.00	98.15	98.85	97.74

CONCLUSION

The present study successfully formulated and evaluated rifaximin-loaded solid lipid nanoparticles using the ionotropic gelation technique. Among the prepared batches, formulation F3 exhibited the most desirable characteristics, including the smallest particle size (65.45 nm), highest entrapment efficiency (78.85%), excellent drug content, and a stable zeta potential (−38.12 mV), indicating strong colloidal stability. In vitro drug release studies confirmed a sustained and controlled release of rifaximin over 12 hours, following Zero-order kinetics, which is ideal for maintaining consistent therapeutic levels. Stability studies further demonstrated that the optimized formulation remained physically and chemically stable for up to three months under both refrigerated and room-temperature conditions. Overall, the findings suggest that the developed nanoparticles offer a promising delivery system for enhancing the solubility, stability, and sustained release of rifaximin, potentially improving its therapeutic efficacy and patient compliance.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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