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FORMULATION, DEVELOPMENT AND EVALUATION OF RIFAXIMIN LOADED SOLID LIPID NANOPARTICLES

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ABSTRACT

This research investigates the formulation, development, and evaluation of solid lipid nanoparticles (SLNs) loaded with rifaximin, an antibiotic known for its effectiveness against gastrointestinal infections. The primary objective was to enhance the bioavailability and therapeutic efficacy of rifaximin through the use of SLNs, which are designed to provide controlled release and improved stability. Various formulations were developed by varying lipid matrices and surfactants, and thorough characterization was performed to assess their physical and chemical properties. The characterization included measurement of particle size, entrapment efficiency, and drug content. The optimized formulation (F14) demonstrated a particle size of 215.45 nm and an impressive entrapment efficiency of 83.32%, indicating a successful incorporation of rifaximin within the lipid matrix. Additionally, the drug content was consistently high across formulations, further confirming the reliability of the preparation method. In vitro drug release studies were conducted to evaluate the cumulative release profile of rifaximin from the nanoparticles. The results indicated a sustained release characteristic, with 98.12% of the drug released over a period of 12 hours. This sustained release pattern aligns with zero-order kinetics, suggesting that the release rate of rifaximin from the SLNs is constant over time, which is beneficial for maintaining therapeutic levels of the drug in the systemic circulation. Overall, the findings of this study underscore the potential of rifaximin-loaded SLNs as an effective drug delivery system. The enhanced bioavailability and controlled release characteristics of the formulation could significantly improve patient compliance and therapeutic outcomes in managing gastrointestinal disorders.

Keywords: Rifaximin, Solid Lipid Nanoparticles, Drug Delivery, Bioavailability, Sustained Release, Formulation Development, Particle Size, Entrapment Efficiency, Kinetics.

INTRODUCTION

Rifaximin is a semi-synthetic antibiotic primarily used to treat gastrointestinal infections, including traveler's diarrhea and hepatic encephalopathy. Despite its efficacy, rifaximin's clinical use is limited by its poor solubility and bioavailability. To enhance its therapeutic potential, innovative drug delivery systems such as solid lipid nanoparticles (SLNs) have emerged as promising solutions. SLNs offer several advantages, including improved drug solubility, controlled release, and enhanced stability compared to conventional formulations.

Solid lipid nanoparticles are colloidal carriers composed of solid lipids that encapsulate the drug, providing a protective environment and facilitating targeted delivery. Their small size enhances cellular uptake and allows for improved penetration across biological membranes, making them particularly suitable for delivering poorly soluble drugs like Rifaximin [1-2].

The formulation of Rifaximin-loaded SLNs involves the selection of appropriate lipid matrices, surfactants, and preparation techniques. Various methods, including high-pressure homogenization and solvent emulsification, have been employed to fabricate these nanoparticles effectively [3]. The successful formulation and optimization of Rifaximin-loaded SLNs can lead to improved pharmacokinetic profiles, reduced dosing frequency, and enhanced patient compliance.

This study aims to formulate, develop, and evaluate Rifaximin-loaded solid lipid nanoparticles, assessing their physicochemical characteristics, drug release profiles, and antibacterial efficacy. Through this approach, we hope to establish a novel delivery system that optimizes rifaximin's therapeutic effects while minimizing its limitations.

MATERIAL AND METHODS

Material:

The formulation development of solid lipid nanoparticles (SLNs) for rifaximin involved various chemicals sourced from reputable suppliers. Rifaximin, the active pharmaceutical ingredient, was procured from Bioplus Life Sciences Pvt. Ltd., Bangalore. Key buffering agents included potassium bromide and disodium hydrogen phosphate, both from S. D. Fine Chem. Ltd., Mumbai, along with di-potassium hydrogen orthophosphate and sodium chloride for maintaining the required pH levels. Organic solvents such as methanol, ethanol, and chloroform, also from Qualigens Fine Chemicals, Mumbai, were utilized in the extraction and formulation processes. The lipid matrix primarily comprised glyceryl tripalmitate from S. D. Fine Chem. Ltd., while soy lecithin and Pluronic F-68

from Hi Media, Mumbai, served as surfactants to stabilize the nanoparticles. Additional stabilizers like stearyl amine, sourced from Loba Chemie Pvt. Ltd., and hydrochloric acid, sodium hydroxide, and potassium dihydrogen phosphate from S. D. Fine Chem. Ltd. and Loba Chemie, respectively, were used to optimize the formulation's properties. Together, these materials contributed to the successful development of rifaximin-loaded SLNs.

Methods

Preparation of Rifaximin loaded solid lipid nanoparticles

Solid lipid nanoparticles were prepared by using microemulsion technique[4] and o/w microemulsions were initially prepared. The oil phase, lipophilic surfactant and continuous phase used are glyceryl tripalmitate, soy lecithin and pluronic F-68 (hydrophilic surfactant) respectively. The lipid and soy lecithin were melted at 70^{o}C and the drug was added with constant stirring. 10 ml of aqueous surfactant solution containing pluronic F-68 heated at the same temperature was added to the melted lipid with mechanical stirring for 15 min. A clear microemulsion was obtained at a temperature close to the melting point of the lipid used. Stearyl amine was used as a positive charge inducer and added to melted lipid. Solid lipid nanoparticles were obtained by dispersing the warm o/w microemulsion which is added drop wise into ice cold water in a beaker under continuous stirring. After completion of stirring, the Solid lipid nanoparticles dispersion was subjected to ultrasonication for 15 min.

Study on the effect of lipid quantity

The effect of lipid quantity on the particle size was studied by varying one parameter, keeping the others constant. Three different batches of Solid lipid nanoparticles were prepared corresponding to varying concentrations of lipid such as 50, 100 and 200 mg keeping the amount of soy lecithin (1% w/w), stearyl amine (1% w/w), pluronic F-68 (1% w/v), stirring time (3 hours) and stirring speed (1500 rpm) constant.

Study on the effect of formulation process variables

The effect of formulation process variables such as stirring time, stirring speed, surfactant concentration on the particle size was studied. From the results obtained, optimum level of those variables was selected and kept constant in the subsequent evaluations.

Effect of stirring time

Five different batches of Solid lipid nanoparticles were prepared corresponding to 1, 2, 3, 4, 5 hours of stirring time keeping the lipid concentration (50 mg), soy lecithin (1% w/w), stearyl amine (1% w/w), pluronic F-68 (1% w/v) and stirring speed (2000 rpm) constant [4].

Effect of stirring speed

Four different batches of Solid lipid nanoparticles were prepared corresponding to 1000, 1500, 2000 and 2500 rpm of stirring speed keeping the lipid concentration (50 mg), soy lecithin (1% w/w), stearyl amine (1% w/w), pluronic F-68 (1% w/v) and stirring time (4 hours) constant.

Effect of surfactant concentration

Four different batches of solid lipid nanoparticles were prepared corresponding to

0.5%, 1%, 1.5% and 2% w/v of pluronic F-68 keeping the lipid concentration (50 mg), soy lecithin (1% w/w), stearyl amine (1% w/w), stirring time (4 hours) and stirring speed (2000 rpm) constant.

Preparation of drug loaded Solid lipid nanoparticles batches

One optimized formulation of drug loaded Solid lipid nanoparticles were prepared by microemulsion method.

Table 1: Composition of solid lipid nanoparticles by varying amount of Lipid

Components	**Formulation code**		
	F1	**F2**	**F3**
Drug	**50**	100	200
Soy lecithin	**1**	1	1
Stearyl amine	**1**	1	1
Pluronic F-68 (1% w/v)	**1**	1	1
Stirring speed (rpm)	**1500**	1500	1500

Stirring time (hrs)	**3**	3	3

Table 2: Composition of Solid lipid nanoparticles by varying Stirring time

Components	**Formulation code**				
	F4	**F5**	**F6**	**F7**	**F8**
Drug	50	50	50	**50**	50
Soy lecithin	1	1	1	**1**	1
Stearyl amine	1	1	1	**1**	1
Pluronic F-68 (1% w/v)	1	1	1	**1**	1
Stirring speed (rpm)	2000	2000	2000	**2000**	2000
Stirring time (hrs)	1	2	3	**4**	5

Table 3: Composition of Solid lipid nanoparticles by varying Stirring speed

Components	**Formulation code**			
	F9	**F10**	**F11**	**F12**
Drug	50	50	**50**	50
Soy lecithin	1	1	**1**	1
Stearyl amine	1	1	**1**	1
Pluronic F-68 (1% w/v)	1	1	**1**	1
Stirring speed	1000	1500	**2000**	2500
Stirring time	4	4	**4**	4

Table 4: Composition of Solid lipid nanoparticles by varying amount Surfactant

Components	**Formulation code**			
	F13	**F14**	**F15**	**F16**
Drug	50	**50**	50	50
Soy lecithin	1	**1**	1	1
Stearyl amine	1	**1**	1	1
Pluronic F-68 (1% w/v)	0.5	**1**	1.5	2
Stirring speed	2000	**2000**	2000	2000
Stirring time	4	**4**	4	4

Table 5: Composition of optimized batch

Components	Formulation code (F14)
Drug	50
Soy lecithin	1
Stearyl amine	1
Pluronic F-68 (1% w/v)	1
Stirring speed	2000
Stirring time	4

Evaluation of nanoparticles

Particle size and zeta potential

Particle size and zeta potential of the Solid lipid nanoparticles were measured by photon correlation spectroscopy using a Malvern Zetasizer the results shown in table [5].

Entrapment efficiency

Entrapment efficiency was determined by dialysis method. Solid lipid nanoparticles entrapped Rifaximin were separated from the free drug by dialysis method. The above said formulations were filled into dialysis bags and the free Rifaximin dialyzed for 24 hours into 50 ml of phosphate buffer saline pH 7.4. The absorbance of the dialysate was measured at 272 nm against blank phosphate buffer saline pH 7.4and the absorbance of the corresponding blank phosphate buffer saline pH 7.4 was measured under the same condition. The concentration of free Rifaximin could be obtained from the absorbance difference based on standard curve. Standard curve was made by measuring the absorbance at 433 nm for known concentrations of Rifaximin solution. The entrapment efficiency of the drug was defined as the ratio of the mass of formulations associated drug to the total mass of drug [6].

Total drug content:

From the prepared Solid lipid nanoparticles formulation 1ml of suspension is dissolved in the 10 ml of phosphate buffer saline pH 7.4 and ethanol mixture. The amount of Rifaximin was determined using UV spectrophotometer at 433nm. The placebo formulation prepared similarly to drug loaded Solid lipid nanoparticles is used as blank. The total drug content was calculated [7].

***In vitro* drug release**

The prepared Solid lipid nanoparticles delivery system was evaluated for *in vitro* drug release. The drug release studies were carried out using USP XXII paddle type Dissolution test apparatus. The dissolution study was carried out in 900 ml dissolution medium which was stirred at 100 rpm maintained at 37±0.2°C. A weighed quantity of formulation (100 mg) was spread over the surface of dissolution media (900 ml) at 37±0.2°C. Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 10ml by PBS (pH 7.4). The samples withdrawn were assayed spectrophotometrically at 433.0 nm for Rifaximin and using UV visible spectrophotometer. The release of Rifaximin was calculated with the help of Standard curve of Rifaximin[8].

RESULTS AND DISCUSSION

Table 6 presents the particle size, entrapment efficiency, and drug content of various formulations of rifaximin-loaded solid lipid nanoparticles (SLNs). Formulation F7 exhibited the smallest particle size of 233.36 nm, along with the highest entrapment efficiency at 85.45%. Conversely, formulation F4 had the largest particle size at 278.85 nm and the lowest entrapment efficiency at 69.98%. All formulations demonstrated high drug content, with F7 and F14 both achieving drug contents greater than 99%. This suggests that the formulations were effective in encapsulating the drug while maintaining favorable particle characteristics.

Table 7 highlights the optimized formulation (F14), which achieved a particle size of 215.45 nm, an entrapment efficiency of 83.32%, and a zeta potential of -36.48 mV. The relatively small particle size is advantageous for enhanced bioavailability, while the negative zeta potential indicates good stability, minimizing aggregation.

Table 8 summarizes the cumulative percentage of drug release over time for the selected formulations (F1, F7, F11, F14). Notably, formulation F14 showed the slowest initial release, with only 3.12% released after 1 hour, compared to F1, which released 11.65% in the same timeframe. Over 12 hours, F14 achieved a cumulative release of 98.12%, indicating a sustained release profile. In contrast, F1 reached near-complete release at 99.45%, suggesting a faster release mechanism.

Table 9 presents the regression analysis data for formulation F14, demonstrating a high R^2 value of 0.983 for zero-order kinetics and a lower value of 0.814 for first-order kinetics. This indicates that

the release of rifaximin from the optimized SLNs follows zero-order kinetics, suggesting a controlled and consistent release rate over time.

The results from these analyses indicate that the optimized formulation F14 not only achieved desirable particle size and entrapment efficiency but also demonstrated a favorable drug release profile, adhering to zero-order kinetics. These characteristics position F14 as a promising candidate for further development in targeted drug delivery systems, particularly for rifaximin, thereby enhancing therapeutic efficacy and patient compliance. Further studies on in vivo performance and stability will be essential for clinical application.

Table 6: Result for Particle size, Entrapment efficiency and drug content of drug loaded solid lipid nanoparticles

Formulation Code	Particle size	Entrapment Efficiency	Drug Content
F1	**245.65**	**83.32**	**98.78**
F2	269.98	78.85	98.85
F3	265.58	73.32	98.12
F4	278.85	69.98	97.85
F5	258.98	81.12	98.85
F6	254.45	77.78	96.65
F7	**233.36**	**85.45**	**99.12**
F8	245.65	73.15	99.45
F9	269.98	71.15	99.05
F10	258.74	68.85	99.45
F11	**220.25**	**83.32**	**97.78**
F12	274.45	73.32	96.65
F13	263.32	63.32	98.74
F14	**215.45**	**83.32**	**99.45**
F15	236.65	81.12	98.78
F16	245.78	87.74	98.78

Table 7: Particle size and Entrapment efficiency of Optimized solid lipid nanoparticles

Formulation Code	Particle size (nm)	Entrapment Efficiency	Zeta potential
F14	215.45	83.32	-36.48

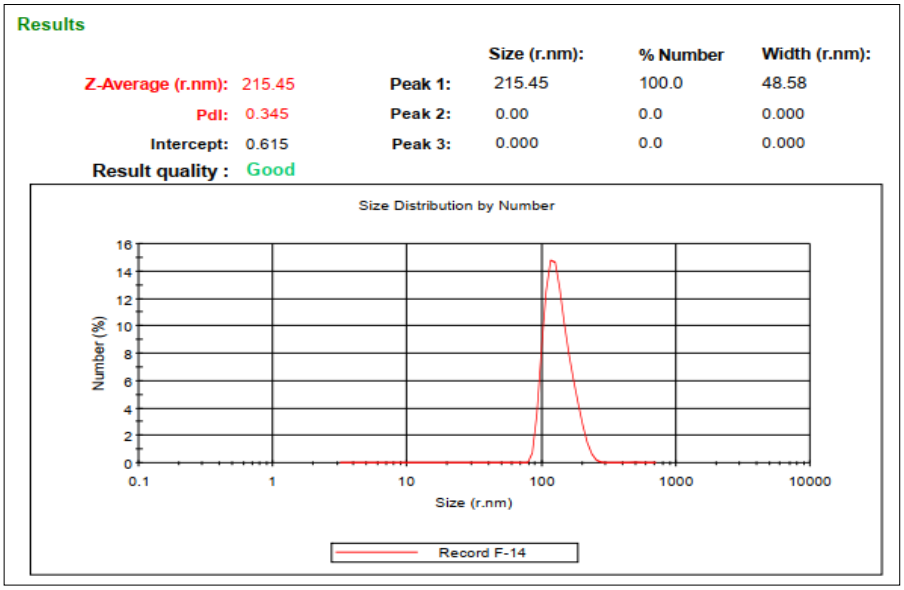

Figure 1: Particle size of Optimized solid lipid nanoparticles formulation F14

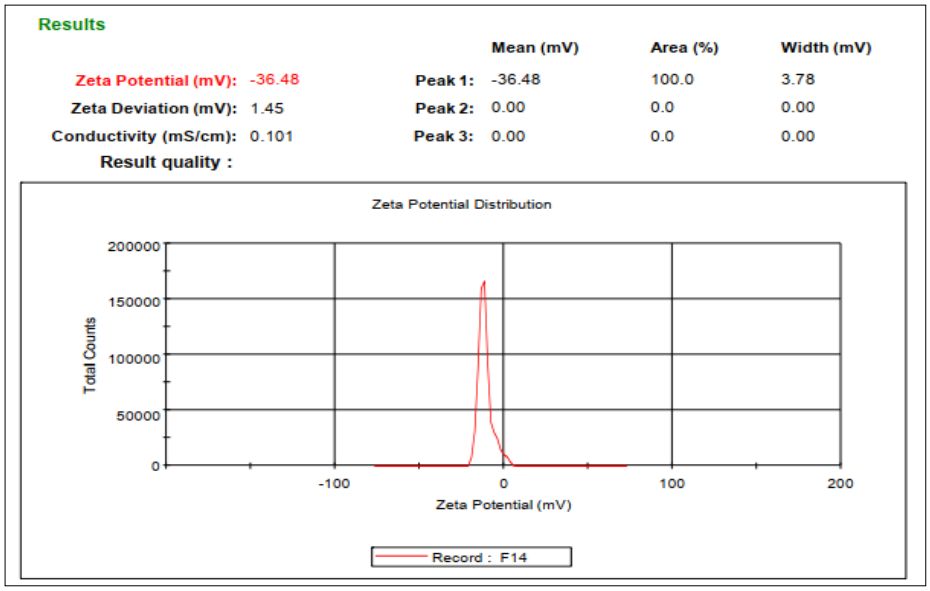

Figure 2: Zeta potential of Optimized solid lipid nanoparticles formulation F14

Table 8: Cumulative % drug release

S. No.	Time (hrs)	% Cumulative Drug Release

		F1	F7	F11	F14
1	1	11.65±0.15	8.85±0.22	6.45±0.31	3.12±0.15
2	2	19.98±0.32	16.65±0.15	13.32±0.33	8.98±0.33
3	3	39.98±0.18	35.45±0.18	29.98±0.45	14.65±0.25
4	4	49.98±0.22	46.65±0.36	43.32±0.26	20.32±0.14
5	5	56.65±0.13	53.32±0.45	50.23±0.35	34.45±0.78
6	6	69.98±0.22	61.14±0.85	59.98±0.47	41.32±0.36
7	7	88.85±0.36	79.98±0.74	67.74±0.58	58.98±0.25
8	8	98.85±0.45	89.98±0.69	84.45±0.41	65.32±0.45
9	9	99.05±0.22	98.85±0.32	97.85±0.36	78.85±0.25
10	10	99.12±0.18	99.12±0.33	98.15±0.25	88.98±0.14
11	12	99.45±0.32	99.65±0.74	99.45±0.14	98.12±0.32

Table 9: Regression analysis data of optimized formulation F14

Batch	Zero Order R^2	First Order R^2
F14	0.983	0.814

CONCLUSION

The study successfully formulated and evaluated solid lipid nanoparticles (SLNs) for the delivery of rifaximin, highlighting the potential of this approach in enhancing the drug's bioavailability and therapeutic efficacy. The optimized formulation (F14) demonstrated favorable characteristics, including a particle size of 215.45 nm and an entrapment efficiency of 83.32%, indicating effective drug incorporation within the lipid matrix. In vitro release studies revealed a sustained release profile, with a cumulative drug release of 98.12% over 12 hours, aligning with zero-order kinetics. This sustained release mechanism is particularly advantageous for maintaining therapeutic drug levels, potentially leading to improved patient compliance and treatment outcomes.

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