International Journal of Multidisciplinary Research and Growth Evaluation.

Development and optimization of ribavirin-loaded solid lipid nanoparticles

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Article Info

ISSN (online): 2582-7138
Volume: 03
Issue: 06
November-December 2022
Received: 28-10-2022;
Accepted: 19-11-2022
Page No: 464-468

Abstract
The Ribavirin has low solubility and permeability which give rise to limited and variable bioavailability; its low stability makes it difficult to develop stable aqueous liquid formulations, the aim of this study was to investigate the effectiveness of a strategy based on the development of solid lipid Nanoparticles as an innovative formulation of Ribavirin with improved therapeutic efficacy. The Ribavirin solid lipid nanoparticles were prepared by emulsification solvent evaporation technique by applying ultrasonic energy through Sonicator, The different formulations with various ratios of drug-lipid and surfactant were evaluated and optimized. The method used for the formulation of Ribavirin containing soya lecithin solid lipid nanoparticles was solvent evaporation method followed by sonication to reduce the particle size. The prepared nanosuspensions were characterized for particle size, surface morphology by SEM, drug excipient compatibility by FTIR and *in-vitro* drug release studies. Formulation (F-4) showed the highest encapsulation efficiency. In this research, a drug encapsulation efficiency as high as 81% has been achieved. It was found that as the concentration of soya lecithin increased, the % of encapsulation efficiency was also increased. The present study revealed that solvent evaporation technique followed by sonication can be used as an effective tool for preparation of Ribavirin solid lipid nanoparticles.

Keywords: Ribavirin drug, solid lipid Nano Particles, Solvent Evaporation, lipid, FTIR, *in vitro* drug release

1. Introduction
Solid lipid nanoparticles (SLN) introduced in 1991 represent an alternative carrier system to tradition colloidal carriers such as - emulsions, liposomes and polymeric micro and nano particles [1]. Nanoparticles made from solid lipids are attracting major attention as novel colloidal drug carrier for intravenous applications as they have been proposed as an alternative particulate carrier system.SLN are sub-micron colloidal carriers ranging from 50 to 1000 nm, which are composed of physiological lipid dispersed in water or in aqueous surfactant solution. SLN offer unique properties such as small size, large surface area, high drug loading and the interaction of phases at the interface and are attractive for their potential to improve performance of pharmaceuticals [2, 3]. In order to overcome the disadvantages associated with the liquid state of the oil droplets, the liquid lipid was replaced by a solid lipid, which eventually transformed into solid lipid nanoparticles. The reasons for the increasing interest in lipid-based system are many-fold and include Lipids enhance oral bioavailability and reduce plasma profile variability [4]. Better characterization of lipoid excipients. An improved ability to address the key issues of technology transfer and manufacture scale-up. Solid lipid nanoparticles are one of the novel potential colloidal carrier systems as alternative materials to polymers which is identical to oil in water emulsion for parenteral nutrition, but the liquid lipid of the emulsion has been replaced by a solid lipid [5]. They have many advantages such as good biocompatibility, low toxicity and lipophilic drugs are better delivered by solid lipid nanoparticles and the system is physically stable. Solid lipid nanoparticles (SLNs) are considered to be the most effective lipid based colloidal carriers, introduced in early nineties [6].

This is the one of the most popular approaches to improve the oral bioavailability of the poorly water-soluble drugs. SLNs are in the submicron size range of 50-1000 nm and are composed of physiologically tolerated lipid components which are in solid state at room temperature. Control and / or target drug release. Excellent biocompatibility [5]. Improve stability of pharmaceuticals [7]. High and enhanced drug content. Easy to scale up and sterilize. Better control over release kinetics of encapsulated compounds. Enhanced bioavailability of entrapped bioactive compounds [8]. The aim was to improve oral bioavailability either by increasing GI absorption or bypassing the first-pass metabolism Several drugs have been incorporated in to SLN formulations for oral administration and enhancement of oral bioavailability of Ribavirin. Preparation of solid lipid nanoparticles by two methods solvent emulsification followed by evaporation method followed by ultrasonication method and optimization of the sonication time for the desired particle size.

Optimization of surfactant and emulsifier concentration for maximum yield and higher entrapment efficiency to evaluate prepared SLN for physico-chemical characteristics such as % drug content, size distribution, percent entrapment efficiency, Invitro drug release and stability studies

2. Materials and Method

2.1 Materials

Ribavirin was collected as a gift sample from Aurobindo labs, Hyd, polymers and other excipients were purchased from AR Chemicals, Hyd.

2.2 Methodology

Compatibility studies Fourier transform infrared (FTIR) analysis

Drug excipient compatibility studies

Drug excipients compatibility studies were performed to know the compatibility of excipient with drug at accelerated conditions. The study was conducted by preparing homogenous mixture of excipients with drug and filled in high density polyethylene bags and low density poly ethylene bags. Glass vials were exposed to 60^0C and 40^0C/75 % relative humidity for 4 weeks and low density polyethylene bags were exposed to 40^0C±75 % relative humidity for 4 weeks. Samples were observed periodically for any physical change [9].

Method of preparation of Ribavirin loaded nanoparticles

HCL loaded SLN were prepared by solvent emulsification/ evaporation method. The composition of all the formulations 20 mg of drug was dissolved in 10 mL methanol, and Phosphatidylcholine was dissolved in 20 mL chloroform separately; drug and lipid solutions were mixed together. The organic solvent mixture was completely evaporated at 70°C using rotary evaporator to remove the of organic solvent. Drug embedded lipid layer was then poured into 100 mL of aqueous solution containing poloxomer 407 surfactant and the mixture was Sonicated for 15 minutes by using Sonicator followed by homogenized for 15 minutes at different homogenization speed using high speed homogenizer. The suspension was then allowed to cool at room temperature. The suspension was filtered through membrane filter. The filtrate was centrifuged (1000 rpm for 10 minutes) and nano particles was collected [10, 11]

Table 1: Composition of Ribavirin for preparation of solid lipid nanoparticles

Ingredients	F1	F2	F3	F4
Ribavirin	25	25	25	25
Phosphatidylcholine	25	50	75	100
Poloxamer 407	10	20	30	40
Solvent(Methanol)	10	10	10	10
Chloroform	20	20	20	20

Evaluation of Ribavirin loaded nanoparticles [12, 13, 14]

Particlesize

All the prepared batches of nanoparticles were viewed under microscope to study their size. Size of Nano particles from each batch was measured at different location on slide by taking a small drop of nanoparticle dispersion on it and average size of nanoparticles were determined.

SEM analysis

The morphology of NPs was studied by a scanning electron microscope. For this purpose, the sample was lyophilized and placed on aluminum stubs and the surface was coated with a layer of gold particles using a sputter coater. The shape of the NPs was determined by scanning electron microscopy (SEM) (XL30, Philips, the Netherlands) at 15 kV and 750 mA.

Drug encapsulation efficiency

Lyophilized nanoparticles 50mg were dissolved in 100ml of phosphate buffer and the drug amount was determined by UV analysis. The encapsulation efficiency was determined as the mass ratio of entrapped Ribavirin in nanoparticles to the theoretical amount of the drug used in the preparation. The entrapment of the Ribavirin nanoparticles was expressed as loading capacity.

In-vitro drug release studies

The release studies were carried out by franz diffusion cell. It containing 10 ml Phosphate buffer. Phosphate buffer pH 7.4 (100 ml) was placed in a 10 ml of beaker. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37±5^{0}C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped Ribavirin dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. 1ml of aliquots were withdrawn at specific intervals, filtered after withdrawal and the apparatus was immediately replenished with same quantity of fresh buffer medium.

Percentage of drug release was determined using the following formula.

Stability studies [15]

Selected Formulation was subjected to stability studies as per ICH guidelines.

Following conditions were used for Stability Testing.

1. 25^{0}C/60% RH analyzed every month for period of three months.
2. 30^{0}C/75% RH analyzed every month for period of three months.
3. 40^{0}C/75% RH analyzed every month for period of three months.

3. Results and Discussion

In the present study 4 formulations with variable concentration of lipid were prepared and evaluated for

physic-chemical parameters, *in vitro* release studies and stability studies.

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.

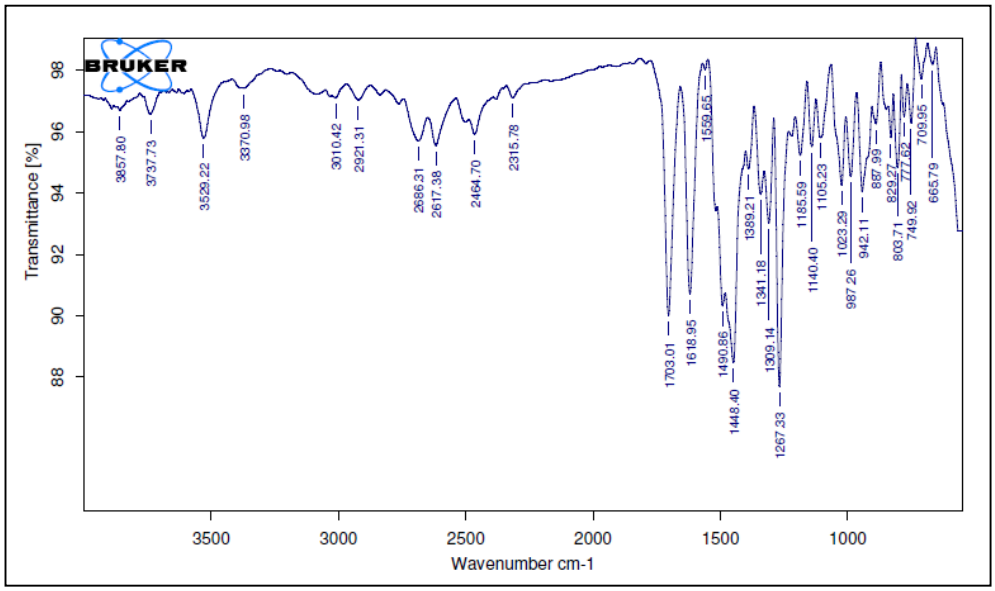

Fig 1: FT-IR Sample for Ribavirin

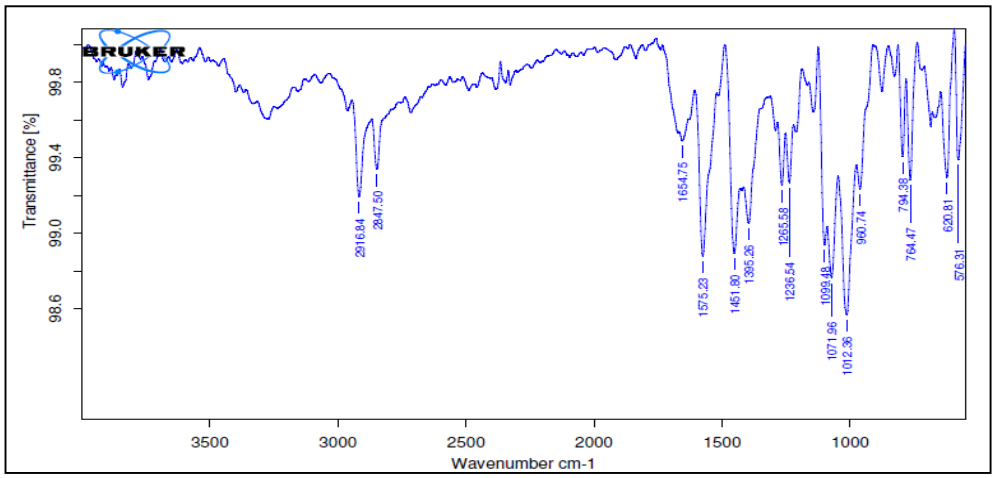

Fig 2: FT-IR Sample for Optimized Formulation

Evaluation Parameters

The solid lipid nanoparticles prepared were evaluated as per the following parameters-

- Particle size and SEM analysis
- Entrapment efficiency
- *In vitro* release study
- Stability studies

Particle size

The particle size increased with increasing of lipid concentration. Based on particle size distribution and entrapment efficiency.

Surface morphology

Scanning electron microscopy (SEM) SEM revealed that the Rabavirin solid lipid nanoparticles were smooth and spherical without any aggregation.

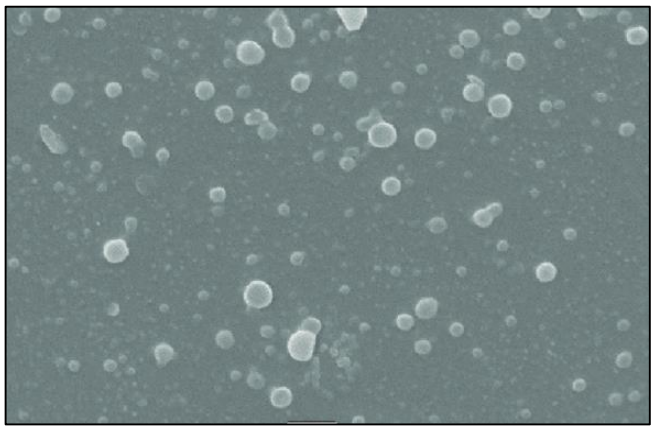

Fig 3: SEM analysis of Optimized Solid lipid solid lipid nanoparticle

Drug entrapment efficiency

The first part of the plan of work was to optimize the concentration of Lipid to be used in the formulation of solid lipid nanoparticles. The optimization of lipid concentration was done on the basis of particle size and entrapment efficiency of solid lipid nanoparticles obtained.

Table 2: Evaluation Studies of Prepared solid lipid nanoparticles: Entrapment Efficiency and Particle size

Batch No	Particle size (nm)	Entrapment Efficiency (%)
F1	332	81
F2	362	80
F3	375	79
F4	395	90

***In vitro* drug release studies**

Results indicate that the formulation showed initial burst release followed by sustained release of the drug for a prolonged period of time. The rapid initial release may be attributed to the fraction of Ribavirin on the surface of solid lipid nanoparticles. The *in vitro* drug release results revealed that the prepared Ribavirin solid lipid nanoparticles would be able to control drug release for extended period of time.

Table 3: Drug release study profiles for all formulations

Time (hrs)	F_1	F_2	F_3	F_4
0	0	0	0	0
1	28.55	26.45	26.55	29.55
2	35.25	34.26	31.6	38.5
3	43.82	34.7	42.55	48.12
4	52.65	53.54	55.55	55.65
5	61.28	62.85	65.58	65.55
6	69.25	72.8	72.9	77.2
7	78.85	81.63	83.52	83.85
8	88.56	90.55	91.29	95.55

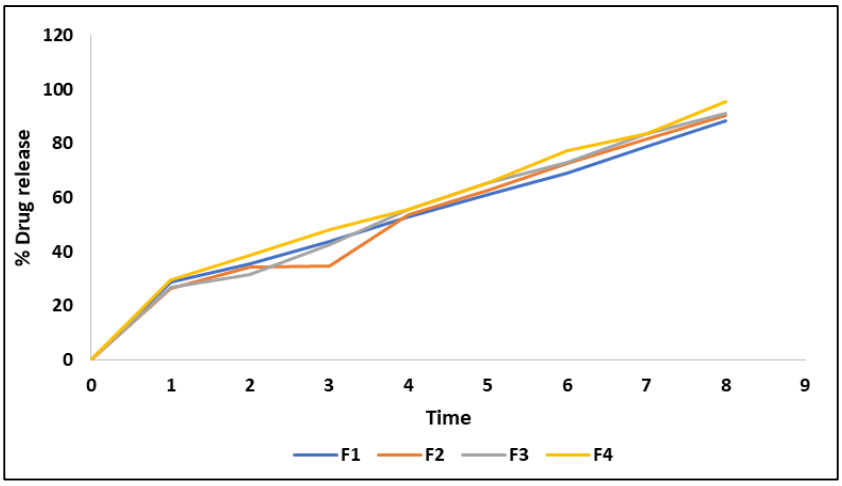

Fig 4: *In vitro* drug release studies for all formulations

The *in vitro* diffusion studies were performed in pH 7.4 buffer using Dialysis membrane for 8 hours. Initially the release of drug from all the three batches was found to be about 25-30% in 8 hours. This was due to the release of adsorbed drug from the surface of Solid lipid solid lipid nanoparticles. Later on a constant and slow drug release was observed for 8hrs. F4 formulation which had lipid and surfactant ratio was decided to be the optimized formulation.

Stability studies

There was no significant change in physical and chemical properties of the nanoparticles of formulation F-4 after 3 months. Parameters quantified at various time intervals were shown

Table 4: Results of stability studies of optimized formulation F-4

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-4	25^0C/60%RH % Release	95.55	95.41	95.38	95.34	Not less than 85 %
F-4	30^0C/75% RH % Release	95.55	95.45	95.37	95.32	Not less than 85 %
F-4	40^0C/75% RH % Release	95.55	95.50	95.35	95.30	Not less than 85 %

4. Conclusion

The present research proposed a novel formulation Ribavirin solid lipid nanoparticles for controlled release. Investigation of the preparation, characterization and in-vitro release of the solid lipid nanoparticles was carried out. The different formulations of with various ratios of drug-lipid and surfactant were evaluated and optimised. In this research, a drug encapsulation efficiency as high as 81% has been achieved. The method used for the formulation of Ribavirin containing soya lecithin solid lipid nanoparticles was solvent evaporation method followed by sonication to reduce the particle size.solid lipid nanoparticles formulations showed good results in terms of the assayed drug content and encapsulation efficiency. This indicates that the method used for the formulation produced good yield and it was suitable and reproducible in nature. Formulation (F-4) showed the highest encapsulation efficiency. It was found that as the concentration of soya lecithin increased, the % of encapsulation efficiency was also increased. Permeation studies with dialysis membrane were carried out as per the

method reported. The formulations showed good drug release from the lipid, the *in vitro* drug release profiles of all the formulations showed an initial burst effect, and followed by a slow drug release. The burst release of drug is associated with those drug molecules dispersing close to the solid lipid nanoparticle surface, which easily diffuse in the initial incubation time. The Ribavirin release was faster for those solid lipid nanoparticles with higher drug content.

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