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Formulation Development of Ritonavir loaded solid lipid Nanoparticles for Targeted Drug Delivery

Swapna Velivela*[1], D.Varun, Veena Gadicherla, D.Naga Latha

1. Sri Indu Institute of Pharmacy, Sheriguda (V), Ibrahimpatnam (M), R.R. Dist-501510.

ABSTRACT

Solid lipid nanoparticles (SLNs) have emerged as a remarkable nano-colloidal system for drug delivery. This research work aims at developing and optimizing the Ritonavir loaded solid lipid nanoparticles for targeted drug delivery by solvent emulsification-evaporation method. The produced solid lipoid Ritonavir nanoparticles were characterized for various physicochemical in terms of size, surface charge, % entrapment efficiency (EE) and in-vitro drug release studies. The entrapment efficiency (%) range of solid lipid nanoparticles (SLNs) is between 16.23% to 54.23% with polydispersity index (PDI) of 0.2. The mean size of particles was found between 189.6 to 271.4 nm. This indicates particles are in uniform distribution. The zeta potential was found in the range of -36.54 to -41.57 mV for prepared solid lipid nanoparticles. The most EE% was about 54.23% achieved in the presence of Polysorbate 20. It was found that addition of Polysorbate 20 in optimized concentration in the process led to increased entrapment efficiency and particle size when compared with Span 20. In this study, we showed the SLNs have more encapsulating Ritonavir with optimized drug release.

Keywords: Ritonavir, solid lipid nanoparticles (SLNs) entrapment efficiency, polydispersity index (PDI)

*Corresponding Author Email: sanjayswamy317@gmail.com

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INTRODUCTION

In the past few years, there has been a fascinating development in nanoscale drug delivery technologies. Majorly biocompatible polymers and lipid excipients have been on the rise, which efficiently incorporated in the lipid nanosystems [1,2]. These lipids are usually physiological lipids possessing little acute and chronic toxicities. Solid lipid nanoparticles (SLNs) offer an amalgamating effect of several carrier systems such as liposomes and niosomes. Similar to other carrier systems, SLNs are also constituted by biocompatible excipients that are physiologically accepted and homology to polymeric nanoparticles [3,4,5].The solid matrix in SLNs also proposes to protect the loaded therapeutic molecules against the rough biological environment and also shield the other chemical degradations with maximum feasibility to alter the release profiles of the therapeutic molecule. Altogether, these proficiencies make the SLNs an exceptional carrier system [6,7]. Solid lipid nanoparticles (SLN) are carriers with a Nano-size ranging from about 40–1000 nm and have a spherical morphology, which structurally includes solid lipids and surfactants [8]. Due to the presence of physiological lipids in the structure of SLN, they are biocompatible, biodegradable, and have very low toxicities. Furthermore, SLN formulations have a high loading capacity for water-soluble and water-insoluble drugs, as well as long-term stability, feasible scale-up capability, and low production cost [9]. Moreover, due to their small size, SLN well penetrates into the lower layers of the skin and, in addition, forms an occlusive layer of fabric on the surface of the skin, which has high covering properties and restores the damaged part of the skin [10].

Most anti-viral drugs which are in use suffers drawbacks of frequent administration, short half-life, peak plasma concentration fluctuations, high first pass metabolism which leads to low patient compliance. There is always a need of development of controlled and sustained drug delivery systems with site specificity to achieve effective plasma concentration without significant plasma drug concentration fluctuations.

Different Ritonavir formulations like pro-liposomal, injectable stealth liposomes, solid dispersion, and nanoparticles [11-14] have been reported to direct the medication to the lymph system, boosting its absorption. Several oral drugs suffer from bioavailability issues due to low solubility, low dissolution and bioavailability [15].

Ritonavir is a protease used as antiretroviral agent for the treatment of HIV-infection alone or in combination with other protease inhibitors [16]. Biological half-life of Ritonavir is 3-5 hours, leads to higher peak plasma concentration fluctuations in the form of conventional dosage form. Moreover it is primarily absorbed from stomach. Preparation and evaluation of nanoparticles

Ritonavir was done for improving the drug bioavailability by prolongation of gastric residence time [17,18].

MATERIALS AND METHOD

Materials

Ritonavir was obtained as a gift sample from Hetero Pharmaceuticals Pvt. Ltd. Compritol 888 ATO (mg) and Precirol ATO 5 were procured from Gattefosse, Mumbai, India. Polysorbate 20, Span 20, Ethanol and Chloroform (analytical grade) were procured from Merck, Mumbai. All other solvents and chemicals used for this work are of analytical grade.

Preparation of ritonavir-loaded SLNs

Solid lipid nanoparticles of Ritonavir were prepared by solvent emulsification and evaporation technique. Ritonavir and lipid were dissolved in 10 ml of chloroform: ethanol (9: 1) ratio. This solution was injected gradually into 50 ml of aqueous medium containing surfactant in specified quantities and then homogenized by high-shear homogenizer at 25,000 rpm around 10 min in ice bath to obtain a nanoemulsion. The organic solvents were then evaporated using rotary evaporator to produce solid lipid nanoparticles of Ritonavir SLN. The obtained Ritonavir SLNs were then centrifuged at 25,000rpm for 1 hr at 10 °C. The precipitated pellets were re-suspended in 2 ml of aqueous solution for further use [19-21].

Table 1: Formulation of Ritonavir Solid lipid nanoparticles

Ingredient	RT1	RT2	RT3	RT4	RT5	RT6
Ritonavir (mg)	20	20	20	20	20	20
Compritol 888 ATO (mg)	1%	1%	1%	---	---	---
Precirol ATO 5(mg)	---	---	---	1%	1%	1%
Polysorbate 20(mg)	0.25%	0.5%	0.75%	0.25%	0.5%	0.75%

Evaluation parameters:

Drug excipient compatibility studies by Fourier transform infra-red (FT-IR) spectroscopy Analysis [22]

IR spectral analysis of pure drug Ritonavir and polymers was carried out and observation was made whether changes occurred in chemical constitution of drug after combining it with polymers. The samples were crushed with KBr to get pellets by applying pressure on 600 Kg/cm^2 and scanned with the IR instrument (Shimadzu, 8400 Series, Tokyo, Japan) from 400-4000 cm^{-1} .

Surface charge of the nanoparticles [23]

The zeta potential of a nanoparticle is commonly used to characterize the surface charge property of nanoparticles. Nanoparticles with a zeta potential above (±) 30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles. The size distribution of the nanoparticles was determined using the particle size analyzer equipped with a dry accessory system. Sample was diluted with water and temperature maintained at 25°C.

Particle size [24]

The particle size (PS), and Polydispersity Index (PI) of the formulated R-SLNs were determined by using a particle size analyzer. The sample was prepared by diluting the formulations to one hundred times with double distilled water to determine the above said parameters.

Entrapment efficiency (EE) [25-27]

The EE of RN-SLNs was analyzed using the ultra-centrifugation technique. The prepared SLN formulations were placed in ultracentrifuge and spun at 50,000 rpm for 45 min. A supernatant was obtained and was diluted with diluted buffer solution and assayed spectrophotometrically (Shimadzu-1800, Japan) to get the amount of un-entrapped medication at 242 nm.

Thermal analysis [28]

The optimized RN-SLNs formulation and ritonavir were subjected to differential scanning calorimetry (DSC) measurement. The samples containing about 10 mg of RN-SLNs formulations were sealed in an aluminium pan. A calorimeter with differential scanning was used to heat the samples at a rate of 10 °C/min, and the thermal analysis was carried out. The temperature range was 20–200 °C. The inert nitrogen atmosphere was supplied during the study, and an empty pan was utilized as a point of departure.

Surface topography [29]

The optimal RN-SLN shape was examined using a scanning electron microscope. The sample was placed on aluminium stubs and covered with gold in an argon environment using double-sided sticky tape. Several magnifications were used to observe the sample under a voltage gradient of 15,000 V.

Differential scanning calorimetry[30]

Lyophilized samples of ritonavir-loaded SLNs, blank SLNs and components of formulation were analysed differential scanning calorimeter (Mettler-Toledo, Greifen- see, Switzerland). Approximately, 5 mg of samples was weighed in aluminium pans and analysed in the range of 0–240 °C in heating rate of 10 °C/min.

Calibration curve of Ritonavir

Ritonavir solution in 0.1N HCl was scanned in at 200-400nm by using UV-Visible spectrophotometer. It was found that λmax of Ritonavir was found be 256 nm in 0.1N HCl. A linear relationship was established between the concentrations (5-25 µg/ml range) on X-axis v/s absorbance on Y-axis with R^2 value of 0.998.

***In vitro* drug release studies [31-32]**

In vitro release of Ritonavir solid lipid nanoparticles was conducted by a dialysis membrane having pore size of 2.4 mm with 75 ml of pH 1.2 hydrochloric acid with 0.2%SLS at 37°C. Briefly in a 100 ml beaker 75 ml of pH 1.2 was taken. A 2 ml of formulation was taken into a dialysis bag and dipped into the buffer solution. The dialysis membrane was activated prior using by soaking in 1% w/v NaOH overnight. The flask was kept on a magnetic stirrer. Stirring was maintained at 250 rpm and the temperature of the buffer was maintained at 37°C. Sampling was done by withdrawing 5 ml of aliquots from a beaker. Immediately 5 mL of fresh buffer was added to maintain the sink condition. Samples were analyzed after adequately diluting with methanol by using a UV-Visible Spectrophotometer at a wave length of 256 nm.

RESULTS AND DISCUSSION

Solubility of Ritonavir (RIT)

Ritonavir has shown highest solubility in 0.1N HCl and found to be 0.38 mg/ml. Solubility of Ritonavir in water is 0.05 mg/ml where as in case of pH 4.5 and 6.8 Phosphate buffer is 0.1 and 0.09 respectively.

Drug Excipient Compatibility Studies: FT-IR

No prominent difference was observed in the principal IR peaks of Drug Excipient mixtures and optimized formulation (RT5) upon comparison with the peaks of drug and polymer alone, which may considered that drug and polymers are compatible enough without any interactions.

Drug entrapment efficiency

Drug entrapment efficiency of all the formulations calculated by using the formula and it is in the range of 26.23% to 54.23%**.** Highest drug entrapment efficiency is found for RT5 i.e. 54.23%.

***In-Vitro* Drug Release Studies:**

The drug release of prepared solid lipid nanoparticles with different combination of lipids like Compritol 888 ATO (1%) with polysorbate 20 (0.25,0.5 and 0.75%) formulation code RT1-RT3 showed 65 to 72% of drug release for a period of 24hrs.

The drug release of prepared solid lipid nanoparticles with different combination of lipids like Precirol ATO 5 (1%) with polysorbate 20 (0.25, 0.5 and 0.75%) formulation code RT4-RT6 showed 39 to 48% of drug release for a period of 24hrs.

Among all the formulations RT5 which containing Precirol ATO 5(1%) with 0.5% Polysorbate 20 has shown controlled release of drug release of around 35% for a period of 24hrs. It was also observed that drug entrapment and drug loading also affect the drug release from nanoparticles.

Table 2: Evaluation Parameters of Ritonavir Solid lipid nanoparticles

Parameter	RT1	RT2	RT3	RT4	RT5	RT6
Entrapment Efficiency (%)	16.23±11.2	30.23±10.5	22.01±9.5	35.62±8.9	54.23±5.2	34.245±6.8
Particle Size (nm)	245.7±13.2	271.4±11.7	196.5±8.5	232.4 ± 6.3	189.6±9.8	201.3±10.7
PDI	0.253±0.02	0.281±0.05	0.242±0.06	0.252±0.04	0.273±0.03	0.261±0.02
Zeta potential (-mV)	36.54	32.31	41.57	38.97	40.27	36.57

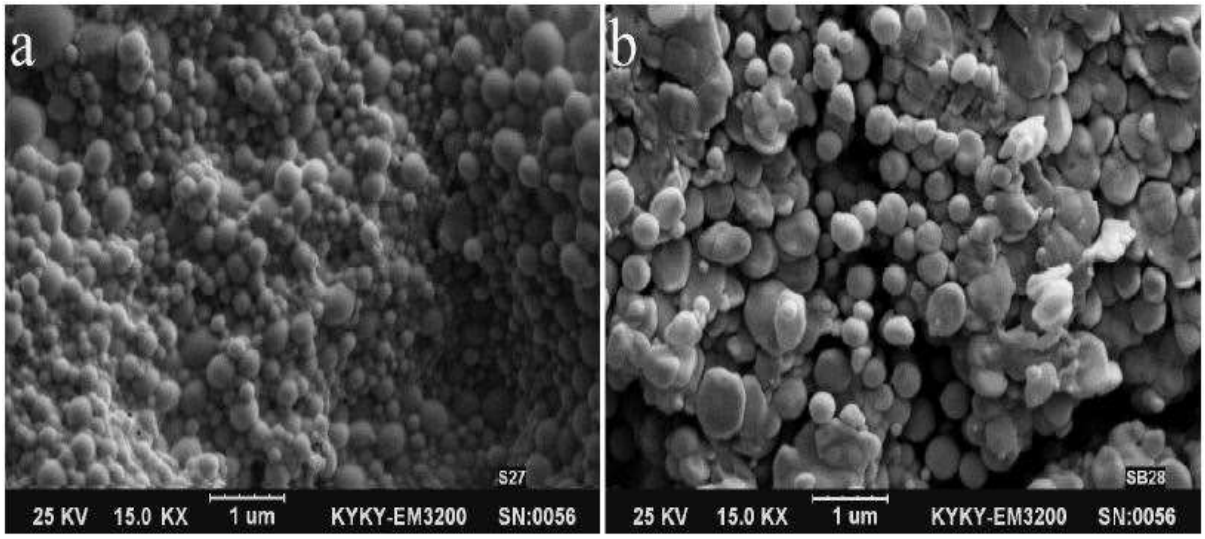

Figure 1: SEM images of Ritonavir Solid lipid nanoparticles

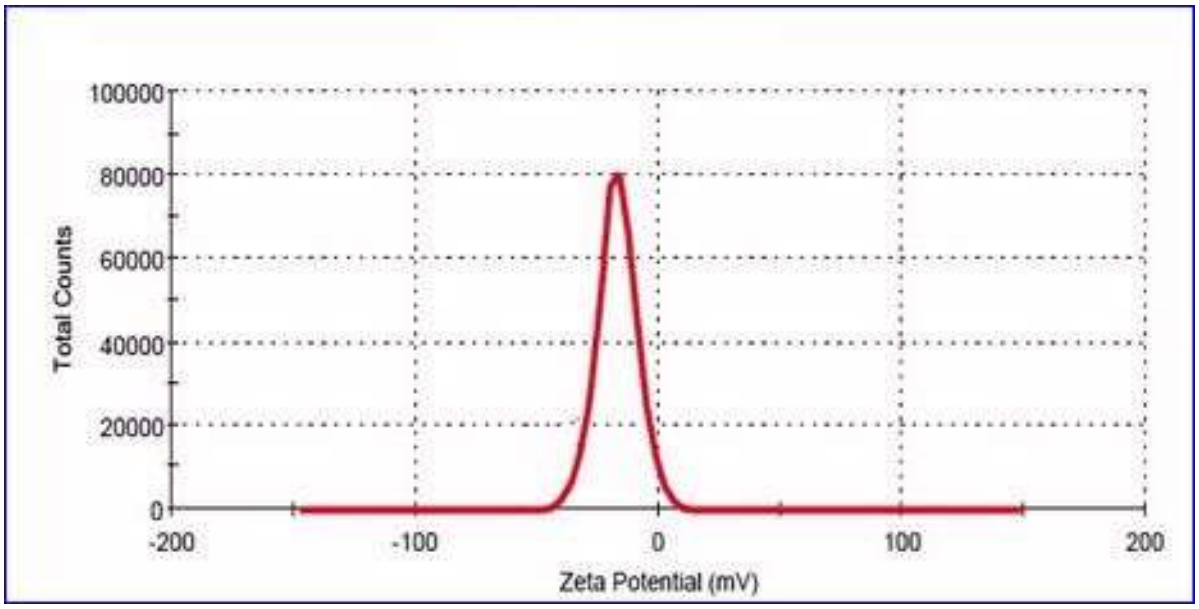

Figure 2: Zeta potential of Ritonavir Solid lipid nanoparticle RT5

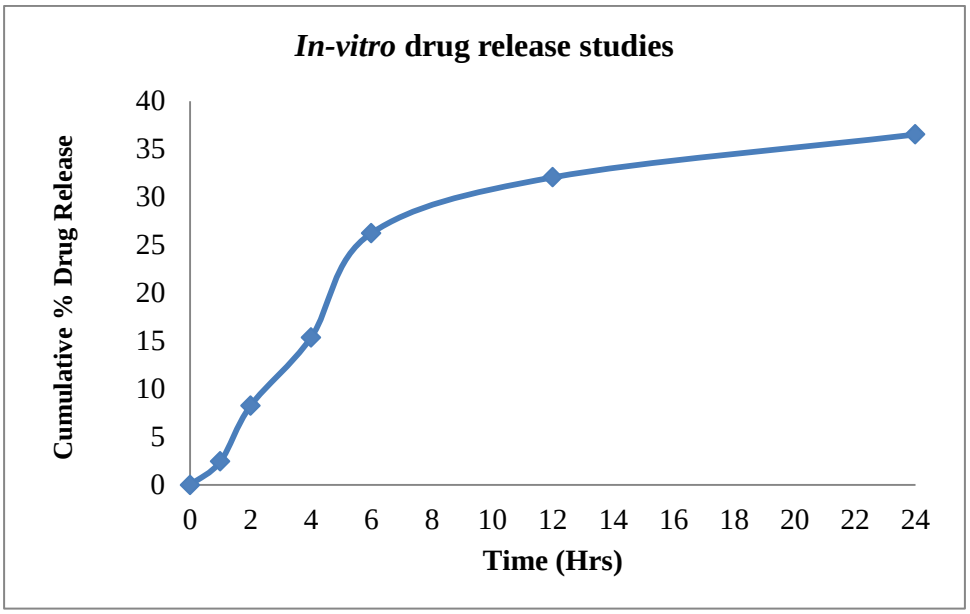

Figure 3: *In-vitro* drug release of RT5 formulation

CONCLUSION:

SLNs have radically gained the attention of several researchers with its exceptional properties and benefits over other conventional dosage forms, and other colloidal counterparts of SLN have proved to be a significant discovery in nanotechnology because of their effective performance and as a safe vehicle in pharmaceutical drug delivery. SLN as a colloidal drug carrier puts together the advantages of polymeric nanoparticles and fat- based emulsions. Ritonavir solid lipid nanoparticles are prepared by solvent emulsification –evaporation techniques. Ritonavir solid lipid nanoparticles are prepared by using Compritol 888 ATO, Precirol ATO 5 and Polysorbate 20 as surfactant. Among all the formulations RT5 which containing Precirol ATO 5(1%) with 0.5% Polysorbate 20 has shown controlled release of drug release of around 35% for a period of 24hrs. It was also observed that drug entrapment and drug loading also affect the drug release from nanoparticles.

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