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DEVELOPMENT, OPTIMIZATION AND CHARACTERIZATION OF ROSUVASTATIN SOLID LIPID NANOPARTICLES EMPLOYING 3² FACTORIAL DESIGN

P. Prakash *, K R Sai Lakshmi [1]

*, [1] Department of Pharmaceutics, Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupati, 517503.

Abstract:

The purpose of this study was to development, optimization and characterization of rosuvastatin solid lipid nanoparticles employing 3² factorial design. rosuvastatin loaded solid lipid nanoparticles, was prepared by hot homogenization technique followed by ultra sonication method. Factorial design was introduced to optimize the formulation of solid lipid nanoparticles Results: Differential scanning calorimetry & Powder X-Ray Diffractometry studies indicate that the excipients added were compatible with the drug. The value of zeta potential Zeta potential value > ± 30 mV is essential for effective stability and to inhibit aggregation of particles. The low polydispersity index in all the formulations indicated the homogeneity of the particle size. Highest entrapment efficiency of 97.4% was observed for tristearin based SLN. The scanning electron nanometer-size and spherical in shape and mono-dispersed SLN with spherical shape.

Key words: Solid lipid nanoparticles, ultra sonication, Factorial design & Differential scanning calorimetry.

Corresponding author:

P. Prakash,

Sri Padmavathi School of Pharmacy

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INTRODUCTION:

Nanoparticles offer several advantages in drug delivery owing to their small particle size, large surface area and the capability of changing their surface properties. In general, nanoparticles can be used to target the delivery of drugs, to sustain its effect, to improve bioavailability, to solubilize it for intravascular delivery and to improve its stability against enzymatic degradation[1-3].

Based on the type of the inactive ingredient used, there are four classes of nanoparticles: Lipid based nanoparticles, polymeric nanoparticles, metal based nanoparticles and biological nanoparticles. The shortage of safe polymers and their high cost have limited the wide spread application of nanoparticles to clinical medicine. To overcome these restrictions of polymeric nanoparticles, lipids have been put forward as an alternative carrier, particularly for lipophilic pharmaceuticals. These lipid nanoparticles are known as solid lipid nanoparticles (SLN), which are attracting wide attention of formulators world-wide. SLN are colloidal carriers developed in the last decade as an alternative system to the existing traditional carriers (emulsions, liposomes and polymeric nanoparticles)[4-6].

MATERIALS AND METHODS:

Materials

Rosuvastatin gift sample was obtained from Dr. Reddy's Laboratories Ltd., Hyderabad, India. Trimyristin, tripalmitin, tristearin, soya lecithin and poloxamer 188 gift samples were obtained from Sigma-Aldrich, Mumbai, India. Sodium dihydrogen orthophosphate, Acetonitrile, Methanol and Ortho phosphoric acid from M/s. Qualigens Fine Chemicals. All other reagents are of either laboratory/analytical grade as per the requirement.

Methods

Rosuvastatin loaded SLN were prepared with three different triglycerides namely trimyristin, tripalmitin and tristearin by hot homogenization technique followed by ultrasonication method. These three triglycerides were procured from Sigma Aldrich with brand names Dynasan 114 (trimyristin), Dynasan 116 (tripalmitin) and Dynasan 118 (tristearin) and hereafter in the thesis Dynasan 114, Dynasan 116 and Dynasan 118 will be referred as trimyristin, tripalmitin and tristearin respectively. Rosuvastatin, triglyceride and soya lecithin were heated above the melting temperature of lipid around 56-68°C and mixed rapidly with glass rod in hot molten condition. Poloxamer 188 dissolved in water heated to equal temperature and was added to the molten lipid phase and homogenization was carried out (Gambhire et al., 2011 & Arjun & Kishan, 2013). Hot homogenization was carried out for three minutes at 5000 rpm in order to get coarse emulsion. Finally the obtained pre- emulsion was subjected to ultrasonication.[7-12]

A 3^2 randomized full factorial design was used in this study and 2 factors were evaluated, each at 3 levels, experimental trials were performed at all 9 possible combinations. Amount of poloxamer188 (X1) and soya lecithin (X2) were selected as two independent variables which were varied at three levels, low level (-1), medium level (0), high level (+1). Amount of drug Rosuvastatin (40 mg), triglyceride (400mg) concentrations and dispersion medium water 20 mL were kept constant. Particle size (Y1), zeta potential (Y2) and entrapment efficiency (Y3) were selected as dependent variables. Values of variables and formulation codes are shown in the Table 3.1 & 3.2. Design-Expert® DX 8.0.7.1 software trial version was used for the generation and evaluation of statistical experimental design[13-15].

Table 1: 3^2 Full factorial design: factors, factor levels and responses

Factors (Independent variables)	Factor levels used		
	Low (-1)	**Medium (0)**	**High (+1)**
Amount of poloxamer 188 (X_1)	100	150	200
Amount of soya lecithin(X_2)	100	150	200
Responses (Dependent variables)			
Y_1= Particle size (nm).			
Y_2= Zetapotential (mV)			
Y_3= Entrapment efficiency (%)			

Table 4.2: Compositions of Rosuvastatin loaded SLN

Formulation	Poloxamer 188 (mg)	Soya lecithin (mg)
F1(-1,-1)	100	100
F2(-1,0)	100	150
F3(-1,+1)	100	200
F4(0,-1)	150	100
F5(0,0)	150	150
F6(0,+1)	150	200
F7(+1,-1)	200	100
F8(+1,0)	200	150
F9(+1,+1)	200	200

Determination of particle size distribution, polydispersity index (PDI) and zeta potential of SLN

The particle size distribution, polydispersity index, and zeta potential of Rosuvastatin loaded SLN were measured using a Malvern Zetasizer (Nano ZS90, UK). About 100µL of the prepared SLN dispersion was diluted to 5 mL with double distilled water and analyzed with Zetasizer (Silpa *et al.*, 2012). Photon correlation spectroscopy is the most widely used technique for measurement of particle size and zeta potential. The principle of dynamic light scattering at a scattering angle of 90 degrees is used to measure particle size[16-18].

Determination of % drug content:

1mL of SLN was dissolved in the chloroform: methanol (1:1) mixture. Final dilution was made with acetonitrile and Rosuvastatin content was determined by UV.

Determination of percentage entrapment efficiency (%EE):

The percentage of drug entrapped in the lipid is determined by measuring the concentration of the drug in the aqueous phase by ultrafiltration method using centrisart devices (Arjun & Kishan, 2013). Centrisart consist of filter membrane (Molecular weight cut off 20,000 daltons) at the base of sample recovery chamber. About 1 mL of undiluted sample is placed in the outer chamber on the top of the sample holder. The unit is centrifuged at 3,500 rpm for 15 – 20 min. The solid lipid nanoparticles along with the encapsulated drug remain in the outer chamber and the aqueous phase is moved into the sample recovery chamber through membrane. The amount of drug in the aqueous phase is estimated by UV[19].

In vitro dissolution:

The in vitro release studies of Rosuvastatin loaded solid lipid nanoparticles were carried out by using modified Franz diffusion cell. Dialysis membrane having pore size 2.4 nm with molecular weight cut off 10,000 daltons was used. Membrane was soaked in double distilled water for 12 hours before mounting in Franz diffusion cell. Rosuvastatin loaded 2 mL of SLN dispersion equivalent to 4 mg was applied to the donor compartment. And the receptor compartment was filled with 12 mL of dialysis medium of 6.8 phosphate buffer (Mayank, 2010). Samples (100 µL) were withdrawn from receiver compartment through side tube at regular time intervals and the same was replaced with fresh dialysis medium maintained at same temperature. In the similar way pure drug equivalent to 4 mg was also added to the 2 mL of distilled water and release studies were performed for comparison[20].

Differential scanning calorimetry:

DSC analysis was carried out on Pyrus DSC Perklin Elmer. Heating rate of 10°C/min was employed in the range of 20–240ºC. Analysis was performed under a nitrogen purge at a flow rate of 50mL/min (Suresh et al., 2007). The sample size was 5 mg for each measurement.

Powder X-Ray Diffractometry (PXRD):

Powder X-ray diffractometer (Siemens) was used for diffraction studies. PXRD studies were performed on the sample by exposing them to CuKα radiation (50kv, 34 mA) and scanned from 3

to 45°, 2θ at a scan step of 0.02° and step time of 3°/min (Vinay Kumar et al., 2012)[19]

Transmission electron microscopic studies of SLN:

A drop of SLN dispersion was applied on a carbon film-covered copper grid. Excess dispersion was blotted from the grid with filter paper to form a thin-film specimen. The sample was then stained with 2% uranyl acetate, air dried and examined under transmission electron microscope (Hitachi H-7500) at a magnification of 50000X (John et al., 1998)[20-21].

RESULTS & DISCUSSION:

Particle size distribution (nm), zeta potential (mV) and polydispersity index:

The mean particle size was in the range of 98.9±0.6 to 210.6±1.7 nm, 93.1±0.2 to 213.5±0.1nm and 96.4±0.2 to 214.5±0.1 nm for trimyristin, tripalmitin and tristearin based formulations respectively after 20 min time of ultrasonication time. The polydispersity index (PDI) was in the range of 0.175±0.2 to 0.445±0.5. Zeta potential values of SLN ranged from -22.9±0.6 to -45.1±4.1 mV, -18.3±1.1 to -48.7±0.4 mV, -20.4±0.5 to -44.9±3.2 mV for trimyristin, tripalmitin and tristearin based formulations respectively. The zeta potential value was found to be > ± 30 mV for almost all the formulations prepared. For any liquid dosage form surface charge is essential for its stability. Zeta potential value > ± 30 mV is essential for effective stability and to inhibit aggregation of particles. As the poloxamer 188 concentration increased particle size was decreased. In three formulations optimum size was obtained at 200 mg of poloxamer 188 concentrations. The low polydispersity index in all the formulations indicated the homogeneity of the particle size. The formulations showed negative zetapotential since solid lipid nanoparticles have negative charge on their surface (Schwarz 1999).

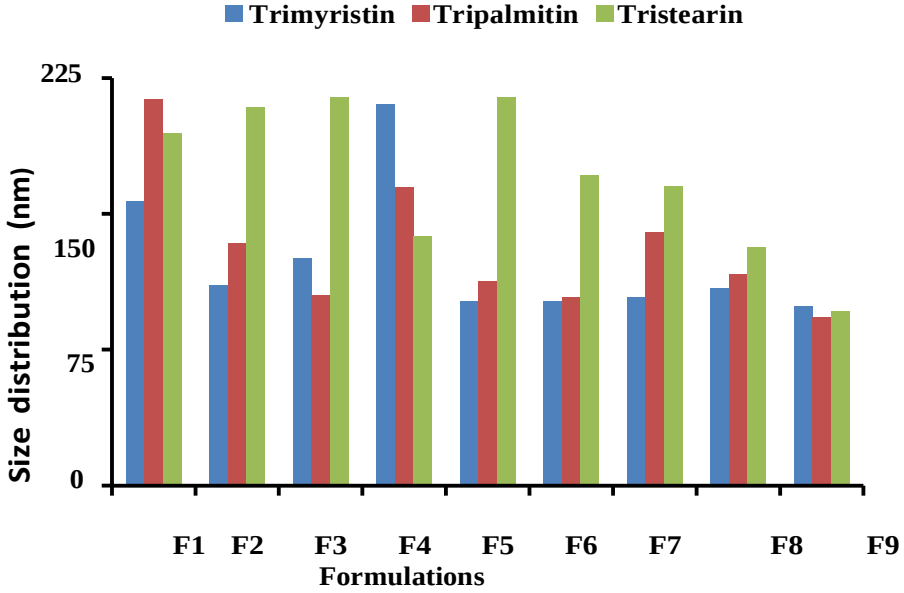


Fig. 1: Comparative particle size distribution of SLN

Determination of percent entrapment efficiency (%EE):

The percent entrapment efficiency of SLN was determined after separating entrapped and unentrapped drug by ultra-filtration. The percent entrapment efficiency varied from 48.6% to 97.4% for all the formulations. Highest entrapment efficiency of 97.4% was observed for tristearin based SLN. The lowest entrapment effeciency was observed when the independent variables poloxamer 188 (X1) and soya lecithin (X2) were at 100 mg and 150 mg concentartions for all the SLN formulations prepared with three different triglycerrides.

The highest entrapment effeciency was observed when the independent variables poloxamer 188 (X1) and soya lecithin (X2) were at higher level (200 mg) concentartions for all the SLN formulations prepared with three different triglycerrides. There is no difference in entrapment efficiency, among three triglycerides. This could be due to maximum carbon chain length in the three triglycerides (Vinay Kumar V, 2012).

In vitro release studies

SLN formulations TMF9, TPF9 and TSF9 released 31.78%, 32.55% and 37.17% of Rosuvastatin respectively for 36hrs. 98% of drug release was observed in 36 hrs for pure Rosuvastatin dispersed in distilled water. The results indicated slower release of Rosuvastatin from the prepared SLN compared to pure drug which may be due to the lipid nature of the prepared SLN and homogeneous dispersion of the drug in the lipid matrix. Zhang et al., and Gambhire et al., 2011a reported similar results in which less than 10% of Rosuvastatin was released in 12 hrs and 37.08% in 48 hrs. Hence

further drug release studies were not carried out after 36 hrs.

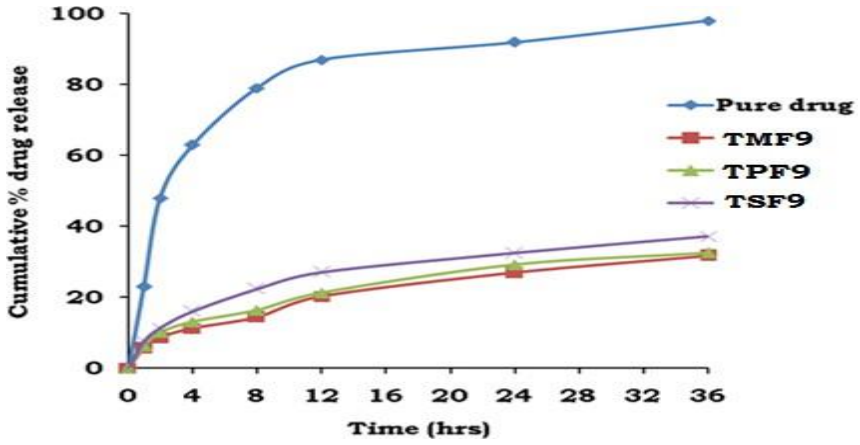
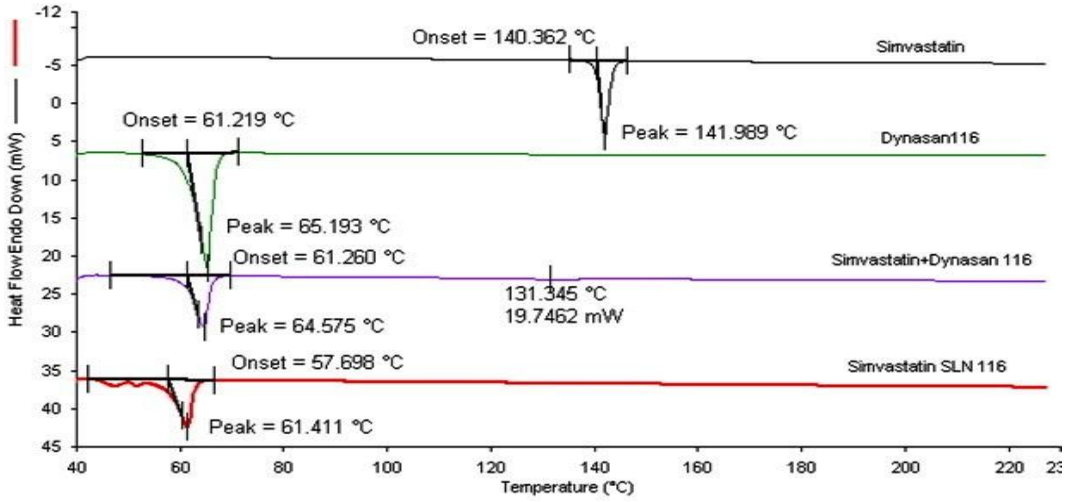


Fig.2: Dissolution profiles of Rosuvastatin released from SLN formulations

Differential scanning calorimetry:

A



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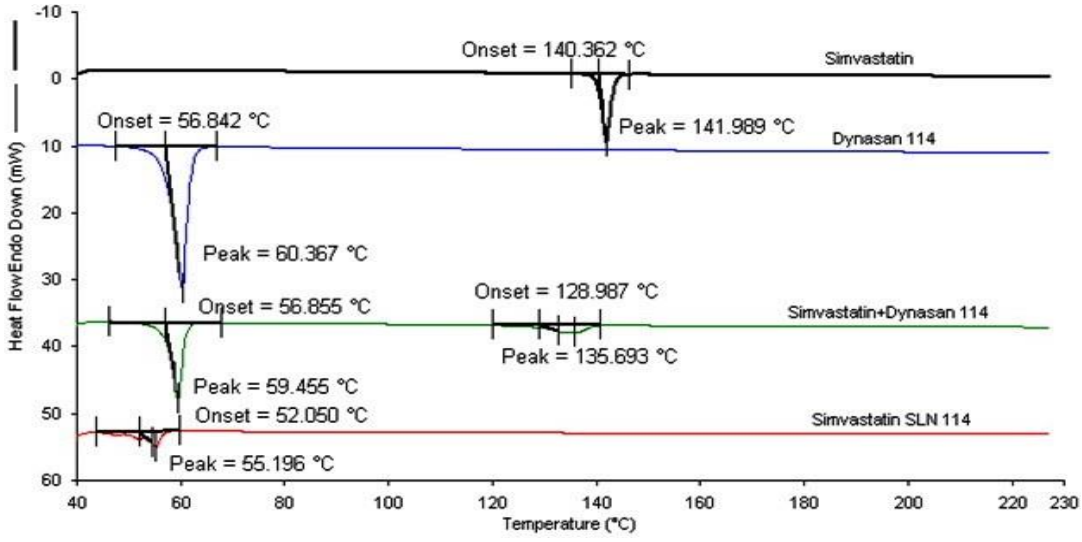

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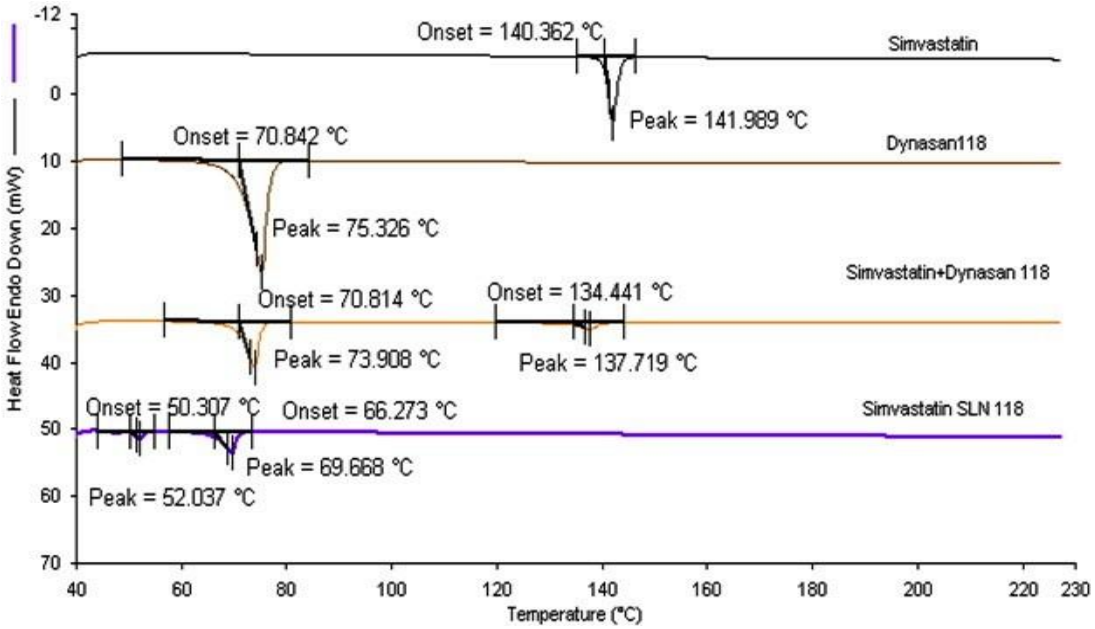

Fig. 3: DSC thermograms A) Trimyristin; B) Tripalmitin and C) Tristearin based optimized formulations

Melting points of trimyristin, tripalmitin and tristearin in SLN form were depressed when compared to melting points of corresponding bulk triglycerides. This melting point depression might be due to small particle size (nanometer range), their high specific surface area and the presence of surfactant (Golmohammadzadeh et al., 2012). This was confirmed by physical mixture where such type of depression was not found.

Powder X-Ray Diffractometry (PXRD):

In the physical mixtures the intensity of the sharp peaks was reduced as compared to the pure drug and triglycerride. The intensities of the peaks were further reduced and broad for Rosuvastatin loaded SLN of trimyristin, tripalmitin and tristearin. This suggests that Rosuvastatin was not in crystalline form in the formulation. However, the characteristic peaks of tristearin were most decreased in SLN, Similarly crystallinity of trimyristin and tripalmitin was also decreased in SLN

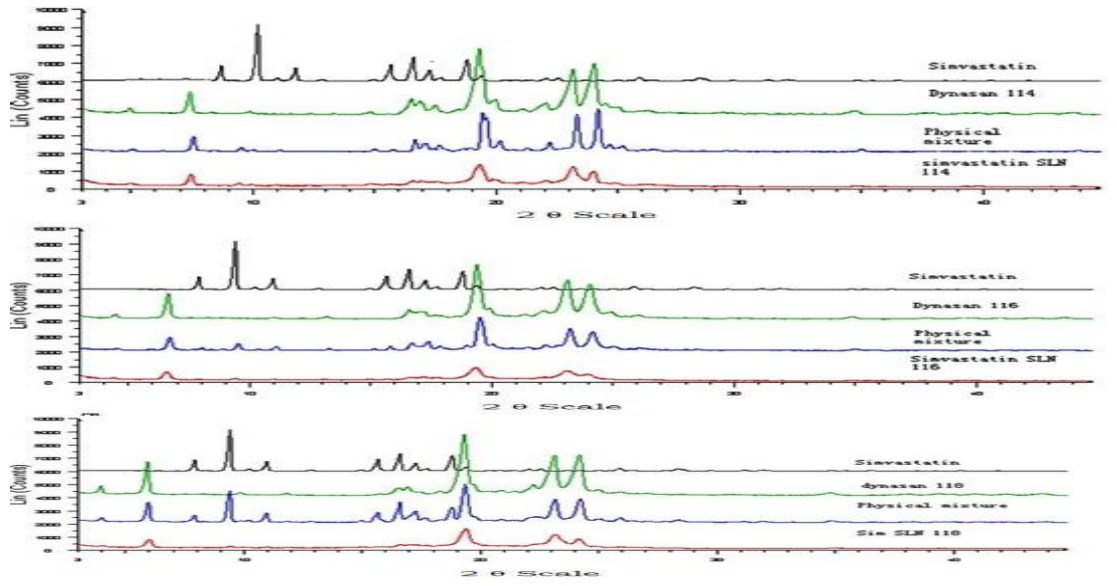

Fig. 4: PXRD of A) Trimyristin; B) Tripalmitin and C) Tristearin based optimized formulations

Transmission electron microscopic studies of SLN

The SLN obtained were in nanometer-size and spherical in shape with well-defined periphery at 50000X magnification. The size of the solid lipid nanoparticles was found to be in agreement with the Malvern Zetasizer particles size distribution for the selected sample.

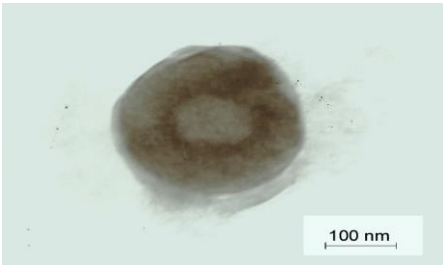

Fig 5: Surface morphology of TSF9 by TEM studies

CONCLUSION:

In the present study SLN improved oral relative bioavailability of the Rosuvastatin by virtue of their unique capability to bypass presystemic hepatic metabolism resulting in enhanced plasma concentration. The particle size of Rosuvastatin was found to be around 100 nm, which might be the reason to enhance the rapid absorption of SLN as compared to pure Rosuvastatin dispersion. These results suggested that solid lipid nanoparticles could be promising delivery systems to enhance the oral bioavailability of Rosuvastatin. The zeta potential around 30 mV, maximum % entrapment (above 95%) and minimum particle size i.e. around 100 nm.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

1. Demirel M, Yazan Y, Muller R H. Kilic F, Bozan B, Formulation and in vitro-in vivo evaluation of piribedil solid lipid micro- and nanoparticles. 2001; 18(3): 359-371.
2. Der VanLinde A. Statistical comment on a new method for noncompartmental pharmacokinetic analysis. Biometrical Journal. 1988; 30: 123-127.
3. Dhiman M K, Yedurkar P D, Sawant K K. Buccal bioadhesive delivery system of 5-fluorouracil: Optimization and characterization. Drug Dev Ind Pharm. 2008; 34: 761–770.
4. Doijad R C, Manvi F V, Godhwani D M, Joseph R, and Deshmukh N V. Formulation and Targeting Efficiency of Cisplatin Engineered Solid Lipid Nanoparticles. Indian J. Pharm. Sci. 2008; 70(2): 203–207.Han C, Qi CM, Zhao BK, Cao J, Xie SY, Wang SL, Zhou WZ. Hydrogenated castor oil nanoparticles as carriers for the subcutaneous administration of tilmicosin: in vitro and in vivo studies. J Veter Pharmacol Ther. 2009 Apr;32(2):116-23.
5. Boistelle R, Fundamentals of nucleation and crystal growth, in: Garti N, Sato K. (Eds.), Crystallization and Polymorphism of Fats and Fatty Acids, Marcel Dekker Inc. New York, Basel, 1988; 189–226.
6. Bunjes H, Siekmann B, Westesen K. Emulsions of super- cooled melts
7. — a novel drug delivery system, in: S. Benita (Ed.), Submicron Emulsions in Drug Targeting and Delivery, Harwood Academic Publishers, Amsterdam, 1998; 175-204.
8. Carlucci G, Mazzeo P, Biordi L, Bologna M. Simultaneous determination of Rosuvastatin and its hydroxyl acid form in human plasma by HPLC with UV detection. J. Pharm. Biomed. Anal. 1992; 10: 693-697
9. Cavalli R, Marengo E, Rodriguez L, Gasco MR. Effect of some experimental factors on the production process of solid lipid nanoparticles, Eur. J. Pharm. Biopharm. 1996; 43: 110–115.
10. Oussoren C, Zuidema J, Crommelin DJ, Storm G. Lymphatic uptake and biodistribution of liposomes after subcutaneous injection.: II. Influence of liposomal size, lipid composition and lipid dose. Biochimica et Biophysica Acta-Biomembranes. 1997 Sep 4;1328(2):261-72.
11. Freitas C, Muller R H. Stability determination of solid lipid nanoparticles (SLNTM) in aqueous dispersion after addition of electrolyte. J. Microencapsul. 1999; 16: 59–71.
12. Freitas RH, Muller, Correlation between long-term stability of solid lipid nanoparticles

(SLN) and crystallinity of the lipid phase. Eur. J of Pharm and Biopharm.1999; 125–132.
13. Freitas C, Muller R H, Spray-drying of solid lipid nanoparticles (SLN).Eur. J. Pharm. Biopharm. 1998; 46: 145–151.
14. Friedrich I, Muller-Goymann C, Characterization of solidified reverse micellar solutions (SRMS) and production development of SRMS- based nano suspensions. Eur. J. Pharm. Biopharm. 2003; 56 (1): 111-119.
15. Frosch P J, Kligman A M. The chamber-scarification test for irritancy., Contact Dermatitis 1976; 2: 314–324.
16. John J Bojjola and Lonnie D Russell. In, Electron microscopy principles and techniques for biologists. 2ndEd. 1998, 132-133.
17. Jumaa M, Muller B W. Lipid emulsions as a novel system to reduce the hemolytic activity oflytic agents: mechanism of protective effect. Eur. J. Pharm. Sci. 2000; 9: 285-290.
.
18. Khan H, Ali M, Ahuja A and Ali J. Stability testing of pharmaceutical products-comparison of stability testing guidelines. Curr Pharm Anal. 2010; 6: 142-150.
19. Angelis D G. Influence of statin characteristics on their safety and tolerability. Int. J. Clin. Pract. 2004; 58; 945–955.
20. Arjun N and Kishan V. Preparation, Characterization and Evaluation of Quetiapine Fumarate Solid Lipid Nanoparticles to Improve the Oral Bioavailability, Journal of Pharmaceutics.2013; 1- 7.
21. Augsten C, Kiselev M A, Gehrke R, Hause G, Mader K. A detailed analysis of biodegradable nanospheres by different techniques–A combined approach to detect particle sizes and size distributions. J. Pharm. Biomed. Anal. 2008; 47: 95-102..