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Research article

FORMULATION DEVELOPMENT AND INVITRO CHARATERIZATION OF ROSUVASTATIN CALCIUM SOLID LIPID NANOPARTICLES

Himavarshini J* and Sumaharshan JR

Department of Pharmaceutics, R R College of Pharmacy, Bangalore 560090, Karnataka, India.

ABSTRACT

The present investigation was aimed at developing Rosuvastatin calcium loaded solid lipid nanoparticles (SLNs). The SLNs were prepared by Hot Homogenization followed by ultrasonication method. Cholesterol, stearic acid, polaxomer 188 & tween 80 were employed as lipid carriers and surfactants respectively. Earlier, Fourier transform infrared studies confirmed no interaction between drug and lipids. Particle size, zeta potential, shape and surface morphology (SEM), % entrapment efficiency and invitro release rates were evaluated for SLNs.

Key Words:-Solid lipid nanoparticles, Rosuvastatin calcium, hot homogenizer, Ultrasonicator etc.

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Corresponding Author

Himavarshini J

Department of Pharmaceutics, R R College of Pharmacy, Bangalore 560090, Karnataka, India.
Email:- himavarshini1993@gmail.com

INTRODUCTION

Solid lipid nanoparticles (SLN) introduced in 1991 represent an alternative carrier system to traditional colloidal carriers such as liposomes, emulsions, and polymeric nanoparticles. Solid lipid nanoparticles are one of the most popular approaches to improve the oral bioavailability of the poorly water-soluble drugs. Solid lipid nanoparticles are having size (50-1000nm) and are composed of lipid components which are in solid state at room temperature. Due to their inherent bioavailability and biocompatibility, lipids are now being extensively investigated as carrier for drugs and proteins. Nanoparticles can protect the drug from degradation, enhance its transport and prolong its release; therefore they may improve the plasma half-life of the drug (Rainer H. Muller, KarstenMader, SvenGohla 2000).

Rosuvastatin is approved for the treatment of high LDL cholesterol (dyslipidemia), total cholesterol (hypercholesterolemia). In February 2010, Rosuvastatin was approved by the FDA for the primary prevention of cardiovascular events. The effect of rosuvastatin on LDL cholesterol is dose related (10-40 mg). Accumulation of cholesterol and fats along the wall of the arteries (a process known as atherosclerosis) decreases blood flow and therefore decreases in oxygen supply to the heart, brain, and other parts of the body. By lowering blood levels of cholesterol and fats with rosuvastatin has been shown to prevent heart disease, angina (chest pains), strokes, and heart attacks (Yang W, Liu JY, Qin L 2013).

MATERIALS AND METHODS

Materials

Rosuvastatin calcium was a generous gift from MSN laboratories Ltd. Hyderabad, India. Other excipients used in research are procured from HiMedia Laboratories Pvt.Ltd., Mumbai, India.

Methodology

Compatibility studies of drug and polymers:

Drug & excipients compatibility studies are done by FTIR spectrophotometer. Infrared spectrum of any compound or drugs gives information about the groups present in that particular compound. A spectrophotometer for recording the spectrum in the infrared region consist of an optical system capable of providing the monochromatic light in the scanning range was 400-4000cm-1 and resolution was 2cm-1. FTIR is used to analyze solid samples for figure print region. A drop of SLN sample is directly place on the stage of (ATR) & scanned from 400-4000cm-1. The infrared spectrum of the sample was obtained using Bruker FTIR & ATR spectrophotometer (Germany) using opus software (Rainer H. Muller et al 2000).

Formulation of rosuvastatin calcium loaded solid lipid nanoparticles

Rosuvastatin calcium loaded SLN were prepared by using hot homogenization followed by ultrasonication**.** The various concentrations of lipids (cholesterol, stearic acid) i.e., 1%, 2% were melted (approximately 5-10°C above its melting point) and to this known weight of rosuvastatin calcium (10mg) was added. The surfactant (0.5%) (Poloxamer 188 and Tween 80) was dissolved separately in methanol and heated to the temperature of lipid mixture. To the clear homogenous hot lipid phase, the hot aqueous surfactant solution was added and homogenization was carried out (at 2000 rpm) by using mechanical stirrer for 30 min. To prevent recrystallization during homogenization, production temperature was kept at least 5°Cabove the lipid melting point. The obtained coarse emulsion is allowed to cool to room temperature and stirred at 400 rpm for 15 min. The prepared nanosuspensionsultrasonicated for 10 min and the SLN dispersion was stored in refrigerator at 4°C (Rajkondwar VV et al 2009).

Characterization of SLNs

Particle size analysis

Particle size and size distribution are the most important characteristics of nanoparticles systems. They determine the *in vivo* distribution, biological fate, toxicity and the targeting ability of nanoparticles systems. In addition, they can also influence the drug loading, drug release and stability of nanoparticles (Rohit and Pal A et al , 2013).

Poly dispersity index

The polydispersity index (PDI) can also be measure from Dynamic light scattering Instruments, PDI is an index of width or speed or variation within the particle size distribution. Monodisperse samples have a lower PDI value, whereas higher value of PDI indicates a wider particle size distribution and the polydisperse nature of the sample (Ramteke K.H et al 2012).

Zeta potential

Zeta potential is the difference in the potential between the surface of tightly bound layer (shear plane) and the electroneutral region of the solution. It is important parameter to analyze long term stability of nanoparticles. It is easily measured because the charge of the potential will move as the suspension I placed between the two electrode that have D.C. voltage across them and the velocity will be proportional to the zeta potential. The technical term for this is electrophoresis (Swathi G et al 2010).

Shape and surface morphology

In the pharmaceutical industry, SEM may be used as a qualitative tool for the analysis of drug substance and drug product in order to obtain information on the shape and surface structure of the material. SEM plays an important role in the characterization of nanoscale and sub-micron particles. It has been used to determine surface topography, texture and to examine the morphology of fractured or sectioned surfaces. The examination of the surface of polymeric drug delivery system can provide important information about the porosity and microstructure of device (Sankha Bhattacharya 2013).

Drug content

From the prepared SLN formulation 1ml of suspension is dissolved in the 10ml of 6.8 pH PBS buffer and ethanol mixture. The amount of drug was determined using UV spectrophotometer. The placebo formulation prepared similarly to drug loaded SLN is used as blank (Mukharjee S et al 2010).

Drug content = test absorbance/standard absorbance×100

Determination of percentage entrapment efficiency

The entrapment efficiency of the compound was determined by measuring the concentration of free rosuvastatin in the dispersion medium. The SLN suspension was ultra centrifuged at 4000 rpm for 30 minutes at 4°C temperature by using remi cooling centrifuge to separate the free drug. The amount of free rosuvastatin was determined in the clear supernatant by UV spectrophotometer against blank at 229 nm. The analysis was made in triplicate. The drug entrapment efficiencies were calculated by using following equation.

Entrapment efficiency (EE%) = amount of drug in SLN/ amount of drug added ×100

***In-vitro* drug diffusion**

A 4-5 cm long portion of the dialysis tubing was made into a dialysis sac by folding and tying up one end of the tubing with thread. It was then filled up with phosphate buffered saline pH 6.8 and examined and 1ml of the ACV liquid nanosuspension was accurately transferred into sacs which served as the donor compartments. The sacs were once again examined for leak and then suspended in the glass beakers containing 50ml phosphate buffered saline,

which become the receptor compartment. At predetermined time intervals, 3ml samples were withdrawn from the receptor compartment and analyzed spectrophotometrically at 229nm. Fresh buffer was used to replenish the receptor compartment at each time interval (Mukherjee S et al 2014).

In vitro drug release kinetics

Different kinetic models such as zero order (cumulative amount of drug released vs time), first order (log cumulative percentage of drug remaining vs time), Higuchi model (cumulative percentage of drug released vs square root of time), korsemeyer-peppas model and Hixson crowell model were applied to interpret the drug release kinetics from the formulations. Based on the highest regression values for correlation coefficients for formulations, the best fit model was decided (Mamdouh M et al., 2004).

The release rate and mechanism of release of drug from the prepared SLN were analyzed by fitting the release data into

Zero order equation

$Q = K_0$ t, Where, Q is the amount of drug release at time, t and K_0 is the release rate constant.

First order equation

Log $Q = K_1$ t, Where Q is the percent of drug release at time, t and K_1 is the release rate constant.

Higuchi's equation

$Q = K_2 t^{1/2}$, Where, Q is the percentage of drug release at time t and K_2 is the diffusion rate constant.

Peppa's equation

$Mt/M\infty = Kt$, Where $Mt/M\infty$ is the fractional release of the drug, t is the release time, K is a constant incorporating structural and geometric characteristic of the release device, "n" is the release exponent indicative of mechanism of release. For non-fickian (anamalous/zero order) release, "n" value is between 0.5 to 1.0; for fickian diffusion, $n<0.5$; for zero order release, n = 1; "n" is estimated from linear regression of log ($Mt/M\infty$) vs log t.

RESULTS AND DISCUSSION

FTIR Studies

The FTIR studies shows that the main functional groups as in pure drug are reproducible in the mixture and formulation, it shows that there is no interaction between the drug an phospholipids and other excipients used in the formulations. It confirm that the compatibility between the drug and phospholipids are good enough for formulation and the character of phospholipids and other excipients will not change both physical and chemical character of rosuvastatin calcium. The results are shown in table 2 and figure 1.

Particle size

The particle size analysis revealed that, all the SLNs formulation was in the nanometer range. The size of the nanoparticles is based on the product and process variables like homogenization time, Concentration of phospholipids and surfactant. The sizes of the loaded SLNs were found to be between 360.4 - 7.5 nm (10 -1000nm). By comparing the formulation F7 shown better and desired particle size of about 19.9nm with polydispersity index of 0.264.this shows good dispersibility of particles throughout the phase. The results are shown in Table 3 and figure 2.

Zeta potential

The stability of formulated SLNs was evaluated by measuring the zeta potential of the SLNs by the Malvern particle size analyzer. The results are shown in Table 3 and figure 3. Zeta potential of rosuvastatin calcium loaded formulations was in the range of -64.4 to -0.2 mV. F7 shown best zeta potential value of -37.2mV, which shows good surface charge potential and stability of particle in continuous phase on long storage condition.

Scanning electron microscopy (SEM)

Shape & surface morphology of the optimized SLNs F7 formulation was observed by scanning electron microscopy. The study revealed that most of the SLNs were fairly spherical in shape, the surface of the particle showed a characteristic smoothness, and that the particle size was in the nanometric range, as depicted by SEM shown in the figure 4.

Drug content

The prepared formulations were analyzed for drug content and the data is reported in table no.3. it was observed that the drug content in the preparation SLNs was satisfactory and the drug was uniformly distributed in all the formulations. The % drug content was highest for F7 formulation was about 92 % and lowest for 84%.

Entrapment efficiency

The percentage yield of the product was calculated after freeze drying the SLN. Entrapment efficiency gives the amount of drug entrapped in the solid lipid nanoparticles. The results are shown in the table no.3. the entrapment efficiency was highest for F7 formulation about 91.90% due to less particle size.

***In vitro* Drug release study**

The invitro drug release studies for all 10 formulations of rosuvastatin loaded SLNs were carried out in pH 6.8 phosphate buffer using dialysis membrane and franz diffusion apparatus. The invitro release profile obtained for F1, F3, F5 and F7 rosuvastatin loaded SLN formulations are shown in figure 4. The cumulative % drug release of rosuvastatin loaded SLNs F7 formulation, after 8 hrs was found to be 45.88%.

From the results it was observed that, all the formulations shows better control release of drug from the SLN. But smaller particles leading to faster drug release due to larger surface area. In general the drug release from all formulation followed a steady pattern. It was observed that the drug release from the formulations decreased as in the tween 80 concentration. In the formulation stearic acid having polaxomer 188 on centration with 0.5% shows the good release. The drug release may be mainly controlled by drug diffusion through the lipid matrix respectively.

The release kinetuics of Rosuvastatin loaded SLNs are evaluated by fitting the data into various kinetic models like first order, zero order, Higuchi, Peppas and Hixon-Crowell equations. The drug release kinetic data of Rosuvastatin loaded F7 SLN was respectively shown in fig.no.5, which shows Zero order model regression R^2 values as 0.9999 respectively. So, it was concluded that F7 formulation follow zero order kinetics, which release the same amount of drug at unit time and it is the ideal method of drug release to achieve pharmacological prolong action. The values of release exponent(n) of all the formulations lies within the range of n = 0.5 – 1 have been observed, which are regarded as Non-fickian diffusion mechanism. Based on the results, the release of Rosuvastatin from SLNs was best fitted in peppas fitting kinetics (Tab.4 and Fig.6).

Table 1. Formulation of Rosuvastatin SLN

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Rosuvastatin	10mg	10mg	10mg	10mg	10mg	10mg	10mg	10mg	10mg	10mg
Cholesterol	100mg	200mg	–	–	100mg	200mg	–	–	100mg	100mg
Stearic acid	–	–	100mg	200mg	–	–	100mg	200mg	100mg	100mg
Polaxomer 188	50mg	50mg	50mg	50mg	–	–	–	–	50mg	–
Tween 80	–	–	–	–	0.5%	0.5%	0.5%	0.5%	–	0.5%
Methanol	20ml	20ml	20ml	20ml	20ml	20ml	20ml	20ml	20ml	20ml

Table 2. Drug excipient compatibility results – Frequencies of figure print region shown in FTIR

Characteristic IR absorption frequencies			Sample frequency range cm^{-1}		
Bond	Compound type	Frequency range	Rosuvastatin(pure drug)	Rosuvastatin +Cholesterol	Rosuvastatin + stearicacid
C-H	Alkynes	3397-3267(s) stretch	3394.53	3397.56	–
C=C	Aromatic rings	1600,1500(w) stretch	1547.45	1601.06	1601.23
C-H	Alkynes	700-610(b) bend	636.94	–	687.38

Table 3. Characterization of Rosuvastatin SLN

Formulation	Mean particle size(nm)	Zeta potential(Mv)	PDI	Drug content (%)	Entrapment efficiency(%)	Inference
F1	171.2	-64.4	1.722	84	83.88	Mean particle size and zeta potential is within the range but on prolong storage phase seperation.
F2	360.4	-48.6	3.813	87	86.91	Particle size and zeta potential are within the range but on prolonged storage it shows phase seperation
F3	19.9	-0.2	7.276	91	90.89	Particle size is within the range but Zeta potential is not within the range
F4	275.6	-41.5	8.315	78	77.90	Particle size and zeta potential are within the range but on prolonged storage it shows phase seperation
F5	7.5	-33.8	15.64	87	87.57	Particle size is not within the range(10-1000nm)

F6	297.3	-41.8	4.561	79	78.91	Particle size and zeta potential are within the range but on prolonged storage it shows phase seperation
F7	154.7	-37.2	0.264	92	91.90	Particle size is less and zetapotential is within the range on prolong storage at 4°C it shows good stability and dispersibility
F8	180.9	-44.5	0.454	89	88.91	Particle size and zeta potential are within the range but on prolonged storage it shows phase seperation
F9	1590.3	-62.3	0.896	88	87.90	Particle size is not within the range(10-1000nm)
F10	1683.1	-0.2	0.909	87	86.89	Particle size is not within the range(10-1000nm)

Table 4. Drug release model of formulation F7

Release model	**Regression**	**Formulation Code**
		F7
Zero order	R^2	0.9994
First order	R^2	0.9982
Hixson Crowell	R^2	0.9863
Higuchi	R^2	0.8782
Peppas	R^2	0.9999
	N	0.9992
Best fit model		Peppas

Figure 1. FTIR spectra for Rosuvastatin, Rosuvastatin + cholesterol and Rosuvastatin+ stearic acid

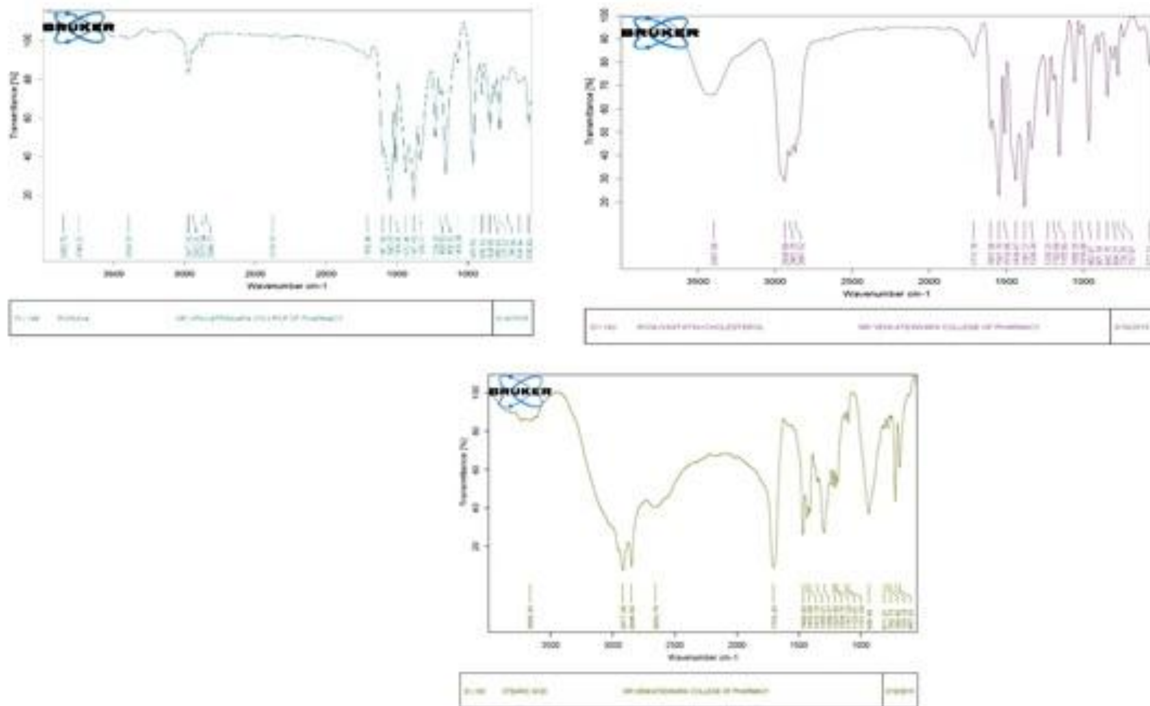


Fig 2. Particle size analysis for formulation F7

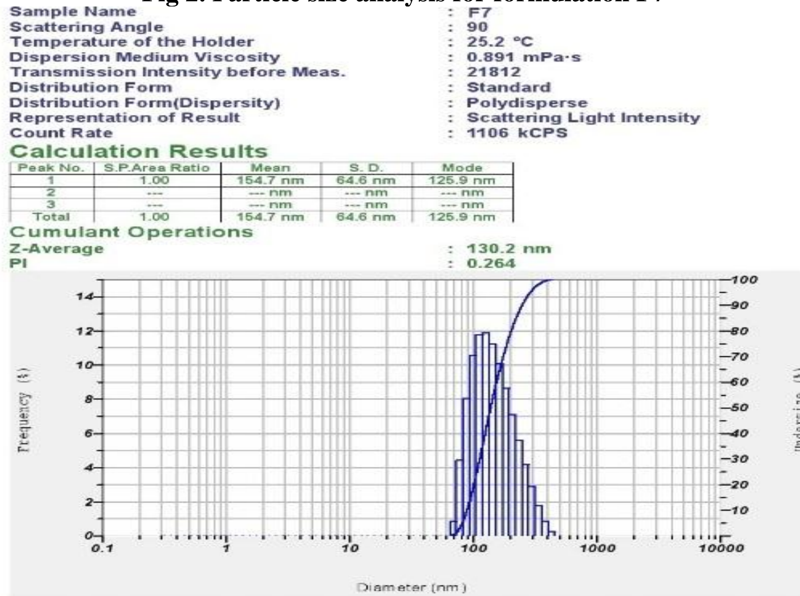


Fig 3. Zeta potential for formulation F7

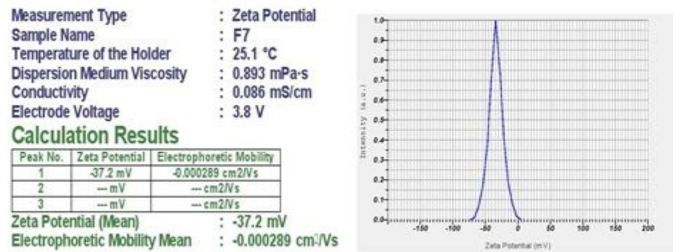


Fig.4. SEM: surface morphology for F7 formulation

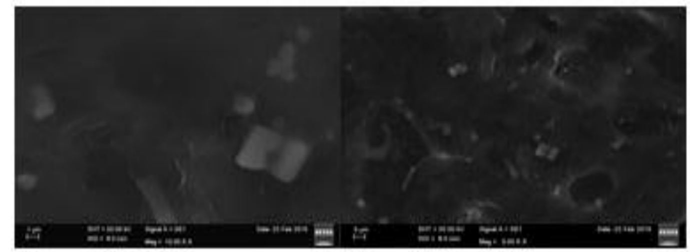


Fig.5. ***in vitro*** **drug release profile of formulation F1, F3, F5 and F7.**

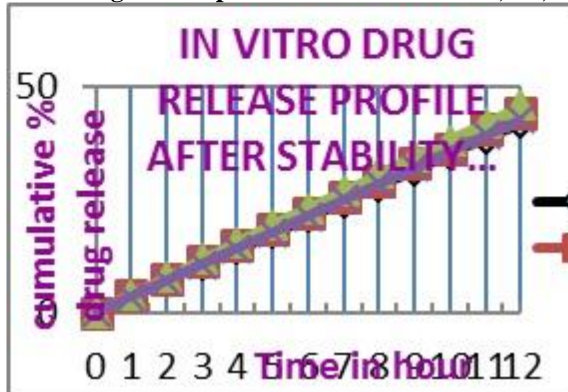
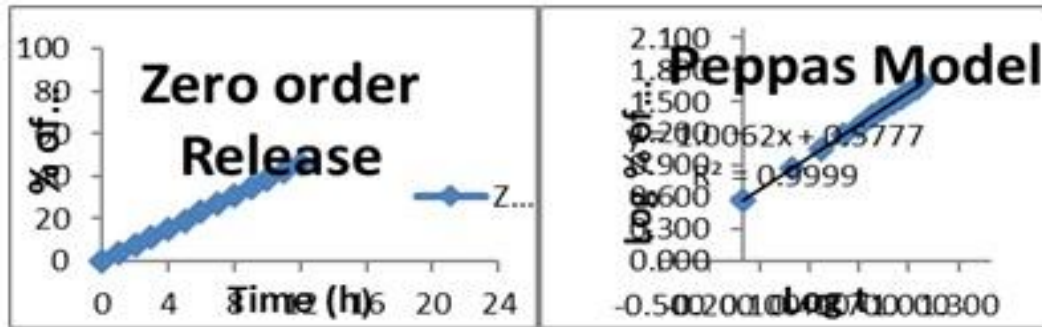


Fig.6. Drug ***in vitro*** **release kinetics profile for zero order and peppas kinetics**



CONCLUSION

The particle size analysis revealed that, F7 SLN formulation closer to the lesser nanometer range of 19.9nm which is approximately between the range of (10-1000nm). The stability of the F7 formulation was evaluated by measuring the zeta potential of the SLNs by Horiba particle size analyzer and found to be -37.2 mv, that showing uniformity in surface charge distribution in the particle and it confirms the good dispersibility of the particles in phase and it conclude that the SLN F7 formulation is the good stable formulation. The percentage drug content, entrapment efficiency was found to be very high in F7 formulation. The F7 formulation show good stability while comparing to other formulation. It was concluded that hot homogenization technique method followed by ultrasonication was an optimized technique for the preparation of SLN nanoparticles containing Rosuvastatin calcium, which lead to better results like high entrapment efficiency, high drug content and polaxomer 188 was a better choice of surfactant to reduce the particle size and leads to uniform distribution of SLN in its phase. So, SLN will be an alternative drug delivery system for Rosuvastatin calcium to enhance the bioavailability and therapeutic response of drug.

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