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International Journal of Nanotechnology in Medicine & Engineering

ISSN 2474-8811

Research Article Open Access

Formulation And In-Vitro Evaluation Of Pravastatin Solid Lipid Nanoparticles

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Citation: S.T. Bhagawati et.al(2017). Formulation And In-Vitro Evaluation Of Pravastatin Solid Lipid Nanoparticles. Int J Nano Med & Eng. 2:6, 113-119.

Received June 14, 2017 **Accepted** July 03, 2017, **Published** July 06, 2017

Abstract

Solid lipid nanoparticles are typically spherical with an average diameter between 1 and 1000 nm. It is an alternative carrier system to tradition colloidal carriers, such as, emulsions, liposomes, and polymeric micro and nanoparticles. Recently, solid lipid nanoparticles have received much attention by the researchers owing to its biodegradability, biocompatibility and the ability to deliver a wide range of drugs. The reason for better treatment with SLNs might be the significant uptake of SLNs by due to smaller size and its lipidic nature.

Pravastatin sodium is an cholesteral lowering agent used in the treatment of hyperlipidemia. It is administered through oral route and the dose is 10mg. The oral bioavailability is 17% and half life is 1-3hrs. It is rapidly excreted through the renal route. The aim is to increase the bioavailability of Pravastatin sodium, solid lipid nanoparticles of Pravastatin sodium are prepared by hot homogenization technique using lipids (Trimyristin, Comprital and glyceryl monostearate) with soylecithin surfactant and poloxamer 188 as stabilizer. The prepared formulations have been evaluated for entrapment efficiency, drug content, in-vitro drug release, particle size analysis, Fourier transform-infrared studies, and stability. The optimization is based upon the range of particle size,zeta potential, and dug release studies. The nanoparticles possess negative surface charge and were enough magnitude for stable preparations. In vitro drug release studies in Phosphate buffer of pH 7.4 exhibited initial burst effect followed by a sustained release of Pravastatin. A solid lipid nanoparticle formulation containing drug pravastatin sodium and lipid Compritol, stabilized with poloxamer 188 as surfactant showed prolonged drug release, smaller particle size, as compared to other formulations with different lipids.

Keywords: Pravastatin sodium, bioavailability, solid lipid nanoparticles, hot homogenization technique,. in-vitro drug release

Introduction

Heart disease refers to various types of conditions that can affect heart function. These types include:Coronary artery (atherosclerotic) heart disease that affects the arteries to the heart Valvular heart disease that affects how the valves function to regulate blood flow in and out of the heart. Coronary heart disease is initially diagnosed by patient history and physical examination. EKG blood tests, and tests to image the arteries and heart muscle confirm the diagnosis. Treatment for coronary heart disease depends upon its severity. Many times lifestyle changes such as eating a heart healthy diet, exercising regularly, stopping smoking and controlling high blood pressure, high cholesterol and diabetes may limit the artery narrowing.1 Hypertension, myocardial infarction, atherosclerosis, arrhythmias and valvular heart disease, coagulopathies and stroke, collectively known as cardiovascular diseases (CVDs)2.

Hyperlipidemia refers to increased levels of lipids (fats) in the blood, including cholesterol and triglycerides. Although hyperlipidemia does not cause symptoms, it can significantly increase your risk of developing cardiovascular disease, including disease of blood vessels supplying the heart (coronary artery disease), brain (cerebrovascular disease), and limbs (peripheral vascular disease). These conditions can in turn lead to chest pain, heart attacks, strokes, and other problems3. Hyperlipidemia is a common risk factor for CVD, with 53.4 percent of adults in the United States having abnormal cholesterol values and 32 percent having elevated low-density lipoprotein (LDL) cholesterol levels4. Hyperlipidemia is a medical condition characterized by an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters, phospholipids and or plasma lipoproteins including very low-density lipoprotein and low-density lipoprotein along with reduced high- density

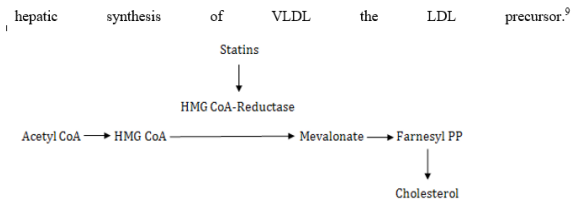
lipoprotein levels. This elevation of plasma lipids is among the leading risk factors associated with cardiovascular diseases5

Cholesterol does not travel freely through the bloodstream. Instead, it is attached to a protein and the two together are called a lipoprotein (lipo=fat). There are three types of lipoproteins that are categorized based upon how much protein there is in relation to the amount of cholesterol. Low-density lipoproteins (LDL) contain a higher ratio of cholesterol to protein and are thought of as the "bad" cholesterol. Elevated levels of LDL lipoprotein increase the risk of heart disease, stroke, and peripheral artery disease, by helping form cholesterol plaque along the inside of artery walls. Over time, as plaque buildup increases, the artery narrows (atherosclerosis) and blood flow decreases. If the plaque ruptures, it can cause a blood clot to form that prevents any blood flow. This clot is the cause of a heart attack or myocardial infarction if the clot occurs in one of the coronary arteries in the heart.8

Hypercholesterolemia is a common disorder and is of major interest since it is one of the risk factor for ischaemic heart disease. For the management of hypercholesterolemia and dyslipidamias, statins are prefered drugs of choice which are proved as the most potent therapies for treating elevated Low Density Lipoprotein-Cholesterol (LDL-C) and congestive heart disease. The widely prescribed statins possess low bioavailability which limits their application in clinical use.6

High-density lipoproteins (HDL) are made up of a higher level of protein and a lower level of cholesterol. These tend to be thought of as "good" cholesterol because they can extract cholesterol from artery walls and dispose of them in the liver. The higher the HDL to LDL ratio, the better it is for the individual because such ratios can potentially be protective against heart disease, stroke, and peripheral artery disease.Very low-density lipoproteins (VLDL) contain even less protein than LDL.Total cholesterol is the sum of HDL, LDL, and VLDL.8

Pravastatin is the generic form of the brand-name drug Pravachol, which is used to lower cholesterol levels.Pravastatin reduces levels of "bad" cholesterol, which is called low-density lipoprotein or LDL.It also raises levels of "good" cholesterol, which is called high-density lipoprotein or HDL, and it lowers levels of harmful triglycerides in the blood.Lowing cholesterol and fats in the blood with pravastatin may prevent heart disease, chest pain, strokes, and heart attacks.Pravastatin is in a group of drugs known as statins, which work by blocking an enzyme that the body needs to make cholesterol7.Pravastatin sodium is a cholesterol lowering agent, which is used in the treatment of hyperlipidemia. Its absolute bioavailability is 17% and average total absorption



Schematic representation of effect of Statins[6]

is 34%. Pravastatin sodium is one of the lower potency statins, and produces its lipid lowering effect in two ways. First as a consequence of its reversible inhibition of HMG-CoA reductase activity. It effects modest reductions in intracellular pools of pravastatoin inhibits LDL production by inhibiting

The aim of this study is to decrease the bioavailability of the drug by prepare and characterize the Pravastatin sodium loaded solid lipid nanoparticles were prepared by hot homogenisation technique, using trimyristin, compritol and glyceryl monostearate as the lipid matrices and soylecithin, poloxamer 188, as stabilizer with a view to improve the bioavailability, which would increase the biological activities.

Materials And Methods

Materials

Pravastatin sodium obtained as a gift sample from (Swapnroop drugs and Pharmaceuticals). Glyceryl monostearate [GMS] (Research Lab Fine Chem,Industries.),Compritol(Himedia Labouratories Pvt.Ltd), Trimyristin(Sasol, Germany), Soylecithin and Polaxamer 188((Himedia Labouratories Pvt.Ltd), Chloroform and Methonol(SD Fine Chem Ltd) were purchased from the local market. All the reagents used were of analytical grade.

Preperation of Pravastatin solid lipid nanoparticles[10,11]

Solid lipid nanoparticles were prepared by using lipid (Trimyristin/ Campritol / Glycerol monostearate) which is first melted by heating and then adding the lecithin (soya lecithin) in a boiling tube and then drug was incorporated in to the lipid– lecithin melt which was then heated at 5 °C temperature above melting point to melt the lipid. Simultaneously in another beaker taken poloxamer 188 was dissolved in water and heated to temperature equal to that of lipid phase, then this aqueous phase is transferred to lipid phase. This mixture is homogenized at 20,000 rpm for 3 min and then immediately placed in probe ultrasonicator at 75% amplitude for 20 min. Blank nanoparticles were prepared in a similar manner omitting the Pravastatin in the preparation.

Table No.1: The composition of different formulations of Pravastatin SLNs (soy lecithin as stabilizer).

FORMULATION CODE	PRAVASTATIN (mg)	TM (mg)	CA (mg)	GMS (mg)	SOY LECITHIN (mg)	POLAXAMER (mg)	WATER (ml)
SLN-TM/S1	-	100	100	100	50	50	9.6
SLN-TM/S2	10	100	100	100	50	50	9.6
SLN-TM/S3	-	200	200	200	100	100	9.6
SLNTM/S4	10	100	100	100	100	100	9.6
SLN-TM/S5	-	200	200	200	150	150	9.6
SLN-TM/S6	10	100	100	100	150	150	9.6
SLN-TM/S7	-	200	200	200	200	200	9.6
SLN-TM/S8	10	100	100	100	200	200	9.6
SLN-CA/S1	-	100	100	100	50	50	9.6
SLN-CA/S2	10	100	100	100	50	50	9.6
SLN-CA/S3	-	200	200	200	100	100	9.6
SLN-CA/S4	10	200	200	200	100	100	9.6
SLN-CA/S5	-	300	300	300	150	150	9.6
SLN-CA/S6	10	300	300	300	150	150	9.6
SLN-CA/S7	-	400	400	400	150	150	9.6
SLN-CA/S8	10	400	400	400	200	200	9.6
SLN- GMS/S1	-	100	100	100	50	50	9.6
SLN- GMS/S2	10	100	100	100	50	50	9.6
SLN- GMS/S3	-	200	200	200	100	100	9.6
SLN- GMS/S4	10	200	200	200	100	100	9.6
SLN- GMS/S5	-	300	300	300	150	150	9.6
SLN- GMS/S6	10	300	300	300	150	150	9.6
SLN- GMS/S7	-	400	400	400	200	200	9.6
SLN- GMS/S8	10	400	400	400	200	200	9.6

Evaluation of Pravastatin-loaded solid lipid nanoparticles 10.11

1)Particle size analysis:

The particle size was determined by dynamic light scattering, using a Malvern system, with vertically polarized light supplied by an argon-ion laser (Cyonics) operated at 40 mW. Experiments were performed at a temperature of 25.0 ± 0.1ºC at a measuring angle of 90º to the incident beam. The zeta-potential of the nanoparticles was measured in distilled water using a Malvern Zeta sizer.

The technique of laser diffraction is based around the principle that particles passing through a laser beam will scatter light at an angle that is directly related to their size. As the particle size decreases, the observed scattering angle increases logarithmically. The observed scattering intensity is also dependent on particle sizes and diminishes to a good approximation, in relation to the particle's cross-sectional area. Large particles therefore scatter light at narrow angles with high intensity, whereas small particles scatter at wider angles but with low intensity.

2)Zeta potential:

Zeta potential analysis was performed to estimate the stability of the nanoparticles. Zeta potential is a measure of effect of electrostatic charges. This is the basic force that causes the repulsion between adjacent particles. Net results are attraction or repulsion depends upon the magnitude of both forces. The thumb rule describes the relation between zeta potential determination responses of the nanoparticles.

3)Percentage Drug entrapment efficiency (% DEE):

About 1ml of solid lipid nanoparticles loaded with pravastatin was taken and placed in outer chamber of the centrisart device and the sample recovery chamber is placed on the top of the sample. The unit is centrifuged at 5000 rpm for 15 min. The solid lipid nanoparticles along with the encapsulated drug remained in the outer chamber and the aqueous phase is moved into the sample recovery chamber through filter membrane (molecular weight cut-off 2 Drug Loading efficiency.

4)Fourier-transform infrared spectroscopy (FT-IR)

Drug-polymer interactions were studied by FTIR spectroscopy. Pure drug and excipients were subjected to FT-IR studies. Also physical mixtures were subjected and the spectra recorded by scanning in the wavelength of 500-4000 cm-1 in a FT-IR spectrophotometer.

5)In vitro drug release

.In vitro drug release studies were carried out in Franz diffusion cell. 1 ml of nanoparticles dispersion was used for diffusion study. Nanoparticles containing drug were placed in donor chamber while the receiver chamber consists of 22 ml of diffusion medium of pH 7.4 (separated by presoaked semi- permeable membrane) maintained at 37 ± 2°C in Franz diffusion cell. The rpm of the magnetic bead was maintained at 50 rpm. 1 ml of the aliquot was withdrawn at predetermined intervals. The solution was analysed for the drug content spectrophotometrically at 260 nm against blank. Equal volume of the diffusion medium was replaced in the vessel after each withdrawal to maintain sink condition. Similarly diffusion of blank formulation is also done to correct the interference. Three trails were carried out for all formulations. From data obtained percentage drug release was calculated and plotted against function of time to study the pattern of drug release.

Table 2: Entrapment efficiency(EE) of pravasatatin solid lipid nanoparticle of different formulations.

Sl.No	Formulation code	EE	
		Absor-bance	EE
1	SLN-TM/S 2	0.015	99.84%
2	SLN-TM/S 4	0.009	99.90%
3	SLN-TM/S 6	0.006	99.93%
4	SLN-TM/S8	0.018	99.81%
5	SLN-CA/S2	0.003	99.96%
6	SLN-CA/S4	0.012	99.87%
7	SLN-CA/S6	0.006	99.93%
8	SLN-CA/S8	0.003	99.96%
9	SLN-GMS/S2	0.001	99.98%
10	SLN-GMS/S4	0.001	99.98%
11	SLN-GMS/S6	0.080	93.15%
12	SLN-GMS/S8	0.113	91.75%

6)Stability studies

Stability studies is carried out for the formulations were taken in glass vials and closed tightly using rubber closures and aluminium cap and kept in room temperature for 90 days.At the end of studies, samples wera analyzed for the particle size.

Results And Discussion

Entrapment efficiency: It is an important parameter for characterizing solid lipid nanoparticles. In order to attain optimal efficiency, several factors were varied, including the type and concentration of the lipid and surfactant material used. The entrapment efficiency of all the prepared SLN formulations is shown in Table 2. The entrapment efficiency of the SLN dispersions

was found to be in the range of 91.75% to 99.98%

In vitro drug release

The in vitro drug release profile of Pravastatin from Campritol SLN formulations is shown in [Figure 1] The in vitro release of Pravastatin from the Campritol SLN dispersion was found to be 93.74% at the end of 12 hours

Table No. 3. The in vitro release profile of different Pravastatin solid lipid nanoparticles prepared using Compritol as lipid carrier and soy lecithin as stabilizer.

s.no	Time(Hrs)	Percentage CDR			
		SLN/CA-S2	SLN/CA-S4	SLN/CA-S6	SLN/CA-S8
1	0	0	0	0	0
2	0.5	1.8	3.9	7.4	9.2
3	1	7.3	13.8	20.3	28.9
4	2	17.2	23.6	35.94	49.2
5	4	31.2	35.6	55.7	61.3
6	6	55.9	66.43	81.7	72.5
7	12	72.8	81.9	98.43	93.74

Fig 1: The in vitro drug release profile of Compritol formulations using soy lecithin as surfactant.

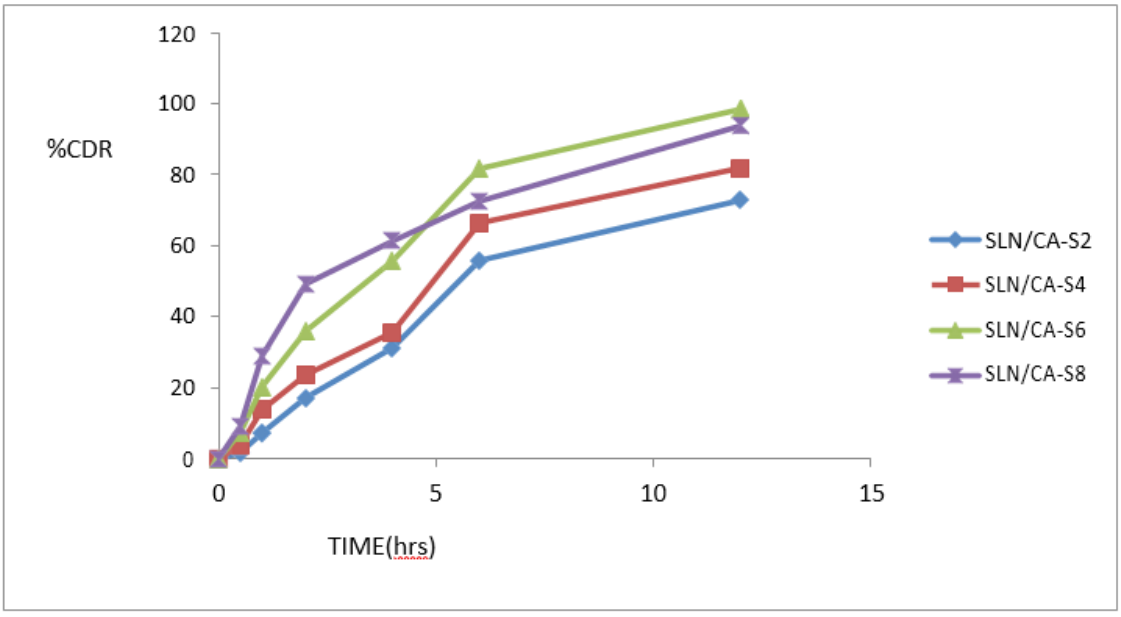


Table No. 4. The particle size, PDI and zeta potential of various formulations (soy lecithin as stabilizer).

Sl. No	Formulation code	Particle size (nm)	PDI	Zeta potential (mV)
1	**SLN-TM/S 2**	144.7	0.228	-28.2
2	**SLN-TM/S 4**	108.8	0.259	-23.3
3	**SLN-TM/S 6**	95.1	0.271	-19.1
4	**SLN-TM/S 8**	96.85	0.272	-26.5
5	**SLN-CA/S 2**	400.8	0.510	-35.4
6	**SLN-CA/S 4**	187.7	0.473	-31.5
7	**SLN-CA/S 6**	100.7	0.390	-21.1
8	**SLN-CA/S 8**	112.6	0.651	-29.1
9	**SLN-GMS/S 2**	335.7	0.493	-29.7
10	**SLN-GMS/S 4**	214.7	0.540	-26.7
11	**SLN-GMS/S 6**	273.2	0.570	-27.3
12	**SLN-GMS/S 8**	192.4	0.690	-31.5

FT-IR studies

Infrared studies were carried out to confirm the compatibility between the lipid, drug, and selected SLN formulation. From the spectra it was observed that there was no major shifting, as well as, no loss of functional peaks between the spectra of the drug, lipid, and drug-loaded SLN. [1670.98 cm–1, and 2340.34 cm–1]. This indicated no interaction between the drug and lipid.

Fig 2: The FTIR spectrum of pure Pravastatin.

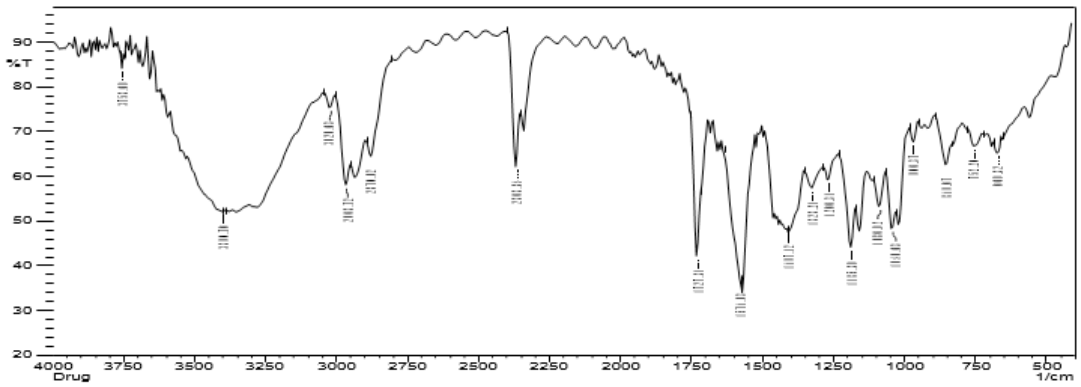
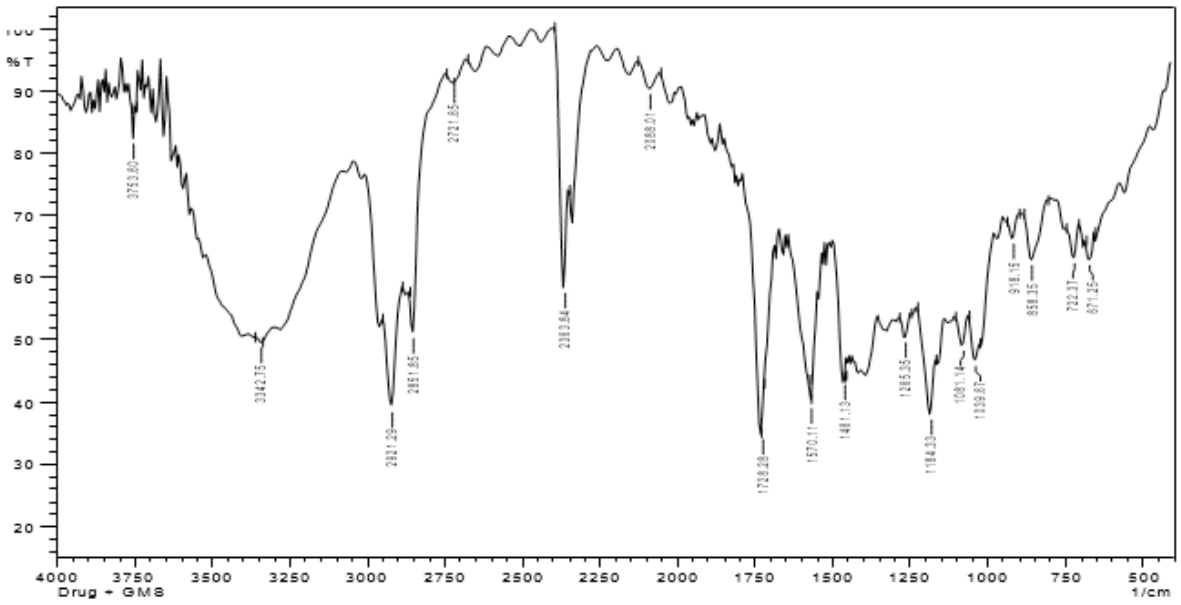


Fig 3: The FTIR spectrum of optimized physical mixture of Pravastatin and Campritol(1:1).



Stability Studies:

After 90 days of stability period at room temperature the formulations were observed under microscope where it was observed absence of particle in micron range which indicates the formulations were stable.

Conclusion

It was observed that the hot homogenization and ultrasound dispersion method was a useful method for the successful incorporation of the drug Pravastatin with high entrapment efficiency. Furthermore, it could be presumed that if the nanometer range particles were obtained, the bioavailability might be increased. Hence, we can conclude that solid lipid nanoparticles provide controlled release of the drug and these systems are used as drug carriers for lipophilic drugs, to enhance the bioavailability of poorly water-soluble drugs through nanoparticles, as a drug delivery system.

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