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Solid- Lipid Nanoparticles: A Newer Approach for Formulation and Optmization of HMG-CoA Reductase Inhibitors

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ABSTRACT

The purpose of the study was to formulate the capsules loaded with the Simvastatin solid lipid nanoparticles using the self emulsifying agent, glyceryl monostearate as the source of lipid for incorporation of Simvastatin to form nanoparticles. Development of solid lipid nanoparticles of Simvastatin and to optimize it for independent variables so as to achieve desired particle size with maximum percent entrapment efficiency and percent cumulative drug release. To achieve the goal and formulations were prepared by hot homogenization method and optimized by 2^3 Factorial design (using MET's STAT software). Optimized formulations were freeze dried and its effect on particle size was evaluated. For their entrapment efficiency, drug content, FTIR, DSC, SEM and Invitro drug release study.

Keywords: Solid Lipid Nanoparticles, Simvastatin, MET's STAT, Glyceryl monostearate

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INTRODUCTION

Drug low solubility and stability in physiological environment constitutes a main hurdle in attaining the appropriate bioavailability. Several polymer based nanotechnologies are being intended in order to optimize the technological (e.g., solubility, stability, bioavailability, etc.) aspects of drugs. Other advantages of lipid excipients, such as biodegradability and cost effectiveness[1], promote their use as novel drug carriers. Simvastatin [butanoic acid, 2,2-dimethyl-,1,2,3,7,8,8a-hexahydro- 3, 7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran- 2-yl)-ethyl]-1-naphthalenyl ester, [1S-[1α,3α,7β,8β(2S*,4S*),- 8aββ]]] lowers blood cholesterol levels through reversible and competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase, an enzyme involved in the biosynthesis of cholesterol. When simvastatin is given orally, it undergoes extensive hepatic first-pass metabolism by cytochrome P450 3A4, which is responsible for its low oral bioavailability[1-3]. Hence in order to overcome with hepatic metabolism and to enhance the oral bioavailability and formulation enabling lymphatic absorption such as nanoparticles and nanoemulsions can be prepared[2]. Solid lipid nanoparticles are considered to be most effective lipid based colloidal carriers. This is one of the popular approaches to improve the oral bioavailability of poorly water soluble drugs[4]. Two different lipid based approaches are known to enhance the lymphatic transport, which includes construction of a highly lipophilic prodrug and incorporation of drug in a lipid carrier[5]. Lipid nanoparticles with a solid matrix, such as solid lipid nanoparticles (SLN), are an alternative nanoparticulate carrier system to polymeric nanoparticles, liposomes and o/w emulsions[6-8]. Lipids can enhance the lymph formation and simultaneously promote lymph flow rate[9]. Transport of drug through intestinal lymphatics via thoracic lumph duct to systemic circulation at the function of jugular and left subclavian vein, avoids presystemic hepatic metabolism and therefore enhances bioavailability. Simvastatin is considered as reasonable substrate for intestinal lymphatic transport because of its high log P value of 4.7[10-13]. Present literature focuses on for enhancement in oral bioavailability of statins using nanoparticulate drug delivery system[14]. In the present work, SIMVA loaded SLN's were successfully prepared by hot homogenization method. The formulations was optimized by MET's stat. the optimized formulations were evaluated for various parameters like particle size, SEM, XRD, invitro release study. Many researchers have optimized nanoparticulate formulations using factorial design12-[15].

MATERIALS AD METHODS

Materials

Simvastatin and glyceryl monosterate were obtained as gift sample from Cipla Pharmaceuticals, Vichroli, Mumbai and Loba Chemicals Ltd respectively. All the other reagents were of analytical grade.

FTIR study

Simvastatin and GMS were kept at room temperature for 30 days. Then the samples were subjected to the FTIR (Shimadzu AZ 13749) studies by using KBr as blank.

Preparation of solid lipid nanoparticles

Simvastatin, span 60, GMS was dissolved in mixture of chloroform and methanol(1:1). Organic solvents were completely removed by heating. Drug embedded lipid layer was melted by heating at 5^0C above the melting point of lipid. An aqueous phase was prepared by dissolving tween 80 in double distilled water and heated to same temperature of melted lipid phase and homogenization (Ika T25, Digital ultra turrax) was carried out at 35000 rpm and temperature maintained 5^0C above the melting point of lipid. Coarse hot oil in water emulsion so obtained was ultrasonicated for further size reduction for 10-30 min.

Optimization of Solid lipid nanoparticles

In order to optimize amount of GMS(X1), concentration of span 60(X2), and concentration of tween 80 (X3) were selected as independent variables. The solid lipid nanoparticles obtained in each of the eight experiments listed in **TableI** were subjected to evaluation for the output variables of particle size and drug entrapment efficiency. Multiple regression analysis was carried out to get equations in the form

$$Y = B0 + B1X1 + B2X2 + B3X3 + B4X1X2 + B5X1X3 + B6X2X3 + B7X1X2X3$$

The value of the coefficients were taken as an indication of the extent of effect that factor had on the output variables i.e. particle size and % entrapment efficiency.

Particle size analysis

The particle size analysis of the solid lipid nanoparticles was carried out using Malvern Mastersizer 2000 MS. The average particle size and size distribution of each simvastatin solid lipid nanoparticle dispersion was recorded.

Particle Morphology

In the study, SIMVA-loaded SLN dispersion was dried in a freeze dryer for 24 hrs. and sputtered with platinum in an ion sputter for 300 s. Images were collected at an acceleration voltage of 15 kV using a back scattered electron detector on Joel JSM 6360 SEM. Analysis was performed at 25±20C.

Drug Entrapment efficiency

For determination of entrapment, the SLNs was expressed as percent of added drug actually entrapped into SLN. For this %ml of chloroform was added to it to solubilize lipid. Chloroform was evaporated to dryness to cause precipitation of lipid. After methanol added again sonication was done for 10 min in bath sonicator. It was filtered using wattman filter paper (0.45 nm) and diluted filterate with methanol. Absorbance of methanolic solution was recorded at 237nm and reffered to calibration equation to get concentration of SIMVA. EE was calculated according to the following equation,

$$\% EE = [(\text{total drug content-unentrapped drug})/ \text{total drug content}] \times 100$$ [16]

Differential Scanning Calorimetry

DSC was performed on Mettler-Toledo DSC 821e (Columbus, OH) instrument, and an empty standard aluminum pan was used as reference. DSC used to characterize material with respect to crystalline behaviour and physical changes.DSC scan for SIMVA was recorded at heating rate of 10°C/min in temperature range 30°-300°C. The degree of crystallinity of lipid was also analyzed by DSC. In this, the freeze dried SIMVA-SLNs, GMS and its physical mixture was weighed into standard aluminium pans using an empty pan as reference. A heating rate of 5˚C/min was applied. The samples were first heated from 30ºC to 100ºC and cooled from 100ºC to 30ºC and again heated from 30ºC to 100ºC under liquid nitrogen[17].

X-ray diffraction

X-ray scattering measurement was carried out on the pure drug (simvastatin), pure glyceryl monostearate and simvastatin solid lipid nanoparticles. XRD study was performed by Philips PAN analytical expert PRO X-ray diffractometer 1780. A Cu Ka radiation source was used, and the scanning rate (2h/min) was 5°/min.

Freeze Drying of Simvastatin Solid Lipid Nanoparticles Dispersion

50 ml aliquots of different batches of the optimized solid lipid nanoparticles were freeze-dried. Lactose (5% w/v) was added as a cryoprotectant to 50 mL aliquots of samples, which were frozen in liquid nitrogen and lyophilized for 48 h at -70 ˚C, at a 0.05 mm Hg pressure. Freeze-dried samples stored at room temperature[18].

***In Vitro* Release Kinetics of Simvastatin From Solid Lipid Nanoparticles:**

In vitro release studies were performed on simvastatin solid lipid nanoparticles using dissolution test apparatus. Dialysis membrane (Himedia, Mumbai) having pore size 2.4 nm, molecular weight cut off between 12,000 and 14,000 was used. The membrane was soaked in double-distilled water for 12 h before use. 1ml of the simvastatin solid lipid nanoparticles

dispersion was filled in the membrane and tied to the paddle of the dissolution test apparatus. The basket was filled upto mark with pH 6.4 phosphate buffer. The paddle was rotated at 50 rpm and temperature maintained at 37 ± 0.5^0C. At fixed time intervals, 10 ml of the sample was withdrawn, diluted suitable and absorbance recorded at 238nm. The amount of drug released was inferred from the relevant calibration equation. Fresh dialysis medium was replaced after each aliquot withdrawal to maintain constant volume[19].

Bulk Properties ofFreeze Dried Powder of Simvastatin Solid Lipid Nanoparticles

Bulk properties of solid SIMVA-SLNs formulation

Loose bulk density (LBD) and tapped bulk density (TBD)

The values for LBD and TBD were found to 0.3999 g/ml and 0.4998 g/ml respectively. Bulk densities of blends were satisfactory. These values may further influence properties such as compressibility and tablet dissolution.

Angle of repose and Compressibility Index

The values of angle of repose and compressibility index of freeze dried SIMVA-SLNs were found to be 22.45%. These values for angle of repose (< 30) indicated good flow properties of powder and this was further supported by passable compressibility index values.

Formulation of Oral Solid Dosage Form of SIMVA-SLNs

The simvastatin solid lipid nanoparticles dispersion was selected for formulation in capsule dosage form depending on its performance in particle size, % entrapment efficiency and in-vitro drug release. The aerosil (10%) was added in capsule formulation to improve the flow properties of solid SIMVA-SLNs. The SIMVA-SLNs were first mixed with aerosil and then it is add to HPMC capsule **(Table II)**

In-Vitro Dissolution Study:

Drug release of simvastatin from optimized plain drug and SIMVA-SLNs capsule formulations was studied by dissolution apparatus. Dialysis membrane (Himedia, Mumbai) having pore size 2.4 nm, molecular weight cut off between 12,000–14,000, was used. Membrane was soaked in double distilled water for 12 hrs before using for dissolution study drug release was studied by incorporating the formulation in dialysis bag in acidic medium and then in phosphate buffer pH 6.8 buffer (900ml) using a USP apparatus II with rotating paddle at 50 rpm and temperature was maintained at 370C±0.50C. The samples of formulations equivalent to 10 mg of simvastatin were used in each dissolution study. 5ml samples were withdrawn by using syringe filter (0.22 nm) at different time intervals till 24 hrs. The samples were assayed at λ max of 238 nm using UV/VIS. Spectrophotometer.

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$$Y = B0 + B1X1 + B2X2 + B3X3 + B4X1X2 + B5X1X3 + B6X2X3 + B7X1X2X3$$

Table I: Details of the eight formulations in the 2^3 factorial design

Formulation number	Drug (%)	Lipid Glyceryl monostearate (%)	Surfactant (% Span-80	Co-surfactant (% Tween-80	Sonication time (min)	X1	X2	X3
F1	10	10	10	10	5	-	-	-
F2	10	100	10	10	5	+	-	-
F3	10	10	40	40	5	-	+	-
F4	10	100	40	40	5	+	+	-
F5	10	10	10	10	15	-	-	+
F6	10	100	10	10	15	+	-	+
F7	10	10	40	40	15	-	+	+
F8	10	100	40	40	15	+	+	+

Table II: Formula for Capsule Dosage Form

Sr. No.	Ingredients	Quantity (mg)
1.	Simvastatin as solid SLN	178.5 (Equivalent to 10 mg of simvastatin)
2.	Aerosil	20 mg (10%)
Total		198.5 mg

RESULT AND DISCUSSION

Optimization of variables using factorial design

When a 23 factorial design was applied for studying the influence of drug: lipid ratio, quantity of surfactant and sonication time, the results as depicted in **table III** were obtained.

Steps involved in calculations of 23 factorial design

Step 1: The first step included transformation of the values i.e. to code the levels of the factors so that the high level of each factor was +1 and the low level was -1. This procedure required a transformation of each of the three variables X1, X2, and X3 to X'1, X'2, X'3. In general, the formula for the transformation was,

$$X' = \frac{X - \text{Average of two levels}}{\text{One - Half the difference of the levels}}$$

Table IV transformed values and also "transformed values for the interactions represented by +1 and -1. The interaction values were obtained by multiplying the appropriate columns. The total column contains only the value +1 and was used to calculate the intercept, β0.

Step 2: In the next step the coefficients for the polynomial equation were calculated. The coefficients for the polynomial equation were calculated as:

$$\beta_0 = \frac{\sum XY}{2^n}$$

Where, X was the total column value (+1) and Y was the response (Y1 or Y2).

$$\beta_1 = \frac{\sum X_1 Y_1}{2^n},$$

Where, X1 was the value of the column X1 and Y1 was particle size. All other coefficients were calculated in the similar manner. The polynomial equation obtained for Y1 was-

Y1 = 250.12 + 32.38X1 + 2.62X2 – 62.38X3 – 5.12X1X2 – 19.88X2X3 + 4.88X1X3 – 22.62X1X2X3

The polynomial equation obtained for Y2 was-

Y2 = 72.25 + 11.5X1 –5.5X2 + 1.25X3 + 2.75X1X2 + 2.0X2X3 – 1.0X1X3 – 1.25X1X2X3

From the polynomial equation for Y1 the following inferences could be drawn-

- As the quantity of lipid is increased, the particle size of the solid lipid nanoparticles increases
- As the quantity of surfactant/cosurfactant increases, the particle size increases but the influence is not very strong.

The sonication time has very significant effect on particle size. As the sonication time increases, the particle size decreases substantially.

Similarly, from polynomial equation for Y2, the following aspects were revealed-

- As the quantity of lipid increases, the % entrapment efficiency increases.
- As the quantity of surfactant/cosurfactant increases, the % entrapment efficiency decreases.
- Sonication time did not have substantial influence on the % entrapment efficiency.

In order to identify the optimum values of the factors those, in combination, were likely to give adequate particle size and % entrapment efficiency, transformed value of X2 was taken as zero and the equations for Y1 and Y2 were solved for transformed values of X1 ranging from -1 to 1 for a range of output variable values. **Table V** gives values of X 3 for assumed values of X1 ranging from -1 to 1 for a range of values of Y1 i.e particle size**.**

Table VI: Values of X3 for different values of X1 when X2 = 0 for output variable Y

From the above tabulated data, it becomes apparent that when the transformed value of factor X2 = 0, and X1 = 1, a value of X3 = 1 can give particle size of 200nm and % entrapment efficiency

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of 84%. Decoding of X1, X2 and X3 gave drug: lipid ratio of 1:10, surfactant concentration of 5% and sonication time of 15min as the optimized variables.

When the ninth experiment was performed using these optimized variables, the results obtained are put forth in **Table VII**

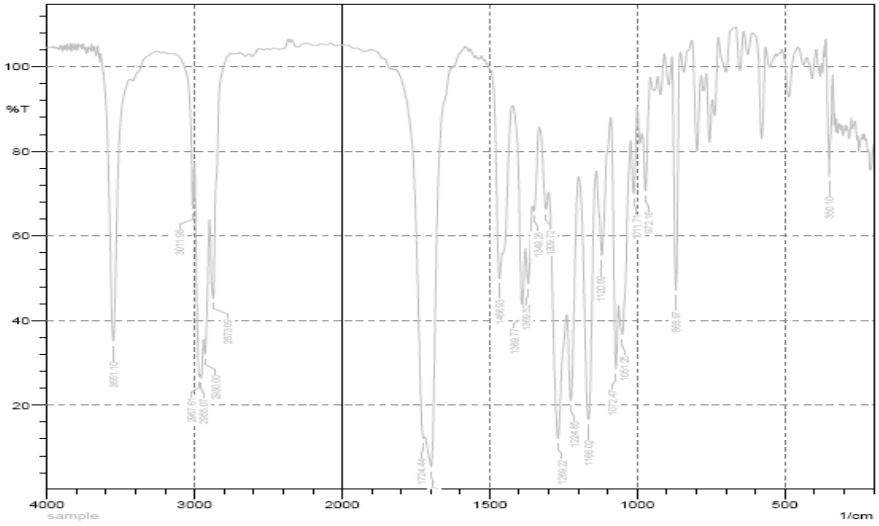

Figure 1: FTIR of Simvastatin bulk drug

Table III: Interpretation of IR simvastatin spectra

Functional Groups	Wavenumber (cm-1)
C=O	(S) 1698.9
C=C	(S) 2012
C-H (Aliphatic)	(S) 2960.73
C-H (Aromatic)	(S) 1381.03
O-H	(S) 3551.4

Optimization of variables using factorial design:

Table III: Results for the optimization batches of solid lipid nanoparticles

Factors				Response	
Formulations	**X1 Drug:lipid ratio**	**X2 Percent of surfactant**	**X3 Sonication time (min)**	**Y1 Particle size (nm)**	**Y2 % entrapment efficiency**
F-1	1:1	2	5	280	80
F-2	1:10	2	5	300	77
F-3	1:1	8	5	290	40
F-4	1:10	8	5	380	65
F-5	1:1	2	15	140	67
F-6	1:10	2	15	270	61
F-7	1:1	8	15	161	68
F-8	1:10	8	15	180	76

ISSN: 0975-8585

Steps involved in calculations of 23 factorial design

Step 1: The first step included transformation of the values i.e. to code the levels of the factors so that the high level of each factor was +1 and the low level was -1. This procedure required a transformation of each of the three variables X1, X2, and X3 to X'1, X'2, X'3. In general, the formula for the transformation was,

$$X' = \frac{X - \text{Average of two levels}}{\text{One - Half the difference of the levels}}$$

Table IV: Transformed values for 23 factorial design along with the responses

Experimental no.	X1	X2	X3	X1X2	X1X3	X2X3	X1X2X3	Total	Y1 (particle size in nm)	Y2 (% entrapment efficiency
F1	-1	-1	-1	1	1	1	-1	1	280	70
F2	1	-1	-1	-1	-1	1	1	1	300	87
F3	-1	1	-1	-1	1	-1	1	1	290	47
F4	1	1	-1	1	-1	-1	-1	1	380	80
F5	-1	-1	1	1	-1	-1	1	1	140	68
F6	1	-1	1	-1	1	-1	-1	1	270	86
F7	-1	1	1	-1	-1	1	-1	1	161	58
F8	1	1	1	1	1	1	1	1	180	82

Step 2: In the next step the coefficients for the polynomial equation were calculated. The coefficients for the polynomial equation were calculated as:

$$\beta_0 = \frac{\sum XY}{2^n}$$

Where, X was the total column value (+1) and Y was the response (Y1 or Y2).

$$\beta_1 = \frac{\sum X_1 Y_1}{2^n},$$

Where, X1 was the value of the column X1 and Y1 was particle size. All other coefficients were calculated in the similar manner. The polynomial equation obtained for Y1 was-

Y1 = 250.12 + 32.38X1 + 2.62X2 – 62.38X3 – 5.12X1X2 – 19.88X2X3 + 4.88X1X3 – 22.62X1X2X3

ISSN: 0975-8585

The polynomial equation obtained for Y2 was-

$$Y2 = 72.25 + 11.5X1 - 5.5X2 + 1.25X3 + 2.75X1X2 + 2.0X2X3 - 1.0X1X3 - 1.25X1X2X3$$

Table V: Values of X3 for different values of X1 when X2 = 0 for output variable Y1

X1/Y1	125	150	175	200	225	250	275
-1	1.04	0.76	0.48	0.2	-0.08	-0.36	-0.69
-0.75	1.14	0.86	0.58	0.29	0.01	-0.27	-0.56
-0.5	1.25	0.96	0.68	0.39	0.1	-0.18	-0.47
-0.25	1.36	1.07	0.78	0.49	0.2	-0.09	-0.38
0	1.48	1.18	0.89	0.59	0.3	0	-0.29
0.25	1.6	1.3	1	0.7	0.4	0.1	-0.2
0.5	1.72	1.42	1.11	0.81	0.5	0.2	-0.11
0.75	1.85	1.54	1.23	0.92	0.61	0.3	-0.01
1	1.98	1.66	1.35	1.03	0.72	0.41	0.09

Table VI gives values of X 3 for assumed values of X1 ranging from -1 to 1 for a range of values of Y2 i.e. % entrapment efficiency.

X1/Y2	80	81	82	83	84	85	86
-1	8.56	9	9.44	9.89	10.33	10.78	11.22
-0.75	8.19	8.69	9.19	9.69	10.19	10.69	11.19
-0.5	7.71	8.29	8.86	9.43	10	10.57	11.14
-0.25	7.08	7.75	8.42	9.08	9.75	10.42	11.08
0	6.2	7	7.8	8.6	9.4	10.2	11
0.25	4.88	5.88	6.88	7.88	8.88	9.88	10.88
0.5	2.67	4	5.33	6.67	8	9.33	10.67
0.75	-1.75	0.25	2.25	4.25	6.25	8.35	10.25
1	15	-11	-7	-3	1	5	9

Table VII: Results for the optimized batch of solid lipid nanoparticles

Experiment mgal no.	Drug(%)	Glyceryl Monostearate	Surfactant(%) Span 60	Co-surfactant(%)	Sonication Time(min)	Y1 Particle size(nm)		Y2 % entrapment efficiency	
						Experimental	Predicted	Experimental	Predicted
F9	10	100	25	25	15	191	200	81.4	84

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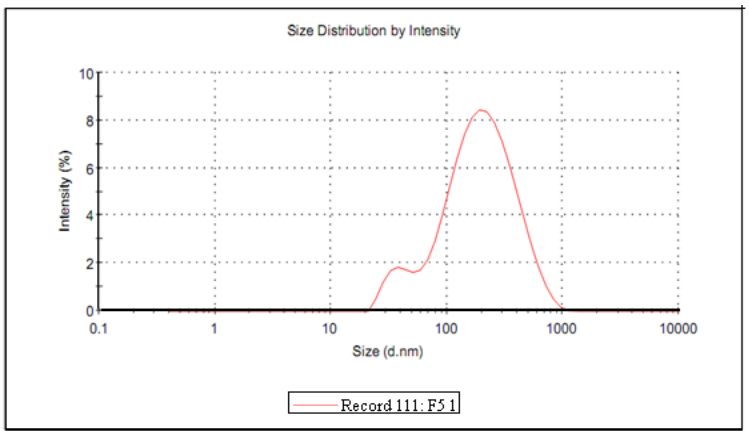

Figure 3: Particle size distribution curve of optimized batch of solid lipid nanoparticles

Particle morphology

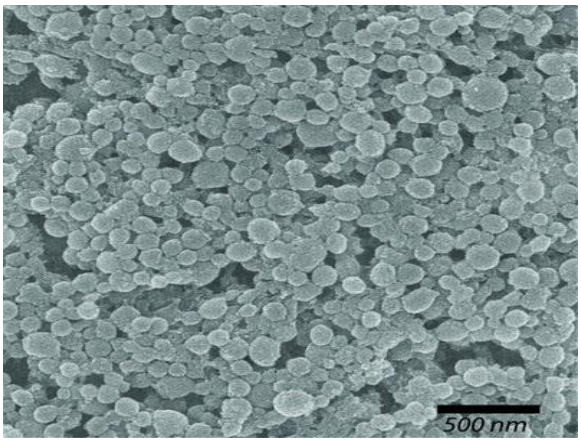

Figure 4: SEM image of solid simvastatin solid lipid nanoparticles

The SEM images revealed that the particle size was in nanometric range (≤400 nm) and the particles had spherical morphology.

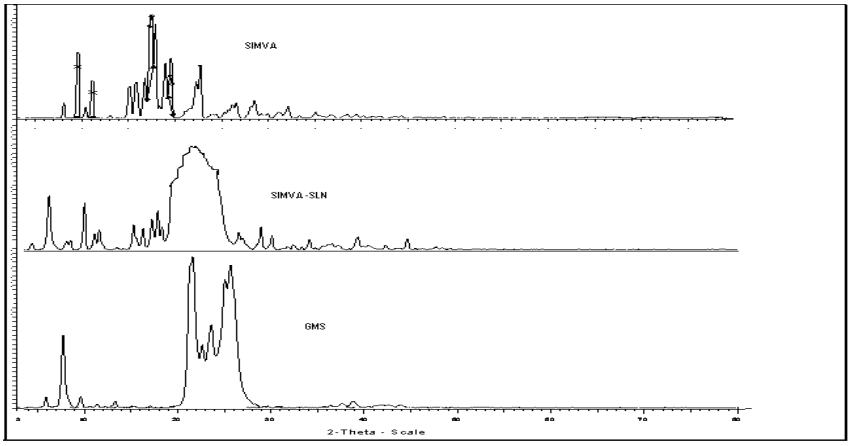

Figure 5: X-ray diffractograms of (a) SIMVA, (b) GMS, (c) SIMVA-SLN.

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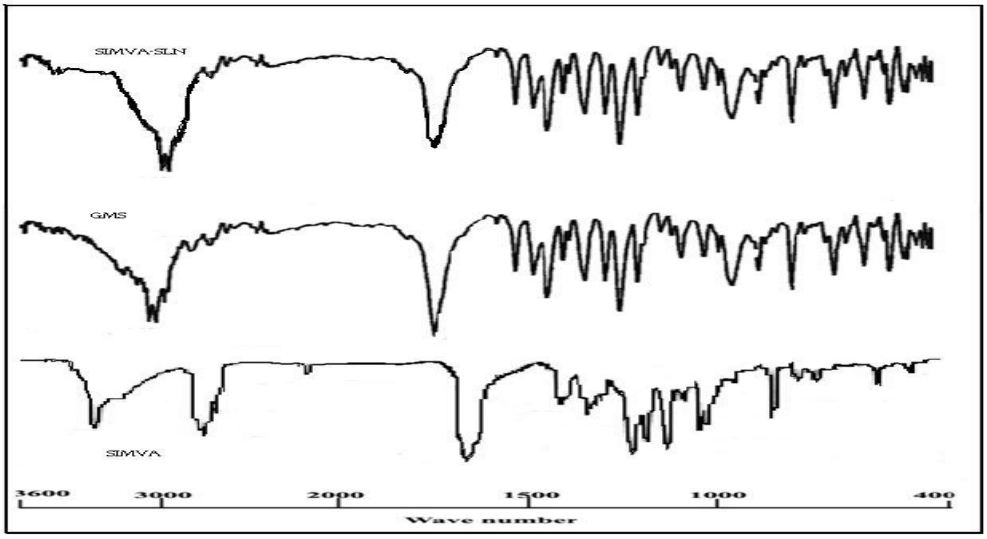

Figure 6: Fourier transforms infrared spectra of (a) SIMVA, (b) GMS, (c) SIMVA-SLN.

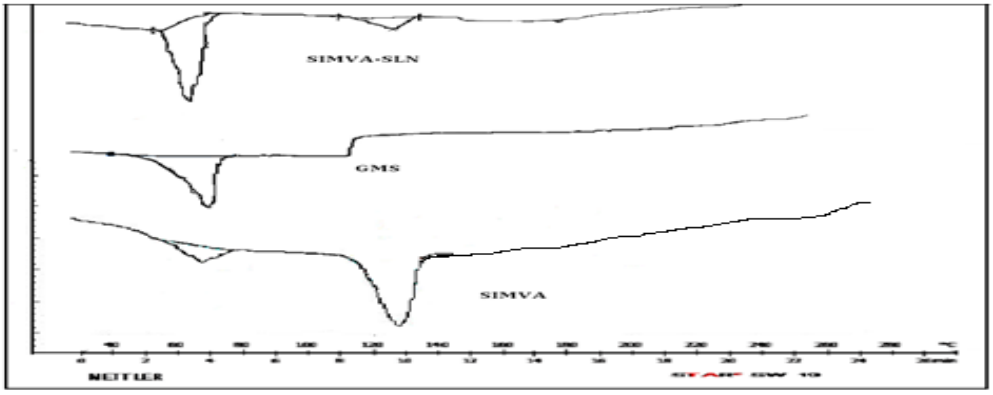

Figure 7: DSC thermograms of (a) SIMVA-SLN, (b) GMS, (c) SIMVA

The bulk material melts between 57.8-63.0˚C with the melting point at 61.2˚ C (**Figure 7**). When cooling the molten lipid down to room temperature, it crystallises between 52.2-49.8˚C with peak at 51.6˚C. A shoulder of the cooling curve at lower temperature indicates the existence of an unstable α- modification. For pure monoglycerides an alpha modification melting at around 50˚C has been reported. Reheating of the lipid leads to an almost identical heating curve with melting peak at 56.6˚C from the high content of monoglycerides (>90%). It can be concluded that glyceryl monostearate as bulk material crystallizes in the beta stable modification. The crystallization behaviour of GMS-SLN differs distinctly from the pure lipid. The cooling scan showed main peak at approx.at 49.0˚C with additional shoulder at 50.0˚C,which has also been found in physical mixture. The main peak could be attributed to β modification and the peak at 50.0˚C to α modification.

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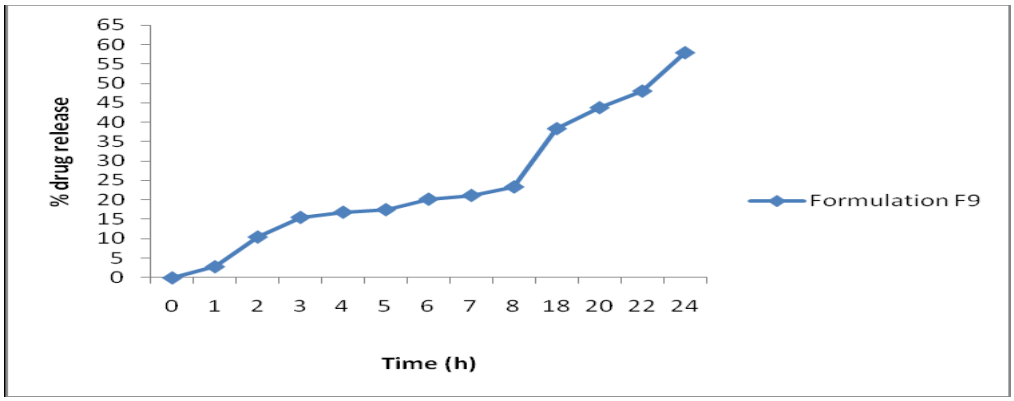

Figure 8: Drug release profile of optimized formulation

Table VIII: *In-Vitro* drug release parameters of optimized formulations

Time(h)	% drug release
Formulation F9	
0	0
1	2.86
2	10.51
3	15.54
4	16.88
5	17.53
6	20.23
7	21.19
8	23.38
18	38.35
20	43.74
22	48
24	57.86

Table IX: *In-Vitro* dissolution study of plain drug and SIMVA-SLNs capsule formulation

Time(h)	% drug release	
	SIMVA-SLN's	Plain drug
1	9.22	10.75
2	11.44	12.37
3	11.73	13.45
4	16.26	17.89
5	18.40	18.73
6	20.03	21.35
7	23.15	25.64
8	25.92	27.05
16	42.09	55.26
24	64.04	78.09

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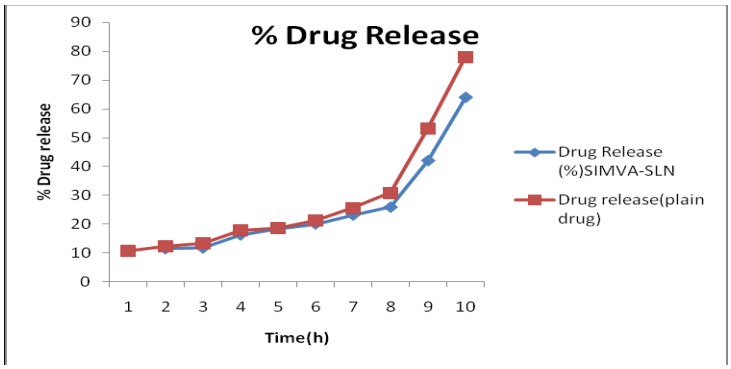

Figure 9: In-vitro dissolution profile of plain drug and SIMVA-SLNs capsule formulation

CONCLUSION

It can be concluded that solid lipid nanoparticles provide controlled release of drug and these systems are used as drug carriers for lipophilic drugs, to enhance bioavailability of poorly water-soluble drugs through nanoparticles as a drug delivery system. Thus, There is need to be further proved by in vivo study in humans/animals. Further the formulation can be subjected for the stability studies as per ICH guidelines. In vitro release experiments exhibited a biphasic release pattern with the burst release at the initial phase followed by sustained release. This system is more suitable for expoiting the lymphatic transport pathway for improving the oral bioavailability of simvastatin.

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