DESIGN AND OPTIMIZATION OF SIMVASTATIN LOADED SOLID LIPID NANOPARTICLES USING FULL FACTORIAL DESIGN

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ABSTRACT

In the present study solid lipid nanoparticles (SLNs) of simvastatin were formulated using glyceryl monostearate and stearic acid in the ratio of 1:1 by applying 3^2 factorial design. The SLNs were optimized to check the effect of melted lipid and surfactant concentration on particle size and entrapment efficiency. A total of 12 formulations were prepared and characterization parameters were studied. The optimized formulation was selected by studying the interaction between the factors using polynomial equations and 3D response plots. Particle size and percentage entrapment efficiency of optimized formulation were found 185.7 nm and 82.53, respectively. A higher drug release was obtained which best fitted to first-order kinetics. Finally it was concluded that glyceryl monostearate and stearic acid in combination helps in improving the quality of simvastatin loaded SLNs.

Keywords: Simvastatin, solid lipid nanoparticles, bioavailability enhancement

INTRODUCTION

Nowadays, most of the population worldwide mainly depends upon the targeted drug delivery systems and there is an urgent need for new strategies for drug delivery of medicines. In the drug delivery system of medicine, patients require higher systemic administration of drugs, but non-specific bio-distribution and rapid metabolism of active drug molecules lead to the less systemic effect. Advancement in monotherapy and nanoencapsulation system has made it possible to target human and animal diseases accurately at their target site and prevent the side effects. The nanoencapsulation system of drugs mainly depends upon the ingredient's rigidity, stability, releasing properties and capability to encapsulate materials with different solubilities[1]. Solubility is the most important reason for decreasing the bioavailability and pharmacological action of the drugs. Solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLC), nanoemulsions, polymeric nanoparticles, metallic nanoparticles and liposomes are the major attempts that have been made to overcome the problems related to solubility, bioavailability and targeted drug release. But some nanoparticles such as metal nanoparticles have some disadvantages like slow degradation in body and toxicity to cells on long term use[2,3]. Solid lipid nanoparticles (SLNs) have become the most promising materials for the effective targeted delivery of different drugs and for their great potential in pharmaceutical research. These are much compatible to biological system when compared to polymeric nanoparticles[4]. SLNs have proved as a propitious system for the delivery of lipophilic as well as hydrophilic drugs. These sub-microns possess anti-hyperlipidemic activity, which was assessed in the hyperlipidemia rat model[5]. SLNs are sub-micron colloidal carriers having a size range of 10 to 1000 nm, composed of lipid dispersed in water or in aqueous surfactant solution[6]. The important parameters which should be evaluated for the SLNs are particle size, charge determination, surface hydrophobicity, entrapment efficiency, drug interaction, *in vitro* drug release, crystallinity of lipid, and drug stability[7].

Simvastatin is an anti-hyperlipidemic drug that belongs to BCS class II which lowers blood cholesterol levels and has lower oral bioavailability when given orally, as it undergoes extensive hepatic first-pass metabolism[8]. It has gained much attention in bone regeneration due to its possible osteoanabolic effect[9]. The lipophilic nature of the drug necessitates the development of an efficient lipid-based drug delivery system which can improve its solubility as well as oral bioavailability[10]. According to a study, PEG-*b*-$PBLG_{50}$ nanoparticles increase the *in vivo* circulation of simvastatin by avoiding its liver phagocytosis[11]. The aim

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of our study was to develop solid lipid nanoparticles of simvastatin using 3^2 factorial design with the help of two independent variables (concentration of lipid melt and surfactant) to obtain small particle size, high entrapment efficiency and improved drug release quality. Two lipids, namely, glyceryl monostearate and stearic acid, were used to improve the entrapment efficiency and drug release of simvastatin.

METHODOLOGY

Materials

Simvastatin was obtained from IPCA Labs Ltd., Mumbai, India. Glyceryl monostearate and stearic acid were procured from Central Drug House (P) Ltd., New Delhi, and Poloxamer 407 was from Sigma Aldrich Ltd., India.

Preliminary studies

Several lipids like fatty acids, mono, di and triglycerides, and waxes are used in the SLN preparation. Glyceryl monostearate (GMS) has some good affinity for simvastatin. In preliminary studies, the addition of stearic acid in GMS showed an increase in percentage entrapment efficiency with an only slight increase in the particle size (data is not shown). To achieve desired particle size with maximum entrapment efficiency, glyceryl monostearate and stearic acid were used in the ratio of 1:1 as lipid melt. Poloxamer 407, which helps to increase the entrapment efficiency, decreases the particle size and prolongs the drug release[12], and was selected as a surfactant for the preparation of solid lipid nanoparticles.

Formulation of simvastatin loaded SLN

Method of preparation

Solid lipid nanoparticles were prepared by the ultrasonic melt emulsification technique with slight modifications[13]. Specified amount of lipid was melted at the temperature of about 10 °C above its melting point on a water bath. Then, the drug was dissolved into melted hot lipid. Simultaneously, the aqueous solution of a specified amount of surfactant was prepared in 20 mL of distilled water and heated up to the same temperature. When both phases reached the same temperature, the aqueous phase was added to the lipid phase with continuous stirring at 400 rpm for 30 min on the magnetic stirrer. Then, the hot emulsion was sonicated for 4 min using a probe sonicator at 40 % voltage efficiency to get the SLNs. SLNs were filtered by using a membrane filter of 0.22 µm and stored at 2-8 °C after sonication[14].

Design of experiment

Based on the preliminary studies, GMS and stearic acid in the ratio of 1:1 were selected as lipid melt and Poloxamer 407 was selected as the surfactant. The effect of two independent variables, namely, lipid melt concentration (X_1) and surfactant concentration (X_2), was studied on particle size (Y_1) and entrapment efficiency (Y_2) using 3^2 factorial design. The 3^2 factorial design along with its factors and levels is mentioned in Table I.

Statistical analysis

The interaction between the factors and their effect on particle size and entrapment efficiency was considered for the optimization. Analysis of variance (ANOVA) was used for the statistical analysis. The P-value of ANOVA gives the direct effect of independent variables on dependent variables. The P-values < 0.05 indicates that model terms are significant and can be considered for the polynomial model. The 3D graphs were constructed to depict the effect of independent variables on particle size and percentage entrapment efficiency.

Table I: 3^2 factorial design for simvastatin SLN

Run	X_1	X_2
1	0	0
2	0	-1
3	1	-1
4	-1	1
5	0	1
6	-1	-1
7	0	0
8	-1	0
9	1	0
10	0	0
11	0	0
12	1	1

X_1= concentration of lipid melt (-1= 200, 0= 400, +1= 600)

X_2= concentration of surfactant (-1= 2%, 0= 4%, +1= 6%)

Evaluation and characterization of simvastatin loaded SLN

Particle size and zeta potential analysis

The particle size, polydispersity index, and zeta potential of solid lipid nanoparticles were determined

after appropriate dilution by using particle size analyzer (Malvern Zetasizer).

Entrapment efficiency

The solid lipid nanoparticle dispersion was centrifuged at 10000 rpm at 10 °C for 60 min. The supernatant was separated and analyzed after proper dilution for free simvastatin at 239 nm spectrophotometrically. The drug entrapped in solid lipid nanoparticles was calculated by using the formula given below[13].

$$(\%)\ \text{Entrapment efficiency} = \frac{\text{Amount of drug added- Amount of drug in the supernatant}}{\text{Amount of drug added}} \times 100$$

Characterization of the optimized batch

Fourier transformation infrared analysis

FTIR spectroscopy of drug, lipids, and selected batch was performed. Disappearance or appearance of the characteristic absorption bands for drugs after incorporation into lipids was investigated by comparing the absorption bands in the FTIR spectra. The sample was mixed with KBr to form a pellet and scanned by using the FTIR spectrophotometer in 4,000–500 cm^{-1} range[15].

Differential scanning calorimetric analysis

The physical state of the drug inside the solid lipid nanoparticles was investigated by DSC. The DSC thermogram of glyceryl monostearate, stearic acid, simvastatin, and the selected batch was obtained by using differential scanning calorimeter (Perkin-Elmer, USA). The selected batch was freeze-dried for solidification before DSC analysis. The sample was sealed in an aluminum pan and heated in the range of 25-300 °C at a heating rate of 10 °C min^{-1} under nitrogen atmospher[16].

Transmission electron microscopy

The morphology of the optimized batch of SLN was examined by using an electronic transmission microscope (TECNAI 200 Kv TEM. Fei, Electron Optics). A drop of the sample was placed on the carbon grid for negative staining. Excess of fluid was drained off and the carbon grid was air-dried and then observed under TEM[17].

In vitro drug release studies

The *in vitro* drug release studies of solid lipid nanoparticles were performed by using the dialysis bag technique. Dialysis membrane of pore size 2.4 nm and molecular weight cut off 12,000 was procured from HiMedia, Mumbai. Before using, the membrane was kept in distilled water for 12 h. Solid lipid nanoparticles equivalent to 5 mg simvastatin were filled in the dialysis membrane and sealed with dialysis clips from both sides. This was tied using a thread in a flask containing 100 mL of phosphate buffer pH 6.8 with 0.03 % sodium lauryl sulphate[18,19]. The flask was kept on the magnetic stirrer by maintaining stirring speed at 50 rpm and temperature at 37±0.5 °C. Sample aliquots of 2 mL were withdrawn at specific intervals from the flask and for maintaining

Table II: Characterization parameters of all formulations

Formulation code	Average diameter (nm) *(Y_1)*	(%)Entrapment efficiency ± SD *(Y_2)*	Polydispersity index	Zeta potential (mV)
F_1	184.9	82.18 ± 3.12	0.147	-24.4
F_2	260	71.38 ± 2.24	0.153	-20.0
F_3	378.6	84.98 ±2.54	0.513	-25.0
F_4	269.8	68.53 ±3.22	0.149	-24.2
F_5	234.6	70.82 ±3.73	0.138	-25.2
F_6	*273.8*	*66.09* ± 2.06	0.241	-18.1
F_7	186.9	82.97 ±2.38	0.181	-23.1
F_8	236.6	62.37 ±2.51	0.456	-17.8
F_9	298.7	79.28 ±3.11	0.412	-25.2
F_{10}	185.7	82.53 ±2.16	0.182	-14.8
F_{11}	185.3	81.98 ±3.27	0.145	-21.0
F_{12}	286.8	77.32 ±2.95	0.154	-20.2

the sink conditions equal volume of buffer solution was added to the flask. All the samples were analyzed by using a UV-visible spectrophotometer at 239 nm against the suitable blank. Data obtained from the *in vitro* drug release studies of the selected batch was fitted to various kinetic equations such as zero-order (% release vs. t), first-order (log % release vs. t), Higuchi's model (% release vs. $\sqrt{t}$) and Korsemeyer-Peppas model (ln Q vs. ln t)[20].

Stability study

The stability study of the powdered sample obtained by freeze-drying of optimized formulation was performed according to ICH guidelines . The physical appearance and entrapment efficiency of optimized formulation were determined at two atmospheric conditions 25 ± 2 °C/60 ± 5 % RH and 40 ± 2 °C/75 ± 5 % RH at specific time intervals (0, 15, 30, 60, 120 and180 days of storage) for six months[21].

RESULTS AND DISCUSSION

Statistical analysis

Two independent variables, the concentration of lipid melt (X_1) and concentration of surfactant (X_2), were studied by using 3^2 factorial design. Particle size and percentage entrapment efficiency were selected as dependent variables. Various experiments were conducted by using X_1 and X_2 at various levels to get the particle size and entrapment efficiency of formulations. The polynomial equations were obtained by regression analysis of entrapment efficiency and particle size. The Dependent variables such as particle size (Y_1) and percent entrapment efficiency (Y_2) showed a wide variation, as shown in Table II.

Particle size

The particle size of the formulations was found to be in the range of 184.9 to 378.6 nm (Table II). The polynomial equation for a full model of particle size including all the significant terms ($p < 0.05$) is:

$$Y = 190.20 + 30.65\ X_1 - 20.20\ X_2 - 21.95\ X_1X_2 + 68.45\ X_1^2 + 48.10\ X_2^2$$

The model was significant with F-value of 68.62. All the model terms were significant with P-values < 0.05 and the signal to noise ratio was found to be 25.581, which represents an adequate signal. The predicted R^2 of 0.8681 is in reasonable agreement with the adjusted R^2 of 0.9685. X_1^2 showed the highest coefficient value, which indicates that it affects the particle size maximum. The value of X_2 was also found to be significant and represented that the particle size decreases with an increase in surfactant concentration, which might be due to the fact that the surfactant decreases surface tension at the interface of particles and thus increases the surface area and decreases the particle size[15]. Besides, the surfactant prevents particle aggregation and helps to stabilize the formulation. The increase in the concentration of lipid melt increases the viscosity of dispersion and thus increases the particle size[22]. The 3D graphs plotted for the effect of independent variables on particle size are shown in Fig. 1. The particle size of the optimized formulation shown in Fig. 2.

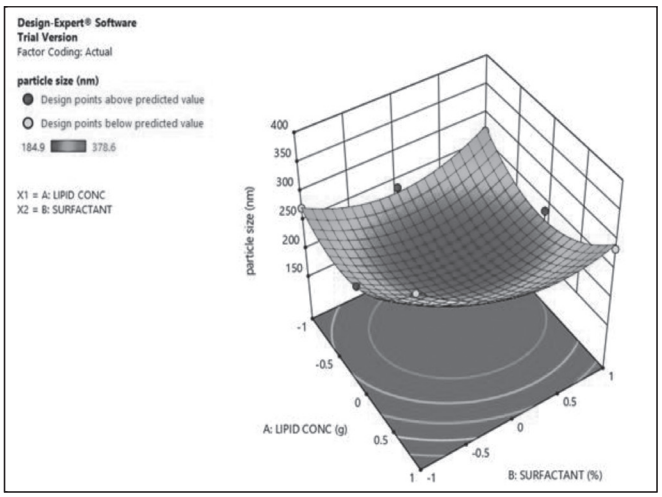

Fig. 1: 3D plot for particle size

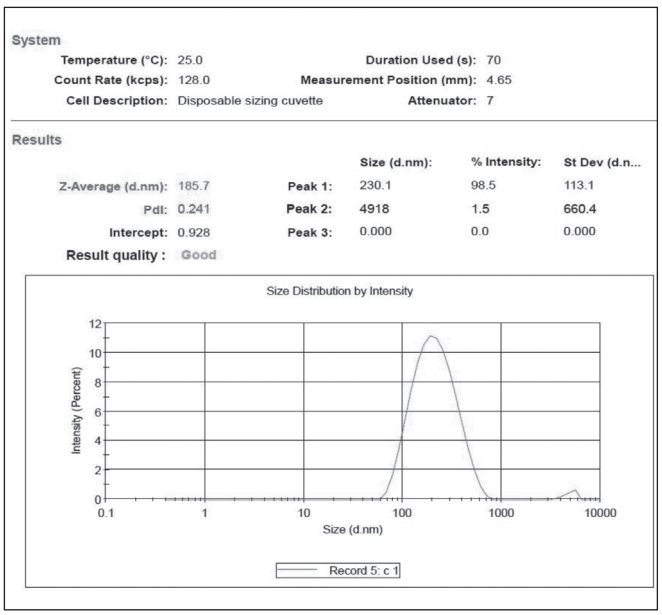

Fig. 2: Particle size of optimized formulation

Entrapment efficiency

The entrapment efficiency was in the range of 62.37 % to 84.98 % (Table II). The polynomial equation for full model of entrapment efficiency including all the significant terms was:

$$Y = 75.87 + 7.43\ X_1 - 0.9633\ X_2$$

The model was found significant with F-value of 4.90. P-value< 0.05 indicated that all the model terms were significant. The difference between predicted R^2 and adjusted R^2 was less than 0.2, with predicted R^2 of 0.2379 and adjusted R^2 of 0.4152. The signal to noise ratio was found to be 5.730, which shows an adequate signal. In the equation, the value of X_1 showed that the more is the amount of lipid the more will be its entrapment efficiency. This may be due to the combination of lipids used which provide enough space to accommodate drug molecules[23]. But on further increase in lipid melt, agglomeration could have led to a slight increase in entrapment efficiency. The optimum entrapment efficiency was achieved by utilizing less raw material, this may be due to the combination of lipids used. The value of X_2 shows that on increasing the concentration of Poloxamer 407, the entrapment efficiency decreases. This may be due to the well-known fact that a large amount of surfactant cannot be absorbed by the SLN and results in the formation of micelles which may also solubilize the drug particles, leading to less entrapment efficiency[24]. The 3D graphs plotted for the effect of independent variables on entrapment efficiency are shown in Fig. 3. The formulation F_{10} with optimum particle size and entrapment efficiency was selected as optimized.

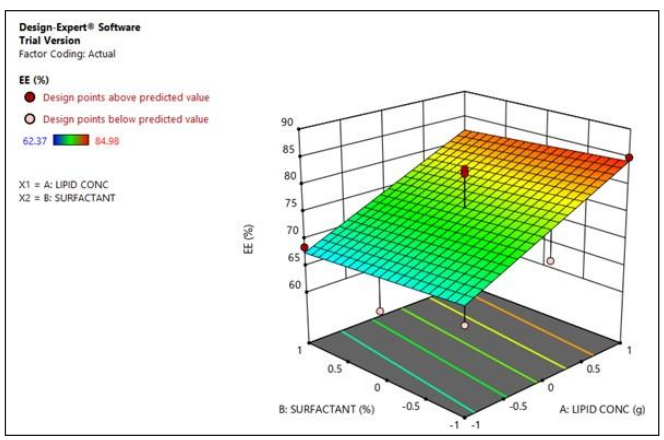

Fig. 3: 3D plot for entrapment efficiency

Zeta potential and polydispersity index

Polydispersity index was in the acceptable range of 0 to 0.5, showing narrow size distribution. Formulations showed negative zeta potential since SLNs have a negative charge on their surface and therefore have sufficient electrostatic repulsion, which prevents aggregation of particles. The zeta potential and polydispersity index of different batches are shown in Table II.

Characterization of optimized batch

The formulation F_{10} was found with the desired values for entrapment, particle size and was observed suitable from the evaluation results and selected for further characterization.

Fourier transformation infrared analysis

FTIR spectra of drug, excipients, and formulation are given below in Fig. 4. The changes were observed in the peaks obtained in the FTIR spectra of F10 for the absorption bands of drugs. The reason might be the transformation of the drug from crystalline to an amorphous state.

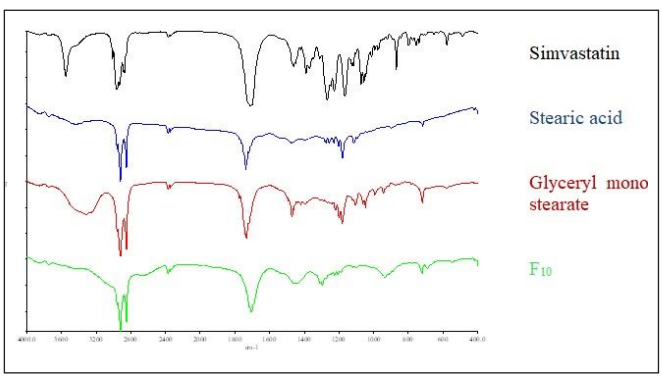

Fig. 4: Overlay of FTIR spectra of drug and lipids

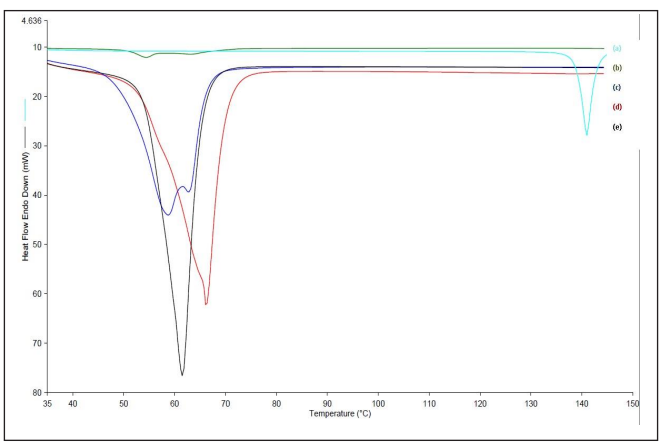

Fig. 5: DSC analysis of (a) Drug (b) Selected batch (c) Physical mixture (d) Glyceryl monostearate (e) Stearic acid

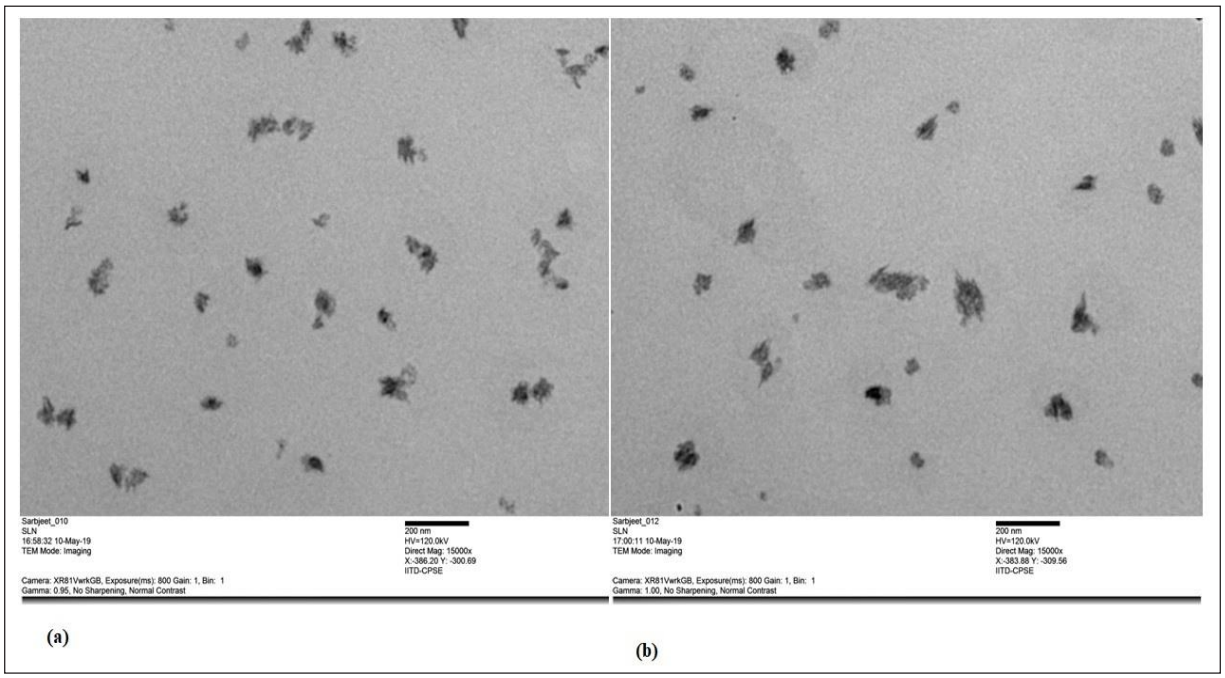

Fig. 6: Transmission electron microscopy images of selected batch

Differential scanning calorimetric analysis

The DSC thermograms of selected batch showed the shifting of the melting peak of simvastatin from 141 °C to 60 °C, as shown in Fig. 5. The absence of peak of simvastatin indicate that the crystalline drug changes to amorphous form and the drug is fully entrapped in the lipid matrix and participated to produced stable SLN. The small particle size of solid lipid nanoparticles and the presence of surfactant may be the reason behind shifting of peaks of lipids. This effect when particles in mixture starts melting at temperature lower than their melting point is known as Kelvin effect[18]. Melting enthalpy drastically depressed in formulations. This might be due to less ordered lattice arrangement in formulations, which requires less energy than the crystalline form in melting. The decrease in enthalpy may results from the change of drug from crystalline form to amorphous form[25].

Transmission electron microscopy

Transmission electron microscopy (TEM) study revealed that simvastatin loaded SLNs were nano-sized and mono dispersed on the surface and contain a dense lipid matrix without aggregation (Fig. 6). It was confirmed that particle size of solid lipid nanoparticles was in desirable range.

In vitro drug release studies

The percent cumulative drug release profiles of optimized formulation and plain drug suspension are given in Fig. 7. The percent cumulative drug release for 12 h was studied. The optimized batch showed biphasic drug release with a high burst release in the first hour followed by sustained release[26]. The higher release was observed due to glyceryl monostearate and stearic acid. The glyceryl monostearate and stearic acid are triglycerides with short chains, which transform extremely fast from the less stable form (α-form) to the more stable form (β-form), further enhancing the drug release. Also, the presence of a surfactant increases the surface area, thus the dissolution of the drug was increased. The correlation coefficient was determined for kinetics models. The values of R^2 shown in Table III indicate that drug release characteristics in SLNs were best fitted to first-order kinetics (Fig. 8). This indicated that the drug release from the solid lipid nanoparticles is dependent on the concentration of nanoparticles[27].

Table III: R^2 value indicated the drug release characterstics for various models

S. No.	Model	R^2
1.	Zero order	0.9679
2.	First order	0.9942
3.	Higuchi equation	0.9926
4.	Korsemeyer Peppas equation	0.9859

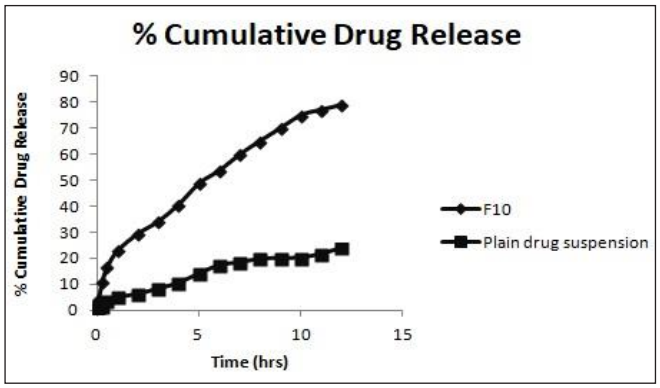

Fig. 7: Drug release profile of optimized formulation and plain drug suspension

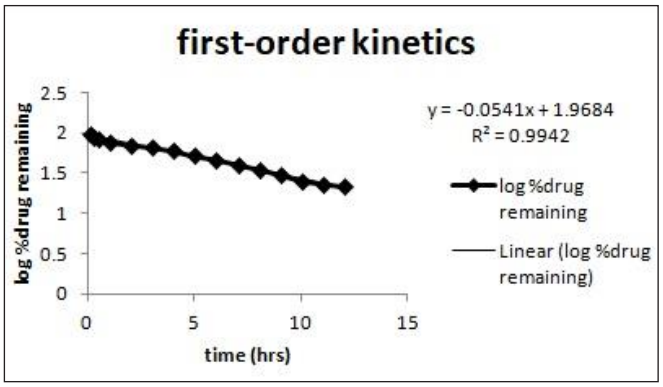

Fig. 8: Drug release kinetics by first-order kinetics

Table IV: Stability study of optimized formulation

		25°C ± 2°C/60% ± 5% RH		40°C ± 2°C/75% ± 5% RH	
Sr. no.	**Time (Days)**	**Physical appearance**	**Entrapment efficiency ± SD (%)**	**Physical appearance**	**Entrapment efficiency ± SD (%)**
1	0	White free flowing powder throughout the study	82.53 ±2.16	White free flowing powder throughout the study	82.53 ±2.16
2	15		82.35 ±2.35		81.96 ±3.19
3	30		81.63 ±1.66		81.21 ±1.57
4	60		81.39 ±2.74		80.55 ±2.38
5	120		81.18 ±3.21		79.78 ±1.89
6	180		80.87 ±2.13		79.16 ±3.46

Stability study

As shown in Table IV, entrapment efficiency was found to be reduced at 40 °C ± 2 °C/75 % ± 5% RH while no change was observed in the physical appearance. The reduction in drug release was observed at accelerated conditions compared to formulation kept at room conditions (data is not shown). This may be due to the degradation of drugs at 40 °C ± 2 °C/75 % ± 5 % RH which indicates that accelerated temperature is not suitable storage condition for solid lipid nanoparticles[28]. Overall, the better condition for SLN storage is the room condition (25±2°C/60 ±5% RH) for a longer period.

CONCLUSION

The solid lipid nanoparticles were successfully prepared by ultrasonic melt emulsification technique with improved quality parameters. The preliminary studies revealed that the addition of stearic acid in GMS, showed maximum entrapment efficiency with a slight change in the particle size, so glyceryl monostearate and stearic acid were used in ratio 1:1 as lipid melt for the preparation of SLN. The interaction between the factors was studied using full 3^2 factorial design of prepared SLN. The increase in the particle size was observed with increased amount of lipid and decreased amount of surfactant. The increasing concentration of lipid melt increase the % EE up to a certain level, on further increase of amount of lipid only a slight increase was observed in entrapment efficiency. Minimum raw material was used to achieve the optimum entrapment efficiency which may be due to the combination of lipids used. Also the higher drug release was observed due to the combining effect of both the lipids. DSC analysis studies of selected formulation showed that drug was efficiently entrapped in lipid matrix. Transmission electron microscopy displayed solid lipid nanoparticles with almost spherical shape with smooth morphology and being mono dispersed. The accelerated stability studies revealed that the degradation of drug only occurs at 40 °C ± 2 °C/75% ± 5%. It could be concluded from the present investigation that there was valuable combining effect of glyceryl monostearate and stearic acid to increase the entrapment efficiency and *in vitro* drug release and overall helped in improving the quality of simvastatin loaded SLNs. The potential use of the developed SLN for a better effective management of hyperlipidemia may be further explored with the help of long term pharmacokinetic and pharmacodynamic studies.

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