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Simvastatin loaded Solid lipid nanoparticles: Formulation optimization using Box Behnken design, characterization and in vitro evaluation.

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Abstract
This study describes a Box-behnken design to optimize the formulation of simvastatin (SIMVA) loaded solid lipid nanoparticles by pre-emulsion ultrasonication technique. The variables drug: lipid ratio, percentage of lipid phase surfactant and sonication time were studied at three levels and arranged in a Box-behnken design, to study the influence on response variables particle size and % entrapment efficiency (%EE). From the statistical analysis of data, polynomial equations were generated. The physical characteristics of SIMVA-SLN were evaluated using particle size analyzer, differential scanning calorimetry and X-ray diffraction. The results of optimized formulation showed average particle size of 245 nm and a drug entrapment of 72.52 %. The *in-vitro* drug release study of SIMVA-SLN using modified Franz diffusion cell showed significantly low release of simvastatin (37.08%) than dispersion of pure drug (97.2 %).

Key Words
Solid lipid nanoparticles, simvastatin, Box- Behnken design.

Introduction
Simvastatin is an antihyperlipidemic drug with poor oral bioavailability (<5%) due to the first pass metabolism[1]. Possible methods to avoid first pass metabolism include transdermal, buccal, rectal and parenteral routes of administration. Oral route is the most commonly used and preferred route of choice for the delivery of drugs, although several factors like pH of GIT, residence time and solubility can affect drug administration by this route. Lymphatic delivery is an alternative choice to avoid first pass metabolism in per oral drug delivery[2]. Enhanced lymphatic transport of drugs reduces the hepatic first-pass metabolism and improves bioavailability, because intestinal lymph vessels drain directly into thoracic duct, further in to the venous blood, thus bypassing the portal circulation[3]. The main function of the lymphatic system is to facilitate absorption of long chain fatty acids via chylomicron formation. 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[4].

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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[5-8]. Aqueous SLN dispersions are composed of a lipid which is solid both at body and room temperature, being stabilized by a suitable surfactant. With regard to developing commercial products for the therapy, SLN possess distinct advantages compared to other carriers, e.g., polymeric nanoparticles. Especially for topical and oral administration, all lipids can be used as matrix material, which are currently in use for creams, ointments, tablets, and capsule formulations including the long list of different surfactants/stabilizers employed in these traditional formulations. Thus, there is no problem with the regulatory accepted status of excipients [9-11]. SLN also enjoy more advantages over other colloidal delivery systems with regard to biocompatibility and scale up[12], also the release of drugs from SLN can be modulated in order to optimize their blood levels[13]. These features together make lipid nanoparticles an interesting carrier system for optimized oral delivery of drugs. Reports on the use of SLN for avoiding first pass metabolism of drugs are scanty. Various researchers have studied the gastro-intestinal uptake and transport to lymphatic circulation of labeled SLNs[14], tobramycin[15], clozapine[16] after duodenal

administration to rats. The objective of this study was to develop a mathematical model using Box-Behnken design in order to deduce the adequate conditions to prepare SLN of desired characteristics. Response surface methodology combined with Box–Behnken design (BBD) was used to ascribe the relationship between the independent variables and the responses. A three-factor, three-level BBD with three replicates at the center point was selected to build response surface models.

Material and Methods

Materials

Compritol 888 ATO was obtained as gift sample from Gattefosse (France). Tween 80, Span 60, glyceryl monostearate (GMS) and poloxamer 188 were provided by LOBA CHEMIE. Simvatsatin was gifted by Glenmark, Nasik. All other chemicals were of reagent grade and used without further purification.

Preparation of SLN

SLN were prepared by pre-emulsion followed by ultrasonication method[17]. Briefly, lipid phase consisted of simvastatin, Compritol 888 ATO and Span 60 maintained at 70 °C. An aqueous phase was prepared by dissolving poloxamer 188 (2% w/v) in distilled water (sufficient to produce 50 ml of preparation) and heated to same temperature as of oil phase. Hot aqueous phase was added to oil phase and homogenization was carried out at 70°C using Omni TH homogenizer (Make Omni USA) at 35000 rpm for 3 min. Coarse hot 'oil in water emulsion' so obtained was subjected to further size reduction using ultrasonicator (make Sonic VCX 750) for 10-30 min.

Experimental design and Statistical analysis

In simultaneous optimization, the experimentation is completed before the optimization takes place. In simultaneous methods, usually called as response surface methodology (RSM), one or more selected experimental responses are recorded for a set of experiments, carried out in a systematic way, to predict the optimum and the interaction effects[18-21]. In this study, a Box- Behnken design was introduced to optimize the formulation of solid lipid nanoparticles. Initial studies were undertaken to decide the excipients and their levels in the experimental design. The choice of lipid was done on the basis of solubility and partitioning of simvastatin in the lipid. Aqueous phase surfactant and lipid phase surfactant were selected on the basis of stability of dispersion prepared by using different surfactants. Three factors, the drug: lipid ratio (X_1), surfactant (lipid phase) concentration (X_2) and sonication time (X_3) were used in the design and the responses were the average particle size (PS) (Y_1) and % Entrapment Efficiency (EE) (Y_2). These three factors that might affect the designed characteristic of nanoparticle formulation were varied over three levels (Table 1) and arranged according to a Box- Behnken experimental design (Table 2).

Evaluation of solid lipid nanoparticles

Particle size analysis

The particle size analysis of the selected formulation was performed using Malvern Mastersizer 2000 MS (Malvern Instruments, Worcestershire, UK) and laser diffraction with beam length 2.40 mm, range lens of 300 RF mm, and at 14.4% obscuration. The d (90) of each batch is recorded in Table 3.

Entrapment efficiency

For determination of entrapment, the SLNs were separated from free drug by Ultra-centrifugation (Beckmann Instruments) at about 25,000 rpm. In which lipid nanoparticles settled at base in pellet form while free drug remained in supernatant liquid. EE was calculated according to the following equation,

$$EE\% = \frac{\text{The amount of entrapped drug in SLN}}{\text{The amount of entrapped drug in SLN + free drug}} \times 100$$

Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) was performed by Mettler-Toledo DSC 821^e (Columbus, OH) instrument and an empty standard aluminum pan was used as reference. DSC scans were recorded at heating rate of 10°C/min in temperature range 30°-300°C. DSC measurements were carried out on pure compritol 888 ATO and simvastatin as bulk material and SLN loaded with simvastatin.

X-ray diffraction

X-ray scattering measurements were carried out with a Philips PAN analytical expert PRO X-ray diffractometer 1780. A Cu Ka radiation source was used, and the scanning rate (2h/min) was 5°C/min. X-ray diffraction measurements were carried out on

pure compritol 888 ATO and simvastatin as bulk material and SLN loaded with simvastatin.

FTIR Studies

A Jasco FTIR spectrophotometer (Perkin Elmer Jasco FTIR- 401, Japan) was used for infrared analysis of samples. About 1-2mg of sample was mixed with dry potassium bromide and the samples were examined at transmission mode over wave number range of 4000 to 400cm^{-1}. FTIR studies were carried out on pure compritol 888 ATO and simvastatin as bulk material and SLN loaded with simvastatin.

***In vitro* release of simvastatin from SLNs**

In vitro release studies were performed using modified Franz diffusion cell. Dialysis membrane having pore size 2.4 nm, molecular weight cut off 12,000–14,000 was used. Membrane was soaked in double-distilled water for 12 h before mounting on a Franz diffusion cell. A volume of 1ml of simvastatin loaded SLN formulation was placed in the donor compartment and the receptor compartment was filled with 10 ml of dialysis medium containing pH 7.0 buffer containing 0.5% sodium dodecyl sulphate in 0.01 M sodium phosphate solution. An aliquot of 0.1ml of sample was withdrawn using micropipette, from receiver compartment through side tube at time intervals of 0.5, 1, 2, 4, 6, 8, 12, 24 and 48 h. Fresh medium was replaced each time to maintain constant volume. Samples were analyzed by using UV-Visible spectrophotometer.

Results and Discussion

Experimental design and statistical analysis

On the basis of the results obtained in the preliminary screening studies, the compritol 888 ATO, Span 60 and Poloxamer 188 were chosen as lipid, lipid phase surfactant and aqueous phase surfactant respectively for further study. For three factors, the Box-Behnken design offers some advantage in requiring a fewer number of runs over the composite central, three level full factorial designs. In full factorial designs, as number of factors increase there is increase in number of trial runs exponentially, such as 3^3= 27, but with Box Behnken design optimization can be completed with 13 experiments. In order to investigate the factors systematically, a Box–Behnken design was employed for simvastatin nanoparticles. The particle size and EE for the 13 batches (R_1 to R_{13}) showed a wide variation 220-798 nm and 41.83- 77.6 % respectively (Table 3). The data clearly indicate that the results of response variables are strongly depend on the selected independent variables.

The coefficients of the polynomial equations generated using MLRA (Design expert 7.1) for particle size and %EE of SIMVA-SLN dispersion studied are listed in (Table 4) along with the values of r^2. Nine coefficients (*a* to *i)* were calculated with k as the intercept.

$$Y = k + aX_1 + bX_2 + cX_3 + dX_1X_2 + eX_1X_3 + fX_2X_3 + gX_1^2 + hX_2^2 + iX_3^2 \quad \ldots. (1)$$

The equation can be used to obtain estimates of the responses. Factor X_1 (drug: lipid ratio) has a positive effect on both the responses, PS and EE as indicated by the positive signs of coefficient *a* (+216.5 and +9.23 respectively) whereas factor X_2 (surfactant concentration) shows negative effect on particle size as shown by the negative sign of coefficient *b*. Factor X_3 (sonication time) did not show significant effect on both the responses. Similarly, effects of different interaction terms such as X_1X_2, X_1X_3 and X_2X_3 on response variables can be seen from the signs and values of *d*, *e* and *f* respectively. X_1^2, X_2^2, X_3^2 terms are second order terms and are useful to estimate non linearity of response. For particle size response, the Model F-value of 9.34 implies the model is not significant. There is only a 4.61% chance that a "Model F-Value" this large could occur due to noise. P value were found to be 0.0461, less than 0.0500 indicate model terms are significant. For %EE response, , the Model F-value of 27.04 implies the model is significant. There is only a 1.02% chance that a "Model F-Value" this large could occur due to noise. P value were found to be 0.0102, less than 0.0500 indicate model terms are significant. Since the values of r^2 are quite high for both the responses, i.e., 0.9656 for particle size and 0.9878 for %EE, the polynomial equations form excellent fit to the experimental data and are highly statistically valid. The criteria for selection of suitable feasible region (From the intensive grid search) were primarily based upon the highest possible values of % EE (>65%) and values of particle size which are less than 250 nm. Composition of optimized batches and comparison of the observed responses with that of the predicted responses along with percentage error is listed in Table 5. Three-dimensional response surface plots for response variables are presented in Fig.1 and Fig.2, which are very useful to study the interaction effects of the factors on the responses.

These types of plots are useful in study of the effects of two factors on the response at one time.

Evaluation of Solid Lipid Nanoparticles

Particle Size Analysis

The d (90) values for nanoparticulate dispersions determined using Malvern Mastersizer showed size raging from 220 – 798 nm (Table 3). The effect of lipid concentration on the particle size can be seen from particle size of sample R_3, R_7 and R_{13} (220 nm, 263 nm and 265 nm respectively) with less lipid concentrations (1:3 and 1:5). Surfactant concentration shows negative effect on particle size, as indicated by the coefficient *b* (-20.25). Fig. 1 reveals a decline in the value of particle size with increase in concentration of surfactant (Span 60), the effect of sonication time being less significant. Particle size distribution of optimized batch S_2 is shown in Fig 3.

Entrapment efficiency

A high amount of drug could be incorporated in nanoparticle dispersion. The %EE of different formulations prepared is shown as in Table 3. The influence of drug: lipid ratio is much more pronounced on %EE (Fig. 2) which can be seen as sample no. $R_{10,}$ $R_{11,}$ R_{12} & R_{13} show %EE about 77.6, 75.1, 70.14 & 72.55 % respectively while remaining batches show less %EE (about 41-69%). The partitioning of drug between lipid and water phases during pre-emulsion formation affects drug entrapment in nanoparticles. This in turn depends on amount of lipid, solubility of drug in lipid, process temperature and surfactant concentration affects this partitioning. Therefore the positive influence of lipid content on entrapment is explained. Both surfactant concentration and sonication time show less significant effect on entrapment efficiency as seen from the values of coefficients *b* and *c* respectively.

Differential Scanning Calorimetry

DSC is a highly useful means of detecting drug-excipient incompatibility in the formulation. Compritol 888 ATO alone and in formulation was studied using DSC. For the bulk material of Compritol 888 ATO, the melting process took place with maximum peak at 75 °C and SIMVA show peak at 140°C shown in Fig. 4. DSC thermogram of SIMVA-SLN showed an endotherm at 73.21°C, which can be attributed to melting of Compritol 888 ATO in SLN. The peak of compritol 888 ATO in formulation shows a shift to the lower temperature side. This could be due to reduction in particle size and increase in surface area leading to decrease in melting enthalpy as compared with heat flow through larger crystals, which require more time. The higher melting enthalpy value suggests higher ordered lattice arrangement. For the less-ordered crystal/ amorphous state the melting of the substance requires less energy than the perfect crystalline substance, which needs to overcome lattice force.

X-ray diffraction

X-ray diffraction data listed in following Fig. 5 was good in agreement with results established by DSC measurements. The diffraction pattern of the bulk matrix showed remarkable difference from those of the SLN, as they showed relative sharp peak than the SLN. It was clear that from SIMVA-SLN, the less ordered crystals were majority and the amorphous state would contribute to the higher drug loading capacity as seen previously. There is a significant difference between the diffraction patterns of simvastatin and SIMVA-SLN. It was confirmed that SIMVA existed in amorphous state in the SIMVA-SLN because of the disappeared sharp peak of SIMVA in the diffraction pattern.

FTIR

From FTIR study, the characteristic peaks of drug such as of C=CH (3012 cm^{-1}), aromatic C=O (1698.9 cm^{-1}), aliphatic O-H (3551.4 cm^{-1}) and benzene ring (1466.7 cm^{-1}) disappeared and were replaced by the peak of compritol 888 ATO. Remaining peaks also either shifted or replaced in the IR spectrum of formulation shown in Fig.6. This established drug entrapment in lipid matrix.

***In vitro* release of simvastatin from SLNs**

The *in-vitro* release of simvastatin through dialysis membrane from SIMVA-SLN dispersion and dispersion of pure simvastatin was evaluated using Franz *diffusion* cell. The mean cumulative amount released Q (mg/cm^2) at each sampling time points was calculated (Table 6). The results of diffusion studies are represented graphically as Q vs $t^{1/2}$ (Sec $^{1/2}$) in Figure 7. In present investigation SIMVA-SLN dispersion of optimized formulation showed significantly low release of simvastatin (37.08%) than dispersion of pure drug (97.2 %).

Conclusion

The pre-emulsion followed by ultrasonication technique was used to prepare solid lipid nanoparticles of reproducible sizes in the range of 220 to 798 nm by addressing the effects of

processing parameters. The application of Box Behken design proved to be a useful tool for optimization of SIMVA-SLN. Using the contour plot data from statistical design one can select suitable composition of formulation to obtain SIMVA-SLN in the size range of 220 to 798 nm depending on the application of the system. The results of the *in-vitro* drug release studies demonstrated, significantly low release of simvastatin (37.08%) from SIMVA-SLN as compared to dispersion of pure drug (97.2 %).

Table 1: Independent variables and their selected levels for nanoparticles formulation.

Independent Variable	Coded Levels		
	-1	**0**	**+1**
Drug: lipid ratio	1:3	1:5	1:7
Surfactant (Lipid phase) concentration	1%	3%	5%
Sonication time	10 min	20 min	30 min

Table 2: A Box Behnken Experimental Design Layout.

Formulation Code	Run	Coded Level (Independent variables)		
		X_1	X_2	X_3
R_1	9	0	-1	-1
R_2	12	0	+1	+1
R_3	7	-1	0	+1
R_4	1	-1	-1	0
R_5	11	0	-1	+1
R_6	6	+1	0	-1
R_7	2	+1	-1	0
R_8	13	0	0	0
R_9	8	+1	0	+1
R_{10}	5	-1	0	-1
R_{11}	4	+1	+1	0
R_{12}	10	0	+1	-1
R_{13}	3	-1	+1	0

Table 3: Values of particle size and entrapment efficiency for SLN of simvastatin (R_1- R_{13}) as per Box Behnken design.

Formulation code	Particle size (nm)	% EE
R_1	239	41.83
R_2	798	64.17
R_3	220	46.95
R_4	640	67.33
R_5	299	50.89
R_6	604	69.6
R_7	263	53.06
R_8	713	65.5
R_9	470	68.3
R_{10}	468	77.6
R_{11}	406	75.1
R_{12}	421	70.14
R_{13}	265	72.55

Table 4: Values of the coefficients for the polynomial equations and r^2 for various response variables of the SIMVA-SLN.

Coefficient code	Polynomial coefficient values for response variables	
	Particle size	**Entrapment efficiency**
k	+265	+72.55
a	+216.5	+9.23
b	-20.25	+1.58
c	-4.25	-0.32
d	-34.75	-0.49
e	+35.75	-1.57
f	+3.75	-3.56
g	+119.38	-15.25
h	+89.88	-2.23
i	+85.87	+2.46
r^2	0.9656	0.9878

Table 5: Comparison of experimental results with predicted responses.

Batch Code	Composition D:L ratio/Surfactant concentration/ Sonication time	Response	Predicted value	Experimental value	Percent error
S_1	**-0.4/ 0/ -0.4**	Particle size (nm) Entrapment (%)	218.66 66.69	220 68.2	0.0061 0.0226
S_2	**-0.1/ 0.3/ -0.1**	Particle size (nm) Entrapment (%)	249.13 71.91	245 72.52	-0.0165 0.0085
S_3	**0/ 0.2/ 0**	Particle size (nm) Entrapment (%)	264.54 72.78	268 73.3	0.0130 0.0071
S_4	**0.2/ 0.1/ 0.2**	Particle size (nm) Entrapment (%)	315.34 73.81	310 74.2	-0.0169 0.0053
Mean (± S.E.M.) of Percentage Error					0.00365 ±0.01285

Table 6: In-vitro drug release from SIMVA-SLN & pure simvastatin.

Time in Hrs.	Mean cumulative amount released (mg/cm^2)	
	SIMVA-SLN dispersion	**SIMVA Plain drug**
0	0	0
0.5	0.17	0.29
1	0.25	0.48
2	0.37	0.62
4	0.425	0.87
6	0.46	1.13
8	0.493	1.38
12	0.54	1.75
24	0.67	2.61
48	1.03	2.7

Figure 1: Three-dimensional response surface plots for Particle size.

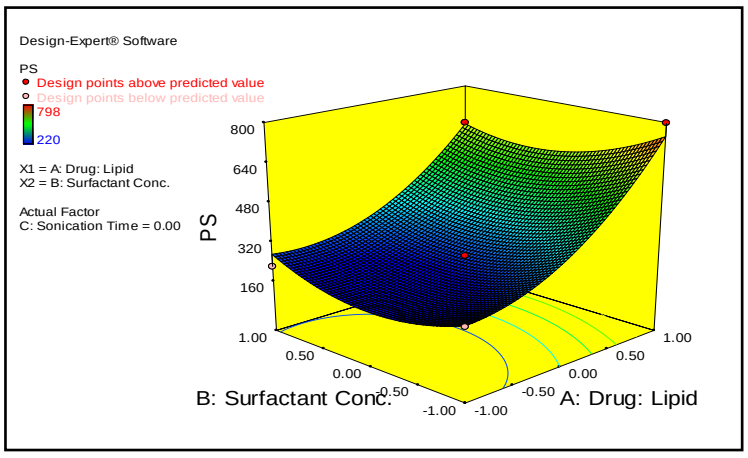


Figure 2: Three-dimensional response surface plots for Entrapment efficiency.

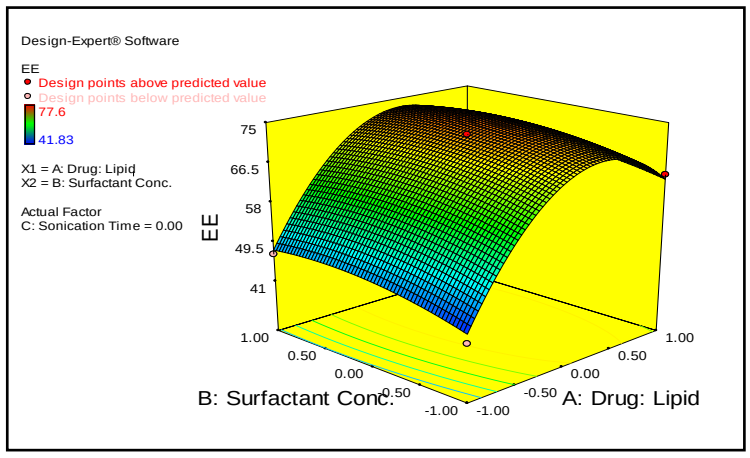


Figure 3: Particle size distribution curve of optimized batch $S_{2.}$

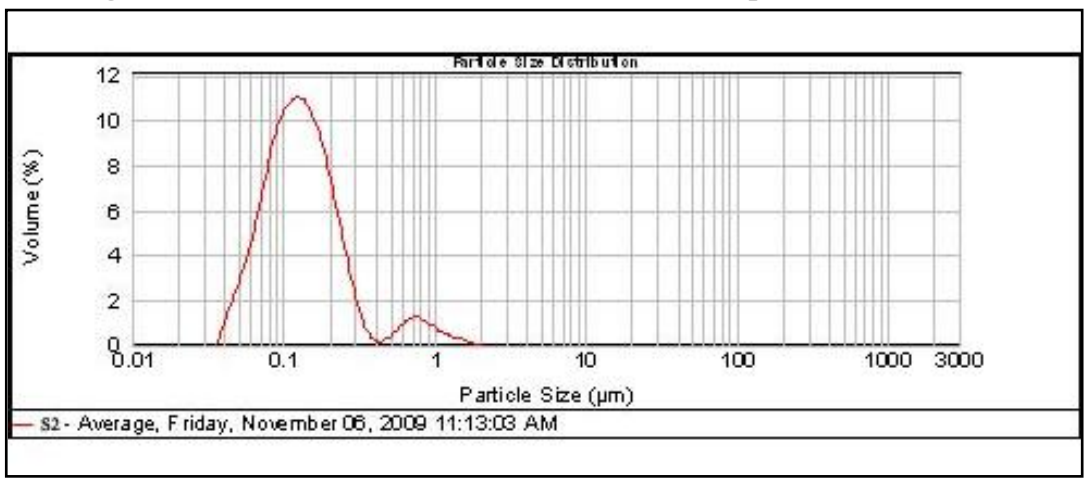


Figure 4: DSC thermogram of simvastatin (SIMVA), Compritol ATO 888 and SIMVA-SLN.

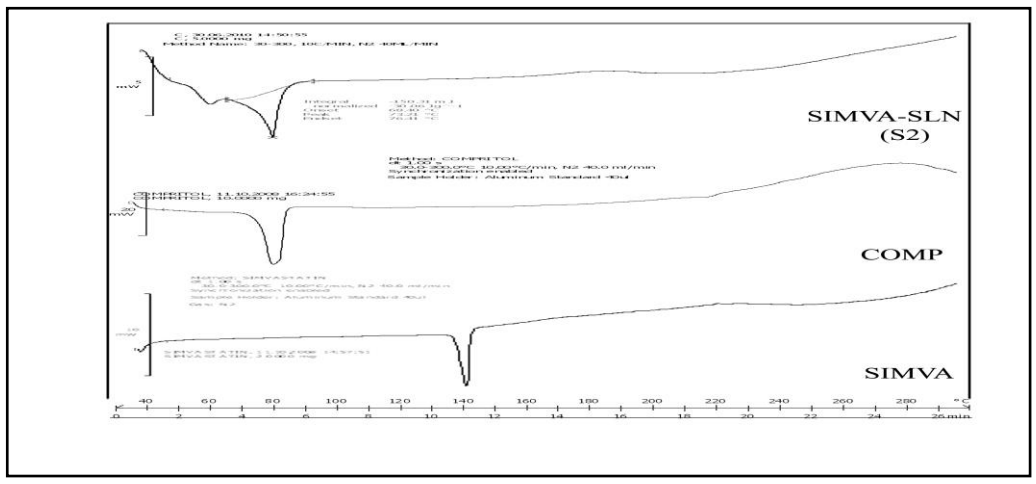


Figure 5: XRD of SIMVA, Compritol ATO 888 and SIMVA-SLN.

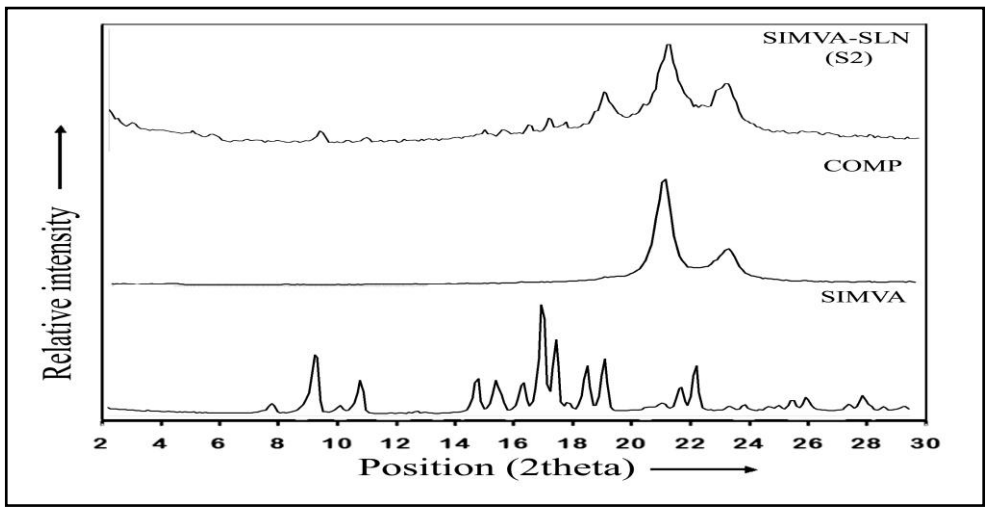


Figure 6: IR spectra of SIMVA, Compritol ATO 888 and SIMVA-SLN.

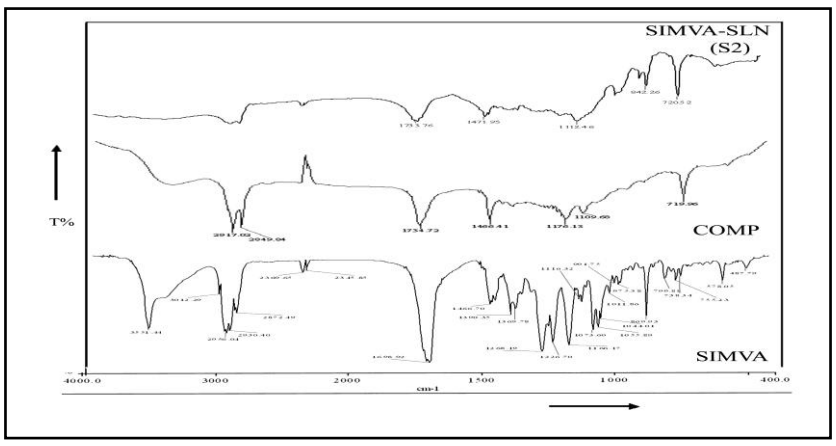
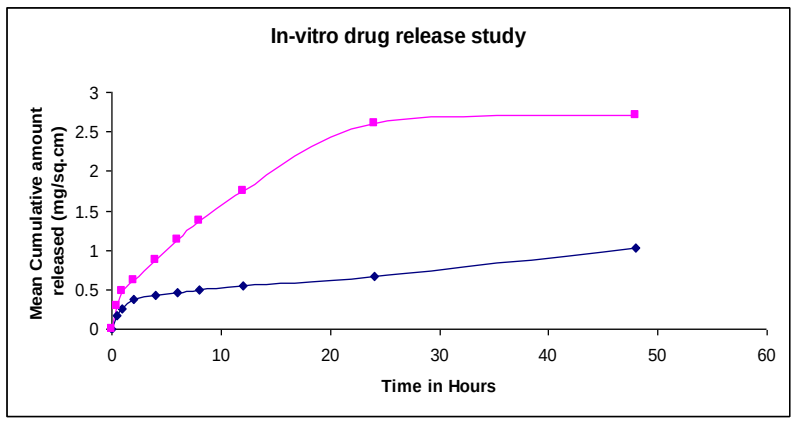


Figure 7: Mean cumulative amount of drug diffused. ■ SIMVA pure drug ♦ SIMVA-SLN.



References

1. Zocor Clinical Pharmacology available from: http://www.rxlist.com/zocor-drug.htm. Accessed on 12th October 2008.
2. Driscoll M C. *Eur. J. Pharm. Sci*. 2002, 15, 405.
3. Porter C J H and Charman W N. *Adv. Drug Deliv. Rev.* 2001, 50, 61.
4. Charman W N and Porter C J H. *Adv. Drug Deliv. Rev.* 1996, 19, 149.
5. Mehnert W and Ma¨der K *Adv. Drug Deliv. Rev.* 2001, 47, 165.
6. Mu¨ ller R H, Mehnert W, Lucks J S, Schwarz C, Zur Mu¨hlen A, Weyhers H, Freitas C and Ru¨ hl D *Eur. J. Pharm. Biopharm.* 1995, 41, 62.
7. Mu¨ ller R H, Ma¨der K and Gohla S *Eur. J. Pharm. Biopharm*. 2000, 50, 161.
8. Souto E B, Mu¨ ller R H, Nanoparticles for Pharmaceutical Applications; Domb A, Tabata Y, Kumar R, Eds.; American Scientific Publishers: 2006, 103.
9. Mu¨ ller R H, Lippacher A, Gohla S, Handbook of Pharmaceutical Controlled Release Technology; Wise D, Ed.; Marcel Dekker, Eastern Hemisphere Distribution: M.A., 2000, 377.
10. E.B. Souto, R.H. Mu¨ ller, Nanoparticulate Drug Delivery Systems: Recent Trends and Emerging Technologies; D. Thassu D, Deleers M, Pathak Y, Eds.; CRC Press: 2007, 213.
11. R.H. Mu¨ ller, W. Mehnert, E.B. Souto, Percutaneous Absorption; Bronaugh L Ed.; Marcel Dekker Inc.; New York, Basel, Hong-Kong: 2005, 719.
12. Muller R H, Mader K and Gohla S *Eur. J. Pharm. Biopharm.* 2000, 50, 161.
13. Zur Mu¨hlen A, Schwarz C and Mehnert W *Eur. J. Pharm. Biopharm.* 1998, 45, 149.
14. Bargoni A, Cavalli R and Caputo O *Pharm. Res* 1998, 15, 745.
15. Cavalli R, Bargoni A, Podio V and Muntoni E *Pharm. Sci.* 2003, 92, 1085.
16. Manjunath K and Venkateswarlu V *J. Control. Release* 2005, 107, 215– 228.
17. Fang J U, Fang C L, Liu C H and Su Y H *Eur. J. Pharm. Biopharm.* 2008, 633.
18. Lewis G A, Mathieu D, Phan-Tan-Luu R, Pharmaceutical experimental design: Drugs and Pharmaceutical Sciences; 1st ed.; Marcel Dekker, 92; New York: 1999, 235.
19. Doornbos C A, Haan P D, Encyclopedia of pharmaceutical technology; Swarbrick J, Boylan J C, Ed.; Marcel Dekker, New York: 1995, 77.
20. Schwartz J B, Connor R E, Modern Pharmaceutics; Banker G S, Rhodes C T, Ed.; 3rd ed.; Marcel Dekker; New York: 1996, 727.
21. Araujo P W and Brereton R G *Trends in Anal. Chem.* 1996, 15, 63.

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