

## IMPLICATION OF CENTRAL COMPOSITE DESIGN IN THE DEVELOPMENT OF SIMVASTATIN-LOADED NANOSPONGES

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### ABSTRACT

**Objective:** The present study's objective was to apply a central composite design to develop the simvastatin-loaded nanosponge formulation to improve its oral bioavailability.

**Methods:** With the help of a design expert (State-Ease version 13.0.1), a central composite design was selected for the formulation of simvastatin-loaded nanospheres by using a defined concentration of Eudragit L-100 (X1) and PVA (X2) as independent variables and particle size (Y1), percent (%) entrapment efficiency (EE) (Y2), *in vitro* drug release (Y3) as dependent variables. Fourteen (SF1-SF14) formulations were prepared using the emulsion solvent evaporation and evaluated for surface morphology, particle size, drug-excipient compatibility, %EE, and % drug release. The optimized model (SF14) obtained from a design expert was evaluated for *in vivo* pharmacokinetics in animal models.

**Results:** SF14 was formulated and evaluated for morphology (shape and size) of the particle, % EE, *in vitro* % drug release, and its kinetics. The formulation showed particle size of 163±0.45 nm, 80.54 %±0.57 of EE, and 97.13%±0.38 of drug release at 8h. The release kinetics followed the zero-order and Higuchi mechanisms with non-fiction diffusion. *In vivo* results showed C<sub>max</sub>, T<sub>max</sub>, AUC<sub>0-t</sub>, AUC<sub>0-∞</sub>, and MRT<sub>0-∞</sub> for nanospheres were 0.175 µg/ml, 6 h, 1.561 µg/mlh, 1.755 µg/mlh, 11.77 h, respectively.

**Conclusion:** The results indicated a significant increase in the bioavailability of the drug in nanospheres compared with standard drugs. The experimentally designed nanosponge formulations have been successfully developed, and evaluated parameters show that the nanosponge formulation of Simvastatin is a promising delivery through the oral route.

**Keywords:** Nanospheres, Eudragit L-100, Surface response method, Particle size, Central composite design

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### INTRODUCTION

Due to its ease of administration and patient compliance, the conventional drug delivery method is a frequently utilized strategy for various medications. However, it has a few drawbacks, like dose frequency, narrow therapeutic index, and fluctuations [1]. Different advanced drug delivery systems, like controlled, sustained, and targeted delivery, including nanotechnology, was focused on enhancing the solubility and improving the release characteristics of drug molecules [2]. Novel drug delivery systems are being used to improve or enhance the bioavailability of the drug and deliver the drug released at a specific site. Nanosponge technology is a novel emerging approach to improving the solubility and bioavailability of poorly water-soluble drugs [3, 4].

Nanosponges are one of the novel formulations in recent trends of nano-drug delivery. Nanospheres can be able to entrap both water-soluble and insoluble drugs. These are non-toxic, porous, stable at high temperatures, and insoluble in water and organic solvents [5-7]. These nanospheres circulate throughout the body, approach the desired location, and release the medication in a planned and controlled manner during parenteral administration.

Hyperlipidaemia is an acquired disorder defined as elevated lipid levels in the body, including HDL, LDL, and VLDL, caused due to diet and genetics [8, 9]. Simvastatin (SIM) is a lipid-lowering drug belonging to the statins class used to lower abnormal lipid levels and reduce the risk of cardiovascular disease by inhibiting cholesterol production in the liver. SIM has less bioavailability (5%) due to low solubility, short half-life (1.9 h), and protein binding is 95% [10, 11]. Due to low solubility and less half-life, and high protein binding (>95%), conventional release formulations may cause adverse events of HMG-CoA reductase inhibitor activity [12].

Novel formulations like liposomes, niosome, liquid crystals, and nanoparticles are formulated [13] to improve solubility and hepatic

availability. Nanosponge is also one of the novel formulations. As per recent reports, a few studies achieved the nanosponge formulations by employing ethyl cellulose, Eudragit S-100 [14] as rate retardant polymers with cyclodextrin [15] and polyvinyl alcohol (PVA) as copolymer. In our earlier studies, we tried to develop a nanosponge of SIM with ethyl cellulose by claiming this technology [16]. However, we have noticed low entrapment efficiency (%EE) with the use of ethyl cellulose combination with PVA. To produce a superior nanosponge formulation, an experimental design approach was adapted by considering two independent variables i.e., Eudragit L-100 as a rate retardant polymer and PVA as a copolymer for the formulation of nanospheres. The formulated nanospheres were evaluated for size, %EE, *in vitro*, *in vivo* drug release, and release kinetics [17, 18].

### MATERIALS AND METHODS

#### Materials

Hetero Drugs, Hyderabad, provided a complimentary sample of SIM and Eudragit-L100. Polymers like PVA, solvents, and other chemicals were procured from SD Fine Chemicals, Mumbai. All chemicals used were analytical grade.

#### Implication of quality by design (QbD) concept

QbD is a systematic approach to drug development that emphasizes understanding the critical process and product parameters that affect product quality and designing a formulation and manufacturing process that ensures consistent quality [19, 20]. When applied to the formulation of SIM nanospheres, QbD can have several implications. QbD can help to select the appropriate raw materials for the nanosponge formulation. By understanding the impact of each raw material on the final product quality, one can choose the most suitable materials to ensure consistent product quality. The steps involved in this procedure include i. identification of Critical Quality Attributes (CQAs) for SIM nanospheres ii. Defining

QTPP (Quality Target Product Profile), iii. Identification of process parameters and material attributes influence the CQAs, iv. Design of experiments, v. Result analysis.

#### Setting of CQAs and failure mode and effects analysis (FMEA)

various features were observed for the QTPP of SIM nanosponges, which should ensure the effect of formulation for treating hyperlipidemia. The CQAs are set based on the literature review mentioned in table 1 and aimed to improve the surface

characteristics of nanosponges and prolonged drug release. Various factors like polymer, copolymer, cross-linker, drug, temperature, method of preparation, and degree of substitution influenced the CQAs. FMEA was used for each CQA, i.e., %EE, particle size, and drug release, to evaluate the failure effects, causes, and control methods to achieve the CQAs and formulate QTPP for SIM nanosponges. In all factors, FMEA identified two significant factors that affect the formulation, Polymer, and copolymer concentration, which were used as independent variables for the design of experiments.

**Table 1: Summary of QTPP and CQAs of SIM nanosponges**

QTPP		CQAs	Justification
Formulation	Nanosponges	%EE	Reduced product loss
Route	Oral	particle size	Enhanced solubility and permeability
		<i>in vitro</i> drug release	Require to increase dose interval.

#### Experimental design for SIM nanosponge formulation

Generally, the design and optimization of the formulations is the crucial step after QbD. In this study, the nanosponge formulations were designed using CCD, a two-level full factorial design with the added center and axial points in the Response Surface Methodology (RSM) [21] by Design Expert-13. Various concentrations of Eudragit L-100 (X1) and PVA(X2) were employed as control variables (process parameters). Particle size (Y1), % EE (Y2), and drug release (Y3) were considered dependent variables. All the possible combinations of formulations were prepared by considering levels-1 and +1 for both controlled variables [22].

#### Preparation of SIM nanosponges

The emulsion solvent evaporation method was employed to formulate SIM nanosponges (SF1-SF13). Different polymer concentrations were selected as per the CCD (table 2). The required concentration of Eudragit L-100 and SIM were dissolved in an organic solvent (dichloromethane) to prepare the organic phase; an aqueous phase was prepared by dissolving PVA in 100 ml of distilled water and stirred at 1000 rpm for 2 h on a magnetic stirrer by drop-wise addition of the organic phase in the continuous aqueous phase. The formed nanosponges were filtered using a vacuum, dried at 40 °C for 24 h, and stored in a desiccator [23].

**Table 2: Formulation of SIM nanosponges**

S. No.	Formulation	Factor-1(Eudragit L100 in mg)	Factor-2 (PVA in mg)
1	SF1	100	100
2	SF2	100	300
3	SF3	275	200
4	SF4	275	200
5	SF5	275	58.57
6	SF6	27.5	300
7	SF7	275	200
8	SF8	450	300
9	SF9	275	200
10	SF10	375	341.42
11	SF11	450	100
12	SF12	522.48	200
13	SF13	275	200
14	SF14	450	149

Note: Drug content taken = 100 mg; Dichloromethane = 10 ml; Water content = 100 ml.

#### Optimization of the model

By following the preliminary investigation, a rotatable CCD experimental design was used to optimize the dependent variables, such as particle size (Y1), %EE (Y2), and drug release (Y3). All studies were carried out in triplicate and random order. Data were examined by design expert-13 (State ease Inc., Minneapolis, MN, USA). The interactions between the essential factors and responses were analyzed using the 2D and 3D response surface plots. The following equation was used to fit the polynomial models with the interacting terms to the experimental findings:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_1^2 X_1^2 + \beta_2^2 X_2^2 + \beta_1 \beta_2 X_1 X_2 \text{ ----- (1)}$$

Where 'Y' is a measured response, ' $\beta_0$ ' is the constant coefficient, and ' $\beta_1$ ' and ' $\beta_2$ ' are interaction coefficients, respectively.  $X_1$  and  $X_2$  were the variables. When one variable was modified at a time, the word  $X_i$  directly impacted a response. The phrase  $X_1 X_2$  showed the interaction between  $X_1$  and  $X_2$ , illustrating how the response would behave when two parameters were changed simultaneously. ANOVA was then used to analyze the model coefficient's statistical significance (p 0.05).

#### Drug-excipient compatibility study

The API and SIM nanosponge samples were subjected to FTIR spectrum measurement (in the region of 400-4000  $\text{cm}^{-1}$ ) using the KBr pellet method [24].

#### Scanning electron microscopy (SEM) analysis

Morphological characterization was performed using a high vacuum mode SEM (Carl Zeiss SEM with Oxford EDX). Digital images were observed at voltages of 30.0 KV [25].

#### Particle size, Polydispersity index (PDI), and zeta potential

The average size of the particle, PDI, and Zeta potential was measured using the Malvern Zeta sizer (Malvern Nano ZS Zetasizer). Every sample was diluted using distilled water and measured at a temperature of  $25 \pm 0.5$  °C.

#### X-ray diffraction study (XRD)

XRD (XRD-7000/Shimadzu) was performed by passing Cu K radiation through the API and witnessing the formulation of SIM

nanosponges. The X-ray diffractograms for the API and selected formulations were recorded [26].

#### Differential scanning calorimetry (DSC) study

DSC (Shimadzu DSC-60) studies for API and SIM nanosponge formulation were carried out to observe the interaction between the drug and excipients during the formulation [27].

#### Entrapment efficiency (% EE) and % drug loading capacity

The amount of drug loaded in the unit weight of the SIM nanosponges was denoted by drug loading capacity. % EE [28] refers to the total amount of drug entrapped in the nanosponge formulation. In 10 ml of phosphate buffer (pH-6.8), approximately 50 mg of SIM nanosponges were completely dissolved. The transparent drug layer was then collected, and its concentration was determined using a UV-visible spectrophotometer. The following formula was used to calculate the drug's % EE and % drug loading capacity in nanosponges [29].

$$\% \text{ EE} = \frac{\text{Total amount of drug} - \text{Amount of drug supernatant liquid}}{\text{Total amount of drug}} \times 100$$

$$\% \text{ Drug Loading} = \frac{\text{Total amount of drug} - \text{Amount of drug supernatant liquid}}{\text{Total wt of nanosponges}} \times 100$$

#### In vitro drug release study

Drug release was estimated using the dissolution apparatus USP-I basket method (Labtronics dissolution apparatus) at 100 rpm, 37±0.2 °C in 900 ml of phosphate buffer (pH-6.8) [30]. A measured quantity of nanosponges was used for drug dissolution. The samples were collected at regular intervals in 1, 2, 3, 4, 6, and 8 h. A UV-visible spectrophotometer was used to quantify the drug content at 247 nm [31, 32].

#### Drug release kinetics

The results obtained from the dissolution studies of each sample were used to fit in the best kinetic models, such as Zero-order, First-order, Higuchi, and Korsmeyer-Peppas equations [33].

#### In vivo release studies

*In vivo*, a drug release study was performed to determine the plasma concentration of the drug in the prepared nanosponge formulation and compare it with the standard drug. Pharmacokinetic parameters

were determined using a PK solver [34]. *In vivo* drug release study was performed using healthy New Zealand rabbits weighing 2-2.5 kg. Rabbits were divided into three groups standard, test, and control. Animals fasted before 24 h of drug administration [35]. The standard drug was administered orally by orogastric gavage for group-I. Nanosponge formulation equivalent to 10 mg/kg was for group-II, and group-III animals for control blood samples were collected for 1, 2, 3, 4, 6, 8, 10, 12, and 24 h from marginal ear vein of rabbit, plasma was separated by micro centrifugation at 5000 rpm and stored at -20 °C.

#### SIM sample preparation and analysis

To the collected sample, protein precipitant (trichloroacetic acid) was added and extracted drug from plasma using centrifugation for 15 min at 4 °C at 5000 rpm. To quantify the concentration of SIM in the extracted drug solution, the supernatant solution was put into HPLC [36]. The analysis was conducted using a C18 column at 1 ml/min flow of 2 mmol ammonium acetate: methanol (20:80 %v/v) with an injection volume of 5 µl. Pure SIM was used to prepare the calibration curve (0.04-0.16 µg/ml). All the samples of SIM solutions were collected and analyzed at 247 nm [37].

#### Stability studies

The samples were subsequently stored in stability chambers that maintained the storage conditions of 40±0.5 °C/75% relative humidity. The formulations were analyzed for six months for their physical appearance and *in vitro* drug release studies [38].

## RESULTS

#### Experimental design-fitting response surface curve

As per the QbD approach, the formulated SIM nanosponges were tested for particle size, % EE, and drug release in a triplicate manner and mean±SD values mentioned in table 3. The surface response approach predicted all the responses dependent on the controlled variables. The data from the formulations were analyzed statistically to get the most accurate prediction of the independent variables. The dependent variables' regression coefficients (r<sup>2</sup>), coded equations, and regression results (p-values) were summarised in table 4. The significance of developed quadratic and linear polynomial models was assessed using ANOVA (table 5). The interaction between the two independent variables was also identified using 3D surface plots (fig. 1).

Table 3: Statistical data for dependent variables

Formulation	Factor-1	Factor-2	Particle size (nm)*	EE (%)*	Drug release*
SF1	100	100	201.56±0.23	22.45±0.46	99.2±0.92
SF2	100	300	198.48±0.43	50.69±0.63	94.3±0.17
SF3	275	200	205.72±0.45	49.12±0.74	95.4±0.37
SF4	275	200	210.89±0.54	39.41±0.36	96.23±0.48
SF5	275	58.578	163.25±0.64	24.65±0.58	98.1±0.74
SF6	27.51	200	142.45±0.35	9.48±0.69	99.69±0.46
SF7	275	200	220.13±0.34	49.65±0.34	96.6±0.61
SF8	450	300	284.14±0.67	87.36±0.42	87.7±0.45
SF9	275	200	217.24±0.62	55.46±0.28	93.8±0.65
SF10	275	341.42	295.23±0.81	85.45±0.21	89.7±0.47
SF11	450	100	195.36±0.24	43.54±0.74	99.12±0.59
SF12	522.487	200	275.45±0.37	89.54±0.27	85.54±0.69
SF13	275	200	200.43±0.38	49.67±0.45	96.54±0.74
SF14	459	149	164±0.45	80.54±0.57	97.19±0.38

\*Data compressed as mean±SD, n=3

Table 4: Equations, probability, regression values, and the final models

S. No.	Dependent variable	Coded equation, r <sup>2</sup> value, P-value and F-value
1	Particle size	214.31+0.6862(A)+54.08(B) 0.8545, P-value: <0.0001, F-value-29.38
2	EE	49.00+4.41 (A)+28.75 (B), R <sup>2</sup> value: 0.7963, P-value: <0.0001, F-value-19.62
3	Drug release	94.94-0.0003(A)-0.05408(B), R <sup>2</sup> value: 0.8348, P-value: <0.0001, F-value-25.26

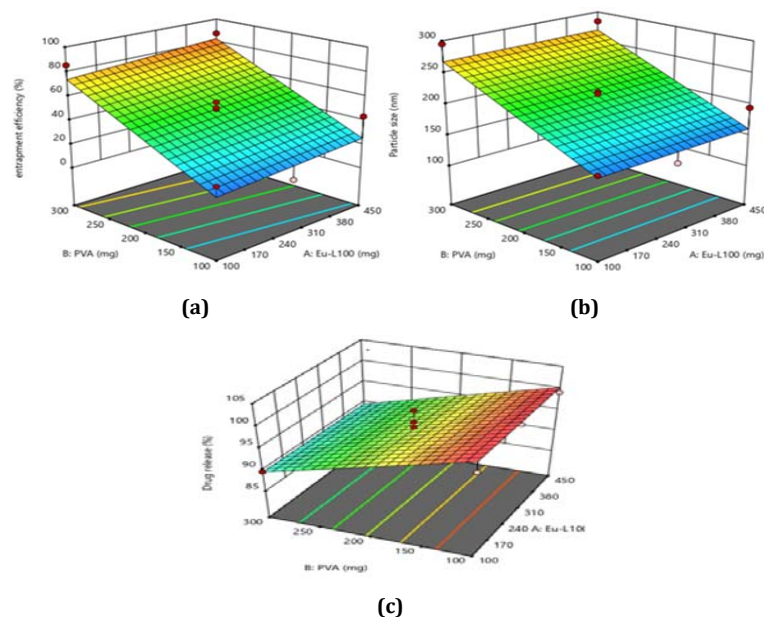


Fig. 1: Surface plots showing an interaction between Eudragit L100 and PVA on (a) size of the particle, (b) % EE (c) Drug release at 8 h

Table 5: ANOVA for three dependent variables

Independent variables		Particle size		% EE		Drug release	
		F-value	P-value	F-value	P-value	F-value	P-value
Main Effect	A	0.0091	<0.0001	1.03	<0.0001	0.0091	<0.0001
	B	50.52	<0.0001	38.20	<0.0001	50.52	<0.0001

#### Validation of the model

The goals for optimizing SIM nanospheres were maximizing the %EE, reducing particle size, and maximizing drug release. The software provided seven solutions, out of which one gave 0.608 desirabilities and the formula for batch SF14. Therefore, it was considered the best formulation that would provide improved %EE and drug release with reduced particle size.

#### Evaluation of optimized formulation

The statistically optimized formulation (SF14) is shown in table 6. It was assessed for its evaluation parameters, and the resulting experimental values with mean $\pm$ SD were indicated in table 7.

#### Drug-excipient compatibility study

The FTIR spectrum of pure SIM (fig. 2A) showed distinctive peaks at 3629  $\text{cm}^{-1}$  (OH stretch), 2968  $\text{cm}^{-1}$  (CH stretch), and 1708  $\text{cm}^{-1}$  (C=O stretch of carbonyl groups). The physical mixture showed prominent peaks (fig. 2B) at 3529  $\text{cm}^{-1}$  (OH stretch), 2956  $\text{cm}^{-1}$  (CH stretch),

and 1708  $\text{cm}^{-1}$  (C=O stretch of carbonyl groups). The interpreted FTIR bands in formulations indicated no drug interaction with the polymer mixture.

Table 6: Optimized formulation (SF14)

Ingredients	Quantity
API	100 mg
Dichloromethane	10 ml
EuL100	450 mg
PVA	149 mg
Water	100 ml

#### SEM analysis

As per the SEM studies, all the formulations maintained a uniform particle size, and drug crystals were not observed on the surface of the nanospheres. The SEM of images of a few formulations (SF4, SF7, SF8, SF12) are shown in fig. 3.

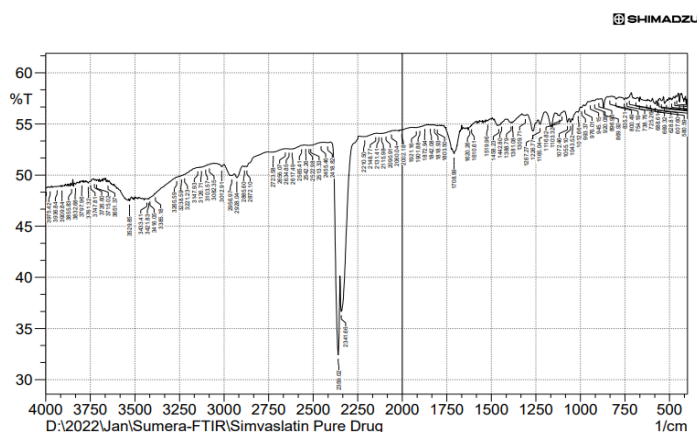


Fig. 2(A): FTIR spectra of SIM standard drug

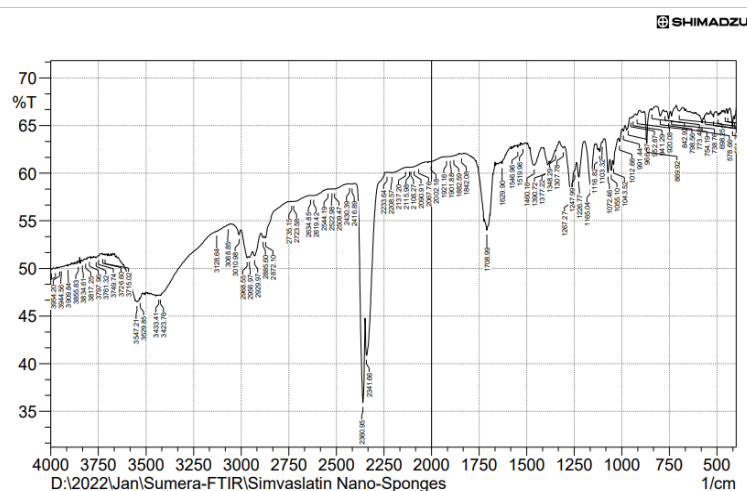


Fig. 2(B): FTIR spectra of SIM nanosponges (SF14)

Table 7: Predicted and observed values for formulation (SF14)

Responses	Predicted	Observed*
Particle size	187 nm	163 nm±0.45
EE	39.58%	80.54%±0.57
Drug release (at 8 h)	97.57%	97.13%±0.38

\*Data compressed as mean±SD, n=3

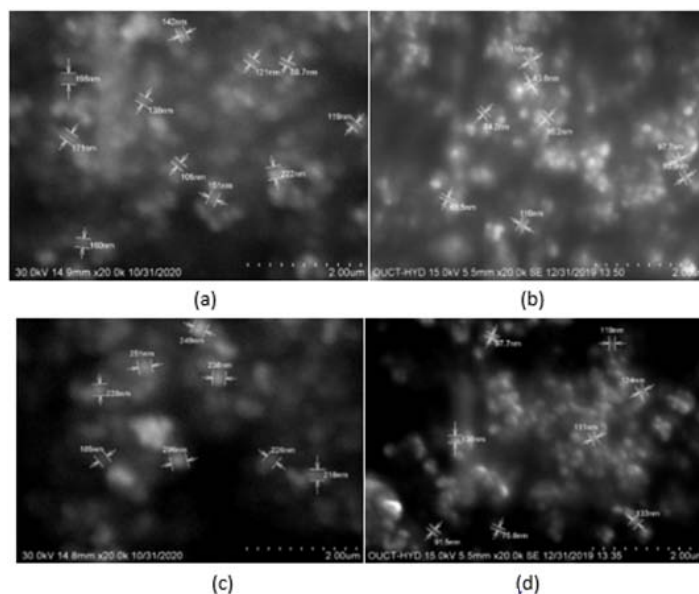


Fig. 3: SEM images of prepared nanosponges formulations (magnification scale 2.00 μm) a) SF4 b) SF7 c) SF8 d) SF12

#### XRD study

XRD for the SIM standard drug (fig. 4A) and nanosponges (fig. 4B) was recorded, the pure drug showed distinctive peaks representing the crystalline nature of the drug, and the SIM nanosponges showed the smoothening of the curve when compared to the standard, which stated the encapsulation of SIM in amorphous nanosponge complex.

#### DSC study

The DSC thermogram of the SIM standard drug showed a prominent melting endothermic peak at 138.07 °C. In SIM nanosponges, it

showed the endothermic peak at 254.81 °C, which was nearer to Eudragit L-100, which showed the endothermic peak at 234.33 °C (fig. 5) stated that the encapsulation of SIM in nanosponge complex.

#### Particle size, Zeta potential, and PDI

The formation of nanosponge (SF1-SF13) was achieved with particle size ranging from 142.45±0.35-295.23±0.81 nm. The optimized formulation (SF14) has an average particle size of 163.2±0.45 nm with 0.394 PDI (fig. 6). SF14 indicated a zeta potential of -6.6 mV (fig. 7). These results showed that the particles were nano in size and separated by repulsive forces.



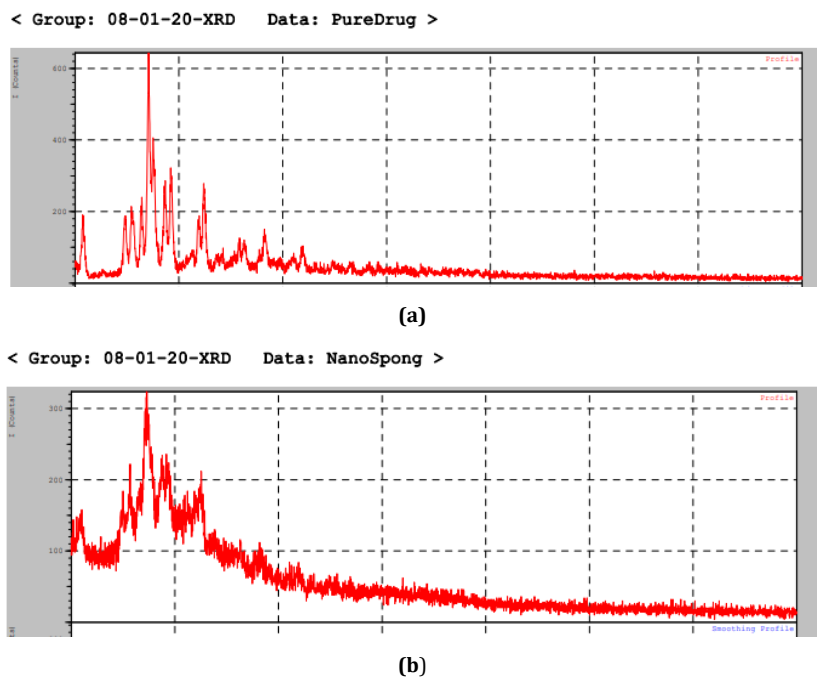


Fig. 4: (a) XRD of the standard drug; (b) XRD of nanosponges (SF14)

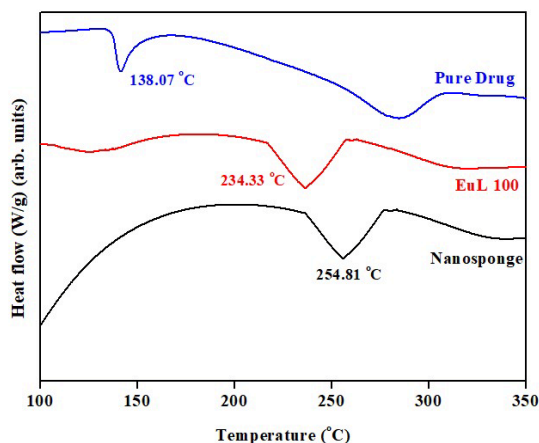


Fig. 5: Overlain DSC thermogram of pure drug, Eudragit L-100, and SIM nanosponge (SF14)

Calculation Results

Peak No.	S.P Area Ratio	Mean	S. D.	Mode
1	1.00	276.5 nm	105.5 nm	262.3 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	276.5 nm	105.5 nm	262.3 nm

Histogram Operations

Size (Median)	: 257.9 nm
Mode	: 262.3 nm
% Cumulative (1)	: 5.0 (%) - 136.9 (nm)
% Cumulative (2)	: 10.0 (%) - 154.1 (nm)
% Cumulative (3)	: 20.0 (%) - 181.1 (nm)
% Cumulative (4)	: 30.0 (%) - 206.0 (nm)
% Cumulative (5)	: 40.0 (%) - 231.1 (nm)
% Cumulative (6)	: 50.0 (%) - 257.9 (nm)
% Cumulative (7)	: 60.0 (%) - 287.6 (nm)
% Cumulative (8)	: 70.0 (%) - 322.3 (nm)
% Cumulative (9)	: 80.0 (%) - 366.1 (nm)
% Cumulative (10)	: 90.0 (%) - 429.9 (nm)

Cumulant Operations

Z-Average	: 163.2 nm
PI	: 0.394

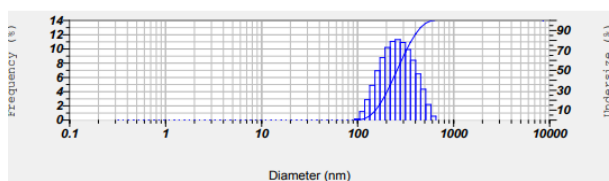


Fig. 6: Particle size and PDI for optimized formulation (SF14)

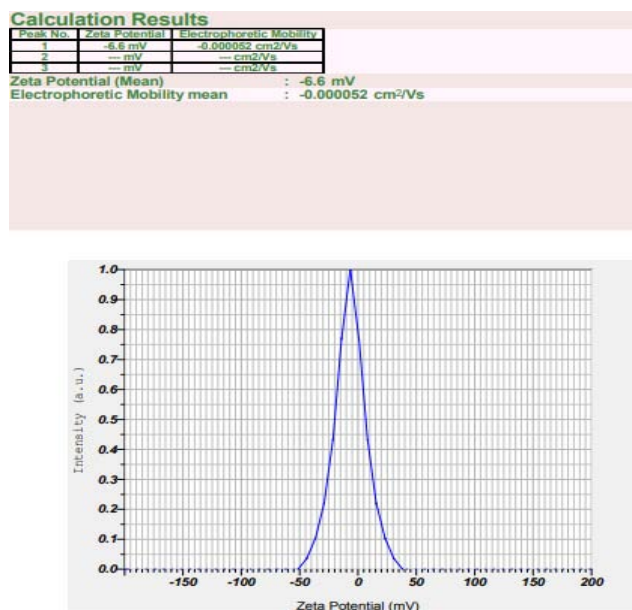


Fig. 7: Zeta potential measurement for optimized formulation (SF14)

#### %EE and %drug loading capacity

% EE of the SIM nanosponge formulations SF1-SF13 was in the range between  $9.48 \pm 0.69$ – $89.54 \pm 0.27\%$  and the % drug loading capacity  $10.24 \pm 0.56$ – $34.54 \pm 0.67\%$ .

#### In vitro drug release study

*In vitro* drug release study exhibited  $87.7 \pm 0.45$  to  $99.77 \pm 0.23\%$  of drug release for all SIM nanosponges (SF1-SF13) up to 8h (table 3). The optimized formulation (SF14) showed  $97.13 \pm 0.38\%$  of drug release in 8h (table 8). The drug release pattern of optimized formulation (SF14) was graphically demonstrated in fig. 8, which indicated a linear relationship with a correlation coefficient ( $r^2$ ) of 0.9928.

Table 8: Cumulative % drug release profile for optimized formulation (SF14)

Time (h)	% drug release
0	0
0.5	$12.16 \pm 0.21$
1	$21.42 \pm 0.32$
2	$28.46 \pm 0.45$
4	$48.54 \pm 0.32$
6	$74.97 \pm 0.65$
8	$97.13 \pm 0.38$

Data compressed as mean $\pm$ SD (n=3)

#### Drug release kinetics

Drug release kinetics for the optimized formulation (SF14) fitted for dissolution models the best, and the coefficient ( $r^2$ ). It showed zero-order kinetics with the Higuchi diffusion mechanism.

#### In vivo release studies

The optimized formulation SF14 was considered for *in vivo* drug release study as it has shown good particle size, ideal % EE, and *in vitro* drug release. The calibration curve was drawn using different concentrations (0.04, 0.06, 0.08, 0.1, 0.12, 0.14, 0.16  $\mu\text{g/ml}$ ), and the regression value was 0.9979. A pharmacokinetic study was

conducted on healthy rabbits with the approval of the animal ethical committee (06/IAEC/VIPER/Ph. D/2021-22/II). The Pharmacokinetic parameters ( $C_{\text{max}}$ ,  $T_{\text{max}}$ , and AUC) were evaluated using plasma concentration profiles for standard drug, and SIM nanosponges (SF14) were administered in oral dosage form (fig. 10). Results indicated that SIM nanosponge (SF14) has better absorption than standard drugs.  $C_{\text{max}}$  of the standard drug and SF14 were 0.161  $\mu\text{g/ml}$  and 0.175  $\mu\text{g/ml}$ , and the  $T_{\text{max}}$  of the standard drug and SF14 was 2 and 6 hr, respectively.  $\text{AUC}_{0-t}$  for standard drug and SF14 was 0.645  $\mu\text{g/mlh}$  and 1.561  $\mu\text{g/mlh}$ ,  $\text{AUC}_{0-\infty}$  0.735  $\mu\text{g/mlh}$  and 1.755  $\mu\text{g/mlh}$ ,  $\text{MRT}_{0-\infty}$  for standard drug and SF14 5.82 h and 11.77 h, respectively. The pharmacokinetic parameters for standard drugs and SF14 were shown different values, and the AUC values showed a significant increase in the bioavailability of the drug in nanosponges compared with standard drug.

#### Stability studies

SIM nanosponges showed good physical stability for six months. Due to particle aggregation, there was a modest increase in particle size during storage, but no colour change was detected for up to six months. Drug release was regulated, as demonstrated in *in vitro* investigations (fig. 11).

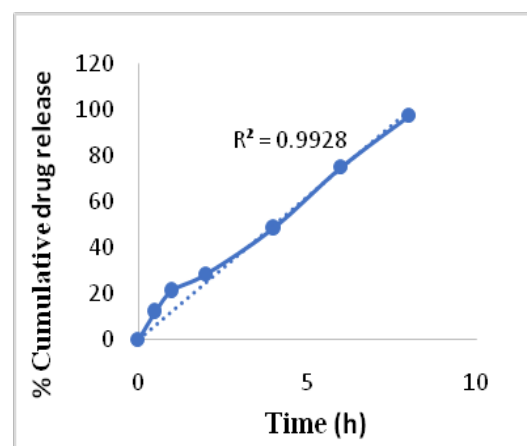


Fig. 8: Cumulative % drug release study for SF14

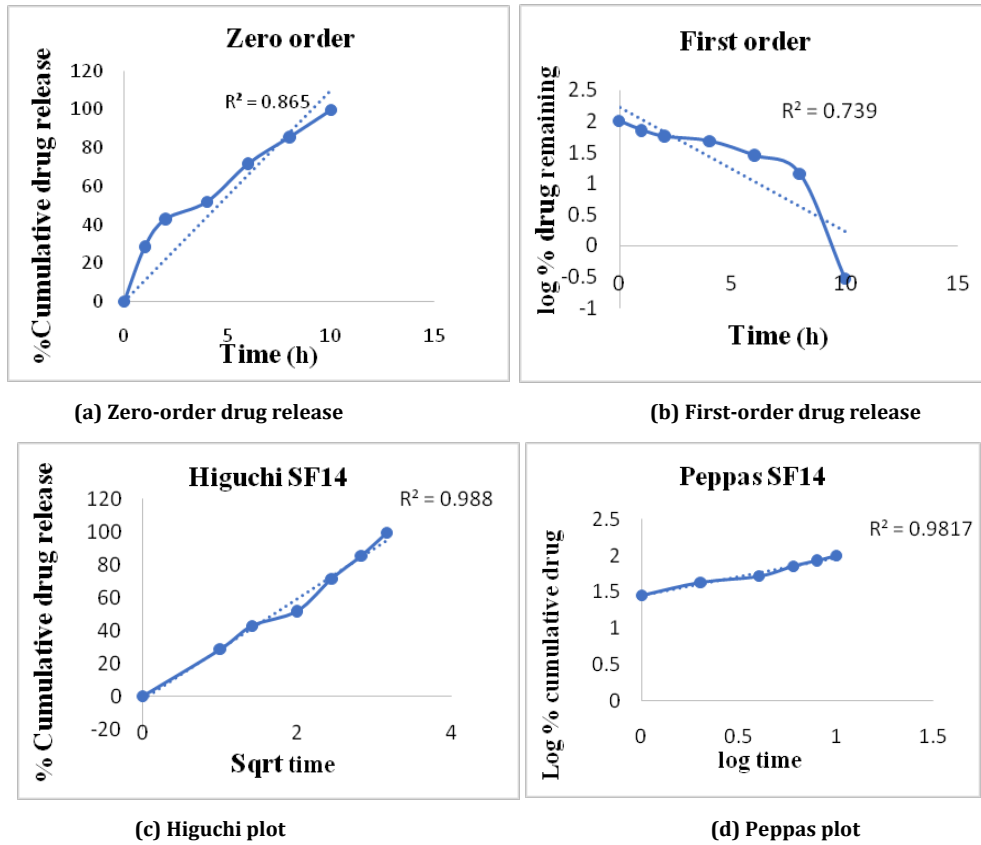


Fig. 9: Drug release kinetics for optimized formulation (SF14)

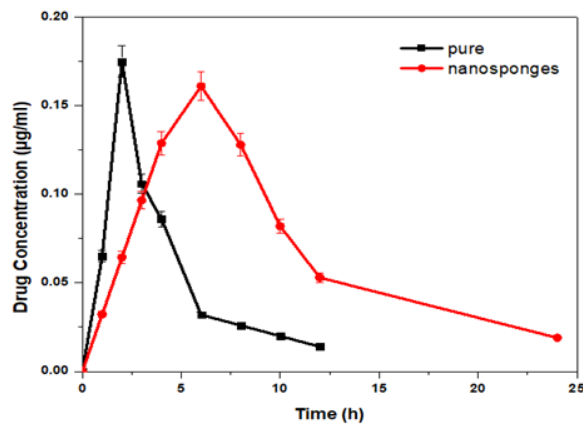


Fig. 10: *In vivo* study of standard drug and SIM nanosponges (SF14), n=3

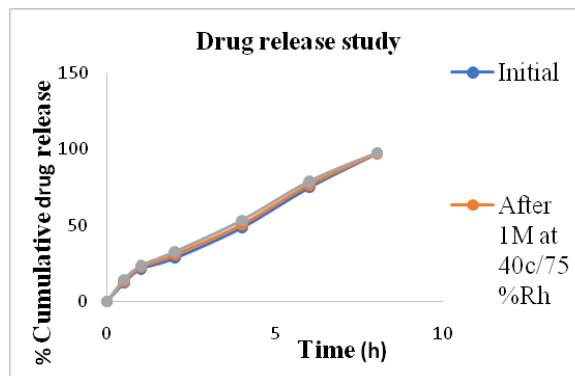


Fig. 11: Stability study for optimized formulation (SF14)



## DISCUSSION

Simvastatin (SIM) is a lipid-lowering drug with less bioavailability (5%) due to low solubility and short half-life (1.9 h). CCD in the surface response methodology approach was used to predict all the responses, dependent on the independent factors. The *p*-values and *F*-values of all model terms in table 4 indicated that the dependent variables were influenced significantly [39]. According to the findings, the rate retardant polymer (Eudragit L100) substantially affects the size of particles, % EE, and drug release. Formulations prepared using a low concentration of polymers show small size, low EE, and high drug release. As per the experimental data, there was no significant difference between the observed and predicted values for all responses in the statistical analysis.

Drug and excipient compatibility was established based on the predictions by design and compared the characterization data such as the FTIR spectrum, where the API and formulation had shown distinctive absorption peaks at 3629 cm<sup>-1</sup> (OH stretch), 2968 cm<sup>-1</sup> (CH stretch), and 1708 cm<sup>-1</sup> (C=O stretch of carbonyl groups). The formulation showed prominent peaks at 3529 cm<sup>-1</sup> (OH stretch), 2956 cm<sup>-1</sup> (CH stretch), and 1708 cm<sup>-1</sup> (C=O stretch of carbonyl groups) stated that the absorbance shifts within statutory limits (<100 cm<sup>-1</sup> absorbance shifts). Furthermore, the XRD of the pure drug showed distinctive peaks representing the crystalline nature of the drug, and the SIM nanosponges showed the smoothening of the curve when compared to the standard, which stated the encapsulation of SIM in amorphous nanosponge complex [40] and DSC analysis indicated no drug and excipient interaction in the formulations. The average particle size of all formulations (SF1-SF13) was maintained between 142.45±0.35-295.23±0.81 nm, whereas the optimized formulation (SF14) was found to be 163±0.45 nm in size with 0.394 PDI and -6.6 mV Zeta potential.

The %EE for all formulations was 9.48±0.69-89.54±0.47%, whereas the optimized formulation (SF14) showed 80.54±0.57. All formulations (SF1-SF13) showed maximum drug release (87.7±0.45 to 99.77±0.23 %) for 8 h, stating that a higher amount of drug release was observed due to the porous structures of nanosponges. The drug release is high in an alkaline buffer because the rate retardant polymer was Eudragit L-100 (a pH-dependent polymer) [41]. The optimized formulation (SF14) showed 97.13±0.38 % of drug releases in 8 h, which was much better than other formulations. The release kinetics for SF14 followed zero-order drug release. Non-fickian diffusion mechanism was suggested by the Higuchi diffusion model. *In vivo* drug release profile for the standard drug and SF14 showed a C<sub>max</sub> of 0.161 µg/ml and 0.175 µg/ml, respectively, whereas the T<sub>max</sub> of the standard drug and SF14 was 2 and 6 h, respectively [37]. AUC<sub>0-t</sub> for the standard drug and SF14 was 0.645 µg/mlh and 1.561 µg/m<sup>3</sup>h, respectively, whereas AUC<sub>0-∞</sub> was 0.735 µg/mlh standard and 1.755 µg/mlh for the formulation, MRT<sub>0-∞</sub> for standard drug and SF14 5.82 h and 11.77 h. The AUC values stated a significant increase in the bioavailability of the drug in the form of nanosponges when compared with standard drug.

## CONCLUSION

An experimental approach was employed for designing various formulations of nanosponges by using Design Expert-13. The CCD in the surface response methodology was employed to determine the effect of independent variables. The SIM nanosponges were successfully developed with the help of the emulsion solvent evaporation technique. The formulated SIM nanosponges were characterized with various methods for drug excipient compatibility and particle size, PDI and Zeta potential. These nanosponge formulations of SIM possessed optimum % EE and drug loading capacity with site-specific drug release at test conditions over the earlier formulation. The optimized formulation (SF14) tested for further *in vivo* results concluded that the formulation of nanosponges was promising and had good scope to develop more effective and easily accessible localized delivery systems for SIM.

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## ETHICAL APPROVAL

Ethical approval was taken by IAEC in VIPER 2022. (06/IAEC/VIPER/Ph. D/2021-22/II).

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Nil

## ABBREVIATIONS

QTPP: Quality Target Product Profile, FTIR: Fourier Transform Infrared Spectroscopy, HPLC: High-performance Liquid Chromatography, ANOVA: Analysis of variance, CCD: Central composite design, SRM: Surface response methodology

## AUTHORS CONTRIBUTIONS

Each author significantly contributed to the article's idea and design, data collection, analysis, interpretation, and drafting or critical revisions for imperative intellectual content.

## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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