Ind. J. Pharm. Edu. Res., 2024; 58(3):784-793.
https://www.ijper.org

Original Article

Development of Nanosponge Formulations of Rosuvastatin for Oral Delivery Using a Central Composite Design

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ABSTRACT

Background: Rosuvastatin (ROS) is an anti-hyperlipidaemic drug which reduces cholesterol levels, having poor solubility and low bioavailability (<20%). The objective of the present study was to increase ROS bioavailability by formulating nanosponges. **Materials and Methods:** Important quality features were identified using the Quality by Design (QbD) method. Central Composite Design (CCD) was utilized to design formulations. Eudragit L-100 (EL-100) and Polyvinyl Alcohol (PVA) were used as polymers and surfactants, respectively. Nanosponges were produced using emulsion solvent evaporation (RF1-RF15). The final formulations were assessed based on parameters including drug-excipient interaction, particle size, surface morphology, Entrapment Efficiency (%EE), and *in vitro* drug release. The Design Expert-13 (DOE) produced the optimized Formulation (RF16), which was utilized in the *in vivo* drug release **Results:** All Formulations (RF1-RF15) showed particle size of 99±0.84 nm to 305±0.26 nm, %EE 17.8±0.42 to 84.69±0.45, and drug release was 94.33±0.45% to 99.77±0.56% in 4 hr. Optimized Formulation (RF16) showed a particle size of 295±0.35 nm, % EE of 78.54±0.26 %, and drug release study of 95.13±0.63% in 3.5 hr. The *in vivo* studies showed $C_{max,}$ T_{max}, AUC_{0-t}, $AUC_{0-\alpha}$, $MRT_{0-\alpha}$ of the pure drug and RF16 of 7.123µg/mL and 14.787 µg/mL, 1.5 and 2.5 hr, 19.56 µg/mL*hr and 25.71 µg/mL*hr, 23.91 µg/ml*h, and 48.85 µg/mL*hr, 5.04 hr and 3.91 hr, respectively. **Conclusion:** The pharmacokinetic parameters RF16 demonstrate a 2-fold enhancement in the bioavailability of ROS nanosponges compared to the pure drug.

Keywords: Rosuvastatin, *In vivo* drug release, Central Composite Design, Eudragit L-100, Quality by Design.

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Received: 04-11-2023;
Revised: 26-02-2024;
Accepted: 05-05-2024.

INTRODUCTION

Nanotechnology is one of the widely used technologies in the present times in physical and biological sciences.[1] Numerous uses of nanotechnology have been explored in nanomedicine and the development of nano-based drug delivery systems.[2] Within biomedicine, nanotechnology has been utilized in drug delivery, biosensors, nanobiotechnology, and tissue engineering.[3,4] Liposomes and micelles are examples of first-generation nanoparticle-based systems employed in these applications, followed by new-generation formulations like nanoparticles, nano-lipid carriers, and nanosponges used to deliver various drugs.[5] Targeting drug delivery mechanisms has long been a goal to get the desired result to avoid the vital issue of burst release in conventional delivery. Initially, the Nanosponge drug delivery system was only available as a topical administration method; however, in the twenty-first century, Nanosponges can be employed orally and Intravenously (IV). The nanosponge delivery method allows for regulated drug release. As a result, nanosponge delivery technology has gained popularity to improve therapeutic efficacy at the target site.[6]

ROS is an HMG-CoA reductase inhibitor and anti-hyperlipidaemic drug that reduces cholesterol synthesis through HMG-CoA conversion to mevalonic acid.[7,8] However, it has poor solubility and less than 20% bioavailability. Different formulations, such as solid dispersions and solid lipid nanoparticles, have been explored to improve ROS bioavailability; these preparations are complicated and may present multiple stability concerns.[9]

Pharmaceutical development using the Quality by Design (QbD) methodology emphasizes on recognizing and managing process and product variability within established limits to provide high-quality products.[10] QbD was utilized in the formulation of ROS nanosponges to determine the Quality Target Product Profile (QTPP), identify Critical Quality Attributes (CQA), specify essential process parameters, and assess and control risk using Failure Mode and Effects Analysis (FEMA).[11] Following

DOI: 10.5530/ijper.58.3.86

the identification of CQAs, the ROS nanosponge Formulations (RF) were designed using a central composite design approach within response surface methodology (RSM), and an optimized formulation was selected based on how independent factors (X) affect the responses (CQAs) (Y) and subsequently evaluated for *in vivo* release profile.[12,13] The current study aims to design and develop drug-loaded nanosponges to increase the drug's solubility and bioavailability. Different ratios of surfactant and polymer were used to generate nanosponges and further evaluated pharmacokinetic parameters.

MATERIALS AND METHODS

Materials

The API was acquired as a gift sample from Hetero Drugs, Hyderabad. Eudragit L-100 was procured as a gift sample from Lee Pharma Limited., Visakhapatnam, India. Solvents, Poly Vinyl Alcohol, and other chemicals (AR grade) were procured from SDFCL, Mumbai.

Methods

Determination of CQA and QTPP

CQAs are the biological, chemical, physical, or microbiological characteristics or features that should be controlled within specific parameters to ensure the desired quality of a pharmaceutical formulation. CQAs are identified based on scientific knowledge, experience, and regulatory guidelines. These attributes directly or indirectly affect the efficiency and safety of the drug.[14] The QTPP represents a drug product's desired characteristics and attributes that will ensure its safety, efficiency, and overall quality. It is a comprehensive summary of the quality criteria a drug formulation should meet to be considered for its intended application.

Screening factors and risk assessment

Screening factors involve the systematic evaluation of different factors or variables that could impact the quality or performance of a product or process. These factors include process parameters, raw materials, formulation components, equipment, and environmental conditions. Screening factors aim to identify the parameters which significantly impact the desired outcomes or pose risks to product quality. Risk assessment is a systematic approach to identifying, evaluating, and prioritizing potential risks associated with a product, process, or activity. Various materials and process parameters attributes were developed using the Ishikawa fishbone illustration,[15] which may cause a variance of CQAs to lead to product failure. From the literature review, material attributes like the concentration of the polymer and copolymer and process parameters like stirring speed were considered screening factors; particle size, %EE, and *in vitro* drug release are considered CQAs for QTPP.

Experimental design

After identifying the Critical Quality Attributes (CQAs) and variables, the formulation must be optimized and refined. Nevertheless, it is critical to conduct multiple tests, and addressing interaction studies that incorporate variables can be pretty complicated. Design Expert-13.[16] software's Central Composite Design (CCD) function was utilized to create the nanosponge formulations for this study. It improves the formulation and identifies the interaction effects of the factors on the responses in an efficient manner. Dosages of ROS drug release (Y_3), EL100 (X_1), PVA (X_2), and swirling speed (X_3) were taken into account during the design of the nanosponge. Y represents the response, while X denotes the independent variable. Considering the levels -1 and +1 for both independent variables, every conceivable formulation combination was generated.[17]

Optimization of the model

After the first study, a rotatable CCD in Response Surface Methodology (RSM) was utilized for the optimization of the dependent variables: drug release (Y_3), %EE (Y_2), and particle size (Y_1). Three repetitions of the examinations were performed in a random order. Checkpoint formulations were prepared to validate the design space. The experimental results were fitted to polynomial models that included interactive terms, as per the following equation:

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{12}X_{12}+\beta_{22}X_{22}+\beta_1\beta_2X_1X_2 \quad [1]$$

The statistical significance ($p<0.05$) of the model coefficient was analyzed by performing an Analysis of Variance (ANOVA).

Preparation of ROS nanosponges (RF)

ROS nanosponges (RF1-RF15) were prepared using the emulsion solvent evaporation technique. EL100 was employed as a polymer, and PVA was used as a surfactant. CCD specified the choice of polymer and surfactant concentrations.[18] First, the organic phase was produced by dissolving appropriate amounts of EL100 and ROS in dichloromethane. PVA was dissolved in distilled water (100 mL) to prepare the aqueous phase. The two phases were combined by adding an organic phase dropwise into the continuous aqueous phase and stirring for 2 hr at 1000 rpm. The formed nanosponges were vacuum-filtered and dried at 40°C for 24 hr before being stored in a desiccator.[19]

Compatibility study of drug-excipient

The spectra of the ROS and RF samples were determined using FTIR (Shimadzu FTIR-8400S) within the 400-4000 cm^{-1} range. The KBr pellet method is used. The pellet-forming process was achieved by combining a minute quantity of the substance with potassium bromide under pressure.[20,21]

SEM Analysis

The ROS was morphologically characterized using scanning electron microscope (Carl Zeiss SEM with Oxford EDX) in a high vacuum mode.[22]

Particle size, Zeta potential, and Polydispersity Index (PDI)

Zetasizer (Malvern Nano ZS) was used to determine the average particle size, PDI, and surface charge of RF. distilled water was added to each sample for dilution before analysis and analysed at 250.5°C.[23]

XRD study

The XRD (XRD-7000/Shimadzu) was used to study the formulation of ROS nanosponges by exposing the API to Cu Kα radiation.[24]

DSC study

In order to identify the interaction between the drug and excipient during formulation, Differential Scanning Calorimetry (Shimadzu DSC-60) tests were performed on the ROS nanosponge formulation and API.[25]

% EE and drug loading capacity (%DL)

The percentage of drug entrapped inside the nanosponge formulation is referred to as %EE.[26] To determine the %DL and % EE, 50 mg of RF was dissolved in 10 mL of phosphate buffer (pH 6.8), and the sample was agitated until complete dissolution. The resulting transparent drug layer was collected for analysis. The amount of ROS in the nanosponges was evaluated utilizing a UV-visible spectrophotometer, and the % EE of the ROS was calculated.[27]

$$\%EE = \frac{Total\ amout\ drug - Free\ drug\ in\ solution}{Total\ amount\ of\ drug} \times 100$$

$$\%DL = \frac{Total\ amount\ of\ drug - Free\ drug\ in\ solution}{Total\ wt\ of\ nanosponges} \times 100$$

In vitro drug release study

The dissolving apparatus USP-II (paddle method, Labtronics dissolution apparatus) was used to estimate the drug release within a temperature range of 37±0.2°C. Phosphate buffer of 900 mL at a pH 6.8 and100 rpm was used.[28] Nanosponges, equivalent to 20 mg of the drug, were measured, packed into a diffusion sachet, and placed into a dissolution beaker for drug dissolution testing. UV-vis spectral analysis at 237 nm evaluated the drug's concentration after samples were taken at specific intervals ranging from 1 to 8 hr.[29] The release pattern of RF's medication was examined by fitting the results of each dissolving sample into the most appropriate kinetic models.[30,31]

In vivo release study

When the plasma concentrations of the nanosponge formulation and the purified drug were compared, an *in vivo* drug release study revealed comparable outcomes. A PK solver 2.0.[32] was employed to determine the pharmacokinetic parameters. For an *in vivo* drug release experiment, healthy rabbits weighing between 1.5 and 2.5 kg were divided into the following three groups: standard (group I), test (group II), and control (group III). Animals are subjected to fasting for 24 hr before the drug administration.[33] Group I was administered the drug solution in its most purified form, while Group II was administered a nanosponge formulation containing 10 mg/kg of ROS. Group III included the control animals. At 1, 2, 3, 4, 6, 8, 10, and 12 hr, blood was taken from the rabbit's marginal ear vein. After undergoing micro centrifugation at 5000 rpm, the plasma was chilled to -20°C.

The plasma was separated from the blood sample by centrifuging for 5 min at 5000 rpm. A protein precipitant (0.2 mL of 20% perchloric acid) was combined with the collected plasma sample, and the drug was extracted from the plasma by centrifugation at 4000 rpm for 10 min at 4°C. Valsartan (an internal standard) was added to the plasma sample. The ROS and valsartan were injected into HPLC (Shimadzu SPD-20A LC-20AD) to measure the ROS in the extracted drug solution. Various concentrations of ROS solutions (0.4 -1.6 µg/mL) were prepared for calibration at 237 nm.[34] The column used for chromatography was the C18 (Cosmos) column. The mobile phase used for the separation is Acetonitrile: 5 mM sodium acetate buffer (70:30), the flow rate is 1mL/min, and the Injection volume is 5 µL.

Stability study

The samples were preserved in stability chambers characterized by a relative humidity of 75% and a temperature of 40±0.5°C. Physical examination and *in vitro* drug release assays were performed on the formulations over six months.

RESULTS

Determination of QTPP and CQAs for rosuvastatin nanosponges

The first phase of QbD for product development is determining the QTPP. The QTPP is a quality attribute set that ensures the efficacy and safety of the product. The QTPP outline is presented in Table 1. The Second phase of the QbD involves choosing the CQAs. The Critical Quality Attributes (CQAs) derived from the QTPP impact the finished product; hence, monitoring and researching this effect is essential.

Determination of CMAs, CPPs, and screening of factors

It was determined which CPPs and CMAs impacted the product quality. The concentrations of the polymer and stabilizer were thought to be the main CMAs determining the effectiveness

Table 1: QTPP of ROS nanosponges.

QTPP	Target	Justification
Formulation	Nanosponge	Particle nanonization is used to improve medication solubility while also achieving predictable drug release.
Particle size	<500 nm	Varying particle size affects the drug loading and release rate.
% EE	Maximum	Low %EE leads to loss of drugs in the system.
Percent yield	-	Process-related problems are indicated.
Drug release	Maximum	The particle's porosity impacts the drug release.

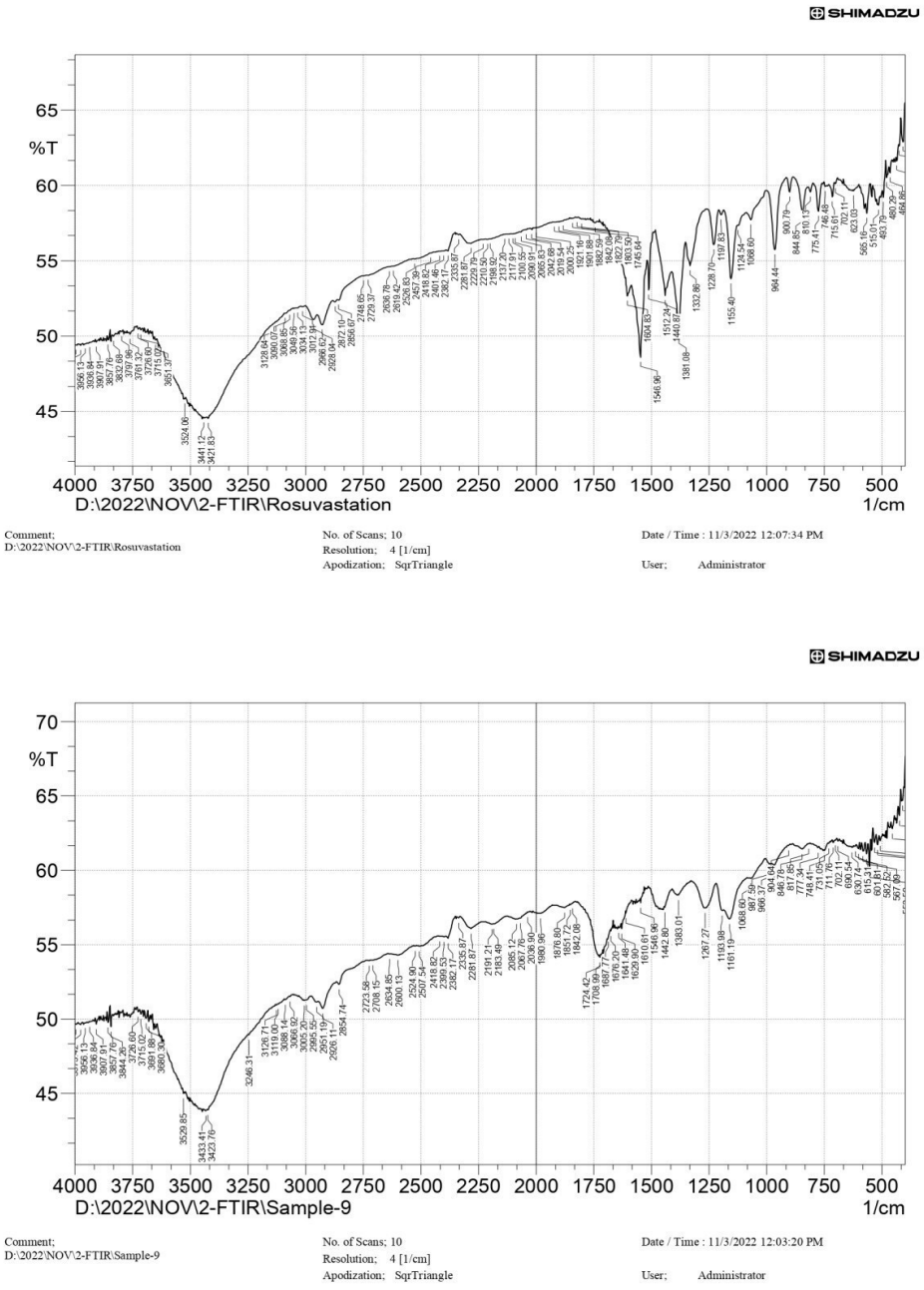


Figure 1: FTIR spectra of Pure drug (a) and ROS nanosponge (R16) (b).

of particle entrapment and particle size. The stirring rate and duration were identified as the process parameters that would have affected the nano formulation's particle size distribution and entrapment effectiveness. Various material variables and critical process parameters are represented in the Ishikawa illustration. From the literature review and preliminary studies, the polymer concentration (EL100), stabilizer concentration (PVA), and stirring speed (RPM) are considered critical factors in the present study.

Drug-excipient compatibility study

The FTIR spectrum of pure ROS and its nanosponge sample showed distinctive peaks for OH stretching at 3421 and 3433 cm^{-1}, C=O 1745 and 1842 cm^{-1}, C=C at 1604 and 1527cm^{-1}, C=N stretching at 1546 and 1629 cm^{-1}, C-F and S=O bending1332 and 1383 cm^{-1}, and so on. The two spectrums showed no interaction between pure drug and nanosponges, and the difference between the peaks of the pure drug and a nanosponge was less than 100 cm^{-1} (Figure 1).

SEM analysis

As per the SEM analysis, the nanosponge Formulation (RF1-RF15) achieved particle size ranging from 99.24±0.84 to 305.35±0.26 nm (Table 2). As shown in Figure 2a, the nanosponge surface had no trace of any crystalline medication particles, and the particle diameters of all formulations remained constant.

Particle size, PDI, Zeta Potential

The optimal formulation (RF16) contained particles that were nanosized and maintained in separation by repulsive forces, as shown in Figure 2b: the average particle size was 294±0.35 nm, the zeta potential was +16.1 mV, and the PDI was 0.489.

XRD study

The confirmation of the formation of ROS nanosponges was illustrated in Figure 2c by observing a smoother XRD curve for the ROS nanosponges relative to a purified substance.

DSC study

An endothermic peak for melting was observed at 138.07 °C on the DSC thermogram of the ROS pure substance. The inclusion of Rosuvastatin in the amorphous nanosponge core is indicated in Figure 2d, as the endothermic peak of the nanosponge was 254.81°C, which is in closer proximity to the 234.33°C peak of EL100.

%EE and %DL

The drug %EE and %DL capacity of all the nanosponges (RF1-RF15) were observed between 17.8±0.42 to 84.69±0.45% and 9.12±0.68 to 34.54±0.56%, respectively, and are shown in Table 2.

In vitro dissolution study

The ROS nanosponges (RF1-RF15) dissolution study was conducted using a USP-II dissolution apparatus at 37±0.5°C with 100 rpm using phosphate buffer (pH-6.8). Samples were collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4 and 6 hr. Drug release rates for all formulations ranged from 94.33±0.45% to 99.77±0.56% within 4 hr (Figure 3). Findings from the study of release kinetics using the Higuchi diffusion mechanism showed that the formulations exhibited zero-order kinetics.

Experimental design-Fitting response surface curve

The ROS nanosponges were assessed regarding particle size change, drug release, and %EE. Statistical analysis was performed on the formulation data to ascertain the model that most accurately corresponds to the independent variables. Compiled were regression results (*p*-values), coded equations, and regression coefficients (R^2) about the dependent variables. The significance of the constructed linear polynomial models was assessed through Analysis of Variance (ANOVA) (Table 3). Three-dimensional plots were used to study how the two independent variables interacted with one another (Figure 4).

Effect of Independent Variables on Particle Size, %EE, and Drug Release

The ANOVA results indicated that the linear model was the most appropriate for response 1, which concerns particle size. X1 and X2 were shown to be significant factors with agonistic impacts on particle size, but X3 did not show statistical significance. %EE demonstrated the linear model as the most suitable model. The significant factors X1 and X2 had a notable impact on the %EE. The linear model identified X1, X2, and X3 as crucial components that interacted in opposition to each other in drug release.

Validation of the model

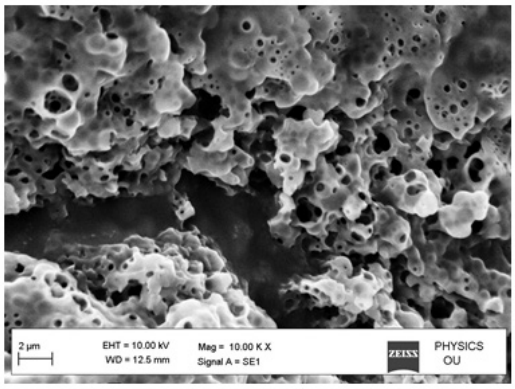
ROS nanosponges optimization aimed to increase the percentage of EE, decrease the particle size, and optimize drug release. Among the twenty-eight responses provided by the program, one produced a desirability of 0.462; this value served as the formula for batch RF16. In conclusion, the formulation in sample RF16 was deemed to be preferable.

Evaluation of Optimized formulation

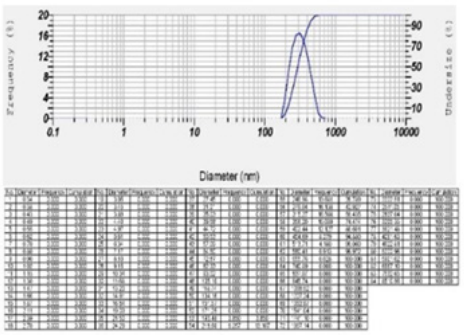
Table 4 exhibits RF16, the statistically optimized optimum formula, and provides an account of the parameter evaluation outcomes for the modified formula.

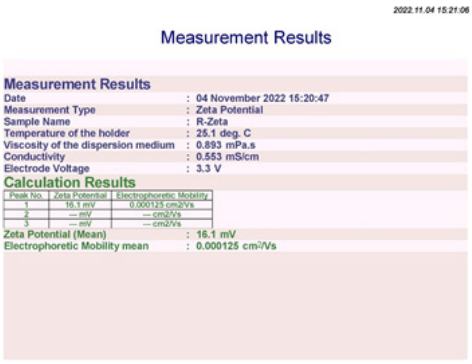
In vivo release study

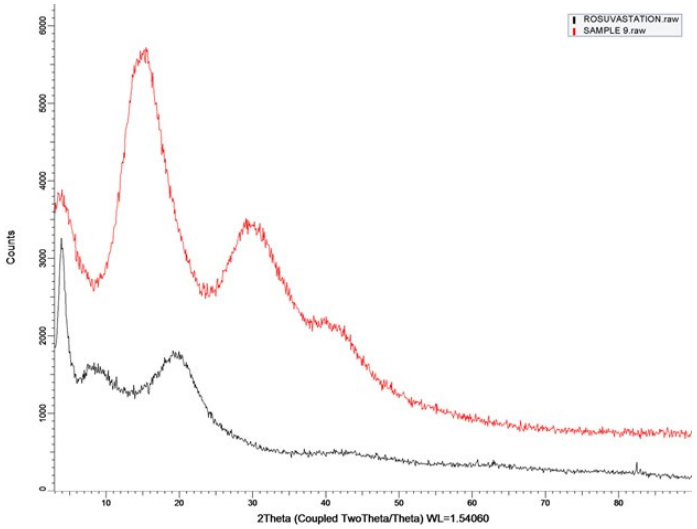
The investigation of drug release *in vivo* employed the optimized formulation RF16, characterized by exceptional particle size, an ideal percent efficacy, and drug release *in vitro*. Extracted drug samples were estimated for the concentration of the drug



(a) SEM Image for RF16







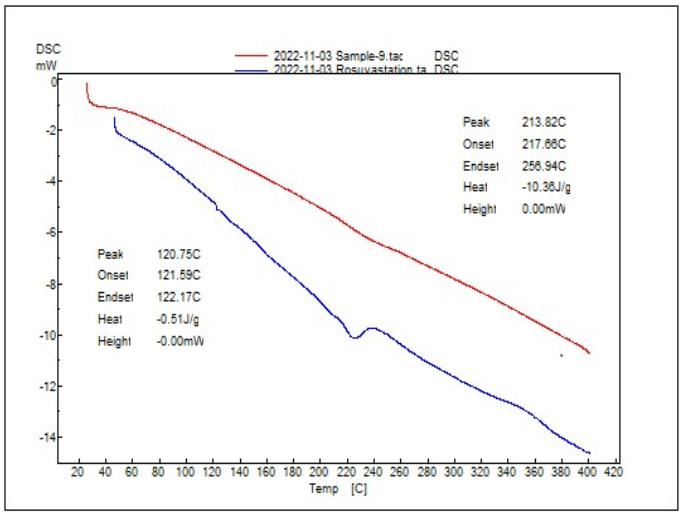


Figure 2: Characterization of nanosponges (a) FTIR (b)Particle size and zeta potential (c) XRD and(d) DSC.

using HPLC. C_{max}, T_{max}, and AUC the three pharmacokinetic parameters were evaluated and are are reported in Figure 5 which shows the levels of the medicine and ROS nanosponges (RF16) in the blood after they were taken orally. The results indicate that the C_{max} and T_{max} values for the purified substance and RF16 were 7.123 µg/mL, 14.787 µg/mL, and 1.5 and 2.5 hr, respectively. The $MRT_{0-\alpha}$ value for pure drug and RF16 were 5.04 hr and 3.91 hr, respectively. The $AUC_{0-\alpha}$ values for RF16 were 48.85 µg/mL*hr, while the $MRT_{0-\alpha}$ values were 19.56 µg/mL*hr and 25.71 µg/mL*hr for pure drug. The AUC results demonstrated that the drug was much more bioavailable in the nanosponges compared to the pure drug. Additionally, distinct pharmacokinetic characteristics were observed between the two groups.

Table 2: Drug loading (%) capacity for all formulations.

Formulation	Particle size (nm)	% EE	% DL
RF1	206	33.66±0.45	20.45±0.35
RF2	156	26.66±0.34	15.49±0.54
RF3	99	17.8±0.42	9.12±0.68
RF4	192	46.14±0.23	25.35±0.32
RF5	206	44.16±0.35	23.65±0.43
RF6	187	42.3±0.56	22.24±0.45
RF7	136	20.3±0.46	12.65±0.56
RF8	103	19.4±0.67	11.68±0.25
RF9	197	39.5±0.56	21.46±0.52
RF10	205	32.7±0.43	19.56±0.26
RF11	295	64.46±0.35	32.45±0.43
RF12	305	84.69±0.45	34.54±0.56
RF13	289	73.76±0.38	22.67±0.65
RF14	258	46.4±0.46	25.56±0.54
RF15	197	34.48±0.54	20.56±0.48

Table 3: Equations, probability, regression values, and the final models.

Sl. No.	Dependent Variable	Coded Equation	R^2 Value	*p*-value	F-value
1	Particle Size	202.07+43.80(A)+45.75(B)+2.21(C)	0.957	<0.0001	83.19
2	%EE	41.76+11.80(A)+13.83(B)+1.25(C)	0.84	<0.0001	19.24
3	%Drug release	95.77-2.56(A)-3.53(B)-0.2953(C)-2.83(AB)-0.355(AC)-0.82(BC)	0.9281	0.0004	17.20

Table 4: Formulation and evaluation of optimized formulation.

Ingredients	RF16	Responses	Predicted	Observed
Eudragit L100 (mg)	361.33	Particle size	252.75 nm	295±0.35 nm
PVA (mg)	472.14	%EE	58.52%	78.54±0.26%
RPM	1500	Drug release (at 4 hr)	90.08%	96.13±0.63%

DISCUSSION

A wide range of nanosponge formulations (RF1-RF15) were experimentally designed utilizing the Design Expert-13 software. The assessment of external variables' effects on CCD is achieved by implementing the surface response approach. The surface response method showed that polymer (EL100) has more influence on the size and %EE of the ROS nanosponges when compared with surfactant (PVA). The nanosponge formulation achieved particle size ranging from 99.24±0.84 to 305.35±0.26 nm for all the Formulations (RF1-RF15), and the optimized Formulation (RF16) showed 294±0.35 nm, the PDI was 0.489, and the zeta potential was 16.1 mV, indicating that the nanosized particles and separated by repulsive forces.

Predictions generated throughout the design process facilitated the identification of the compatibility between the excipient and the medication. Based on the characterization data, including FTIR spectra, it was observed that the API and formulation exhibited distinct absorption peaks. Furthermore, the absorbance shifts remained within the acceptable range of 100 cm^{-1} absorbance variations, effectively ruling out any potential incompatibility between the excipients and the medication. The XRD patterns of purified drug and ROS nanosponges were notably dissimilar. The gentler trajectory of the former indicated that the drug was encapsulated in an amorphous nanosponge complex. In contrast, the distinct peaks of the latter suggested that the drug existed in crystalline form. An endothermic apex for melting was observed at 138.07°C on the DSC thermogram of the pure substance of the ROS. Because the endothermic peak of EL100 occurred at 234.33°C and the endothermic peak of nanosponges occurred at 254.81°C, it was determined that the amorphous nanosponge core comprised Rosuvastatin.

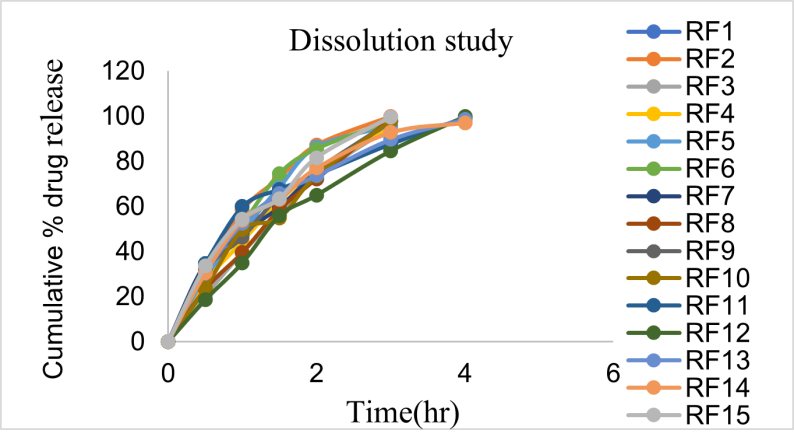


Figure 3: *In vitro* drug release profile for ROS formulations (RF1-RF15).

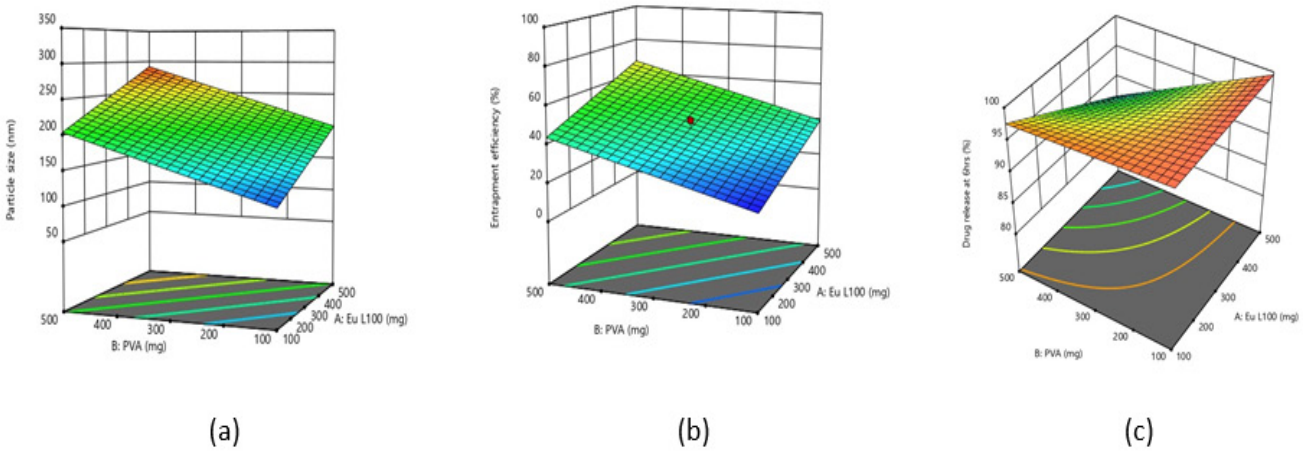


Figure 4: Surface response plots showing an effect between Eudragit L100 and PVA on particle size (a), % EE (b), and Drug release at 4 hr (c).

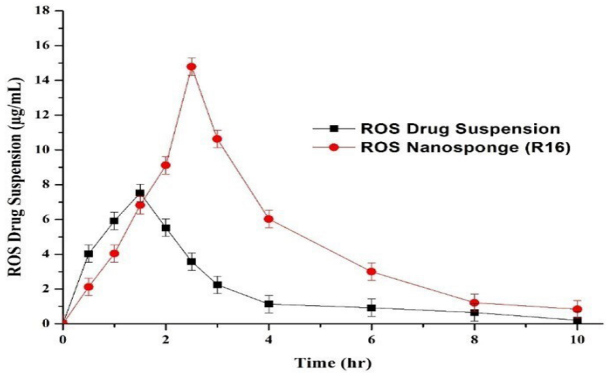


Figure 5: *In vivo* study of pure drug and ROS nanosponges (RF16).

The drug %EE of all the nanosponges (RF1-RF15) was observed between 17.8±0.42 to 84.69±0.45%, and the optimized formulation showed %EE 78.54±0.26. *In vitro* drug release study of optimized formulation results indicate that ROS enclosed in the nanosponge (RF16) absorbs better than pure drug. The time to maximum concentration (C_{max}) for the pure drug was 1.5 hr and for RF16 it was 2.5 hr, according to the *in vivo* release kinetics. The C_{max} values for the two substances were 7.123 µg/mL and 14.787 µg/mL, respectively. The AUC_{0-t} for the pure drug was 19.56 µg/mL*hr, while for RF16 it was 25.71 µg/mL*hr. The $AUC_{0-\alpha}$ for the pure drug was 23.91 µg/ml*h, while for RF16 it was 48.85 µg/mL*hr. The $MRT_{0-\alpha}$ for the pure drug was 5.04 hr and

for RF16 it was 3.91 hr. The *in vivo* pharmacokinetic properties of the purified drug and RF16 were dissimilar, indicating that RF16 exhibited a twofold drug release enhancement compared to the pure drug. The area under the curve data also demonstrated that the medication's bioavailability was much improved in nanosponges compared to the pure drug.

CONCLUSION

An experimental approach was used to design different nanosponge formulations using Design Expert-13. The surface response curves showed that polymer A (EL100) affects the size and EE (%) of ROS nanosponges more than surfactant (PVA). All the formulated ROS nanosponges exhibited high EE (%), drug loading capacity, and % drug release. The optimized formulation (RF16) was used in *in vivo* experiments. *In vivo* studies indicated that the optimized formulation (RF16) showed a 2-fold increase in drug bioavailability than the pure drug. As a result, the current investigation led to the conclusion that the nanosponge formulation exhibited potential and could be utilized to develop ROS delivery systems that are more accessible and efficient.

ACKNOWLEDGEMENT

The authors wish to extend their sincere appreciation to the Principal and Management of the KL College of Pharmacy, KL (Deemed to be University), Vaddeswaram, for their support to conduct this study. The authors would like to sincerely thank the University College of Technology, Osmania University, Hyderabad, India, for facilitating the analytical characterization data.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception and design of the study. Sadhana N, BVS Praveen, and Vishnu P performed material preparation, data collection, and analysis. Sadhana N and Narender M wrote the manuscript's first draft, and all authors commented on earlier versions. All authors read and approved the final manuscript.

ETHICAL APPROVAL

All animal studies were conducted with prior approval from the Institutional Animal Ethical Committee (approval no. 06/IAEC/VIPER/Ph.D/2021-22/II).

ANIMAL STUDIES

All institutional and national guidelines for the care and use of laboratory animals were followed.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FTIR: Fourier-transform infrared spectroscopy; **DSC:** Differential Scanning Calorimetry; **XRD:** X-ray Diffraction; **SEM:** Scanning Electron Microscopy.

SUMMARY

In the present work, the Rosuvastatin (ROS) nanosponges were formulated using the emulsion solvent evaporation method by employing the QbD approach and CCD in response surface methodology in the Design of Experiments (DoE). When assessing the synthesized nanosponges, several factors were considered, including particle size, Entrapment Efficiency (%EE) percentage, *in vitro* drug release research, and *in vivo* drug release. The produced nanosponges exhibited a particle size range of 99±0.84 to 305±0.26. The percentage of Effective Release (%EE) varied between 17.8±0.42 and 84.69±0.45. The substance showed a 4 hr release range of 94.33±0.45 to 99.77±0.56. The drug release study yielded results for the enhanced Formulation (RF16) in 3.5 hr: a rate of 95.13±0.63%, a particle size of 295±0.35 nm, and an efficiency evaluation of 78.54±0.26%. *In vivo* study indicated the 7.123 µg/mL and 14.787 µg/mL, 1.5 and 2.5 hr, 19.56 µg/mL*hr and 25.71 µg/mL*hr, 23.91 µg/ml*h and 48.85 µg/mL*hr, 5.04 hr and 3.91 hr of C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\alpha}$, $MRT_{0-\alpha}$ for the pure drug and RF16, respectively. The present study confirmed that the nanosponge formulation was the most suitable approach to enhance the solubility of ROS.

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Cite this article: Noothi S, Narender M, Pulavarthy V, Praveen BVS. Development of Nanosponge Formulations of Rosuvastatin for Oral Delivery Using a Central Composite Design. Indian J of Pharmaceutical Education and Research. 2024;58(3):784-93.