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Formulation and Optimization of Nanosuspensions for Enhancing Simvastatin Dissolution Using Central Composite Design

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ABSTRACT

Simvastatin is a poorly water-soluble drug, and bioavailability from its crystalline form is very low. The purpose of this investigation was to increase the solubility and dissolution rate of simvastatin by the preparation of nanosuspensions with Pluronic F127 and zirconium oxide (ZrO_2) beads using a wet-milling technique at the laboratory scale. Prepared nanosuspensions were evaluated for particle size and in vitro dissolution. A 3^2 central composite design was employed to study the effect of the independent variables (i.e., amount of Pluronic F127, X_1, and amount of ZrO_2, X_2) on the dependent variables (i.e., particle size [nm] and percentage of drug released after 10 min, Q_{10}). The relationship between the dependent and independent variables was further elucidated using multiple liner regression analysis (MLRA) and contour plots. The results show that nanosuspensions prepared with the higher concentrations of Pluronic F127 and the higher quantities of ZrO_2 (up to 8 g) reduced the particle size and enhanced the dissolution rate of the formulation. The dissolution rate of the optimized nanosuspension was enhanced (64% in 10 min) relative to that of a micronized suspension of simvastatin (3.5% in 10 min), mainly because of the formation of nanosized particles. These results show that the preparation of simvastatin-loaded nanosuspensions significantly improved the in vitro dissolution rate, thus possibly enhancing the fast onset of therapeutic drug effect.

INTRODUCTION

The design and formulation of a dosage form require consideration of the physical, chemical, and biological characteristics of all the drug substances and pharmaceutical ingredients to be used in its preparation. An important property of a drug substance is solubility, especially aqueous system solubility (*1*).

The solubility–dissolution behavior of a drug is a key factor to its oral bioavailability. An improvement in the solubility of poorly water-soluble drugs remains one of the most challenging tasks of drug development. The techniques that can generally overcome the problem of solubility are salt formation, micronization, use of surfactant, and use of prodrugs. However, all these techniques have certain limitations. Over the last ten years, nanoparticle engineering processes have been developed and reported for pharmaceutical applications (*2, 3*).

Various approaches have been developed to improve bioavailability by increasing drug dissolution rate and solubility. For example, nanosizing techniques have been used to enhance dissolution rate by increasing drug surface area, thereby improving the oral bioavailability of poorly water-soluble drugs (*4, 5*). Nanosuspensions are submicron colloidal dispersions of pure drug particles in an outer liquid phase. Nanoparticle engineering enables poorly soluble drugs to be formulated as nanosuspensions either alone or with a combination of pharmaceutical excipients. The nanosuspension engineering processes currently used are precipitation (*6*), high-pressure homogenization (*7*), and pearl milling (*8*), either in water or in mixtures of water and water-miscible liquids or in nonaqueous media (*9*).

In the present study, a wet-milling technique was used to prepare nanosuspensions; an aqueous suspension was formulated with ZrO_2 as a milling medium. As the beads rotated, they flew to the grinding vial interior and impacted against the sample on the opposite grinding vial wall. The combination of frictional forces and impact forces led to a high degree of particle size reduction. Cavitation fields generated inside the chambers also contributed to particle size reduction. The main advantage of this technique is that no hazardous solvents are used (*10, 11*).

Simvastatin (SIM) is a lipid-lowering agent derived synthetically from a fermentation product of *Aspergillus terreus*. After oral ingestion, SIM, an inactive lactone, is hydrolyzed to the corresponding β-hydroxy acid form. This is a principal metabolite and an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme-A (HMG Co-A) reductase, the enzyme that catalyses an early and rate-limiting step in the biosynthesis of cholesterol (*12*). SIM is a white, crystalline, nonhygroscopic powder, insoluble in water and 0.1 N HCl (30 µg/mL and 60 µg/mL, respectively). It is generally considered that compounds with very low aqueous solubility will show dissolution-rate-limited absorption. Improvement of aqueous solubility in such cases is a valuable goal to improve therapeutic efficacy. The dissolution rate is a function of the solubility and the surface area of the

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drug; thus, dissolution rate will increase if drug solubility and surface area are increased (*13, 14*).

Central composite design (CCD) is one of the tools used to study the effect of different variables on the dependent variables of any formulation. Based on the principle of design of experiments, CCD was employed to investigate the effect of two independent factors. Design of experiments encompasses the use of various types of experimental designs, generation of polynomial equations, and responses over the experimental domain to determine the optimum formulation. Multiple linear regression analysis of results leads to equations that adequately described the influence of the independent variables on the selected responses (*15*).

The purpose of this study was to optimize the wet-milling method for the preparation of nanosuspensions. A central composite design was applied to investigate the combined effect of two formulation variables, the amount of Pluronic F127 (X_1) and the amount of small-sized ZrO_2 spheres (X_2). The particle size (nm, Y_1) and the percentage of drug released after 10 min (Q_{10}, Y_2) were taken as responses. Multiple linear regression analysis (MLRA) was employed to construct polynomial equations relating each response to the factors affecting it. Counter plots were drawn, and an optimum formulation was selected using the desirability function. Dissolution studies of both prepared nanosuspensions and micronized suspensions were performed using distilled water as dissolution medium, and the drug release profiles of both suspensions were compared.

MATERIALS AND METHODS

Materials

Simvastatin, Pluronic F127, and Lutrol F68 were obtained as gift samples from Torrent Pharmaceutical Ltd., Ahmedabad, India. Tween 80, polyvinyl alcohol, and PVP-K 30 were gift samples from S. D. Fine Chemicals. Bidistilled water was prepared in the laboratory. All materials used for this study conformed to *USP* standards.

Preparation of Nanosuspension

Nanosuspensions were prepared using wet milling. Suspensions of 20 mg SIM in 10 mL bidistilled water were prepared in 20-mL vials. Nanosuspensions of simvastatin were prepared in distilled water using ZrO_2 beads (0.4–0.7 mm) as a milling medium and Pluronic F127 as stabilizer. Nanosuspensions were prepared by dissolving different concentrations of stabilizer in distilled water. Drug was then added directly into the stabilizer solution. These were comminuted with different amounts of ZrO_2 beads on a magnetic stirrer for 16 h. Nanosuspensions were separated from the ZrO_2 beads by decanting the suspension followed by washing the beads with water.

Particle Size Determination

Particle size was determined by photon correlation spectroscopy (PCS) using a Zetasizer 3000 (Malvern Instruments, UK). This analysis yields the mean diameter (*z*-average, measuring range 20–1000 nm). All data presented are the means of the average values of three independent samples produced under identical production conditions. To obtain suitable concentrations, samples were diluted with deionized water before measurement.

Table 1. Formulation and Dissolution Characteristics of Central Composite Design Batches[a]

	Coded values		Actual values		Dependent Variables	
Run	X_1	X_2	X_1	X_2	Y_1	Y_2
H_1	-1	-1	15	4	999.0	33.5
H_2	-1	0	15	6	840.0	41.0
H_3	-1	1	15	8	1011.0	34.0
H_4	0	-1	25	4	859.1	43.0
H_5	0	0	25	6	525.0	56.0
H_6	0	1	25	8	572.0	50.5
H_7	1	-1	35	4	888.3	42.0
H_8	1	0	35	6	421.0	59.0
H_9	1	1	35	8	333.0	64.0

Y_1: particle size (nm).
Y_2: percentage release of SIM after 10 min.
X_1: amount of stabilizer.
X_2: amount of ZrO_2.
[a]Each batch contains 20 mg of simvastatin; total nanosuspension volume was 10 mL. Standard deviation of the responses did not exceed 3% of the measured value.

Optimization of Formulation Using Central Composite Design

A central composite design (CCD) for two factors at three levels each was selected to optimize the response of the variables. The two factors, amount of stabilizer (Pluronic F127) (X_1) and amount of ZrO_2 beads (X_2), were varied, and the factor levels were suitably coded. Particle size and percentage of drug released after 10 min (Q_{10}) were taken as the response variables. In this design, two factors are evaluated, each at three levels, and experimental trials are performed for all nine possible combinations (Table 1). All other formulation variables and processing variables were kept invariant throughout the study.

Optimization, Data Analysis, and Desirability Function

Various response surface methodology (RSM) computations for the current optimization study were performed employing Design-Expert software (Version 7.0.1, Stat-Ease Inc., Minneapolis, MN). Polynomial models including interaction and quadratic terms were generated for all the response variables using a multiple linear regression analysis (MLRA) approach. In addition, 2-D contour plots were constructed using the output files generated by the Design-Expert software.

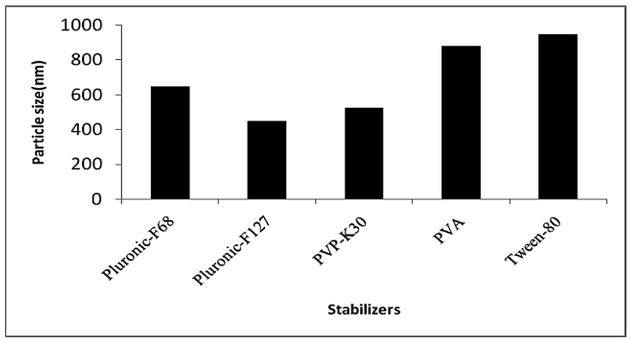

Figure 1. Influence of type of stabilizers on SIM with 6 g ZrO_2 beads.

After the fitting of the mathematical model, the desirability function was used for the optimization. During the optimization of a multivariable formulation, such as a nanosuspension, the responses are combined to find a product having the desired characteristics. The desirability function combines all the responses into one variable to predict the optimum levels for the independent variables. A desirability value of 0 represents an unacceptable value for the responses, and a value of 1 represents the most desired value for the responses.

Dissolution Study

In vitro drug release studies were performed in USP Apparatus 2 (paddle) at a speed of 50 rpm. Dissolution was carried out in 900 mL of distilled water as the medium at 37.0 $\pm$ 0.2 °C. Five milliliters of sample was withdrawn periodically (every 10 min) and replaced with an equal volume of fresh distilled water up to 60 min. Samples were diluted suitably and filtered through filter paper (0.22 µm, Whatman Inc., USA). The dialyzate was then subject to UV analysis versus a blank (distilled water). Percent cumulative release of SIM was calculated based on the standard UV calibration curve at 233 nm (Systronic 2203, Japan).

RESULT AND DISCUSSION

Simvastatin is a BCS Class II drug having low solubility and high permeability. Thus, it is challenging to enhance the dispersion of simvastatin particles in an aqueous solution. The presence of stabilizer improves the wettability of poorly soluble drug particles, and zirconium oxide beads provide particle size reduction. The combined effect of stabilizer and ZrO_2 beads can produce drug nanoparticles during media milling. The influence of stabilizer type at a fixed concentration in the media-milling method was investigated in terms of particle size with a fixed quantity of ZrO_2 beads and amount of drug. In the prescreening study, Pluronic F68, Pluronic F127, PVP-K 30, PVA, and Tween 80 were selected as stabilizers. Before the statistical experimental design was employed, two factors (amount of stabilizer and amount of ZrO_2 beads) were prescreened by varying one factor at a time. For the effective size reduction of the drug particles, the prescreening study was based on water-soluble stabilizers at a fixed quantity of ZrO_2 beads.

From this study, it was found that Pluronic F127 gave the desired particle size (450 nm) with a lower polydispersivity index (0.227) compared with the other stabilizers. Pluronic F127 provided efficient steric stabilization by forming adsorption layers on drug nanoparticles. An important function of Pluronic F127 was that it formed a substantial mechanical and thermodynamic barrier at the interface that retards the approach and coalescence of individual particles. Therefore, Pluronic F127 was selected as the stabilizer for further investigation. It was observed that the extent of size reduction was mainly governed by the amount of zirconium oxide beads rather than other operative conditions in media milling. In the prescreening study, media milling of drug suspension was done with 6 g and 8 g of ZrO_2 beads at a fixed amount of Pluronic F127.

A larger particle size with high polydispersivity occurred when 6 g of ZrO_2 beads was used. On the other hand, a smaller particle size with high monodispersivity was obtained with 8 g of ZrO_2 beads. One of the possible reasons was that the suspension undergoes intense media grinding and passes through a small gap in the presence of 8 g of ZrO_2 beads. A larger quantity of ZrO_2 beads has fewer voids, leading to smaller particle size. These induce cracks in the particles by a combination of impact and attrition induced by bead rotation at 250 rpm on high-speed shakers. The freshly created particulate surfaces are immediately coated by a layer of Pluronic F127, which prevents the broken particles from agglomerating (Figure 1).

Central Composite Design

CCD for two factors at three levels with $\alpha = 1$, equivalent to a 3^2 factorial design, was chosen as the experimental

Table 2. Regression Equations for the Responses

Coefficients	β_0	β_1	β_2	β_3	β_4	β_5	R^2
Y_1	530.87	-201.28	-138.48	-141.82	96.68	181.73	0.9931
P-Value	0.000008	0.000024	0.000074	0.000126	0.0011	0.00017	
Y_2	54.83	9.41	5	5.375	-4.25	-7.5	0.9913
P-Value	0.029	0.036	0.010	0.077	0.380	0.238	

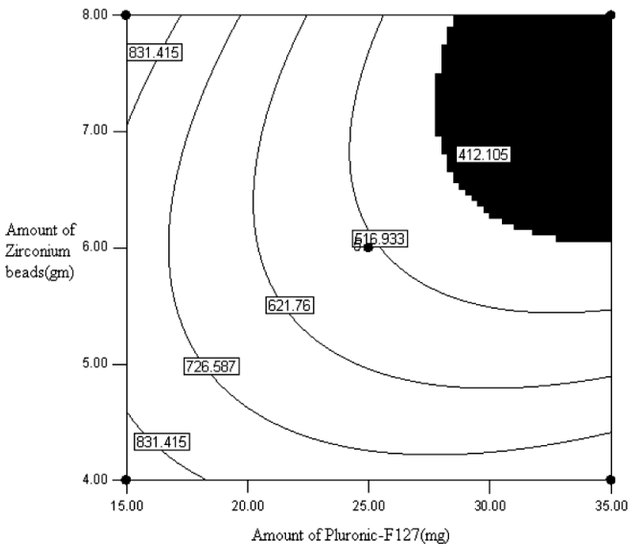

Figure 2. Counter plot showing the influence of Pluronic F127 and ZrO_2 beads on particle size.

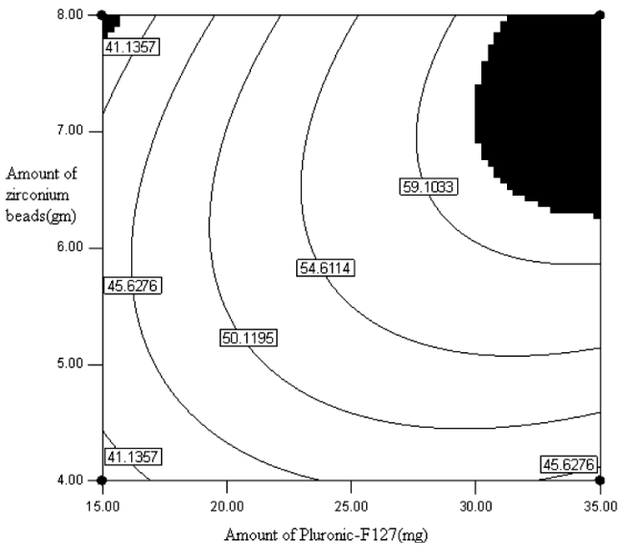

Figure 3. Counter plot showing the influence of Pluronic F127 and ZrO_2 beads on Q_{10}.

design. This is an effective second-order experimental design associated with a minimum number of experiments to estimate the influence of individual variables (main effects) and their second-order effects. Further, this design has an added advantage of determining the quadratic response surface, not estimable using a factorial design at two levels (*15*).

To investigate the factors systematically, a central composite design was employed. As shown in eq 1, a statistical model incorporating interactive and polynomial terms was used to evaluate the responses.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2 + \beta_6 X_1 X_2^2 + \beta_7 X_2 X_1^2 \quad (1)$$

Seven coefficients (β_1 to β_7) were calculated representing β_0 as the intercept, and β_3 to β_7 various quadratic and interaction terms. Mathematical relationships generated using MLRA for the studied response variables are expressed as equations. The polynomial equations comprise the coefficients for intercept, first-order main effects, interaction terms, and higher-order effects. The sign and magnitude of the main effects signify the relative influence of each factor on the response.

Concerning particle size, the results of multiple linear regression analysis show that both coefficients β_1 and β_2 bear a negative sign ($R^2 = 0.9797$). It can be concluded from eq 2 that X_2 (amount of ZrO_2 beads) is more effective compared with X_1 (amount of Pluronic F127).

$$Y_1 = 530.87 - 201.28 X_1 - 138.48 X_2 - 141.82 X_1 X_2 + 96.68 X_1^2 + 181.73 X_2^2 \quad (2)$$

The coefficients β_1, β_2, and β_3 were significant at $P < 0.05$ (Table 2). Therefore, increasing the amount of Pluronic F127 or the amount of ZrO_2 beads is expected to decrease the particle size. A smaller amount of stabilizer induces agglomeration or aggregation, and too much stabilizer promotes Oswald's ripening (a phenomenon in which small crystals, more soluble than large ones, dissolve and re-precipitate onto larger particles). Particle size was decreased because increasing the quantity of ZrO_2 beads led to fewer voids among them. Therefore, the drug particles were reduced to micro- and nanoparticles, which were efficiently shielded by stabilizer to prevent agglomeration. Figure 2 shows a sharp decline in particle size as the content of Pluronic F127 or amount of ZrO_2 beads was increased.

Concerning Q_{10}, the results of MLRA show that both coefficients β_1 and β_2 have a positive sign ($R^2 = 0.9913$). It can be concluded from eq 3 that X_1 (amount of Pluronic F127) was more effective than X_2 (amount of ZrO_2 beads). The coefficient β_1 only was found to be significant at $P < 0.05$ (Table 2).

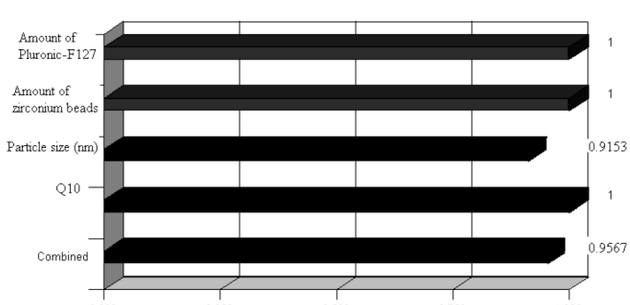

Figure 4. Bar graph showing individual desirability values of various objective responses and their associated overall desirability.

Table 3. Experimental and Predicted Responses for Optimized Formulation

Run	Observed (Y_1)	Predicted (Y_1)	% Bias (Y_1)	Observed (Y_2)	Predicted (Y_2)	% Bias (Y_2)
H_9	333.0	336.0	0.89	64.0	62.9	-1.71

$$Y_2 = 54.83 + 9.41\ X_1 + 5\ X_2 + 5.73\ X_1\ X_2 - 4.25\ X_1^2 - 7.5\ X_2^2 \quad (3)$$

Slow dissolution can be partly attributed to hydrophobicity as evidenced by poor wetting of the drug surface. This causes the particles to aggregate rather than disperse. Dissolution rate in the nanosuspension is improved because of increased surface area, which enhances the strong hydrophilic character of drug toward Pluronic F127 because of the formation of intermolecular hydrogen bonds and improved wettability of hydrophobic SIM. Figure 3 shows a nearly linear ascending pattern for the values of Q_{10} as the content of Pluronic F127 or ZrO_2 beads increases. The effect was much more prominent with Pluronic F127 than with ZrO_2 beads.

Formulation Optimization Using the Desirability Function

The aim of pharmaceutical formulation optimization is generally to find the levels of the variable that affect the chosen responses and determine the levels of the variable from which a robust product with high quality characteristics may be produced (*17*). All the measured responses that may affect the quality of the product should be taken into consideration during the optimization procedure. Upon "trading off" different response variables, the following criteria were adopted: $Y_1 \leq 350$ nm and $Y_2 \geq 60\%$.

Using the desirability function, all the defined responses can be combined into one overall response, the overall desirability (Figure 4). After a comprehensive evaluation of feasibility and the subsequent use of the desirability function, the formulation composition with stabilizer levels of Pluronic F127 (35 mg) and ZrO_2 beads (8 g) fulfilled maximum requisites of an optimum formulation because of smaller particle size (333 nm) and higher drug release after 10 min (64%) (Table 3).

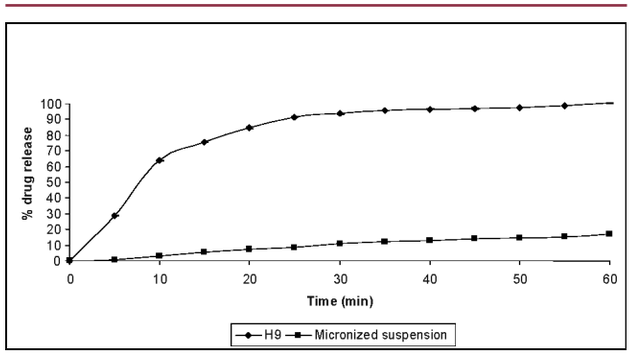

Figure 5. Dissolution profiles of (-♦-) simvastatin nanosuspension (H_9) and (-■-) the suspension of micronized simvastatin.

The in vitro dissolution of a drug is an indirect method to predict its bioavailability from a formulation. As shown in Figure 5, optimized nanosized simvastatin (H_9) showed a dramatic increase in rate and extent of dissolution compared with micronized simvastatin. The rate of dissolution of the optimized nanosuspension was enhanced (64% in 10 min) relative to a micronized suspension of simvastatin (3.5% in 10 min). The slope of the dissolution profile is especially different for the nanosuspension in the initial stage (first 10 min) and is maintained throughout the experiment compared with micronized simvastatin. Drug nanoparticles of simvastatin had a higher surface area than micronized particles in suspension.

CONCLUSION

Simvastatin nanosuspension can be prepared using a wet-milling method with Pluronic F127 as hydrophilic polymer stabilizer at the laboratory scale. In experimental design, careful selection of the amount of ZrO_2 beads and Pluronic F127 as stabilizer are critical to achieve a particle size close to 350 nm and greater than 60% drug dissolved in 10 min. Nanosized simvastatin dissolved much more rapidly than the micronized form. Clearly, these results indicate the suitability of the formulation procedure for the preparation of simvastatin nanosuspension with significant improvement of the in vitro dissolution rate, and thus possibly improved oral bioavailability.

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