International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.1, pp 155-161, Jan-Mar 2010

Optimization of Formulation Parameters on Famotidine Nanosuspension Using Factorial Design and the Desirability Function

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Abstract: The aim of this work is to study the optimization of formulation parameters in the preparation of nanosuspension loaded famotidine (FAM) by solvent evaporation technique. A 3^2 full factorial design and the desirability function were designed to study the effects of the amount of stabilizer (Lutrol F-68) and stirring speed (800, 1000 and 1200) on the particle size (Y_1), cumulative percentage FAM released after 10 min (Y_2), and cumulative percentage FAM released after 120 min (Y_3). Optimization was performed using a desirability function to obtain the levels of X_1 and X_2, which close to 500 nm, 30% and 90% for Y_1, Y_2 and Y_3 consequently. The optimized nanosuspension were predicted to yield particle size of 478.1 nm, drug release after 10min (Q_{10}) of 31.73%, and drug release after 120min (Q_{120}) of 92.66%, which were remarkably close to the experimental values of 495.4 nm, 31.71%, and 94.25% consequently.

Keywords: Quality by design, Famotidine, Nanosuspension, Factorial design, Optimization.

Introduction

Quality by design refers to the achievement of certain predictable quality with desired and predetermined specifications. As different techniques of drug nanosuspensions involve many interacting variables and operating conditions, experimental design methods are extensively being used in the nanosuspension studies. To understand the variables and their interactions, many statistical experimental designs have been recognized as useful techniques. Optimization through experimental design (including factorial design) and response surface methodology is a common practice in biotechnological and pharmaceutical processes.[1-2]

Famotidine (FAM) is a histamine H_2-receptor antagonist. It is widely prescribed in gastric ulcers, duodenal ulcers, Zollinger- Ellison syndrome and gastro-esophageal reflux disease. FAM is reported to be 7.5 and 20 times more potent than ranitidine and cimetidine, respectively. In spite of the great therapeutic interest of this drug, the bioavailability after oral dosing is low (20–40%) with a higher variability. The incomplete and variable bioavailability of famotidine has been attributed to its poor aqueous solubility. In recent years, much attention has been focused on drug nanosuspensions for the bioavailability improvement of water insoluble drugs.[3-4]

Nanosuspension engineering processes currently used are precipitation, pearl milling and high pressure homogenization, either in water or in mixtures of water and water-miscible liquids or non-aqueous media.[5-6]

Solvent evaporation method presents numerous advantages, in that it is a straightforward technique,

rapid and easy to perform. Solvent evaporation, however, require a large number of experiments to describe the effect of excipients and experimental condition on the formulations characteristics. The present study, therefore, deals with the optimization of formulation variables to design the best product under conditions of competitive objectives, because interactive effects via a trial-and-error approach are time-consuming and often unsuccessful. Mathematical optimization by means of an experimental design is most helpful in shortening the experimental time.[7]

The objective of the present work was to apply 3^2 factorial design with desirability function for understanding the quality and optimization of FAM nanosuspension. The independent variables for the present study are the following: amount of stabilizer namely Lutrol F-68 (X_1) and stirring speed (X_2). As part of the optimization process, the main effects and interaction effects of the formulation parameters were investigated. Stabilizer, stirring speed and their interactions were evaluated for their effect on the particle size, cumulative percentage, Q_{10}, and Q_{120}.

Experimental

Famotidine was obtained from Cadila Pharmaceutical Ltd. as a gift sample (Ahmedabad, India). Lutrol F-68 was obtained as a gift sample from Torrent Pharmaceutical Ltd. (Gandhinagar, India). Methanol was obtained as a gift sample from Chemdyes Corporation. (Rajkot, India). Double distilled water was prepared in laboratory for study.

Preparation of nanosuspensions

Nanosuspensions were prepared by the solvent evaporation technique. FAM was dissolved in a methanol (6 ml) at room temperature. This was poured into 10 ml water containing different amount of Lutrol F-68 maintained at a temperature of 30–40°C and subsequently stirred at ranging agitation speed for 2 hrs to allow the volatile solvent to evaporate (Remi, High speed stirrer, India.). Addition of organic solvents by means of a syringe positioned with the needle directly into surfactant containing water. Organic solvents were left to evaporate off under a slow magnetic stirring of the nanosuspensions at room temperature for 3 hrs.

Particle size and scanning electron microscopy

The particle size of the produced nanosuspension was analyzed by photon correlation spectroscopy (PCS) using a Zetasizer 5000 (Malvern Instruments Ltd., UK). All samples were measured appropriately after diluted with bidistilled water. The nanoparticle surface appearance was analyzed by scanning electron microscopy (SEM).

Drug release studies from nanosuspension

FAM release from nanosuspension was taken in modified diffusion cell apparatus (Fig 1). The drug release from nanosuspension was determined using a dialysis tube (donor compartment) containing the known quantity (10 ml) of the nanosuspension in a water-jacketed beaker containing 300 ml of 0.1N HCl (pH 1.2) at 37 ± 1°C for 2 hrs. The contents of the beaker were agitated on a magnetic stirrer. Samples were withdrawn periodically and replaced with an equal volume of fresh 0.1N HCl (pH 1.2). Samples were diluted suitably and filtered through a filter paper (0.22 μm). Famotidine content was determined by UV method at 267nm (Systronic 2203, Japan).

Experimental design and desirability function

A two-factor, three-level full factorial design was applied for the optimization procedure using Design expert 7.1.6 software (Stat Ease, Inc. Minneapolis, MN). The independent factors and the dependent variables used in this design are listed in Table 1. The amounts of stabilizer and stirring speed were used to prepare each of the 9 formulations are given in Table 1. These high, medium, and low levels were selected from the preliminary experimentation. After generating the polynomial equations, relating the dependent and independent variables, the process was optimized for the particle size (Y_1), Q_{10} (Y_2), Q_{120} (Y_3). After the fitting of the mathematical model, the desirability function was used for the optimization. During the optimization of a multivariable formulation, such as nanosuspension the responses have to be combined in order to find a product, which the formulator defines as having the desired characteristics. The application of the desirability function combines all the responses into one variable and leaves the possibility to predict the optimum levels for the independent variables.[8]

Result and discussion

Solvent evaporation with homogenization has been employed to produce nanosuspension of FAM. The different formulative variables (1) amount of stabilizer (2) stirring speed were contribute much towards the change in particle size in nanosuspension preparation. Factorial design was applied in this study to optimize the FAM nanosuspension with constraints on the particle size, Q_{10} and Q_{120}. From the preliminary experimentation, higher variability was found for the amounts of drug released from the smaller particle size than from the larger ones. Accordingly, in order to reduce this variation, optimization was performed using a desirability function to obtain the levels of X_1 and X_2, which close to 500 nm, 30% and 90% for Y_1, Y_2 and Y_3 consequently.

The observed responses for the 9 formulations are given in Table 3. In order to investigate the factors systematically, a factorial design was employed. As shown in equation 1, a statistical model incorporating interactive and polynomial terms was used to evaluate the responses.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad \text{..... (1)}$$

Where Y_i (Y_1, Y_2 and Y_3) are the dependent variables, namely, particle size, Q_{10} and Q_{120}, b_0 is the arithmetic mean response of the 9 runs, b_1 and b_2 are the estimated coefficients for the factors X_1 and X_2, respectively. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction term (X_1X_2) shows how the response changes when two factors are simultaneously changed. The polynomial terms (X_{12} and X_{22}) are included to investigate nonlinearity. The fitted equations (full models) relating the responses to the transformed factor are shown in table 2. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (i.e., positive or negative). A coefficient with positive sign represents a synergistic effect of the factor on the response, while a negative sign indicates an antagonistic effect. The mathematical relationship in the form of factor's coefficients, its corresponding *P*-values for the measured responses and correlation coefficient are listed in Table 4. Coefficients with *P*-value less than 0.05 had a significant effect on the prediction efficacy of the model for the measured response. The high values of correlation coefficient for the dependent variables indicate a good fit.

Concerning particle size, the results of multiple linear regression analysis showed that both the coefficients b_1 and b_2 bear a positive sign (R^2=0.9831). It can be concluded from the equation (2) that X_1 (amount of Lutrol F-68) showed the largest positive effect compare to X_2 (stirring speed).

The coefficients b_1, b_2, and b_{22} were found to be significant at $P < .05$ (table 4). Therefore, increasing the amount of Lutrol F-68 or stirring speed is expected to increase the particle size. Less amount of stabilizer induces agglomeration or aggregation and too much stabilizer promotes Oswald's ripening. Particle size was increased because of Lutrol F-68 was formed thick adsorption layer onto particles. This was lading to formation of aggregation of particles and increase in particle size.

In order to obtain a formulation having particle size close to 500nm, factorial design was used to determine the levels of these factors. The polynomial equation relating the response Y_1 and the independent variables was shown in equation 2.

$$Y_1 = 560.122 + 84.43\,X_1 + 48.48\,X_2 + 17.57\,X_1X_2 + 17.86\,X_1^2 - 130.48\,X_2^2 \quad \text{.....(2)}$$

The values of X_1 and X_2 were substituted in the equation to obtain the theoretical values of Y_1. To assess the reliability of the model, a comparison between the experimental and predicted values of the responses is also presented in terms of % bias in table 5.

$$\% \text{ Bias} = \frac{[\text{Predicted Value} - \text{Experimental Value}]}{\text{Predicted Value}} * 100$$

..... .(3)

Furthermore, low value of % bias for all batches showed good agreement between predicted and experimental values.

Concerning Q_{10}, the results of multiple linear regression analysis showed that both the coefficients b_1 and b_2 bear a negative sign (R^2=0.9984). It can be concluded from the equation (4) that X_1 (amount of Lutrol F-68) showed the more effective than X_2 (stirring speed). The coefficients b_1, b_2, b_{11} and b_{22} were found to be significant at $P < .05$.

$$Y_2 = 30.28 - 0.35\,X_1 - 0.64\,X - 0.16\,X_1X_2 - 4.41\,X_1^2 + 2.09\,X_2^2 \quad \text{.....(4)}$$

The results of multiple linear regression analysis reveal that, on increasing either amount of surfactant (Lutrol F-68) or stirring speed, a decrease in FAM release after 10 min. During the dissolution experiments, it was noticed that more amount of Lutrol F-68 were retard the drug release due to formation of viscous block on to particle surface. Therefore increasing the concentration of Lutrol F-68 is expected to decrease the drug release after 10min. From the multiple regression analysis, both the coefficients b_1 and b_2 bear a negative sign for FAM release after 120 min. From equation (5), X_1 (amount of Lutrol F-68) showed the less pronouncing than X_2 (stirring speed). The coefficients of b_1 and b_{22} were significant at $P < .05$. Higher agitation speeds made easier the evaporation of the solvent, with the concomitant rapid precipitation of the drug upon contact with the aqueous phase and a partial coalescence of particles in larger aggregates. Due to this, particles were taken more time for escaping drug into dissolution media. That means increasing stirring speed was decrease Q_{120}.

$$Y_3 = 92.66 - 1.5\,X_1 - 0.5\,X_2 - 0.7\,X_1X_2 - 0.88\,X_1^2 + 2.91\,X_2^2 \quad \text{.....(5)}$$

Optimization of the formulation using the desirability function

The aim of the optimization of pharmaceutical formulations 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. All the measured responses that may affect the quality of the product should be taken into consideration during the optimization procedure. Optimization was performed using a desirability function to obtain the levels of X_1 and X_2 which target Y_1 in terms of achieving particle size at near to 500 nm with more or close to 25% and 90% for Y_2 and Y_3 respectively. Using the desirability function, all the defined

responses can be combined into one overall response, the overall desirability (Fig 2). The results of the desirability analysis are presented in Table 6. SEM image with its particle size distribution for nanosuspension of F_{13} is shown with Lutrol F-68; 25mg and Stirring speed: 1200 rpm (Fig 3 and 4). Based on Equations (2, 4 and 5), this should give a particle size of 478.1 nm, drug release after 10min (Q_{10}) of 31.73%, and drug release after 120min (Q_{120}) of 92.66% respectively. These calculated values were in close accordance with the experimental results obtained. The experimental results led to particle size of 495.4 nm, Q_{10} of 31.71%, and Q_{120} of 94.25% respectively.

Conclusion

A nanoprecipitation method was developed to prepare famotidine (FAM) nanoparticles using Lutrol F-68 as stabilizer. FAM loaded nanosuspension was successfully formulated using factorial design and desirability function. The particle size, drug release after 10min (Q_{10}) and drug release after 120min (Q_{120}) were highly dependent on the amount of stabilizer (Lutrol F-68), and stirring speed for the preparation of FAM loaded nanosuspension. Amount of stabilizer (Lutrol F-68) and stirring speed had a positive effect on particle size and negative effect on Q_{10} and Q_{120}. The particle size should be tailor made depending on the therapeutic requirements and purpose. Additional work with concept of mucoadhesion into drug nanoparticles with this nanoprecipitation method is currently under investigation.

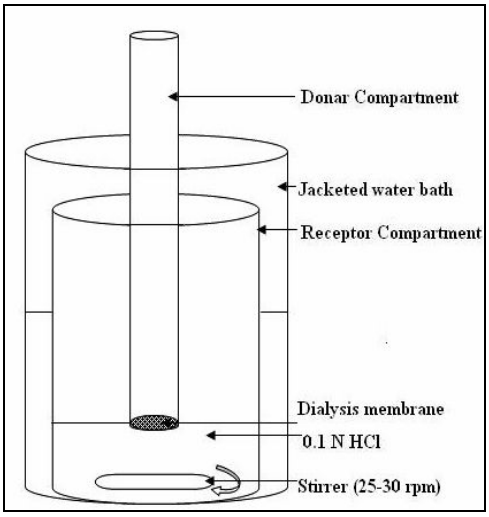

Figure 1: Schematic representation of modified diffusion cell apparatus

Table 1: Variables in 3^2 factorial design

Independent Variables	**Levels**		
	Low (-1)	Medium (0)	High (+1)
X_1: Amount of stabilizer (mg)	15	25	35
X_2: Stirring Speed (rpm)	800	1000	1200
Dependent variables	**Constraints**		
	Low	**High**	**Goal**
Y_1: Particle size (nm)	348.6	669.6	500
Y_2: Cumulative percentage release of FAM after 10 min (min)	25.33	32.96	≥30
Y_3: Cumulative percentage release of FAM after 120 (min)	90.38	97.52	≥90

Table 2: Experimental Matrix for the factorial Design

Run	Amount of stabilizer (mg) **(X_1)**	Stirring Speed (rpm) **(X_2)**
F_8	15	800
F_9	15	1000
F_{10}	15	1200
F_{11}	25	800
F_{12}	25	1000
F_{13}	25	1200
F_{14}	35	800
F_{15}	35	1000
F_{16}	35	1200

Table: 3: Observed responses for the 9 formulations of factorial design

Run	**Y_1(nm)**	**Y_2 (%)**	**Y_3 (%)**
F_8	348.6	28.75	95.33
F_9	480.9	26.33	93.66
F_{10}	390.2	27.75	96.85
F_{11}	358	32.96	97.52
F_{12}	566	30.35	92.2
F_{13}	495.4	31.71	94.25
F_{14}	472.6	28.52	93.88
F_{15}	669.2	25.33	90.38
F_{16}	584.5	26.88	92.59

Y_1: Particle size (nm); Y_2: Amounts of FAM released after 10 min; Y_3: Amounts of FAM released after 120 min.
*Standard deviation of the responses did not exceed 3% of the measured value.

Table 4: Regression equations for the responses

Co efficients	**bo**	**b_1**	**b_2**	**b_{12}**	**b_{11}**	**b_{22}**	**R^2**
Y_1	560.122	84.43	48.48	17.57	17.86	-130.48	0.9831
P-Value	0.0006	0.0029	0.0140	0.2239	0.3529	0.0040	
Y_2	30.28	-0.35	-0.64	-0.16	-4.41	2.09	0.9984
P-Value	0.00001	0.0135	0.0025	0.1458	0.0003	0.0003	
Y_3	92.66	-1.5	-0.5	-0.7	-0.88	2.91	0.8929
P-Value	0.0001	0.0557	0.3791	0.3281	0.3803	0.0392	

Table 5: Experimental and predicted responses obtained for the studied parameters

Run	Observed (Y_1)	Predicted (Y_1)	% Bias(Y_1)	Observed (Y_2)	Predicted (Y_2)	% Bias(Y_2)	Observed (Y_3)	Predicted (Y_3)	% Bias (Y_3)
F_8	348.6	332.1	-4.95	28.75	28.79	0.14	95.33	96.07	0.77
F_9	480.9	493.5	2.56	26.33	26.22	-0.42	93.66	97.15	3.60
F_{10}	390.2	393.9	0.96	27.75	27.83	0.29	96.85	90.29	-7.27
F_{11}	358	381.1	6.07	32.96	33.01	0.15	97.52	89.67	-8.75
F_{12}	566	560.1	-1.05	30.35	30.28	-0.23	92.2	92.66	0.50
F_{13}	495.4	478.1	-3.61	31.71	31.73	0.06	94.25	92.66	-1.72
F_{14}	472.6	465.9	-1.44	28.52	28.41	-0.38	93.88	91.16	-2.98
F_{15}	669.2	662.4	-1.02	25.33	25.52	0.74	90.38	91.16	0.86
F_{16}	584.5	597.9	2.25	26.88	26.81	-0.26	92.59	91.16	-1.57

Table 6: Optimum Levels for the Formulation Variables

Formulation variable	Optimum values
Amount of Lutrol F-68	25 mg
Stirring speed	1200 rpm

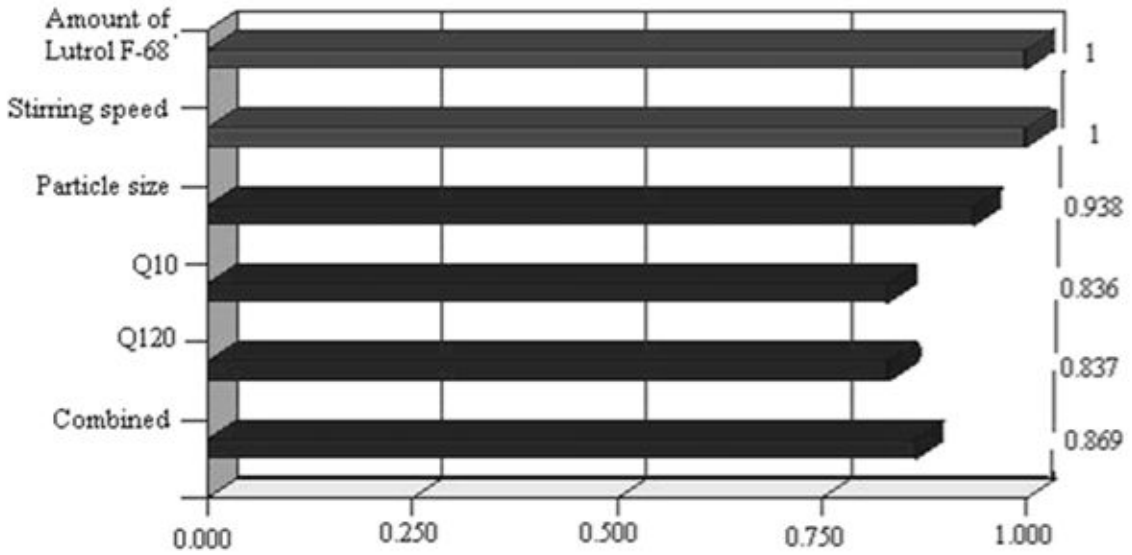

Figure 2: Bar graph showing individual desirability values of various objective responses and their association overall desirability.

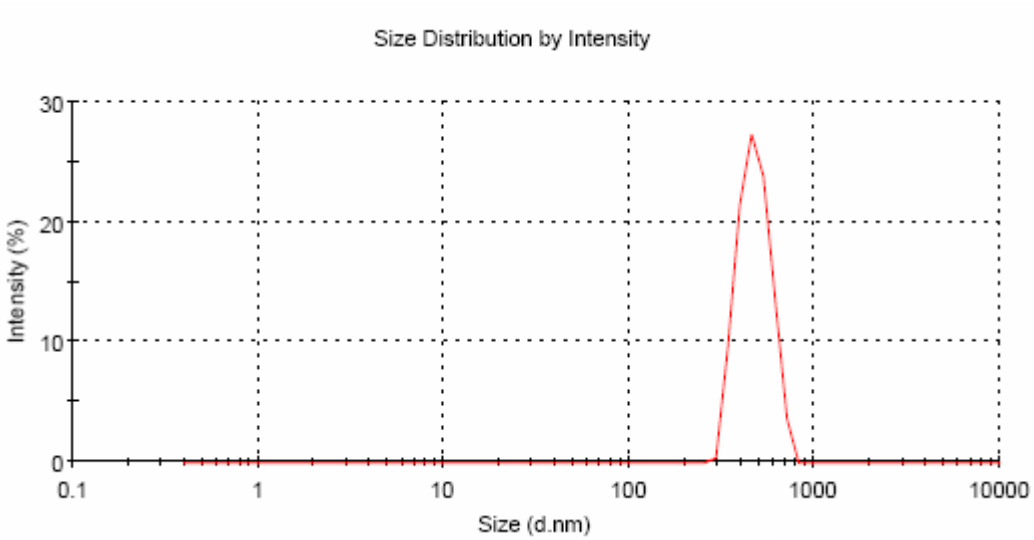

Figure: 3 Particle size distribution of batch F_{13}

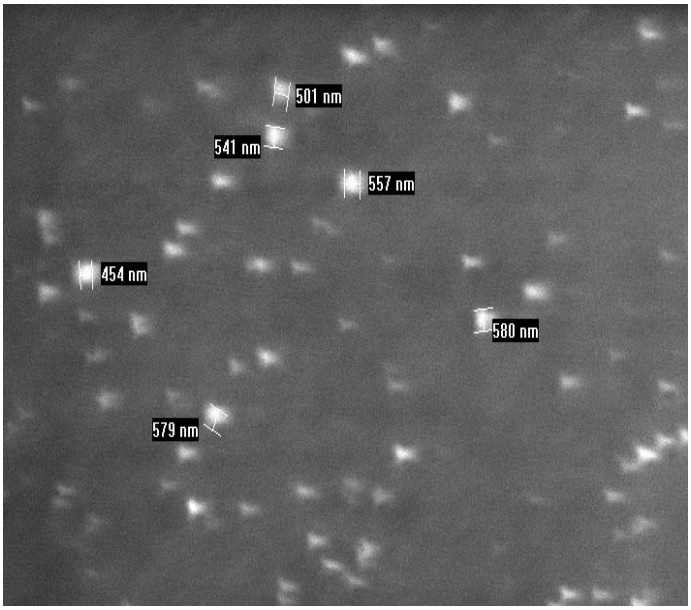

Figure: 4 Scanning electron micrograph of nanosuspension of F_{13} at Lutrol F-68; 25mg, stirring speed; 1200 rpm).

Acknowledgements

The authors wish to thank Kiranbhai Chudhary for skillful technical help and linguistic support.

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