Department of Pharmaceutics, Faculty of Pharmacy, Health Science Center, Kuwait University, Kuwait

Studies on development of insoluble drugs as pharmaceutical suspensions by response surface methodology

I. S. KHATTAB

Received February 20, 2008, accepted March 17, 2008

Dr. Ibrahim S. Khattab, Assistant Professor of Pharmaceutics, Faculty of Pharmacy, Kuwait University, Kuwait.
Khattab@hsc.edu.kw/ib.khattab@gmail.com

Pharmazie 63: 726–730 (2008) *doi: 10.1691/ph.2008.8049*

The preformulation of insoluble drugs, trimethoprim and nitrofurantoin, was studied in order to achieve a suspension with desirable requirements. The objective of the formulator is to avoid the irreversible aggregation called "caking", and to obtain a suspension with an airy, large volume sediment easily redispersible and with suitable rheological properties. An experimental design useful to determine optimal properties is a Box-Behnken design. The surfactant, thickener and electrolyte at different proportions were the three factors studied. This strategy allows to point on the main significant effect and to determine the concentrations of each product leading to optimal properties of the suspensions.

1. Introduction

Suspensions are dispersions of solids in liquids. Pharmaceutical suspensions are coarse dispersions of solid particles of different sizes (0.1–10 μm) in a liquid medium, generally an aqueous solution. They are energetically unstable systems and are showing in some situations problems as aggregation of the single particles leading to "caking" (after the settling of particles to a closely packed sediment impossible to redisperse). Pharmaceutical suspensions have to be homogeneous and stable when the therapeutic doses are taken, so their formulation requires to avoid irreversible aggregation (cake) and to maintain the sediment in a flocculated state easy to disperse before dispensation. For this purpose, different agents can be used: surfactants for the wetting of the solids which improve their dispersion, thickeners for rheological requirements or electrolytes to modify the conditions of the electrolytic medium and increase the stability of the particles against aggregation (Attwood and Florence 1983; Swarbrick and Boyland, 1996). The final aspect of the preparation, where rapid clearance of the supernatant is undesirable, is closely linked to the relative proportions of the constituents.

The aim of this work was to study the formulation of two insoluble drugs, an antimicrobial agent (trimethoprim) and urinary tract antibiotic (nitrofurantoin), in order to achieve oral suspensions with optimal properties. It is unrealizable to examine the effects on the suspension properties of all components at various levels. Experimental designs offer an excellent approach to reduce the charge of time and money by limiting the number of experiments, furthermore this methodology gives high quality information. To determine the optimal experimental conditions, the response surface method can be employed (Nazzal et al. 2002; Huang et al. 2004). The optimization procedure involves systematic formulation designs to minimize the number of trails, and analyze the response surfaces in order to realize the effect of causal factors and to obtain the appropriate formulations. Box-Behnken design in three factors is a statistical technique that used for optimizing multivariable systems (Solanki et al. 2007; Dayal et al. 2005; Karnachi and Khan 1996). For this purpose, several constituents were fixed, and the percentages of electrolyte, surfactant and thickener are the three main factors, of which the influence on the behavior of suspensions is examined. These products (NaCl as electrolyte, Polysorbate 80 as surfactant and Avicel RC-591, a combination of microcrystalline cellulose and carboxymethylcellulose sodium, as thickener) have been chosen according to the literature (Zietsman et al. 2007; Gallardo et al. 1990) and to medicines on the marketplace.

2. Investigations, results and discussion

The wide variation of responses (Y_1–Y_4) indicated that the factor combinations resulted in different properties of the suspensions. The causal factor and response variables were related using polynomial equation with statistical analysis through Design-Expert® software (Vaughn et al. 2000). As shown in Table 1, the approximations of response values (Y_1–Y_4) based on the quadratic model was most suitable based on R^2 and PRESS values. The values of the coefficients X_1, X_2 and X_3 are related to the effect of these variables on the response.

The contour plots illustrating the simultaneous effect of the causal factors on individual and combined response variable are represented in Figs. 1–6. This expression gives an insight into the effect of the different independent variables (response). A positive sign of coefficient indicates a synergistic effect while a negative term indicates an antagonistic effect upon the response.

The larger coefficient means the causal factor has more potent influence on the response. As shown in Table 1, the

Table 1: Optimal regression equation for each response variable

Model	Coefficient	Y_1	Y_2	Y_3	Y_4
	$b_1(X_1)$	−3.36	−0.062	8.60	2.16
	$b_2(X_2)$	2.26	0.69	−30.55	2.83
	$b_3(X_3)$	31.47	−7.50	−261.29	0.96
	$b_{12}(X_1X_2)$	1.69	−1.88	−43.48	−2.06
	$b_{13}(X_1X_3)$	−7.87	−.05	2.63	0.049
	$b_{14}(X_2X_3)$	−3.21	0.00	−3.2	−0.36
Linear	CV	87.25	22.12	67.59	20.62
	R^2	0.6107	0.9051	0.9457	
	Adjusted R^2	0.5208	0.8832	0.6521	0.9332
	PRESS	15375.96	96.75	10963.03	2.32
Quadratic	CV	8.99	13.19	4.45	17.37
	R^2	0.9949	0.9818	0.9937	0.9792
	Adjusted R^2	0.9651	0.9585	0.9855	0.9525
	PRESS	758.55	134.25	1993.0	4.77
Cubic	CV	1.79	5.17	6.26	14.33
	R^2	0.9999	0.9984	0.9993	0.9919
	Adjusted R^2	0.9998	0.9936	0.9971	0.9677
	PRESS	N/A	N/A	N/A	N/A

coefficient of X_3 was largest, showing that the effect of Avicel RC-591 was the main influence factor on the drug suspension. The value of coefficients of X_2 was mostly less than that of X_1 and X_3, indicating that the influence of NaCl (X_2) was less than that of other factors.

2.1. Trimethoprim

The quadratic models describing the responses are:

$$Y_1 = 7.18 - 3.36\,X_1 + 2.26\,X_2 + 31.47\,X_3 + 1.69\,X_1 X_2 - 7.87\,X_1 X_3 - 3.21\,X_2 X_3 - 0.7\,X_1^2 + 44.11\,X_3^2$$

$$Y_2 = 9.2 - 0.062\,X_1 + 0.69\,X_2 - 7.5\,X_3 - 1.88\,X_1 X_2 - 0.5\,X_1 X_3 - 0.01\,X_2 X_3 + 0.96\,X_1^2 - 0.038\,X_2^2 - 2.16\,X_3^2 .$$

An analysis of variance tested the statistical significance of each effect. For both responses (Y_1 and Y_2), one main effect, the effect of the factor X_3 (thickener), is significantly different from zero at the 95% confidence level. F value was 0.0001 for both responses and for other factors X_1 and X_2 (electrolyte and surfactant) F value is greater than 0.1 (0.2128 and 0.3866) respectively.

The R-squared statistic shows that the model as fitted justifies 98.29% of the variability of the sediment volume and the predicted R^2 of 0.7272 is in reasonable agreement with the adjusted one of 0.9609.

In case of caking level R^2 is 97.83%, and the predicted R^2 of 0.6891 is not as close to the adjusted one of 0.9504 as one might normally expect. While, adequate precision which measures the signal to noise ratio was 17.608 indicates an adequate signal.

The graphical illustration of the results permits a good evaluation of what happens (Figs. 1, 2). As the factor X_3 is the most significant, and since the variations of electrolyte and surfactant as a function of this factor give response surfaces with similar shapes, we decided to represent the response surfaces for sediment volume and caking level as a function of electrolyte and thickener. Figure 1 indicates that the maximum dispersed sediment volume was obtained for the highest thickener concentration and the lower electrolyte concentration. Figure 2 shows that the con-

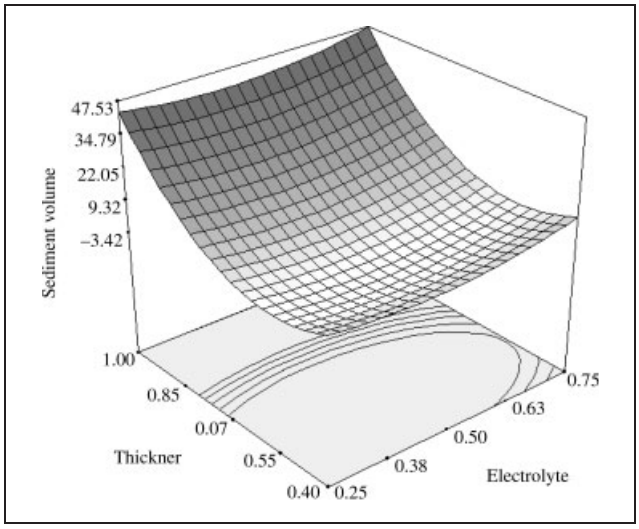

Fig. 1: Contours of estimated response surface for sediment volume trimethoprim suspensions

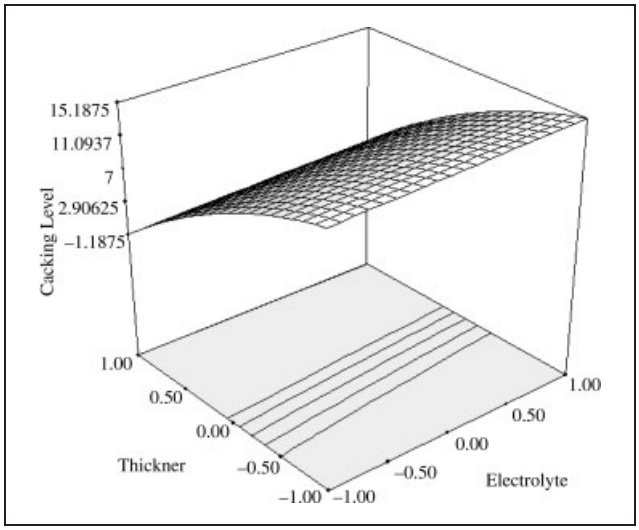

Fig. 2: Contours of estimated response surface for caking level of trimethoprim suspensions

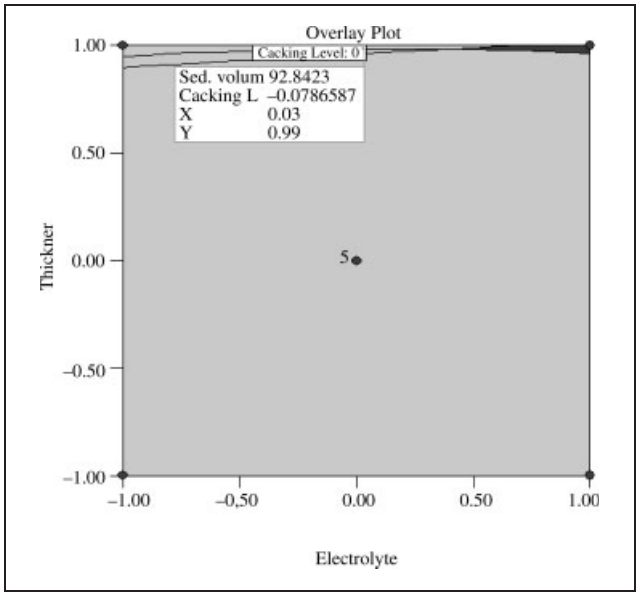

Fig. 3: Overlay plot of the effect of electrolyte (X_2) and thickener (X_3) on the sediment volume and caking level for trimethoprim suspensions

centration of electrolyte had no influence on the caking level, but that a high concentration of thickener limits consequentially the caking level. By superimposing the contour plots of both responses we can delimit the optimal zone to formulate the trimethoprim suspensions (Fig. 3). The caking level should be equal to zero and the volume of dispersed sediment is considered acceptable between 90 and 100%. These conditions should lead to homogeneous, easy to disperse and stable suspensions. An experiment was performed in the optimal zone corresponding to the proportions of surfactant 1.0%, electrolyte 0.03% and thickener 0.99% and the results were conformable to the predicted values which indicated that response surface methodology optimization technique was quite useful for optimizing trimethoprim suspension.

2.2. Nitrofurantoin

The quadratic models describing the responses are:

$$Y_1 = 11.59 + 8.06\,X_1 - 30.55\,X_2 - 261.29\,X_3 - 43.48\,X_1 X_2 + 2.63\,X_1 X_3 - 3.2\,X_2 X_3 + 2.38\,X_1^2 + 3.67\,X_2^2 + 36.98\,X_3^2$$

$$Y_2 = 1.41 + 2.16\,X_1 + 2.83\,X_2 + 0.96\,X_3 - 2.06\,X_1 X_2 + 0.049\,X_1 X_3 - 0.36\,X_2 X_3 - 0.11\,X_2^2 - 0.068\,X_2^2 + 0.32\,X_3^2\,.$$

As for trimethoprim, the analysis of variance give the effect of the factor "thickener" significantly different from zero at the 95% confidence level, and also, no interaction has a significant effect. The R-squared statistics indicate that the model as fitted explains 92.65% of the variability of the sediment volume and 94.9% in the case of the viscosity. The graphical representations show the same kind of results for these responses. Figures 4 and 5 indicate that the concentration of electrolyte has no influence on the caking level (the surfactant gives same result), but that a high concentration of thickener gives the highest sediment volume and of course maximizes the viscosity. By superimposing the contour plots of both responses we can delimit the opti-

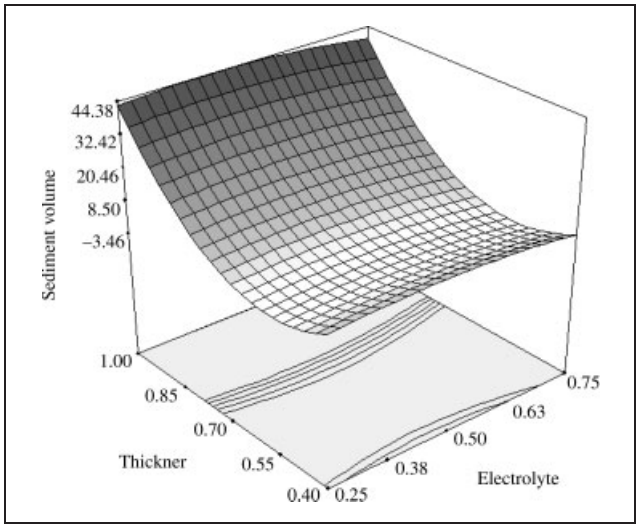

Fig. 4: Contours of estimated response surface for sediment volume of nitrofurantoin suspensions

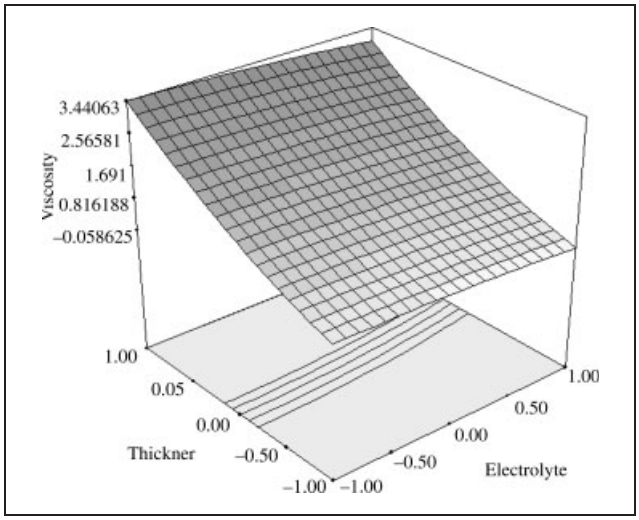

Fig. 5: Contours of estimated response surface for viscosity of nitrofurantoin suspensions

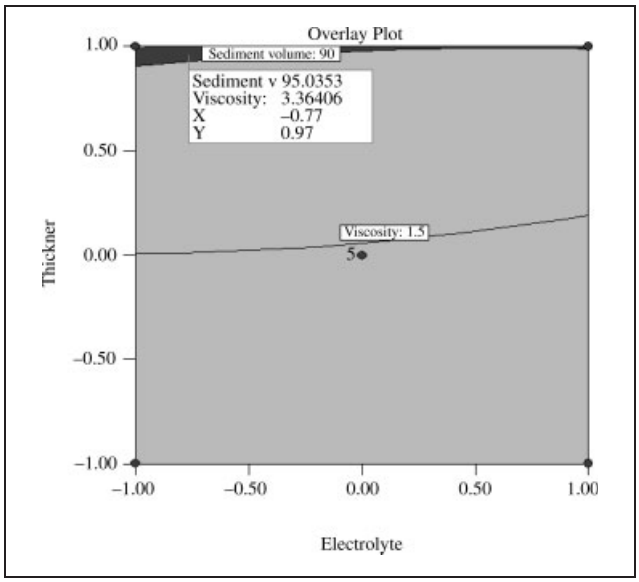

Fig. 6: Overlay plot of the effect of electrolyte (X_2) and thickener (X_3) on the sediment volume and viscosity for nitofurantoin suspensions

mal zone to formulate the nitofurantoin suspensions (Fig. 6). We consider that the volume of dispersed sediment is good from 90 to 100% and an adequate deliverance will be achieved with a value of apparent viscosity between 1.5 and 3.5 Pa.s. This zone has been verified with an experimental point (surfactant 1%, electrolyte 0.19%, thickener 0.97%) which leads to optimal properties.

These results are perfectly related to the properties of Avicel RC-591 in suspensions. This kind of components can protect each particle in suspension and is able to limit the sedimentation. Their role of thickeners explains also the decrease of the sedimentation phenomenon (Nash 1988). The electrolyte and the surfactant have in both cases no significant effect, their effects are certainly masked by the preponderant influence of the Avicel RC-591.

This work has allowed optimizing the formulation of two drugs in suspension. Box-Behnken design, response surface methodology and multiple response optimization using polynomial equation could be suitable to point on the main significant effect and to determine the optimal conditions of a formulation. We can see that the optimized areas were quite small and were situated near the limits of the domain. It would be interesting to develop the research in that region of the domain to make our formulation more robust.

3. Experimental

3.1. Materials

The drugs are sulfadiazine, (Merck, Darmstadt, Germany), and aluminum hydroxide (Prolabo, Paris, France). The non-ionic surfactant used is a polysorbate 80 (Tween 80, ICI, Paris, France), the thickener Avicel RC-591, FMC, USA) and the ionic species is NaCl (Merck).

3.2. Preparation of the suspensions

The vehicle is predetermined and constituted with 15% of saccharose (Prolabo), 0.5% of citric acid in water. The preparation of 200 g of suspensions involved the simple mixing of the components at a temperature of 75 °C. Suspensions were stocked in measuring glasses (vol. 250 mL, precisely graduated) at 25 °C and then observed after 24 h and 48 h in the dark with a light allowing to read by transparency the dispersed sediment volume and the caking height. The rheological properties of suspensions were studied with an Oswald's viscometer.

3.3. Data analysis

A Box-Behnken design was chosen to evaluate the factors that significantly influence suspension properties and what levels of the factors are needed to produce an optimal suspension. This multivariate approach consists of a set of points lying at the midpoint of each edge and the replicated center point of the multidimensional cube (Fig. 7). The first one is used to evaluate the effects of the variables and of their interactions. Then, replicates at the centrepoint of the design allow to calculate the experimental error of the process and to determine response surfaces and the corresponding contour plots. Accordingly, this Box-Behnken design in three variables requires 17 experiments consisting of three four-run, two-level factorials in two factors – with the third factor at its mid-level, and five center points (Box et al. 1978; Cochran and Cox 1992). The three factors are X_1, percentage of electrolyte, X_2, percentage of surfactant and X_3, percentage of thickener and are represented by -1, 0 and $+1$, analogous to the low, middle and high values respectively. A condition for the Box-Behnken design is that these levels are equally spaced to insure orthogonality.

The best fitting mathematical model was selected based on the comparison of several statistical parameters including the coefficient of variation (CV), the multiple correlation coefficient (R^2), adjusted multiple correlation coefficient (adjusted R^2), and the predicted residual sum of square (PRESS), proved by Design-Expert® software.

The following equations describe the response:

Linear model:

$$Yi = b_0 + b_1X_1 + b_2X_2 + b_3X_3 \quad (1)$$

Quadratic model:

$$Yi \,\square = \square\, b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1X_2 + b_{12}X_1X_3 + b_{13}X_2X_3 + b_{14}X_1^2 + b_{15}X_2^2 + b_{16}X_3^2\square \quad (2)$$

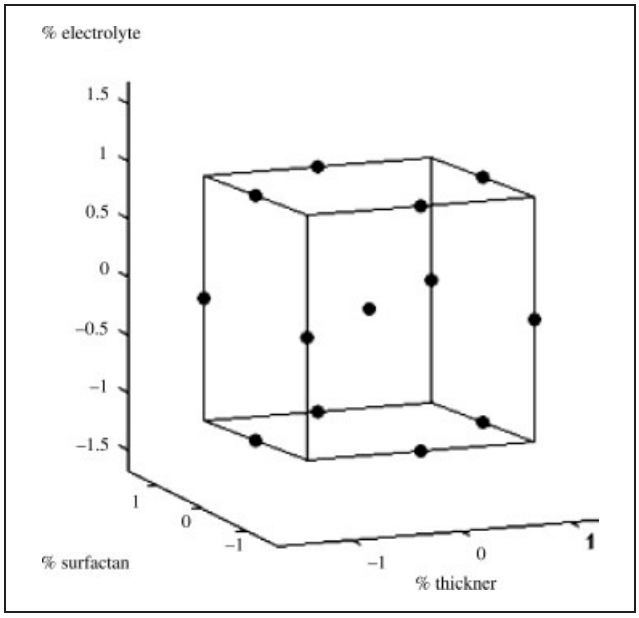

Fig. 7: The geometry of a Box-Behnken design

Cubic model:

$$Yi \,\square = \square\, b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1X_2 + b_{12}X_1X_3 + b_{13}X_2X_3 + b_{14}X_1^2 + b_{15}X_2^2 + b_{16}X_3^2 + b_{21}X_1^3 + b_{22}X_2^3 + \square\, b_{23}X_3^3\, b_{31}X_1^2X_2 + b_{32}X_1^2X_3 + b_{33}X_1X_2^2 + b_{34}X_1X_3^2 + b_{41}X_2^2X_3 + b_{42}X_2X_3^2 + b_{51}X_1X_2X_3 \quad (3)$$

where y is the response, x the factors and b the coefficients of each term calculated by multiple regression analysis. The responses studied for trimethoprim were the volume of the dispersed sediment, Y_1, (in fraction of total volume), measured after 48 h and the caking level, Y_2, (in mL) after 24 h. The viscosity be measured owing to the presence of the caking. For nitrofurantoin, the responses were the volume of the dispersed sediment, Y_3, (in fraction of total volume), measured after 48 h and as no caking appeared after 24 h we used as dependant variable the viscosity, η, measured by Oswald's viscometer. All measurements were made at 25 °C. Viscosity was calculated using the following equation:

$$\eta_{suspension} = \frac{t_{suspension}}{t_{water}} \frac{d_{suspension}}{d_{water}} \times \eta_{water} \quad (4)$$

where, $t_{suspension}$ and t_{water} are the times of flow of the suspension and water, respectively, between the two marks for constant volume on the bulb of the viscometer; $d_{suspension}$ and d_{water} are the densities of the suspension (determined using equation (5) and water (known from literature to be 0.9971×103 kg m^{-3} at 25 °C), respectively; and η_{water} is the viscosity of water at 25 °C (known from the literature to be 0.8937 cP). The density of the solution was determined by:

$$d_{suspension} = \frac{\text{weight of suspension}}{\text{weight of the same volume of water}} \times d_{water} \quad (5)$$

The optimized suspension formulation requires a maximized dispersible sediment volume, an absence of non-redispersible caking and an appropriate viscosity for a good delivery. According to the response requirement,

Table 2: Experimental domains and coding of the variables

Variables	Levels		
	−1	0	+1
Surfactant % (X_1)	0.5	1.0	1.5
Electrolyte % (X_2)	0.25	0.5	0.75
Thickener % (X_3)	0.4	0.8	1.2

Responses
Y_1 Sediment volume (%) for trimethoprim
Y_2 Caking level (ml) for trimethoprim
Y_3 Sediment volume (%) for nitrofurantoin
Y_4 Viscosity (Pa.s) for nitrofurantoin

Table 3: Matrix and responses for trimethoprim and nitrofurantoin

Run	Independent Variables			Responses			
				Trimethoprim		Nitrofurantoin	
	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4
1	1	0	1	85.46	0	96.4	3.22
2	0	1	−1	18.00	14	6.82	0.032
3	−1	−1	0	9.06	7.5	11.8	0.92
4	0	0	0	7.14	9	9.77	1.16
5	1	0	−1	12.3	17	7.15	0.035
6	−1	1	0	9.30	14	9.14	0.92
7	0	−1	−1	6.14	14	10.54	0.04
8	0	0	0	6.84	9	14.6	1.46
9	−1	0	1	93.0	0	39.7	3.18
10	−1	0	−1	11.58	15	6.55	0.034
11	0	0	0	8.03	9	12.3	1.23
12	0	0	0	7.22	9	11.4	1.68
13	0	1	1	97.0	0	93.3	3.2
14	0	−1	1	98.0	0	98.3	3.35
15	0	0	0	6.67	10	9.87	1.5
16	1	1	0	14.27	9	12.6	1.03
17	1	−1	0	7.28	10	37.0	2.06

the preliminary study and a published report (Gallardo et al. 1990), the levels of excipients were set at polysorbate 80 (X_1) from 0.5 to 1.5, NaCl (X_2) from 0.25 to 0.75, and Avicel RC-591 (X_3) from 0.40 to 1.20 (Table 2). The compositions arranged according to Box-Benken design, the responses evaluated by power equation of all model formulations are summarized in Table 3.

References

Attwood D, Florence AT (1983) Surfactant Systems, Chapman and Hall, London.

Box GE, Hunter WG, Hunter JS (1978) Response surface methods. Statistics for experimenters, Wiley & Sons, New York.

Cochran G, Cox G (1992) Experimental Designs, 2nd edition, Wiley & Sons, New York.

Dayal P, Pillay V, Babu RJ, Singh M (2005) Box-Behnken experimental design in the development of a nasal drug delivery system of model drug hydroxyurea: characterization of viscosity, in vitro drug release, droplet size, and dynamic surface tension. AAPS PharmSciTech 6: E573-E585.

Gallardo V, Delgado A, Parera A, Salcedo J (1990) Electrophoretic study of nitrofurantoin in aqueous suspensions. Effect of the addition of a polymeric thickener. J Pharm Pharmacol 42: 225–229.

Huang YB, Tsai YH, Yang WC, Chang JS, Wu PC (2004) Optimization of sustained-release propranolol dosage form using factorial design and response surface methodology. Biol Pharm Bull 27: 1626–1629.

Karnachi, AA, Khan, MA (1996) Box-Behnken design for the optimization of formulation variables of indomethacin coprecipitates with polymer mixtures. Int J Pharm 131: 9–17.

Nash RA (1988) Pharmaceutical suspensions, in: Pharmaceutical dosage forms: Dispersed systems. Dekker, M., New York.

Nazzal S, Nutan M, Palamakula A, Shah R, Zaghloul A, Khan MA (2002) Optimization of a self-nanoemulsified tablet dosage form of ubiquinone using response surface methodology: effect of formulation ingredients. Int J Pharm 240: 103–114.

Solanki AB, Parikh JR, Parikh RH (2007) Formulation and Optimization of piroxicam proniosomes by 3-factor, 3-level Box-Behnken design. AAPS PharmSciTech 8: E 86.

Swarbrick J, Boylan JC (1996) Encyclopaedia of pharmaceutical tecnology, Marcel Dekker, New York.

Vaughn N, Polnaszek C, smith B, Helseth T (2000) Program DESIGN-EXPERT, Stat-Ease Inc.

Zietsman S, Kilian G, Worthington M, Stubbs C (2007) Formulation development and stability studies of aqueous metronidazole benzoate suspensions containing various suspending agents. Drug Dev Ind Pharm 33: 191–197.