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Research Article

Impact of formulation and process variable on the preparation of acyclovir microspheres by spray drying using factorial design

Madhuri L.Shinde*, Virag A. Shah, Dhananjay S. Ghodke, Rohit R. Shah

Department of Pharmaceutics, Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra- 416416, India

ABSTRACT

The aim of this work is to study the impact of polymer ratio and feed flow rate (ml/min) in the preparation of acyclovir microspheres by using spray drying technique. A 3^2 full factorial experiment was designed to study the effects of the polymer ratio and feed flow rate (ml/min) of spray dryer on the percent yield and encapsulation efficiency of microspheres. The result of analysis of variance test for both effects indicated that the test is significant. The effect of factor X_1 (polymer ratio) (SSY_1=120.96; SSY_2=357.38) is higher than factor X_2 (feed flow rate) (SSY_1=79.06; SSY_2=88.94) for optimizing the percent yield and encapsulation efficiency. The optimum polymer ratio (X_1) and feed flow rate (X_2) was found to be 3.68 and 10.00 ml/min respectively for obtaining higher percent yield and maximum encapsulation efficiency of microspheres which was found to be 33.26% and 91.23% respectively.

Keywords: Acyclovir; carbopol; spray dryer; polymer ratio; feed flow rate.

INTRODUCTION

Spray-drying is extensively applied in the pharmaceutical industry to produce raw drugs or excipients or in the microencapsulation process. This technique transforms liquid feed into dry powder in one step and is feasible for the scaling-up of the microencapsulation in a continuous particle processing operation which can be used for a wide variety of materials (Patel, JK et al., 2007). The spray drying technique consists of spraying an emulsion of polymer and drug through the nozzle of a spray dryer apparatus. The solvent evaporates very quickly, leaving behind solid microparticles. The spray drying process involves the following four sequential stages: atomization of the product into a spray nozzle, spray-air contact, drying of the sprayed droplets, and collection of the spray-dried microparticles (Conti, 1992). Hydrophobic as well as hydrophilic drugs can be incorporated into a polymer by spray drying.

Acyclovir , 2-amino-1, 9-dihydro-9- [(2-hydroxyethoxy) methyl]-6H-purin-6-one, is a synthetic purine nucleoside analog that possesses in vitro and in vivo inhibitory activity against Herpes simplex virus (HSV)-1, Herpes simplex virus-2, and Varicella zoster virus (VZV) (Dollery, 1999 & Lindenberg, M et al., 2004) . The main problem with the therapeutic effectiveness of acyclovir is its absorption that is highly variable and dose dependent thus reducing the bioavailability to 10–20%. The drug is most prominently absorbed from the duodenum and jejunum region of the GIT (Dhaliwal, S et al., 2008). The absolute oral bioavailability is also considerably poor because of its low water-solubility (about 0.2%, 25°C) and short half-life (about 2.5 hr) (Chiou, W.L. et al., 1998). Hence, it can be envisaged that increasing the residence time at the absorption site and water solubility can enhance the absorption and oral bioavailability of acyclovir. Different workers have attempted to prepare mucoadhesive microspheres of acyclovir for increasing the bioavailability (Tao, Y et al., 2009 and Stulzer, HK et al., 2009).

In the present work acyclovir microspheres were prepared by spray drying technique using carbopol 974P as mucoadhesive material. A 3^2 factorial design was applied to study the effect of formulation and process variable. The polymer ratio (X_1) and feed flow rate (ml/min) (X_2) were selected as independent variables while the percent yield and encapsulation efficiency were chosen as the dependant variables. The levels for formulation as well as process variable were determined from the preliminary trials.

EXPERIMENTAL

Materials

Acyclovir supplied from Zydus Cadila, Ahmedabad, as a gift sample and carbopol 974P from Dr. Reddy, Hyderabad.

Preparation of Acyclovir microspheres using carbopol 974P

The microspheres were produced by spray drying method. Drug-Loaded mucoadhesive microspheres were

* Corresponding Author
Email: shindemadhuri28@gmail.com
Contact: +91-9767106903
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prepared using carbopol 974P in three different polymer ratio. The drug and polymer were dissolved in distilled water, under stirring at room temperature to obtain the feed solution (Harikarnpakdee, S et al., 2006 and Nagda, C et al., 2009). Acyclovir-loaded microspheres were obtained by spraying the feed-solution with a spray-dryer (Labultima mini spray dryer, India.) using a standard 0.7mm nozzle. The solution was fed to the nozzle with a peristaltic pump, atomized by the force of compressed air and blown together with heated air to the chamber where the solvent in the droplets was evaporated. The dried microparticles were harvested from the apparatus collector. Parameters for the preparation of microspheres are summarized in Table 1.

Table 1: Parameters for the preparation of microspheres

Parameters	Conditions
Inlet temperature	120°C
Outlet temperature	60°C
Aspirator speed	85%
Feed flow rate	10,20,30 (ml/min)
Polymer ratio	3, 4, 5

Full factorial design with coded form and actual form of variables for each batch is described in Table 2 and 3.

Table 2: Full factorial design with coded form of variables for each batch

Batch	Variables levels in coded form	
	X_1	X_2
A	-1	-1
B	-1	0
C	-1	+1
D	0	-1
E	0	0
F	0	+1
G	+1	-1
H	+1	0
I	+1	+1

Table 3: Full factorial design with actual form of variables for each batch

Coded values	Actual Values	
	X_1	X_2
-1	3	10
0	4	20
+1	5	30

X_1 = Polymer ratio; X_2 = Feed flow rate (ml/min)

Characterization of the Microspheres

Percent yield

The percentage of production yield (wt/wt) was calculated from the weight of dried microspheres (W_1) recovered from each of three batches and the sum of the initial dry weight of starting materials (W_2). The formula for calculation of percent yield is as follows (Shin-Shing, S 2002):

$$\text{Percent yield} = \frac{\text{weight of dried microspheres } (W_1)}{\text{Total weight of drug and polymer } (W_2)} \times 100$$

Encapsulation efficiency

A certain amount of microspheres were ground to powder. About 100mg of the powder was mixed with 2ml of 0.4% NaOH solution and 90ml of distilled water. The suspension was ultrasonicated (Lab-Hosp Corporation, LHC-670) for 2 h, and then diluted to 100ml. After filtration through a 0.45µm membrane filter, 1ml of the filtrate was diluted to 10 ml. The acyclovir standard solution (20µg/ml) was taken as reference. The samples were analyzed by HPLC (Jasco-2000) equipped with a HiQ Sil C-18 (4.5mm×250mm). The mobile phase of 8% methanol and 92% distilled water was used at a flow rate of 1ml/min, and acyclovir was detected by UV detector at 254nm. The formula for calculation of encapsulation efficiency is as follows (Tao, Y et al., 2009):

$$\text{Encapsulation efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

Response Surface Analysis

The results are expressed as second order polynomial equation of the following term (Equation 1):

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

Where Y is the predicted response, b_0 is the arithmetic mean response of 9 runs (Table 2). The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low value to its high value. The interaction (X_1X_2) shows how the percent yield and encapsulation efficiency value changes when two factors are simultaneously changed, and the exponential terms (X_1^2 and X_2^2) represent curvature. The coefficients corresponding linear effects (b_1 and b_2), interaction (b_{12}) and the quadratic effects (b_{11} and b_{12}) were determined from the results of the experiment (STAT-EASE, design expert, 8.0, Trial version) (Rawat, M et al., 2007; Gohel, 1999 and Monness, E et al., 2007). 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 4. The formula for calculation of % bias is as follows:

$$\%\ \text{Bias} = \frac{\text{Predicted value} - \text{Actual value}}{\text{Predicted value}}$$

RESULTS AND DISCUSSION

The percent yield (Y_1) and encapsulation efficiency (Y_2) from the 9 experiments was used to generate predictor equations for acyclovir microspheres with independent variables as polymer ratio and feed flow rate. The results of multiple regression analysis and analysis of variance test (ANOVA) are summarized in Table 5.

Table 4: Actual response, Predicted response and % Bias obtained for the studied parameters

Batch	Percent yield			Encapsulation efficiency		
	Actual	Predicted	% Bias	Actual	Predicted	% Bias
A	32.5	30.19	7.62	94.0	93.79	0.22
B	24.2	26.56	8.88	90.0	90.16	0.17
C	25.0	22.93	9.02	85.2	85.24	0.05
D	34.0	34.68	1.96	88.2	88.99	0.88
E	30.0	31.05	3.38	86.0	85.78	0.25
F	29.4	27.42	7.19	81.0	81.29	0.36
G	40.0	39.17	2.10	78.10	77.51	0.75
H	38.32	35.54	7.85	73.80	74.72	1.23
I	30.32	31.91	5.0	71.0	70.66	0.47

The percent yield and encapsulation efficiency of microspheres showed R^2 values for full model are 0.8474 and 0.9952 (Table 5), respectively; indicating good fit and it was concluded that the second-order model adequately approximated the true surface. Furthermore, low value %bias for all batches showed good agreement between the predicted and the actual values as shown in Table 4.

Table 5: Regression analysis data for measured responses

Coefficients	Percent yield (%)	Encapsulation efficiency
	Full model	Full model
b_o	31.06	85.78
b_1	4.49	-7.72
b_2	-3.63	-3.85
b_{11}	-	0.43
b_{22}	-	-3.34
b_{12}	-	-0.64
R^2	0.8474	0.9952
F	27.76	293.01

The fitted model for percent yield is linear model which is expressed in equation 2

$$Y_1 = 31.06 + 4.49X_1 - 3.63X_2 \quad (2)$$

The fitted model for encapsulation efficiency is quadratic model which is expressed in equation 3

$$Y_2 = 85.78 - 7.72X_1 - 3.85X_2 + 0.43X_1X_2 - 3.34X_1^2 - 0.64X_2^2 \quad (3)$$

For the percent yield and encapsulation efficiency of microspheres the calculated F values for full models is 27.76 and 293.01 respectively. The source sum of squares (Source SS) in ANOVA indicated the contribution of factor X_1 (polymer ratio) (SSY_1=120.96; SSY_2=357.38) is higher than factor X_2 (feed flow rate) (SSY_1=79.06; SSY_2=88.94) for optimizing the percent yield and encapsulation efficiency of microspheres. The interaction terms X_1 X_2 indicated insignificant values of individual source sum of squares.

Response surface plot (Figure 1.) indicates the positive effect of polymer ratio on the percent yield. The response observed for this effect is of linear type. With increase in the polymer ratio, the percent yield also increases. This statement is well supported by Motlekar, N et al., 2008, who also reported that the increase in the percent yield may be due to the increases throughput of the polymer slurry and rapid evaporation of the solvent. Response surface plot also (Figure 1.) indicates the negative effect of feed flow rate on the percent yield. The response observed for this effect is also of linear type. With increase in the feed flow rate, the value of percent yield decreases. This is also well supported by Motlekar, N et al., 2008 who suggested that the reduction in yield may be attributed to the incomplete atomization and drying, resulting in the deposition of a large amount of microparticles on the walls of the dessicating chamber and the cyclone separator.

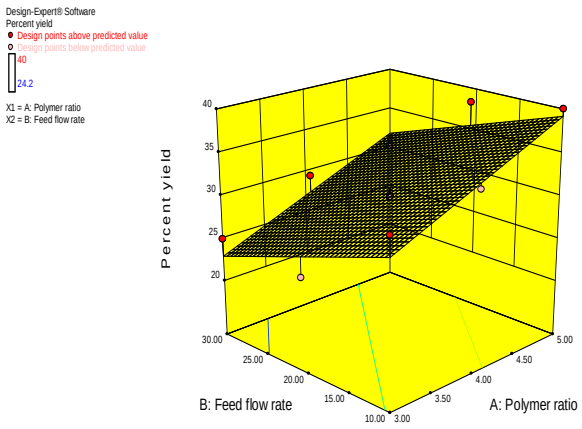

Figure 1: Response surface plot showing the effect of selected variables on the Percent yield

Polymer ratio at higher level(X_1, +1) and feed flow rate at lower level(X_2, -1) yielded microspheres with higher percent yield.

When considering another response term encapsulation efficiency (Y_2) response surface plot (Fig. 2) indicates the negative effect of polymer ratio on the encapsulation efficiency. The encapsulation efficiency of the microspheres decreases when moving from polymer ratio 3 to 5.This is also observed by Nagda, C et al., 2009 and Tao, Y et al., 2009. The reason behind this

effect is unknown. Response surface plot (Figure 2) indicates negative effect of feed flow rate. This is well supported by author Wan, L et al., 1991who suggested that the high pumping rates during the spray drying process result in large volumes of nebulized solutions to be dried. Owing to this heated air may not instantaneously transform the liquid droplets into solid microparticles, leading to the formation of larger/irregular part that are not completely dried and hence may result in decrease in encapsulation.

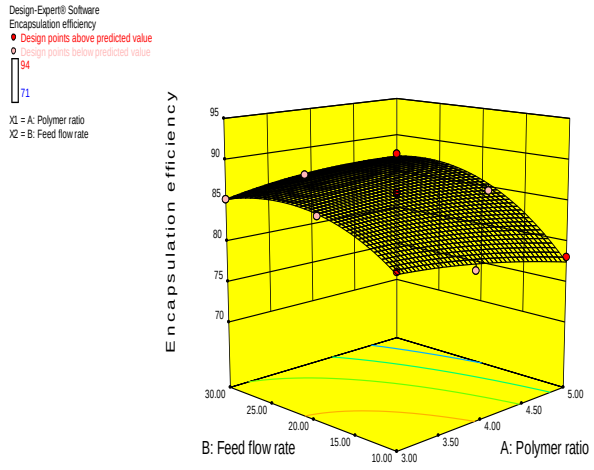

Figure 2: Response surface plot showing the effect of selected variables on the encapsulation efficiency

Polymer ratio at lower level(X_1, -1) and feed flow rate at lower level(X_2, -1) yielded microspheres with maximum encapsulation efficiency.

Figure 1and 2 represent the response surface plot, which shows the effects of the X_1 and X_2 on the percent yield and encapsulation efficiency. The positive coefficient of X_1 in case of Y_1 response (Equation 2) refers to increase in percent yield with increase in drug-to-polymer ratio. Similarly, negative coefficient of X_2 in case of Y_1 response (Equation 2) refers to decrease in percent yield with increase in feed flow rate. While in case of response term Y_2, there is negative coefficient of X_1 and X_2 factors (Equation 3) refers to decrease in encapsulation efficiency of microspheres.

The results from the estimated ridge of maximum response value of Y_1 (percent yield), and maximum response value of Y_2 (encapsulation efficiency) in terms of desirability revealed that optimum Polymer ratio (X_1) and feed flow rate (X_2) was found to be 3.68 and 10.00 ml/min respectively for obtaining desirable response with maximum percent yield and maximum encapsulation efficiency which was found to be 33.26% and 91.23% respectively.

CONCLUSION

The percent yield and encapsulation efficiency of the acyclovir loaded carbopol 974P microspheres was found to be highly dependent on the polymer ratio and feed flow rate. The optimum polymer ratio (X_1) and feed flow rate (X_2) from 3^2 full factorial design was found to be 3.68 and 10.00 ml/min respectively for obtaining higher percent yield and maximum encapsulation efficiency of microspheres which was found to be 33.26% and 91.23% respectively.

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