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Impact of Selected Variables on the Preparation of Aceclofenac Microspheres by Spray Drying using Full Factorial Design

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Abstract: A 3^2 full factorial experiment was designed to study the effects of the drug-to-polymer ratio and feed flow rate (ml/min) of spray dryer on the percent yield, particle size and encapsulation efficiency of aceclofenac loaded Eudragit S100 microspheres. Aceclofenac microspheres were prepared by using Spray dryer. Formulated microspheres were characterized for percent yield, particle size and encapsulation efficiency. Response Surface Analysis data was obtained using Software STAT-EASE, design expert, 8.0, Trial Version. Second order polynomial equations for percent yield, particle size and encapsulation efficiency were generated. The generated equation was studied for main effects; interaction terms and exponential terms. Reliability of the generated models was studied by comparing the experimental and predicted values in terms of %Bias. Low values of %Bias for all responses shows a good agreement between the experimental and predicted values. The result of analysis of variance test for both effects indicated that the test is significant. The effect of factor X_1 (drug-to-polymer ratio) (SSY_1=993.31; SSY_3=388.8) is higher than factor X_2 (feed flow rate) (SSY_1=34.56; SSY_2=24.00) for optimizing the percent yield and encapsulation efficiency of microspheres. While in case of particle size the source sum of squares in ANOVA indicated the contribution of factor X_2 (SSY_2=137.76) is higher than factor X_1 (SSY_2=48.00). The values of all the responses are highly dependent on the factors. The optimum drug-to-polymer ratio (X_1) and feed flow rate (X_2) was found to be 1:5 and 20.00 ml/min respectively for obtaining higher percent yield, smaller particle size and maximum encapsulation efficiency.
Keywords: Factorial design; Microspheres; Spray drying; Aceclofenac; Percent yield; Particle size; Encapsulation efficiency.

Introduction

Spray-drying has been used in pharmaceutical industry since the early 1940s for drying heat-sensitive materials, increasing the solubility of poorly water-soluble drugs, masking the taste, enteric coating, improving the flow properties in tablet production, and coating of some drugs or drug microencapsulation.[1]

Spray drying is a method in which a fluid mixture is usually sprayed into hot dry air. The fluid mixture may be a solution, suspension, emulsion. It is atomized into millions of individual droplets in a nozzle. The solvent is evaporated by the hot air. A fluid mixture is converted into powder in a one step processes. The separation of the dried product from the drying medium occurs in the cyclone and the final product is collected in the collecting vessel. The resulting product properties are dependent on the operating variables such as feed solution composition [2], inlet/outlet air temperature [3], feed flow rate and air flow [4].

Aceclofenac (ACE), a phenyl acetic acid derivative 2-[(2,6-dichlorophenyl)amino] phenyl acetoxy acetic acid, is a novel nonsteroidal anti-inflammatory drug (NSAID) indicated in the symptomatic treatment of pain and inflammation.[5, 6] To reduce the adverse effects and dosing frequency during prolonged treatment, it is necessary to formulate in long-acting dosage form. Different workers have attempted to prepare sustained release oral formulations of ACE like sustained release tablet, microparticulate system and microemulsion. [5, 7]

In this present work aceclofenac microspheres were prepared by spray drying technique using Eudragit S100 as release retarding material.[8] A 3^2 full factorial design was applied to study the effect of selected variables. The drug-to-polymer ratio (X_1) and feed flow rate (X_2) (ml/min) were selected as independent variables while the percent yield, particle size and encapsulation efficiency were chosen as the dependant variables in the present investigation. The levels for these two parameters were determined from the preliminary trials.

Experimental

Materials

Aceclofenac supplied from Emcure Pharmaceuticals, Pune as a gift sample; Eudragit S-100 from Dr. Reddy Laboratories, Hyderabad.

Preparation of Aceclofenac microspheres using Eudragit S100

Microspheres were prepared by using spray dryer (Labultima mini LU-222, India). Drug and polymer were dissolved in acetone and stirred using overhead stirrer at room temperature. Aceclofenac loaded Eudragit S100 microspheres were obtained by spraying the feed-solution with a spray-dryer 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. [9, 10, 11, 12] Parameters for the preparation of microspheres were optimized from preliminary studies and are summarized in Table 1. 3^2 full factorial design with coded form and actual form of variables for each batch is described in Table 2 and 3.

Table 1: Parameters for the preparation of microspheres

Parameters	Conditions
Inlet temperature	80ºC
Outlet temperature	50 ºC
Aspirator speed	80
Drug-to-polymer ratio	1:3, 1:4, 1:5
Feed flow rate	20,30,40 (ml/min)

Table 2: 3^2 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: 3^2 full factorial design with actual form of variables for each batch

Coded values	Actual Values	
	X_1	X_2
-1	1:3	20
0	1:4	30
+1	1:5	40

X_1 = Drug-to-polymer ratio
X_2 = Feed flow rate (ml/min)

Characterization of the Microspheres

Percent yield

The percent yield of each batch of microsphere was obtained on weight basis of microspheres with respect to the total expected weight of drug and polymer.[12]

$$\% \text{ yield} = \frac{\text{weight of microspheres}}{\text{Total weight of drug and polymer}} \times 100$$

Particle size

Particle size analysis of drug-loaded Eudragit S100 microspheres was performed using stereomicroscope which was calibrated using calibrated micrometers. The microscope was equipped with the software Bioplus-55 Video Plan-11UP through a camera. A small amount of dry microspheres were suspended in water (10 ml). The suspension was ultrasonicated for 10 seconds. A small drop was of suspension was placed on a clean glass slide. The slide with specimen was observed under the microscope. The magnification of the microscope used for observations was 100X. An image was taken with the help of camera and the particle size was determined using software. The process was repeated for each batch prepared.[13]

Encapsulation efficiency

Microspheres (10mg) were suspended in 10 ml of methanol. After 24 hrs, the solutions were then filtered through the whatmann filter paper (0.45µm). The absorbance of the solution at 275 nm was taken after suitable dilution.[8] Encapsulation efficiency was evaluated by the following formula:

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

Response Surface Analysis

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

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

Where Y is the predicted response, b_0 is the arithmetic mean response of 9 runs (Table 1). 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, particle size 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).[14, 15, 16] 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:

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

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

Batch	Percent yield			Particle size			Encapsulation efficiency		
	Actual	Predicted	% Bias	Actual	Predicted	% Bias	Actual	Predicted	% Bias
A	56.2	54.89	2.38	23.9	23.36	2.31	93.6	92.92	0.73
B	52.2	53.4	2.24	29.19	30.03	2.79	89.3	89.6	0.33
C	51.3	51.39	0.17	32.6	32.29	0.96	87.2	87.57	0.42
D	37.5	38.93	3.67	18.10	18.88	4.13	86.2	87.6	1.59
E	36.8	36.78	0.27	26.1	25.88	0.85	85.1	84.9	0.23
F	35.5	34.13	4.01	28.15	28.46	1.08	84.3	83.6	0.83
G	30.6	30.46	0.45	17.3	17.05	1.46	76.2	75.47	1.06
H	28.8	27.66	4.12	24.12	24.37	1.02	73.1	73.5	0.50
I	23.1	24.36	5.17	27.3	27.18	0.44	72.5	72.82	0.41

Table 5: Regression analysis data for measured responses

Coefficients	Percent yield		Particle size		Encapsulation efficiency	
	Full model	Reduced model	Full model	Reduced model	Full model	Reduced model
b_o	36.79	38.40	25.58	25.47	84.96	83.68
b_1	12.87	12.87	2.83	2.83	8.05	8.05
b_2	-2.40	-2.40	4.79	4.79	-2.00	-2.00
b_{11}	0.65	-	-0.32	-	-0.67	-
b_{22}	3.75	-	1.33	-	-3.41	-
b_{12}	-0.25	-	-2.20	-	0.64	-
R^2	0.9908	0.9493	0.9893	0.9171	0.9911	0.9132
F	150.64	93.56	128.92	55.33	155.28	52.57

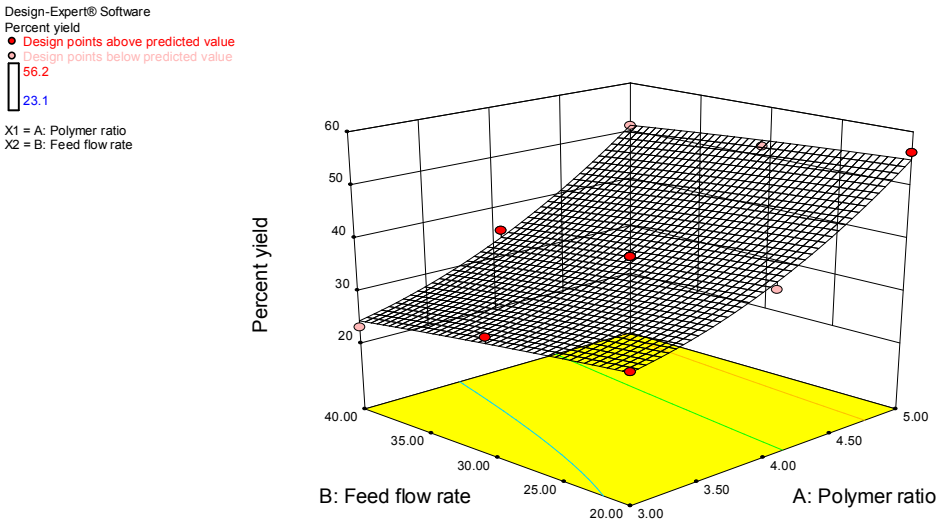

Figure 1: Response surface plot showing the effect of selected variables on the percent yield

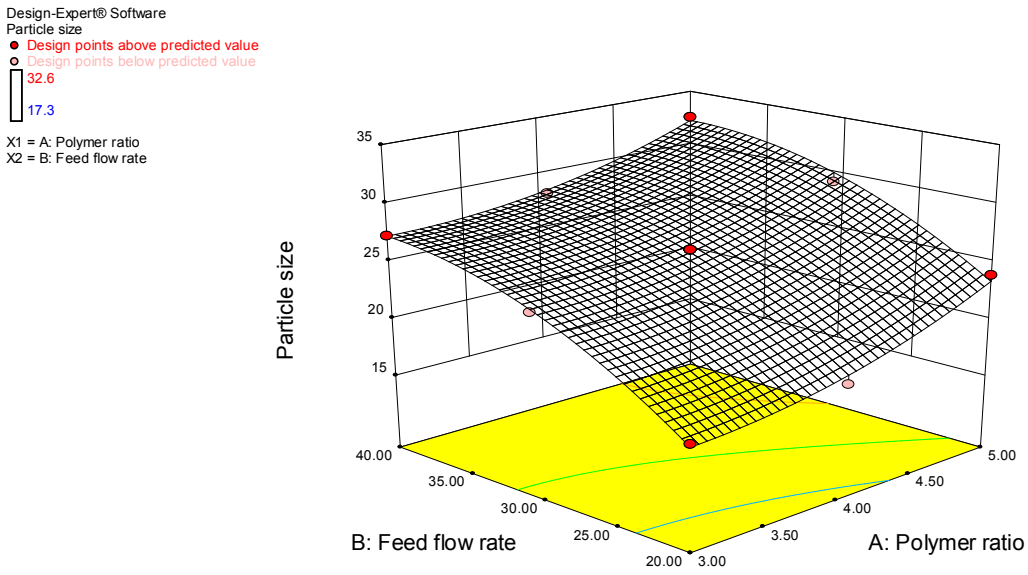

Figure 2: Response surface plot showing the effect of selected variables on the particle size

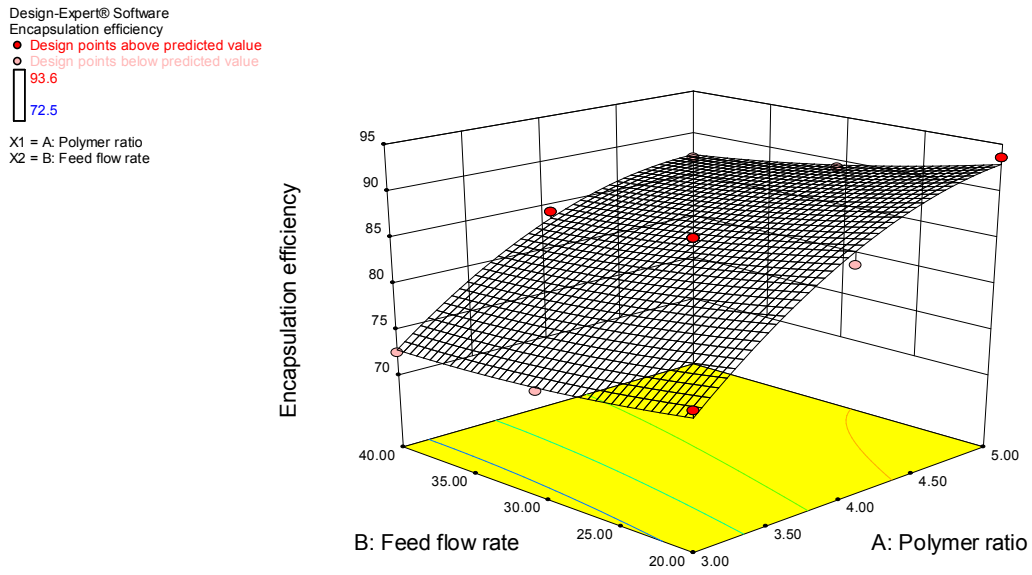

Figure 3: Response surface plot showing the effect of selected variables on the encapsulation efficiency

Results and Discussion

The percent yield (Y_1), particle size (Y_2) and encapsulation efficiency (Y_3) from the 9 experiments were used to generate predictor equations for aceclofenac microspheres with independent variables as drug-to-polymer ratio and feed flow rate. The results of multiple regression analysis and analysis of variance test (ANOVA) are summarized in Table 5.

The percent yield, particle size and encapsulation efficiency of microspheres showed R^2 values for full model are 0.9908, 0.9893 and 0.9911 (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.

The fitted model for percent yield, particle size and encapsulation efficiency are shown in equations 2, 3 and 4 respectively.

$$Y_1 = 36.79 + 12.87X_1 - 2.40X_2 + 0.65X_1X_2 + 3.75X_1^2 - 0.25X_2^2 \quad \text{.....(2)}$$

$$Y_2 = 25.58 + 2.83X_1 + 4.79X_2 - 0.32X_1X_2 + 1.33X_1^2 - 2.20X_2^2 \quad \text{.....(3)}$$

$$Y_3 = 84.96 + 8.05X_1 - 2.00X_2 - 0.67X_1X_2 - 3.41X_1^2 + 0.64X_2^2 \quad \text{.....(4)}$$

For the percent yield, particle size and encapsulation efficiency of microspheres the calculated F values for full models is 150.64, 128.92 and 155.28 respectively. The source sum of squares (Source SS) in ANOVA indicated the contribution of factor X_1 (drug-to-polymer ratio) (SSY_1=993.31; SSY_3=388.8) is higher than factor X_2 (Feed flow rate) (SSY_1=34.56; SSY_2=24.00) for optimizing the percent yield and encapsulation efficiency of microspheres. While in case of particle size the source sum of squares in ANOVA indicated the contribution of factor X_2 (SSY_2=137.76) is higher than factor X_1 (SSY_2=48.00). 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 drug-to-polymer ratio on the percent yield. With increase in the drug-to-polymer ratio, the percent yield also increases. This effect is also supported by Motlekar *et al* [16] who 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. With increase in the feed flow rate, the value of percent yield decreases. This is also well supported by Motlekar *et al* [16] 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.

Drug-to-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 particle size (Y_2), interaction terms are insignificant. Response surface plot (Figure 2.) indicates the negative effect of drug-to-polymer ratio on the particle size. The particle size of the microspheres decreases with decrease in the drug-to-polymer ratio. Nagda *et al* [9] and Motlekar *et al* [16] also reported that there is increase in drug-to-polymer ratio increases particle size which may be due to increased viscosity of feed solution which influence the interaction between disperse phase and dispersion medium that affects the size distribution of particle. Response surface plot (Figure 2.) indicates negative effect of feed flow rate. This may be due to at higher feed flow rate the atomizing air may not be able to penetrate the stream of liquid. As a result, incomplete atomization may lead to wider droplet size distribution.[16]

Drug-to-polymer ratio at lower level(X_1, -1) and feed flow rate at lower level(X_2, -1) yielded microspheres with smaller particle size.

When considering the response term encapsulation efficiency the response surface plot (Figure 3.) indicates the positive effect of drug-to-polymer ratio on the response term. The encapsulation efficiency of the microspheres increases with increase in the drug-to-polymer ratio. Trivedi *et al* [8] also reported that there is increase in encapsulation efficiency with increase in the drug-to-polymer ratio. They reported that the amount of drug remaining and available for encapsulation increases as theoretical drug loading increases. Consequently, the actual drug loading increases. As the molecular weight of the polymer increased, its hydrophobicity increased, leading to better precipitation of polymer at the boundary phase of the droplets. Response surface plot (Figure 3.) indicates negative effect of feed flow rate. This is well supported by Wan *et al* [17] who 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 particles that are not completely dried and hence resulting in decrease in encapsulation.

Figure 1, 2 and 3 represent the response surface plot, which shows the effects of the X_1 and X_2 on the percent yield, particle size and encapsulation efficiency. The positive coefficient of X_1 in case of Y_1, Y_2 and Y_3 (Equation 2, 3 and 4) refers to increase in percent yield, particle size and encapsulation efficiency with increase in drug-to-polymer ratio. Similarly, positive coefficient of X_2 in case of Y_2 (Equation 3) refers to increase particle size with increase in feed flow rate. While in case of response term Y_1 and Y_3, there is negative coefficient of X_2 (Equation 2 and 4) refers to decrease in percent yield and encapsulation efficiency.

The results from the estimated ridge of maximum response value of Y_1 (percent yield), minimum response value of Y_2 (particle size) and maximum response value of Y_3 (encapsulation efficiency) in terms of desirability revealed that optimum drug-to-polymer ratio (X_1) and feed flow rate (X_2) were 1:5 and 20.00 ml/min respectively for the desirable response.

Conclusion

The percent yield, particle size and encapsulation efficiency of the aceclofenac loaded Eudragit S100 microspheres was found to be highly dependent on the drug-to-polymer ratio and feed flow rate of spray dryer. The optimum drug-to-polymer ratio (X_1) and feed flow rate (X_2) was found to be 1:5 and 20.00 ml/min respectively for obtaining higher percent yield, smaller particle size and maximum encapsulation efficiency which is 54.89%, 23.36µm and 92.92% respectively.

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References

1. Zgoulli S., Grek V., Barre G., Goffinet G., Thonart P., Zinner S., Microencapsulation of erythromycin using a spray drying technique, J Microencapsul., 1999, 16, 565-71.
2. Chidavaenzi O., Buckton G., Koosha F., The effect of co-spray drying with polyethylene glycol-4000 on the crystallinity and physical form of lactose. Int J Pharm., 2001, 216, 43-49.
3. Broadhead J., Edmond R.S.K., Hau I., Rhodes C.T., The effect of process and formulation variables on the properties of spray dried β-galactosidase, J Pharm Pharmacol., 1994, 46, 458-467.

4. Stahl K., Claesson M., Lilliehorn P., Linden H., Backstrom K., The effect of process variables on the degradation and physical properties of spray dried insulin intended for inhalation, Int J Pharm., 2002, 233, 227-237.
5. Brogden R., Wiseman L., Aceclofenac: A review of its pharmacodynamic properties and therapeutic potential in the treatment of rheumatic disorders and in pain management, Drugs, 1996, 52, 113-24.
6. Parfitt K., Analgesics anti-inflammatory and antipyretics. In: Reynolds JEF, ed. Martindale: The complete drug reference. London: Royal pharmaceutical society, 1996, 2–12.
7. Mutalik S., Naha A., Usha A., Ranjith A., Musmade P., Manoj K., Preparation, in vitro, preclinical and clinical evaluations of once daily sustained release tablets of aceclofenac, Arch Pharm Res., 2007, 30, 222-34.
8. Trivedi P., Verma A.M.L, Garud N., Preparation and characterization of aceclofenac microspheres, Asian J Pharm Sci., 2008, 4, 110-115.
9. Nagda C., Chotai N.P., Patel U., Patel S. et al., Preparation and characterization of spray-dried mucoadhesive microspheres of aceclofenac, Drug Develop Ind Pharm., 2009, 35, 1155-1166.
10. Jain D., Panda A.K., Majumdar D.K., Eudragit S100 entrapped insulin microspheres for oral delivery, AAPS Pharmascitech, 2005, 6(1), 100-107.
11. Rawat M., Saraf S. et al. Influence of selected formulation variables on the preparation of enzyme-entrapped eudragit S100 microspheres, AAPS Pharmascitech, 2007 8, 1-9.
12. Patil S.S., Kasture P.V., Studies on preparation and evaluation of biodegradable poly (Lactide-Co-Glycolide) microspheres of aceclofenac, CMU J Nat Sci., 2007, 6, 195-204.
13. Rathananand M., Kumar D.S., Shirwaikar A., Kumar R., Kumar D.S., Prasad R.S., Preparation of mucoadhesive microspheres for nasal delivery by spray drying, Indian J Pharm Sci., 2007, 69, 651-657.
14. Monness E., Linsley M.J., Garzon I.E., Comparing different fractions of a factorial design: A metal cutting case study, Appl Stochastic Models Bus Ind., 2007, 23, 117-128.
15. Gohel M.C., Amin A.F., Formulation design and optimization of modified-release microspheres of diclofenac sodium, Drug Develop Ind Pharm., 1999, 25, 247-251.
16. Motlekar N., Youan B., Optimization of experimental parameters for the production of LMWH-loaded polymeric microspheres, Drug Des Devel Ther., 2008, 2, 39-47.
17. Wan L., Hang P., Chia C., Preparation of coated particles using spray drying process with an aqueous system, Int J Pharm., 1991, 77, 183-191.

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