CHAPTER: 11

DEVELOPMENT, OPTIMIZATION AND CHARACTERIZATION OF NASAL SPRAY METHOD

11.1 Introduction

Drug is delivered by nasal route in several dosage forms. The generally preferred one is nasal spray or nasal drop, where drug can be formulated as a solution, emulsion or suspension. Other option for dosage forms are the nasal aerosols and nasal powders. The formulation generally contains water or oil as solvent, drug, mucoadhesive polymer, viscosity increasing agents such as carboxymethyl cellulose/hypromellose to stabilize suspension or increasing residence time of formulation in nasal tract, surfactants for solubilizing drug or increasing the wet ability of drug, tonicity agents to avoid nasal tissue irritation and, some formulation contains , penetration enhancers (1, 2). Though the formulation is stable, delivery device plays a major role in nasal drug delivery, and a good combination of formulation and nasal delivery device will decide the fate of drug in nasal tract. Hence, lead to a very complex process of nasal drug products development, as inconsistency of the formulation and the device have to be taken into account (3) As a result, authorities like Food and Drug Administration (FDA), European Medicines Agency (EMA), Canadian Agency have published guidelines, different test methods and regulations for nasal drug delivery approval which are more stringent than those for solid dosage forms (4). But, in order to obtain consistent and trustworthy results, the test methods need to be validated, and to validate the method one must know the factors affecting the measurements. Many scientist have shown that spray parameters are affected by the formulation characteristics such as viscosity and surface tension, and device design and operation of device by patient (actuation parameters)(5). Along with this technique selected for characterization and measurements set up also have an impact on obtained results and hence they should also be considered during method development.

This chapter explains about the parameters affecting droplet size distribution (DSD), plume geometry, spray pattern, shot weights and priming and repriming. The study comprising of test such as DSD, plume geometry, spray pattern and shot weights using developed nicergoline model formulations and a standard nasal spray pump.

11.2 Material and Methods

Nasal spray pumps ejection 100 µL of formulation per actuation was supplied by Aptar (Mumbai, India). Double distilled Water was used for preparation of micelle formulation, TPGS 2000, Poloxamer F 127 was obtained from BASF (Mumbai, India)

Model Formulations

The model formulation consists of water, TPGS, Poloxamer F 127 and Benzalkonium chloride.

11.3 Optimization of Spray parameters

The process parameters were optimized using a 3^4 full factorial design augmented with 5 center points. Factors considered for optimization and theit coded values along with actual values are tabulated in (Table 11.1 and Table 11.2)

11.3.1 Factors responsible for variation in spray parameters

Spray parameters such as droplet size distribution (DSD), plume geometry, span, and plume angle are totally dependent upon the set up parameters of test method and the handling of the device. Hence, following parameters were considered to study their direct and indirect impact on spray parameters during development of method.

Table 11.1 Various Factors and Responses Used in Optimization

Factors	Distance (cm)
	Force (N)
	Angle (degree)
	Force time (µsec)
Response Parameters	D90, D50, D10 (µm)
	Shot weight (mg)
	Span
	Stable Phase (µs)

Force: It is the force applied in Newton by the spray actuator at the bottom of nasal spray pump.

Distance: It is the distance between nasal spray tip and the laser beam of the diffractor.

Angle: It is angle at which the spray was carried out.

Hold time: Time for which the sprayer was hold at the bottom of nasal spray once the force is applied.

Table 11.2 Coded and Actual Values of Process Parameters

Coded value	**Actual value**			
	Distance (cm)	Force (N)	Angle (Degree)	Hold time (sec)
-1	3	35	30	1
0	5	45	60	2
1	7	55	90	3

RSM was applied using comprehensive software, Design-Expert 8.0.4 (Stat-Ease Inc., MN, USA) to fit second order polynomial equations, obtained by multiple linear regression analysis (MLRA) approach. A full and a reduced model for all variables were established by putting the values of regression coefficients in polynomial equation. Statistical soundness of the polynomial equations was established on the basis of ANOVA statistics (6-12).

Three dimensional response surface plots were established by varying levels of two factors and keeping the third factor at fixed levels at a time (13-15). In this way they are more helpful in understanding the actual interaction amongst the varying factors on the response parameter and are more meaningful. 3-D response surface graphs were constructed using the Design Expert software.

Optimized formulation was derived by specifying goals and importance to the formulation variables and response parameters. Results obtained from the software were further verified by actually performing the experiment and comparing the predicted and actual results.

Applied 3^4 full factorial design matrix is shown in the **Table 11.3** along with the responses. The experiments were performed in a random manner (in the sequence provided in “Run” column and responses were recorded.)

Table 11.3 Design matrix

Std	**Run**	**Factor 1A**	**Factor-2 B**	**Factor-3 C**	**Factor 4 D**	**Response 1**	**Response 2**	**Response 3**	**Response 4**	**Response 5**	**Response 6**
		Distance (cm)	**Force (N)**	**Angle (Degree)**	**Hold time (sec)**	**D_{10} (µm)**	**D_{50} (µm)**	**D_{90} (µm)**	**Shot weight (mg)**	**Span**	**Stable Phase (µsec)**
65	1	0.00	-1.00	0.00	1.00	24.83	40.01	80.24	100	1.8	99
54	2	1.00	1.00	1.00	0.00	42.47	98.67	215.76	105.9	1.5	57
59	3	0.00	0.00	-1.00	1.00	18.9	75.43	156.49	95	0.6	52
16	4	-1.00	1.00	0.00	-1.00	20.12	65.34	131.97	104.5	1.0	52
20	5	0.00	-1.00	1.00	-1.00	22.23	47.39	95.99	95.2	2.8	104
85	6	0.00	0.00	0.00	0.00	21.38	55.09	119.17	102.3	1.2	67
5	7	0.00	0.00	-1.00	-1.00	22.95	62.79	128.94	103.7	0.6	58
44	8	0.00	1.00	0.00	0.00	40.06	90.27	171.29	90.5	1.0	30
51	9	1.00	0.00	1.00	0.00	33.84	80.95	153.4	102.7	1.8	63
69	10	1.00	0.00	0.00	1.00	33.62	74.36	149.18	97.2	1.2	60
37	11	-1.00	-1.00	0.00	0.00	26.96	44.72	85.56	99.6	1.6	107
56	12	0.00	-1.00	-1.00	1.00	22.56	45.82	95.26	98.3	0.8	95
48	13	1.00	-1.00	1.00	0.00	27.59	68.35	127.97	103.9	2.4	60
23	14	0.00	0.00	1.00	-1.00	21.86	53.11	112.93	103.1	1.9	69
2	15	0.00	-1.00	-1.00	-1.00	19.25	47.14	95.25	101.3	0.8	100
49	16	-1.00	0.00	1.00	0.00	31.72	81.54	155.31	100	1.9	45
50	17	0.00	0.00	1.00	0.00	21.87	49.63	98.57	106.6	1.9	68
68	18	0.00	0.00	0.00	1.00	22.18	53.54	114.06	96.45	1.2	64
8	19	0.00	1.00	-1.00	-1.00	26.29	63.85	121.03	104.7	0.5	48

4	20	-1.00	0.00	-1.00	-1.00	14.45	42.24	81.97	107.8	0.6	90
72	21	1.00	1.00	0.00	1.00	42.62	103.28	180.31	105	1.0	53
12	22	1.00	-1.00	0.00	-1.00	36	72.49	148.31	85.3	1.5	88
57	23	1.00	-1.00	-1.00	1.00	28.25	71.32	139.13	98	0.7	58
3	24	1.00	-1.00	-1.00	-1.00	28.25	71.32	133.74	107.7	0.7	58
61	25	-1.00	1.00	-1.00	1.00	18.57	48.01	107.47	106.3	0.5	53
7	26	-1.00	1.00	-1.00	-1.00	19.93	58.31	124.42	106.2	0.5	49
84	27	0.00	0.00	0.00	0.00	26.44	63.88	124.27	98.3	1.2	57
58	28	-1.00	0.00	-1.00	1.00	17.44	43.16	110.63	105.2	0.6	58
24	29	1.00	0.00	1.00	-1.00	39.37	88.7	196.88	104.8	1.8	62
41	30	0.00	0.00	0.00	0.00	23.65	59.75	119.92	92.5	1.2	59
67	31	-1.00	0.00	0.00	1.00	20.5	45.86	95.12	103.1	1.3	64
15	32	1.00	0.00	0.00	-1.00	43.42	101.81	228.91	95.4	1.2	61
75	33	1.00	-1.00	1.00	1.00	29.83	55.93	105.06	104	2.4	98
19	34	-1.00	-1.00	1.00	-1.00	22.23	47.39	95.99	98.5	2.5	104
6	35	1.00	0.00	-1.00	-1.00	28.25	71.32	113.74	104.8	0.5	52
11	36	0.00	-1.00	0.00	-1.00	23.47	41.33	89.11	94.3	1.8	99
28	37	-1.00	-1.00	-1.00	0.00	19.97	44.52	87.42	110.3	0.8	98
25	38	-1.00	1.00	1.00	-1.00	43.98	108.86	195.01	93.5	1.6	26
73	39	-1.00	-1.00	1.00	1.00	22.71	47.18	92.33	98.8	2.5	93
22	40	-1.00	0.00	1.00	-1.00	35.83	99.48	188.81	98	1.9	31
78	41	1.00	0.00	1.00	1.00	30.56	79.23	119.92	105.9	1.8	62
17	42	0.00	1.00	0.00	-1.00	33.2	79.33	144.16	96	1.0	40
42	43	1.00	0.00	0.00	0.00	38.95	84.22	171.65	97.2	1.2	60
53	44	0.00	1.00	1.00	0.00	44.01	89.36	183.18	103.5	1.5	45

55	45	-1.00	-1.00	-1.00	1.00	19.64	43.72	90.68	111.4	0.8	90
74	46	0.00	-1.00	1.00	1.00	22.71	47.18	95.33	99.1	2.8	93
30	47	1.00	-1.00	-1.00	0.00	28.25	75.32	121.72	107	0.7	56
32	48	0.00	0.00	-1.00	0.00	30.49	76.05	154.06	99.2	0.6	65
43	49	-1.00	1.00	0.00	0.00	33.83	89.41	166.24	109.5	1.0	60
45	50	1.00	1.00	0.00	0.00	40.41	101.66	201.22	106.8	1.0	40
13	51	-1.00	0.00	0.00	-1.00	19.98	44.1	92.22	104.1	1.3	63
40	52	-1.00	0.00	0.00	0.00	17.81	43	97.67	96.9	1.3	65
14	53	0.00	0.00	0.00	-1.00	20.12	65.34	133.01	96.8	1.2	51
34	54	-1.00	1.00	-1.00	0.00	18.9	75.43	160.49	105.4	0.5	49
63	55	1.00	1.00	-1.00	1.00	48.42	159.11	310.91	100.2	0.4	48
82	56	0.00	0.00	0.00	0.00	30.83	77.37	146.08	99.4	1.2	87
10	57	-1.00	-1.00	0.00	-1.00	22.31	47.27	99.14	104.3	1.6	103
52	58	-1.00	1.00	1.00	0.00	29.23	79.64	151.01	106.2	1.6	81
26	59	0.00	1.00	1.00	-1.00	57.98	107.18	201.35	107.5	1.5	20
76	60	-1.00	0.00	1.00	1.00	66.12	141.77	274.59	85.4	1.9	56
38	61	0.00	-1.00	0.00	0.00	25.01	38.09	75.21	99.6	1.8	102
47	62	0.00	-1.00	1.00	0.00	24.4	58.63	121.05	88.3	2.5	104
60	63	1.00	0.00	-1.00	1.00	31.43	83.75	179.4	100.7	0.5	59
80	64	0.00	1.00	1.00	1.00	43.46	146.37	270.57	105.6	1.5	23
79	65	-1.00	1.00	1.00	1.00	20.38	63.03	127.28	107.9	1.6	81
83	66	0.00	0.00	0.00	0.00	31.63	75.77	150.45	88.2	1.2	65
27	67	1.00	1.00	1.00	-1.00	43.03	111.25	229.3	108.7	1.5	55
87	68	0.00	0.00	0.00	0.00	29.81	74.62	151.15	87.9	1.2	78
62	69	0.00	1.00	-1.00	1.00	25.19	64.89	136.94	108.5	0.5	65

70	70	-1.00	1.00	0.00	1.00	23.32	72.09	138.23	106.7	1.0	83
86	71	0.00	0.00	0.00	0.00	37.12	92.23	187.5	90.5	1.2	55
71	72	0.00	1.00	0.00	1.00	32.24	75.17	146.7	97.9	1.0	43
9	73	1.00	1.00	-1.00	-1.00	49.99	142.73	300.19	98.23	0.4	42
35	74	0.00	1.00	-1.00	0.00	33.66	73.48	128.11	100.8	0.5	55
1	75	-1.00	-1.00	-1.00	-1.00	20.83	44.92	97.01	109	0.8	102
64	76	-1.00	-1.00	0.00	1.00	22.63	46.61	90.24	100	1.6	103
36	77	1.00	1.00	-1.00	0.00	48.23	132.76	280.21	97.24	0.4	45
77	78	0.00	0.00	1.00	1.00	35.68	92.55	210.96	90.6	1.9	80
18	79	1.00	1.00	0.00	-1.00	40.17	108.24	222.34	106.6	1.0	35
46	80	-1.00	-1.00	1.00	0.00	24.4	58.63	121.04	88.3	2.5	104
66	81	1.00	-1.00	0.00	1.00	27.34	54.93	111.26	95.1	1.5	87
29	82	0.00	-1.00	-1.00	0.00	20.13	42.14	90.14	97	0.8	95
33	83	1.00	0.00	-1.00	0.00	28.48	65.78	135.25	97	0.5	80
21	84	1.00	-1.00	1.00	-1.00	28.54	55.89	103.6	107.5	2.4	98
39	85	1.00	-1.00	0.00	0.00	29.6	63.27	143.11	92.3	1.5	95
81	86	1.00	1.00	1.00	1.00	42.96	124.18	253.78	104.3	1.5	60
31	87	-1.00	0.00	-1.00	0.00	25.18	65.96	134.72	102.9	0.6	85

11.3.2 Determination of Droplet Size Distribution (D10, D50, D90)

The droplet size distribution (DSD) was determined using HELOS BR laser diffraction with SPRAYER-module as well as the force actuator, respectively (Sympatec GmbH, Clausthal Zellerfeld, Germany). The spray angle was varied between 30° and 90°; the actuation force between 35 and 55 N. The distance to the measuring zone ranged from 3 to 7 cm, and Hold time 1 to 3 sec. Time-resolved measurements were performed, and data was analyzed as per Fraunhofer theory. All determinations were performed in triplicate.

Regulatory bodies such as FDA and the EMA propose making use of laser diffraction technique, which is now a day's considered as a standard technique droplet and particle size analysis Laser diffraction use two common light scattering principles, which are Mie- or Fraunhofer-theory (16). The DSD should be measured every 1 ms, over the entire spray event and on the basis of time history profiles (obscuration/DSD versus time), the spray event can be split into three distinct phases: first formation phase, which is depicted by a rapid increase in obscuration and decrease in droplet size, second fully developed phase, where obscuration and droplet size reach a plateau, and, finally, the dissipation phase, designated by a rapid decrease in obscuration and an increase in droplet size (17).

11.3.2.1 Statistical Analysis of Response 1 (D90)

p-values of the different models, p-value for lack of fit in the models, adjusted R^2 value and predicted R^2 values are shown in the following Table 11.4.

Table 11.4 ANOVA Analysis of Different Models for Response 1 - D90

Source	Sequential p-value	Lack of Fit p-value	Adjusted R-Squared	Predicted R-Squared	
Linear	< 0.0001	0.1226	0.4979	0.4514	
<u>2FI</u>	<u>0.0106</u>	<u>0.1635</u>	<u>0.5625</u>	<u>0.4667</u>	<u>Suggested</u>
Quadratic	0.1345	0.1777	0.5805	0.4652	
Cubic	0.2830	0.1916	0.6000	0.2960	Aliased

Based on the p values and agreement between adjusted and predicted R^2 values, highest polynomial was chosen for response evaluation and prediction. Here 2FI model was found to best fit the experimental results. Cubic and higher models were found to be aliased (predicted results would be confounded) and hence were left out. In order to improve the prediction by the selected quadratic model, stepwise regression with alpha in value of 0.100 and alpha out value of 0.100 was performed. Prob> |t| is the probability of getting a 't' value this large if the term does not actually have an effect. In order to keep the term in the model, this value must be less than or equal to the alpha in value and not larger than the alpha out value. Based on the results reduced quadratic model was chosen.

Table 11.5 Factors Selected on Basis of Stepwise Regression Analysis D90

Forced Terms Added	Coefficient Estimate	t for H0 Coeff=0	Prob > |t|	R-Squared	MSE
B-Force	40.16	6.88	<0.0001	0.3576	1840.78
A-Distance	25.62	4.96	<0.0001	0.5031	1440.61
A^2	17.15	2.82	0.0060	0.5466	1330.39
AB	-17.09	-2.94	0.0043	0.5898	1218.41
AC	8.92	1.91	0.0600	0.6074	1180.42
C-Angle	40.16	6.88	<0.0001	0.3576	1840.78

Table 11.6 ANOVA Table for Response Surface Reduced 2FI Model Response 1 - D90

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	1.479E+005	5	29587.99	25.07	< 0.0001	Significant
A-Distance	35455.01	1	35455.01	30.04	< 0.0001	Significant
B-Force	87087.76	1	87087.76	73.78	< 0.0001	Significant
C-Angle	4296.05	1	4296.05	3.64	0.0600	
AB	10588.75	1	10588.75	8.97	0.0036	Significant
AC	10512.40	1	10512.40	8.91	0.0038	Significant
Residual	95613.75	81	1180.42			
Lack of Fit	92051.58	75	1227.35	2.07	0.1815	Non significant
Pure Error	3562.17	6	593.69			
Cor Total	2.436E+005	86				

Table 11.5 shows Stepwise Regression Analysis for D90 and

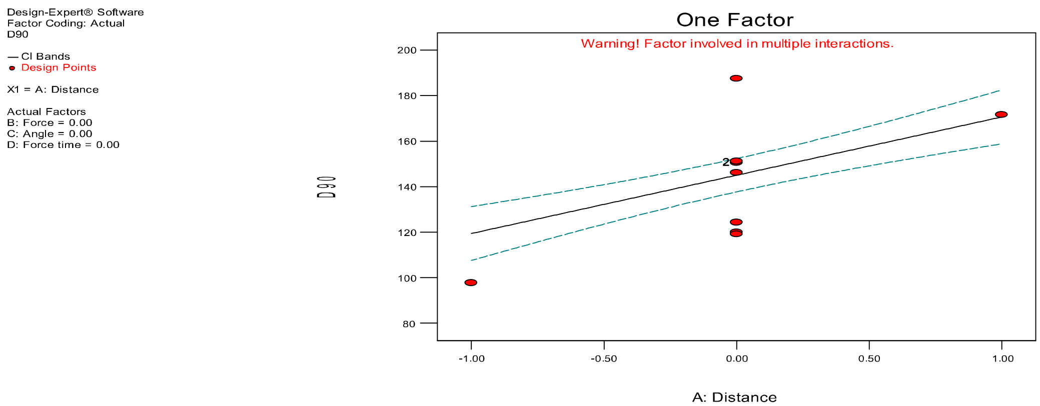
Table 11.6 shows the ANOVA analysis of chosen reduced 2FI model. The Model F-value of 25.07 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case, A, B, C, AB and AC are significant model terms. Distance from the laser beam, Force applied at the base of spray and Angle of spray have significant impact on D90, while two factor interactions AB and AC also have significant effect on D90.

The "Lack of Fit F-value" of 2.07 implies the Lack of Fit is not significant relative to the pure error. Non-significant lack of fit shows that selected model is appropriate.

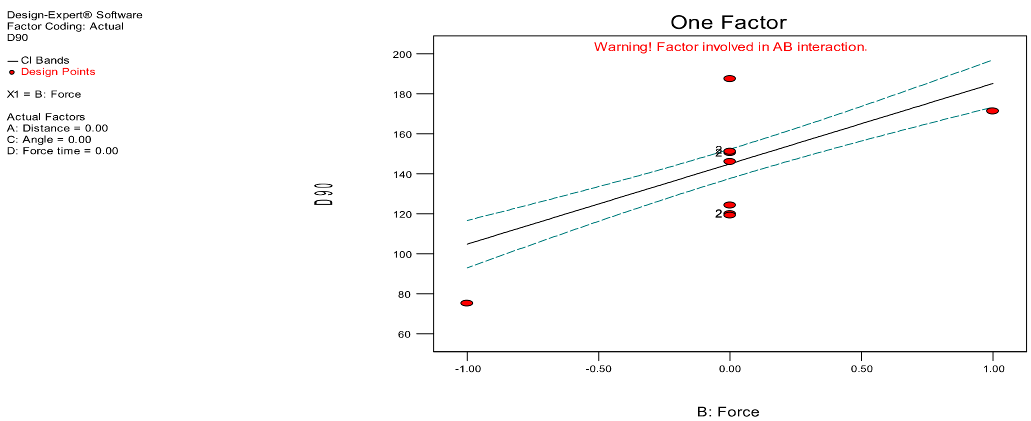
Table 11.7 Summary of ANOVA Results of Reduced 2FI Model Response 1-D90

Parameter	Value
Std. Dev.	34.36
Mean	145.03
C.V. %	23.69
PRESS	1.111E+005
R-Squared	0.6074
Adj R-Squared	0.5832
Pred R-Squared	0.5440
Adeq Precision	18.370

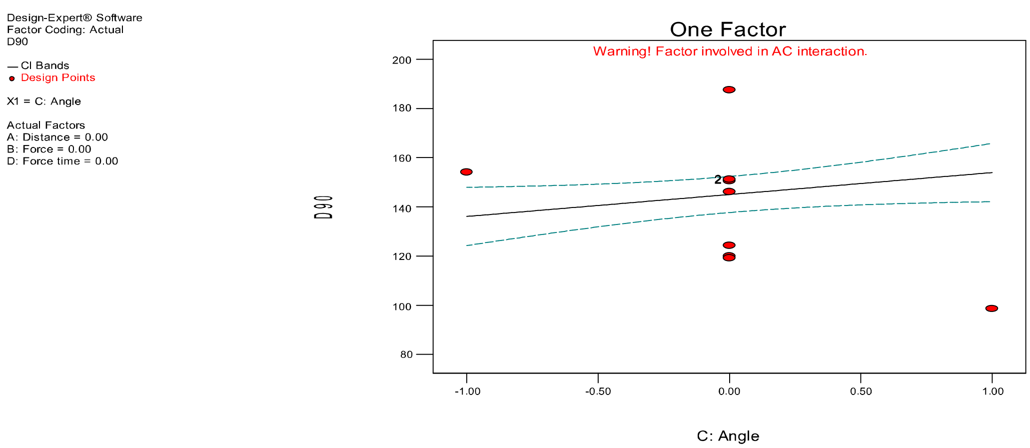
Table 11.7 shows summary of ANOVA results for quadratic model. As it can be seen, the "Pred R-Squared" of 0.5440 is in reasonable agreement with the "Adj R-Squared" of 0.5832 implying adequecy of selected model in predicting responses. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Obtained ratio of 18.370 for reduced 2FI model model indicates an adequate signal. Hence, selected reduced 2FI model can be used to navigate the design space.



1A.

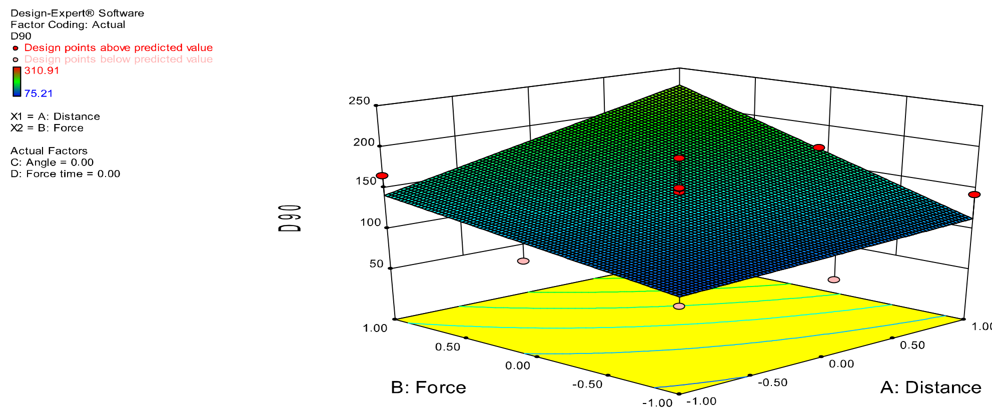


1B.

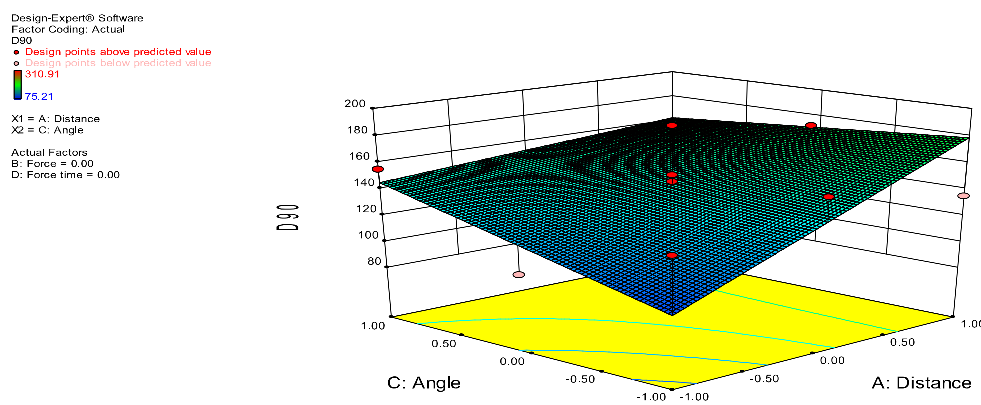


1C.

Figure 11.1 One factor response plots A. Distance Vs D90 B. Angle Vs D90 C. Force Vs D90



2A.



2B.

Figure 11.2 Two factor response surface plots A. Force x distance Vs D90 B. Angle x Distance Vs D90

Figure 11.1 shows one factor effect plots while **Figure 11.2** shows two factor response surface plots. Only plots showing effects of significant factors have been shown. It can be seen that increase in distance from the laser beam, force applied at the bottom of spray and angle of spray bottle increases D90 with highest effect from distance and force. Response surface plots indicate that lowest D90 was observed at lowest levels of force, distance and angle.

Changes in D90 as a result of rotating the actuator (30°, 60° and 90° angles) suggest that the spray formation from a nasal device is unbalanced. It is evident from the images shown in Figure 11.3 that there exists localized greater concentration of droplets (so-called ‘‘hotspots’’) within a nasal-spray pattern, which appear asymmetrically and vary according to pump design and orientation. Such kind of different spray leads to difference in droplet size distribution (DSD).

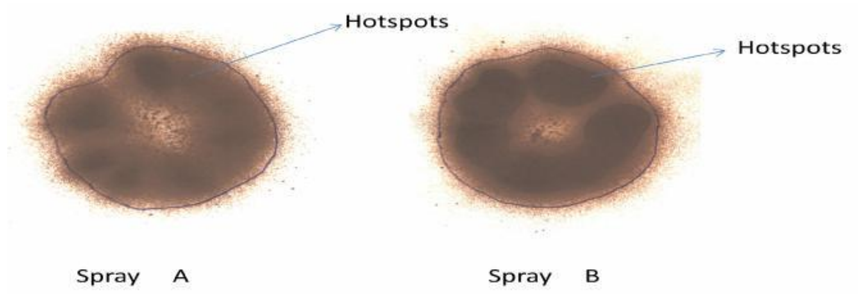


Figure 11.3 Pattern of plume determined by TLC method

The profiles show a DSD pattern of an emitted spray from the initial nasal spray formation, middle stable phase and last dissipation phase. After looking into the data, one can conclude that extreme changes in all spray parameters (D10, D50, and D90) can occur from formation to dissipation phase. It is obvious from Figure 11.4 that D10 and D50 levels were fairly stable throughout the spray period, whereas D90 fluctuated in the dissipation phase.

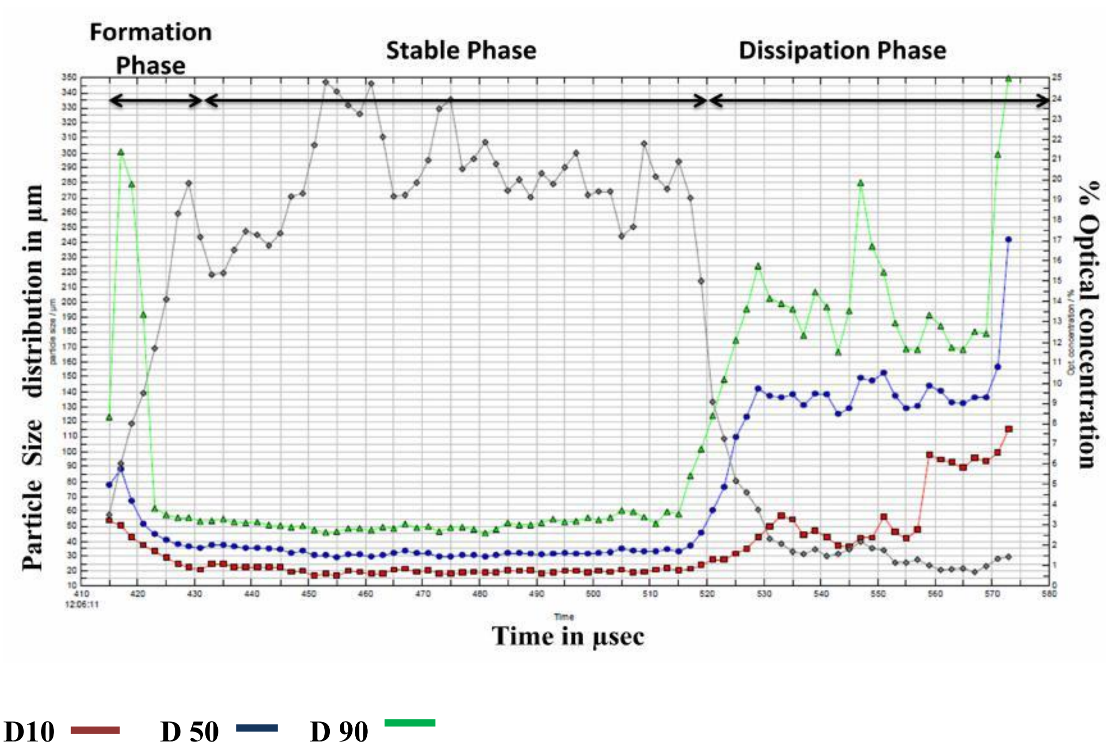


Figure 11.4 Phases of nasal spray development

Predicted response at any factor level can be calculated using following equation:

D90 =+145.02632

+25.62370 * Distance

+40.15889 * Force

+8.91944 * Angle

+17.15028 * Distance * Force

-17.08833 * Distance * Angle

11.3.2.2 Statistical Analysis of Response 1 (D50)

p-values of the different models, p-value for lack of fit in the models, Adjusted R^2 value and Predicted R^2 values are shown in the following **Table 11.8**

Table 11.8 ANOVA Analysis of Different Models (D50)

Source	**Sequentialp-value**	**Lack of Fitp-value**	**AdjustedR-Squared**	**PredictedR-Squared**	
Linear	< 0.0001	0.1524	0.5215	0.4766	
2FI	0.0042	0.2131	0.5952	0.5024	Suggested
Quadratic	0.0929	0.2362	0.6169	0.5080	
Cubic	0.4044	0.2388	0.6227	0.3410	Aliased

Based on the p values and agreement between adjusted and predicted R^2 values, highest polynomial was chosen for response evaluation and prediction. Here 2 factor interaction (2FI) model was found to best fit the experimental results. Quadratic model was not chosen, due to inadequacy of sequencial model p value (0.0929 vs 0/0042). Cubic and higher models were found to be aliased (predicted results would be confounded) and hence were left out. In order to improve the 2FI model, stepwise regression with alpha in value=0.100 and alpha out value of 0.100 was performed. Prob> |t| is the probability of getting a t value this large if the term does not actually have an effect. In order to keep the term in the model, this value must be less than or equal to the Alpha In value and not larger than the Alpha Out value. Based on the results shown in **Table 11.9** reduced 2FI model was chosen.

Table 11.9 Factors Selected on Basis of Stepwise Regression Analysis (D50)

Forced Terms Added	**Coefficient Estimate**	**t for H0 Coeff=0**	**Prob> |t|**	**R-Squared**	**MSE**
B-Force	20.56	7.03	<0.0001	0.3680	461.41
A-Distance	13.12	5.11	<0.0001	0.5178	356.20
AC	-10.32	-3.49	0.0008	0.5796	314.30
AB	7.16	2.50	0.0144	0.6094	295.60
C-Angle	5.39	2.37	0.0204	0.6346	279.92

Table 11.10 ANOVA for Response Surface Reduced 2FI Model (D50)

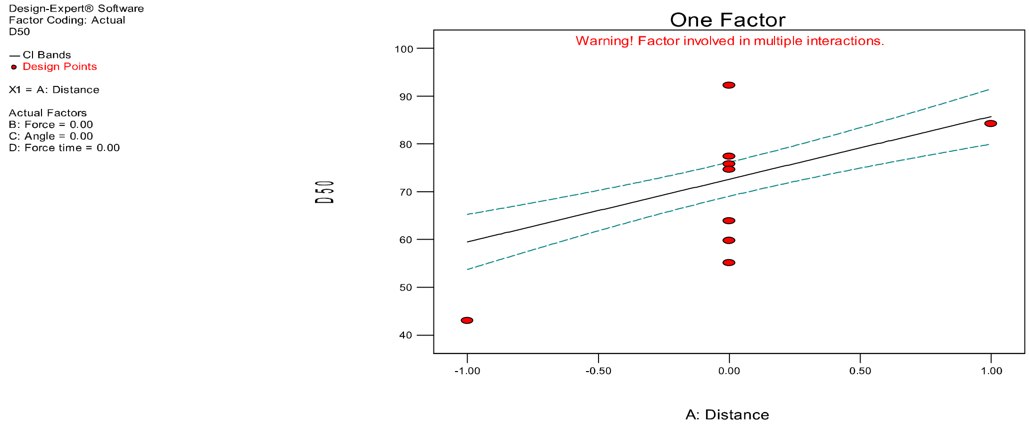
Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	39379.36	5	7875.87	28.14	< 0.0001	Significant
A-Distance	9299.19	1	9299.19	33.22	< 0.0001	Significant
B-Force	22832.70	1	22832.70	81.57	< 0.0001	Significant
C-Angle	1566.01	1	1566.01	5.59	0.0204	Significant
AB	1847.57	1	1847.57	6.60	0.0120	Significant
AC	3833.88	1	3833.88	13.70	0.0004	Significant
Residual	22673.20	81	279.92			
Lack of Fit	21716.09	75	289.55	1.82	0.2323	Non significant
Pure Error	957.11	6	159.52			
Cor Total	62052.56	86				

The Model F-value of 28.14 shown in **Table 11.10** implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, AC are significant model terms i.e. Distance, Force, Angle had significant effect on D50 and AB and AC are the two way interactions that significantly affect the D50 response. The "Lack of Fit F-value" of 1.82 implies the Lack of Fit is not significant relative to the pure error. There is a 21.31% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit shows that selected model is appropriate.

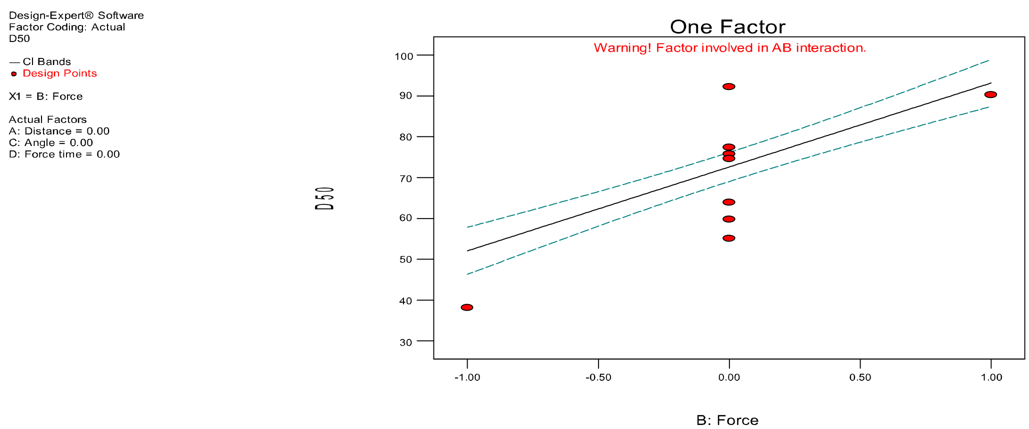
Table 11.11 Summary of ANOVA results of 2FI model (D50)

Std. Dev.	16.73	R-Squared	0.6346
Mean	72.62	Adj R-Squared	0.6121
C.V. %	23.04	Pred R-Squared	0.5741
PRESS	26430.34	Adeq Precision	20.031

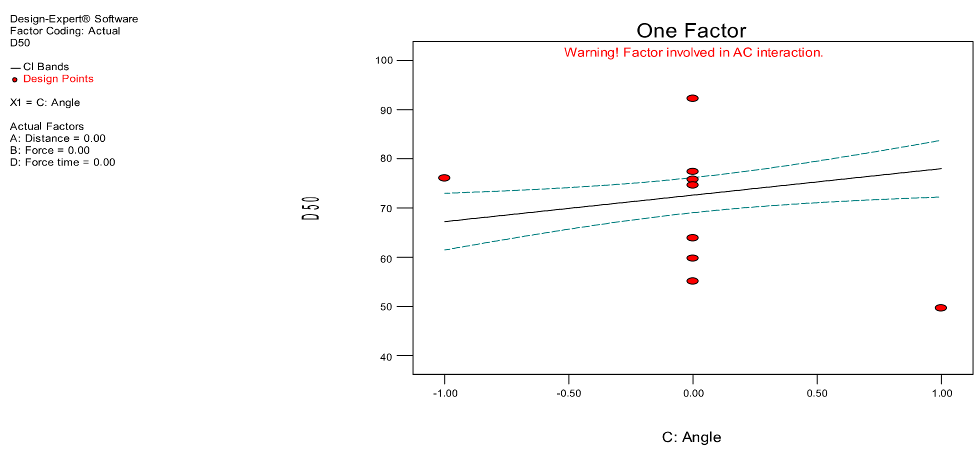
Table 11.11 shows summary of ANOVA results for 2FI model. As it can be seen, the "Pred R-Squared" of 0.5741 is in reasonable agreement with the "Adj R-Squared" of 0.6121 implying adequecy of selected model in predicting responses. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Obtained ratio of 20.031 for 2FI model indicates an adequate signal. Hence, selected 2FI model can be used to navigate the design space.



5A.

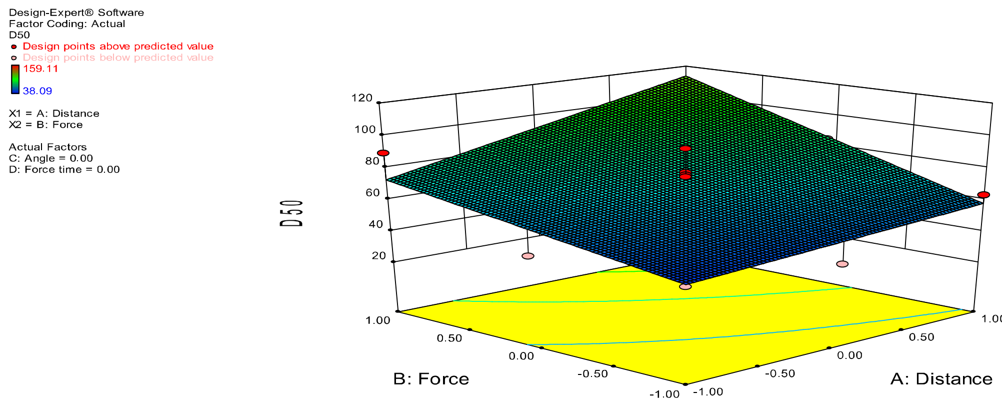


5B.

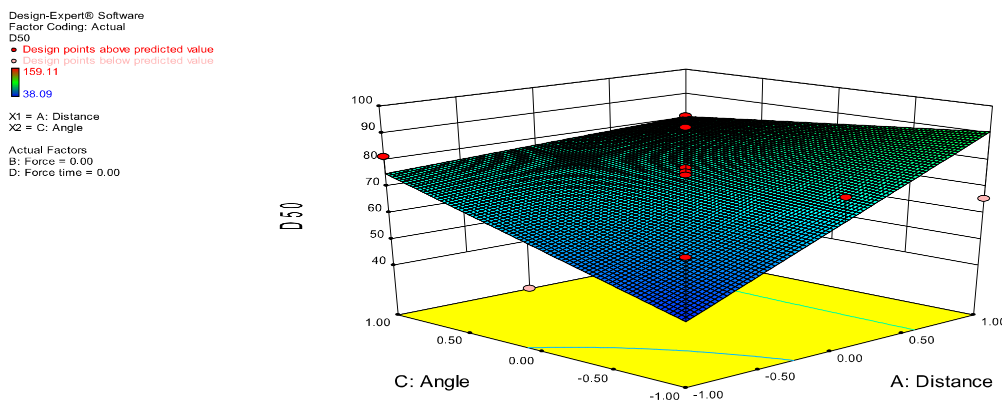


5C.

Figure 11.5 One factor response plots A. Distance Vs D50 B. Force Vs D50 C. Angle Vs D50



6A.



6B.

Figure 11.6 Two factor response surface plots A. Force x distance Vs D50 B. Angle x Distance Vs D50

It can be seen that increase in distance from the laser beam, force applied at the bottom of spray and angle of spray bottle increases D50 with highest effect from distance and force. Response surface plots indicate that lowest D50 was observed at lowest levels of force, distance and angle.

11.3.2.3. Statistical Analysis of Response D10

p-values of the different models, p-value for lack of fit in the models, Adjusted R^2 value and Predicted R^2 values are shown in the following **Table 11.10**.

Table 11.12 ANOVA Analysis of Different Models (D10)

Source	**Sequential p-value**	**Lack of Fit p-value**	**Adjusted R-Squared**	**Predicted R-Squared**	
Linear	< 0.0001	0.1913	0.4362	0.3874	
2FI	0.0069	0.2554	0.5155	0.4163	Suggested
Quadratic	0.5680	0.2481	0.5087	0.3746	
Cubic	0.2945	0.2648	0.5300	0.1728	Aliased

Based on the p values and agreement between adjusted and predicted R^2 values. **Table 11.12**, highest polynomial was chosen for response evaluation and prediction. Here 2FI model was found to best fit the experimental results. Quadratic model was not chosen, due to inadequacy of sequential model p value (0.05680 vs 0.0069). Cubic and higher models were found to be aliased (predicted results would be confounded) and hence were left out. In order to improve the selected prediction by 2FI model, stepwise regression with alpha in value of 0.100 and alpha out value of 0.100 was performed. Prob> |t| is the probability of getting a t value this large if the term does not actually have an effect. In order to keep the term in the model, this value must be less than or equal to the Alpha In value and not larger than the Alpha Out value. Based on the results reduced quadratic model was chosen.

Table 11.13 Factors Selected on Basis of Stepwise Regression Analysis (D10)

Forced Terms Added	**Coefficient Estimate**	**t for H0Coeff=0**	**Prob > |t|**	**R-Squared**	**MSE**
B-Force	5.42	4.47	<0.0001	0.1901	79.55
A-Distance	5.39	5.03	<0.0001	0.3778	61.84
C-Angle	3.61	3.61	0.0005	0.4622	54.09
AC	-3.42	-2.91	0.0046	0.5126	49.62
AB	3.00	2.65	0.0097	0.5515	46.23

Table 11.14 ANOVA table for Response Surface Reduced 2FI Model (D10)

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	4603.93	5	920.79	19.92	< 0.0001	Significant
A-Distance	1567.09	1	1567.09	33.90	< 0.0001	Significant
B-Force	1586.87	1	1586.87	34.33	< 0.0001	Significant
C-Angle	704.96	1	704.96	15.25	0.0002	Significant
AB	324.42	1	324.42	7.02	0.0097	Significant
AC	420.59	1	420.59	9.10	0.0034	Significant
Residual	3744.51	81	46.23			
Lack of Fit	3575.07	75	47.67	1.69	0.2646	Non significant
Pure Error	169.44	6	28.24			
Cor Total	8348.44	86				

Table 11.13 shows the ANOVA analysis of chosen reduced 2FI model. The Model F-value of 19.92 implies the model is significant

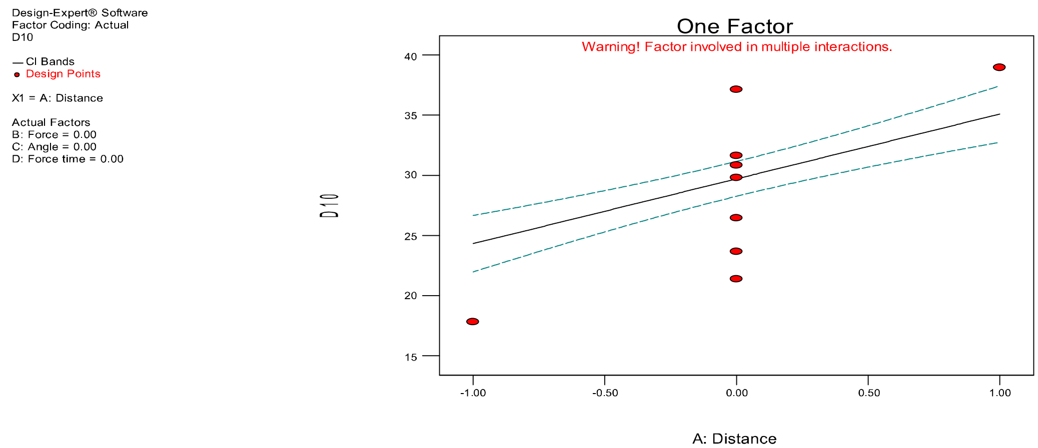
Table 11.14. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case, A, B, C, AB and AC are significant model terms. Distanec, Force and Angle have significant impact on D10, while two factor interactions AB and AC also have significant effect on D10. The "Lack of Fit F-value" of 0.2646 implies the Lack of Fit is not significant relative to the pure error. There is a 26.46% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit shows that selected model is appropriate.

Table 11.15 Summary of ANOVA results of reduced quadratic model

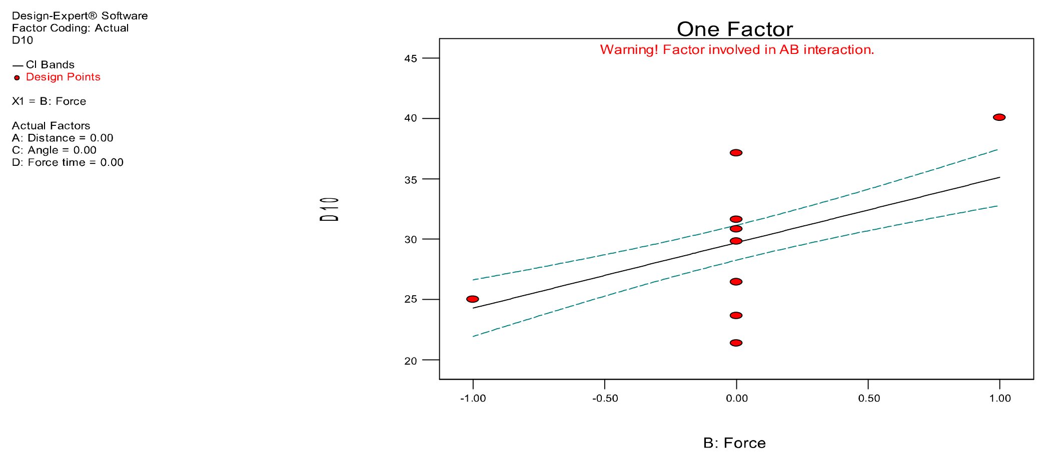
Parameter	**Value**
Std. Dev.	6.80
Mean	29.71
C.V. %	22.89
PRESS	4319.94
R-Squared	0.5515
Adj R-Squared	0.5238
Pred R-Squared	0.4825
Adeq Precision	16.153

Table 11.15 shows summary of anova results for quadratic model. As it can be seen, the "Pred R-Squared" of 0.4825 is in reasonable agreement with the "Adj R-Squared" of 0.5238 implying adequecy of selected model in predicting responses. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Obtained ratio of 16.153 for

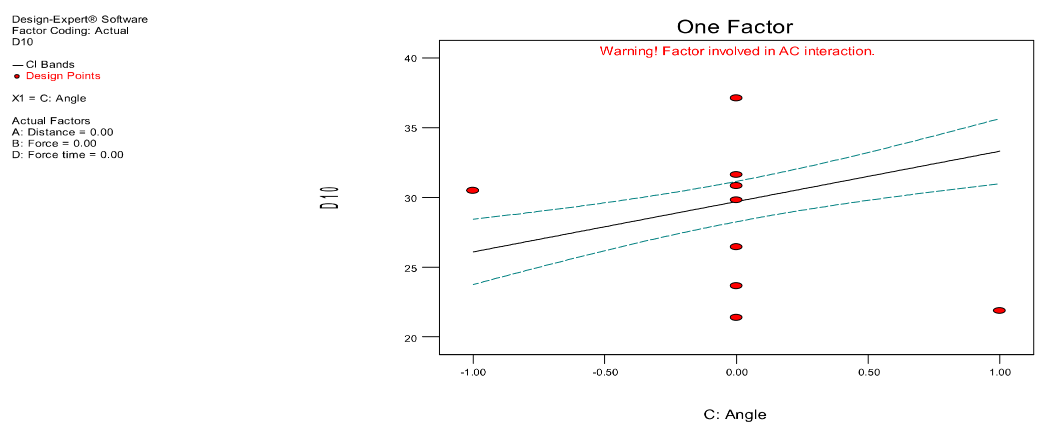
quadratic model model indicates an adequate signal. Hence, selected reduced 2FI model can be used to navigate the design space.



7A.

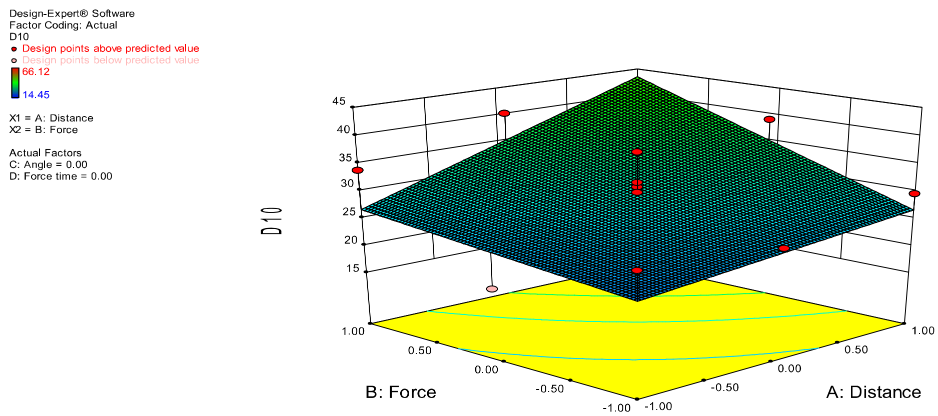


7B.

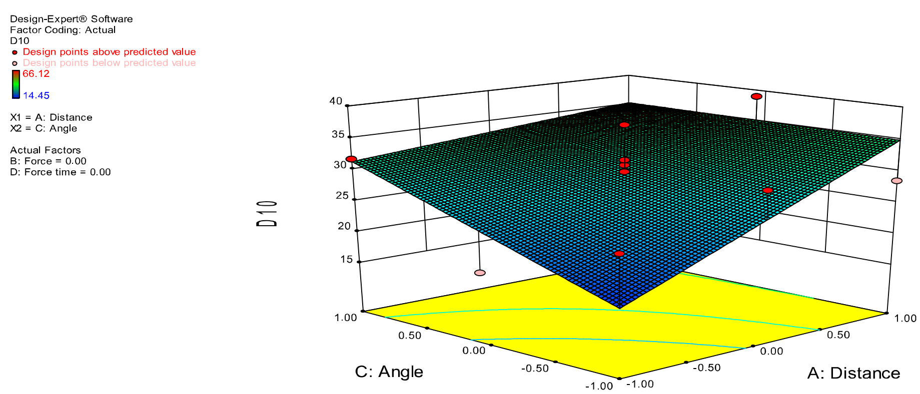


7C.

Figure 11.7 One factor response plots A. Distance Vs D10 B. Force Vs D10 C. Angle Vs D10



8A.



8B.

Figure 11.8 Two factor response surface plots A. Force x distance Vs D10 B. Angle x Distance Vs D10

Figure 11.7 show one factor effect plots while **Figure 11.8** show two factor response surface plot of stable phase. Only plots showing effects of significant factors have been shown. It can be seen that increase in distance, force and angle caused linear increase in D10 showed quadratic effect while angle showed linear effect. Increase in distance led to initial increase followed by prominent decrease in span. Increase in angle linearly and significantly caused rise in span. Highest span value has been predicted at lowest level of force (35 N) and highest level of angle (90^o).

Final Equation in Terms of Actual Factors:

D10 =+29.70609

+5.38704 * Distance

+5.42093 * Force

+3.61315 * Angle

+3.00194 * Distance * Force

-3.41806 * Distance * Angle

11.3.2.4 Impact of distance on Droplet size distribution

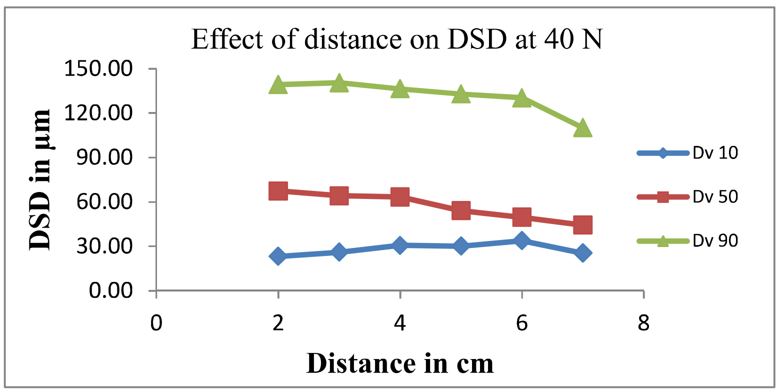


Figure 11.9 Plot showing impact of distance on DSD

FDA recommends study of DSD at two distances ranging from 2 to 7 cm from the nozzle tip , where distance between the two should be 3 cm or more (17). To compare the effect of distance on DSD the data to should be collected only during the fully developed phase and should comprise the droplet size expressed in terms of D10, *D*50 and *D*90 and the span value defined as (*D*90 − *D*10)/*D*50 as an indicator for the width of the distribution and, for NDAs, additionally, the fraction of droplets smaller than 10 µm (18). Distance between the spray nozzle and the laser beam influence the DSD measurement because of diverse settling velocities of the droplets, the plume dynamics and the diverse demonstration of the true DSD in the measurement zone (19, 20).

Many scientists (21) have explained the impact of distance between nasal spray tip and the laser beam on droplet size distribution data. But contradictory results were obtained on the effects of actuation distance on DSD. None of them have explored the effect of distance on DSD from different distances. In our study we examined our developed nasal product and obtained impact of distance of on DSD (**Figure 11.9**). We varied the nasal spray tip to measuring zone distance from 2 to 7 cm and observed decrease in D 50 and D 90 value but D 10 remain unaffected. The Spraytec laser beam has a sampling zone width of 30 cm and diameter of 1 cm. As the distance from the laser beam increases the area of the resultant actuated spray also increases which results in larger percentage of droplets missing the beam. Thus, this is not the actual DSD as fewer droplets represent the DSD of the spray. As a result, actuation of spray from short distance from the laser beam may provide better information of the DSD from nasal sprays. But, analysis at a short distance may lead to multiple scattering, due to the high density of droplets in the measuring zone, which can result in misinterpretation of data of droplet size and, thus, a distance has to be chosen based on the obscuration levels that reduce multiple scattering events (21) FDA draft guidance states that DSD must be checked from two distances. Guidance has specified a maximum distance of 7 cm from the beam and the second distance should be at least 3 cm from the first. So, a short distance should be chosen or vacuum must be applied at the top after the beam of analysis to characterize the droplet distribution. Applied vacuum will reduce the loss of droplets from the measurement zone during flight. Hence, distance selection must be done carefully or experiments must be carried out at fixed force and vacuum but at different distances from laser beam. From the data one must choose two distances which have significantly different DSD (21).

11.3.2.5 Impact of Force on droplet size distribution

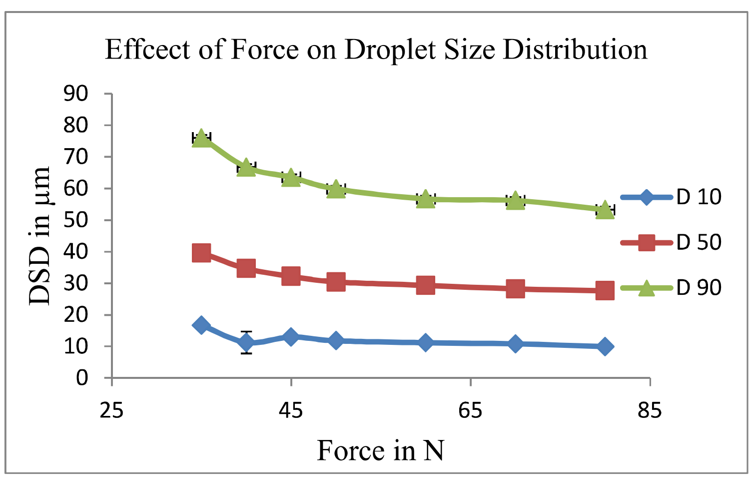


Figure 11.10 Plot showing impact of Force on DSD

The study of impact of force on DSD is carried to represent the force employed by the relevant patient group (21) and compare it in form of in vitro force using machine. As determined by Doughty *et al.* (22) a force of 5.82 kg and 3.37 kg to be utilized for adult and children respectively. As per Dayal*et al* increase in actuation force (3 to 7 kg) lead to a decrease in *D*50 values by 37%. But in our study, the actuation force ranged from 25 to 70 (1 N = mass (kg) × 9.81 m/s2), and the results for DSD are shown Figure 11.10. For our device 30 N force was not sufficient for actuation of device. Hence, we were unable to collect data below 35N actuation force. We finalized 45N as reliable for our device.

11.3.3 Statistical Analysis of Response 2 (Shot weight)

11.3.3.1 Determination of Shot Weights/Validation of Pump Delivery

Nasal spray was filled with 10 ml of developed formulation in order to determine the shot weights. The device was actuated with an automated actuator (SPRAYER-module, Sympatec), and after each actuation of spray, weight of device was taken on an analytical balance (Mettler Toledo) to determine the delivered mass. The actuation parameters were set as follows: the actuation force ranged from 35 to 55 N.

p-values of the different models, p-value for lack of fit in the models, Adjusted R^2 value and Predicted R^2 values are shown in the **Table 11.16**.

Table 11.16 ANOVA Analysis of Different Models on response-2 Shot weight

	Sequential	**Lack of Fit**	**Adjusted**	**Predicted**	
Source	**p-value**	**p-value**	**R-Squared**	**R-Squared**	
Linear	0.1161	0.5160	0.0407	-0.0279	
2FI	0.0090	0.6151	0.1687	0.0404	
Quadratic	0.0001	0.7901	0.3628	0.2192	Suggested
Cubic	0.6224	0.7706	0.3414	-0.0907	Aliased

Based on the p values and agreement between adjusted and predicted R^2 values, highest polynomial was chosen for response evaluation and prediction. Here quadratic model was found to best fit the experimental results. Cubic and higher models were found to be aliased (predicted results would be confounded) and hence were left out. In order to improve the quadratic model, stepwise regression with alpha in value of 0.100 and alpha out value of 0.100 was performed. Prob> |t| is the probability of getting a t value this large if the term does not actually have an effect. In order to keep the term in the model, this value must be less than or equal to the alpha in value and not larger than the alpha out value. Based on the results reduced quadratic model was chosen.

Apart from the terms selected based on stepwise regression analysis **Table 11.17**, term A (distance from the laser beam) was included in the final model to maintain the hierachy to satisfy the significant interaction term of AC.

Table 11.17 Factors Selected on Basis of Stepwise Regression Analysis on Shot weight

Forced Terms Added	Coefficient Estimate	t for H0Coeff=0	Prob > |t|	R-Squared	MSE
AC	3.46	3.61	0.0005	0.1330	33.15
A^2	3.88	3.21	0.0019	0.2280	29.87
C^2	3.38	2.90	0.0048	0.2990	27.45
B-Force	1.85	2.70	0.0085	0.3561	25.52
BC	2.00	2.44	0.0167	0.4003	24.06
B^2	2.08	1.93	0.0577	0.4269	23.28
C-Angle	-1.11	-1.71	0.0904	0.4475	22.73

Table 11.18 ANOVA table for Response Surface Reduced Quadratic ModelResponse 2 -Shot weight

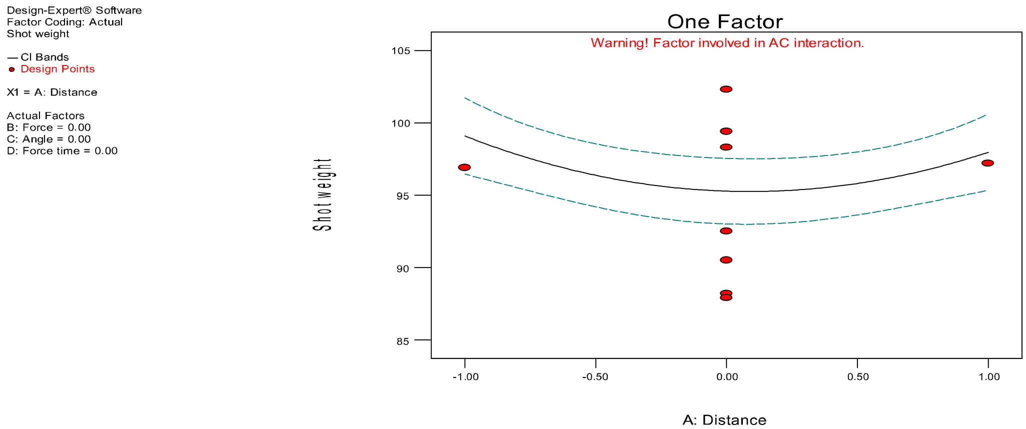
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1471.37	8	183.92	8.06	< 0.0001	Significant
A-Distance	17.04	1	17.04	0.75	0.3901	
B-Force	185.44	1	185.44	8.13	0.0056	Significant
C-Angle	66.82	1	66.82	2.93	0.0909	
AC	432.15	1	432.15	18.95	< 0.0001	Significant
BC	143.72	1	143.72	6.30	0.0141	Significant
A^2	210.36	1	210.36	9.22	0.0032	Significant
B^2	86.36	1	86.36	3.79	0.0553	
C^2	199.00	1	199.00	8.73	0.0041	Significant
Residual	1778.87	78	22.81			
Lack of Fit	1577.15	72	21.90	0.65	0.8208	Non significant
Pure Error	201.72	6	33.62			
Cor Total	3250.24	86				

Table 11.18 shows the ANOVA analysis of chosen reduced quadratic model. The Model F-value of 8.06 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case main effect of Force, two way interactions (Distance+ Angle, Force+Angle) and quadratic effeccts (Distance^2 and Angle^2) are significant model terms indicating their significant effect on shot weight. Distance and angle have quadratic effect on shot weight. The "Lack of Fit F-value" of 0.65 implies the Lack of Fit is not significant relative to the pure error. Non-significant lack of fit shows that selected model is appropriate.

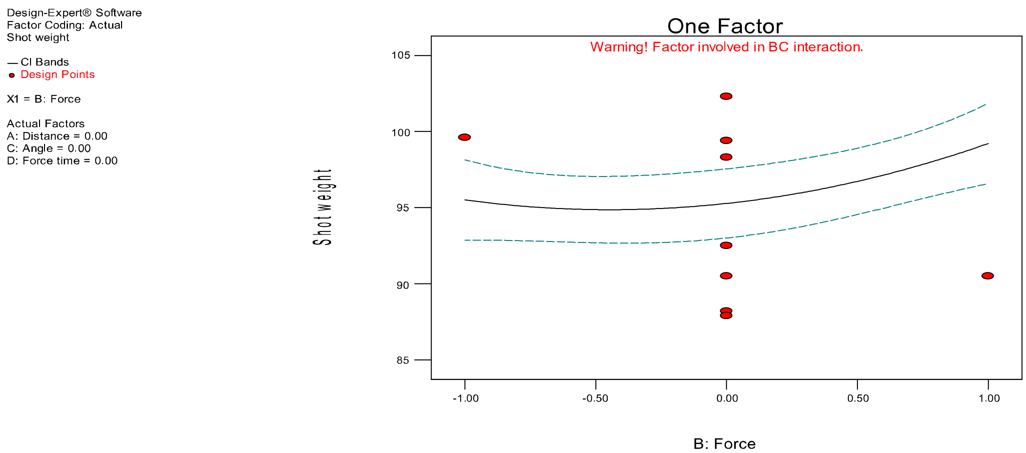
Table 11.19 Summary of ANOVA Results of Reduced Quadratic Model Response 2-Shot weight

Parameter	Value
Std. Dev.	4.78
Mean	100.55
C.V. %	4.75
PRESS	2200.48
R-Squared	0.4527
Adj R-Squared	0.3966
Pred R-Squared	0.3230
Adeq Precision	9.199

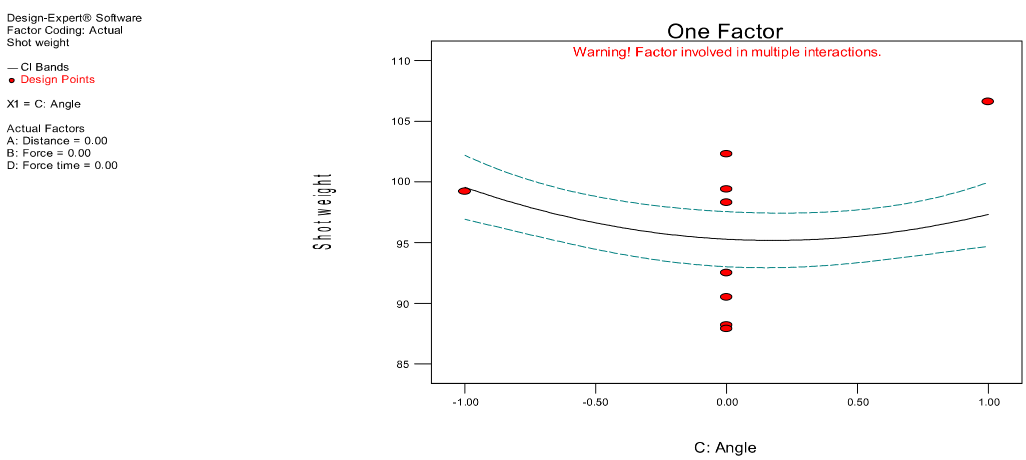
Table 11.19 shows summary of ANOVA results for reduced quadratic model. As it can be seen, the "Pred R-Squared" of 0.3230 is in reasonable agreement with the "Adj R-Squared" of 0.3966implying adequecy of selected model in predicting responses. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Obtained ratio of 9.199for quadratic model model indicates an adequate signal. Hence, selected quadratic model can be used to navigate the design space.



9A.

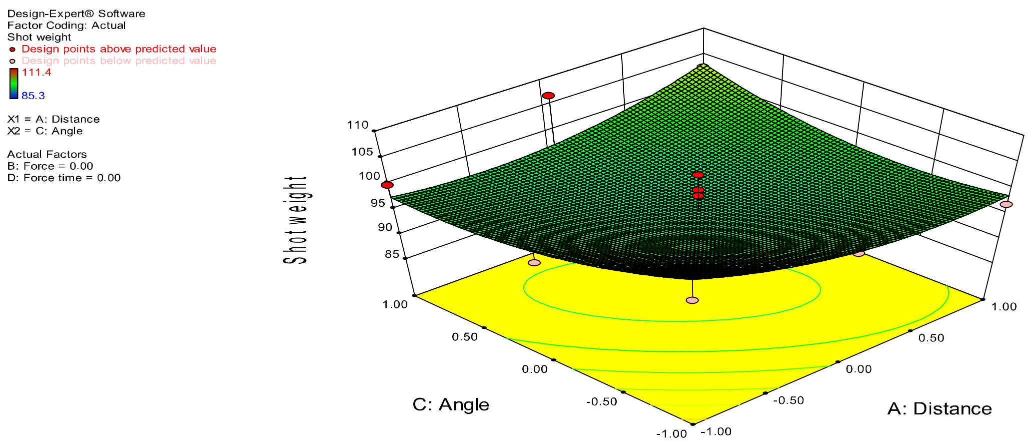


9B.

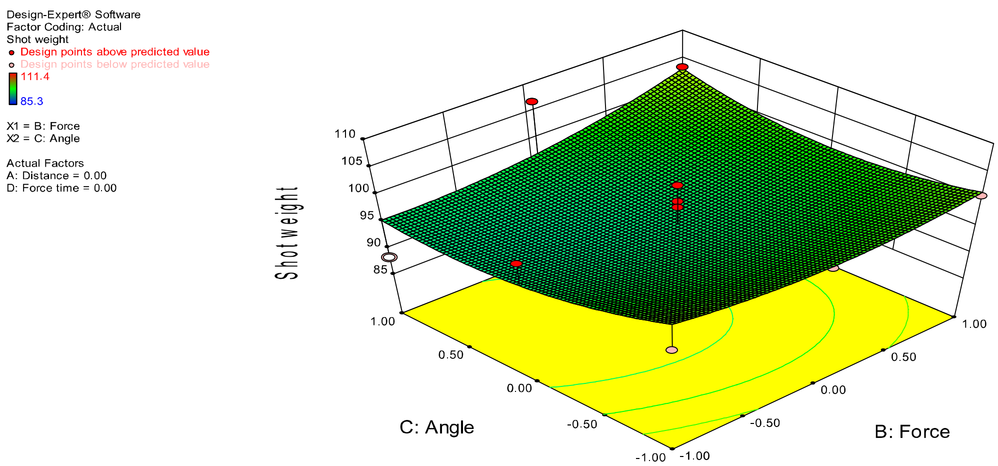


9C.

Figure 11.11 One factor response plots A. Distance VsShot weight B. Angle Vs Shot weight C. Force Vs Shot weight



10A.



10B.

Figure 11.12 Two factor response surface plots A. Angle x distance Vs Shot weight B. Angle x Force Vs Shot weight

Figure 11.11 show one factor effect plots while **Figure 11.12** show two factor response surface effect plots. Only plots showing effects of significant factors have been shown. It can be seen that increase in distance, force and angle show quadratic effects with significant being that of distance and angle. Increase in distance and angle, initially reduces shot weight up to around zero level and then increases shot weight. When the force applied at the bottom of bottle increases, shot weight increases because more is the force, more will be gap covered between nozzle and bottle. The force affects the shot weight mainly by determining the weight of formulation to be pulled into the dip tube and sprayed.

A force higher than the actuation limit of pump may affect the functionality of nasal spray valve. Hence, proper force selection should be done taking care of valve. In case of angle vs shot weight, the shot weight is more at an angle where the dip tube in the nasal spray bottle is completely dipped. In case of conventional bottles which were used in our studies were flat based, but the newer modified bottles have conical base which will help in increasing number of dosing and uniformity of dosing (**Figure 11.13**).

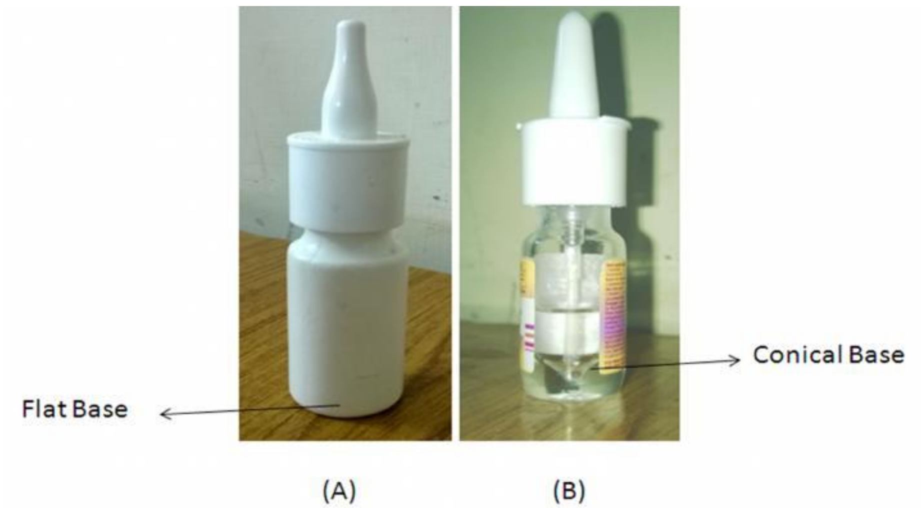


Figure 11.13 A) Conventional Nasal Spray B) Modified Nasal Spray

Predicted response at any factor level can be calculated using following equation:

Shot weight=+95.28301
-0.56167 * Distance
+1.85315 * Force
-1.11241 * Angle
+3.46472 * Distance * Angle
+1.99806 * Force * Angle
+3.24752 * $Distance^2$
+2.08085 * $Force^2$
+3.15863 * $Angle^2$

11.3.3.2 Determination of Shot weight

To measure the shot weight for a nasal spray, weight of device should be taken prior and after each actuation in order to evaluate the release mass. As per FDA acceptance criteria include the weight of the individual sprays to within 85% to 115% of the target weight and mean weight to be within 10% of the target weight (18). Guo*et al.* have studied the impact of surfactant such as Na-CMC and Polysorbate 80 and their concentration on shot weight and found out that the concentration of polymer and surfactant have a very slight effect (3).

Procedure: 5 sprays were tested separately. Five times actuation was carried out in waste before start of each experiment to prime the device, followed by one test actuation. Each vial was weighed before and after actuation separately and individual volume was calculated in µl by formula

$$\text{Shot weight}=(W_1 - W_2)/D$$

where
W_1 – initial weight
W_2- weight after actuation
D – Density of liquid formulation
Density of nasal spray formulation: 1.039 gm/ml

Table 11.20 Effect of priming on shot weight

	Shot weight from different spray pumps (mg)				
Spray number after priming	Pump 1	Pump 2	Pump 3	Pump 4	Pump 5
1	101.79	99.85	100.62	96.61	104.32
2	100.60	97.87	101.46	97.50	92.19
3	99.92	102.92	100.64	98.36	100.31
4	98.62	97.44	99.32	100.25	98.21
5	103.38	98.54	99.66	101.76	100.44
Average	100.9	99.3	100.3	98.9	99.1

11.3.3.3 Priming and Repriming of nasal spray

Weight of spray pump was recorded after each actuation and shot weight was determined. **Table 11.21** shows shot weights after each actuation of a representative spray. Shot weight limit was set between 90 mg to 110 mg i.e. 90% to 110% of the specified quantity of spray formulation. As it can be seen minimum 4 sprays are required for priming and around 80 actuations can be performed while being within the limits specified.

Table 11.21 Number of sprays and their shot weight

Spray Number	Weight in mg	Spray Number	Weight in mg
4 sprays for priming		44	96.17
1	**80.00**	45	95.67
2	**81.00**	46	94.89
3	**82.00**	47	98.60
4	**85.00**	48	105.10
5	100.38	49	99.23
6	96.25	50	102.60
7	94.80	51	107.20
8	95.09	52	100.77
9	94.99	53	104.43
10	103.75	54	97.02
11	101.25	55	103.10
12	99.04	56	102.40
13	99.23	57	101.23
14	100.19	58	99.62
15	98.26	59	103.46
16	96.25	60	101.64
17	94.32	61	94.32
18	96.19	62	103.66
19	102.31	63	102.98
20	102.21	64	102.10
21	101.44	65	91.53
22	100.58	66	108.84
23	105.39	67	100.00
24	102.69	68	100.10
25	95.99	69	103.46
26	102.21	70	100.87
27	103.85	71	96.92
28	94.61	72	93.36
29	97.50	73	93.55
30	96.36	74	96.82
31	96.25	75	98.55
32	97.76	76	98.85
33	95.86	77	100.87
34	96.63	78	101.92
35	95.38	79	96.44
36	94.99	**Total Number of sprays allowed 79**	
37	96.43	80	84.54
38	99.81	81	83.59
39	95.48	82	81.06

40	98.46	83	82.79
41	96.83	84	82.60
42	104.61	85	81.92
43	97.03	86	84.62
		87	80.38

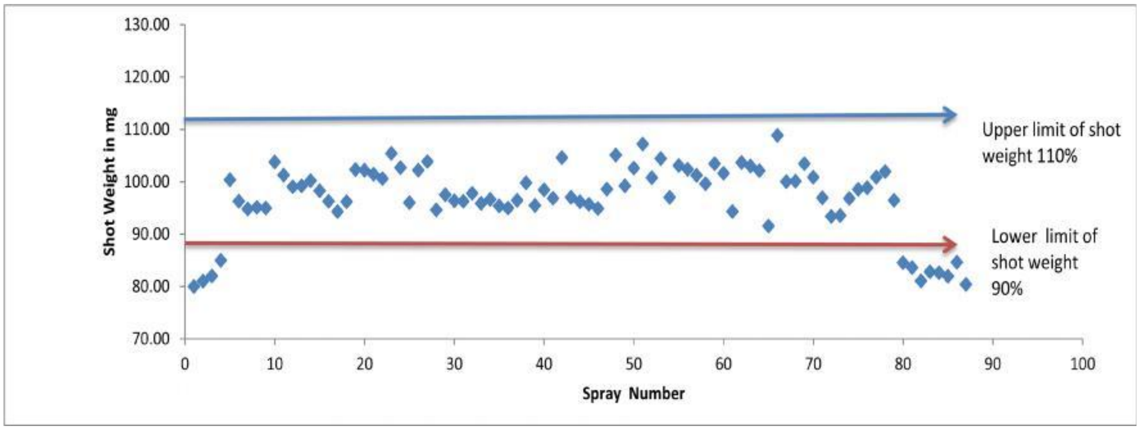


Figure 11.14 Number of sprays Vs shot weight

Limits for passing the test:

The volume of each spray delivered should be between 80 and 120 µl and the average volume is between 90 to 110 µl.

Conclusion: From the above results Figure 11.14, it can be concluded that the delivered volume by the selected spray falls within the specified limit.

11.3.3.4 Effect of Resting Time on Priming, Repriming

Another study to find the effect of resting time on the first spray of unprimed units, followed immediately by the second and the third sprays is recommended.

Procedure: Three units of nasal spray were used for the study.

1. The spray units were primed just before the study initiation.
2. The spray units were kept under resting condition for increasing periods of time (6, 12, 24, 48, 60 hours).

3. After the specified time intervals as mentioned in step 2, uniformity of the shot weight in five actuation of each pump was checked without priming.

Table 11.22 Effect of Resting Time on Priming, Repriming

Spray Rest Time in Hr	Actuation 1	Actuation 2	Actuation 3	Actuation 4	Actuation 5	Mean(n=5)	SD
6	103	99	96	101	97	99	2.4
12	98	97	101	99	104	100	3.9
24	100	101	105	97	98	100	3.2
36	83	89	97	101	99	94	3.0
48	81	85	84	89	85	85	2.8
60	78	80	93	88	82	84	2.9
72	80	85	84	87	88	85	3.4

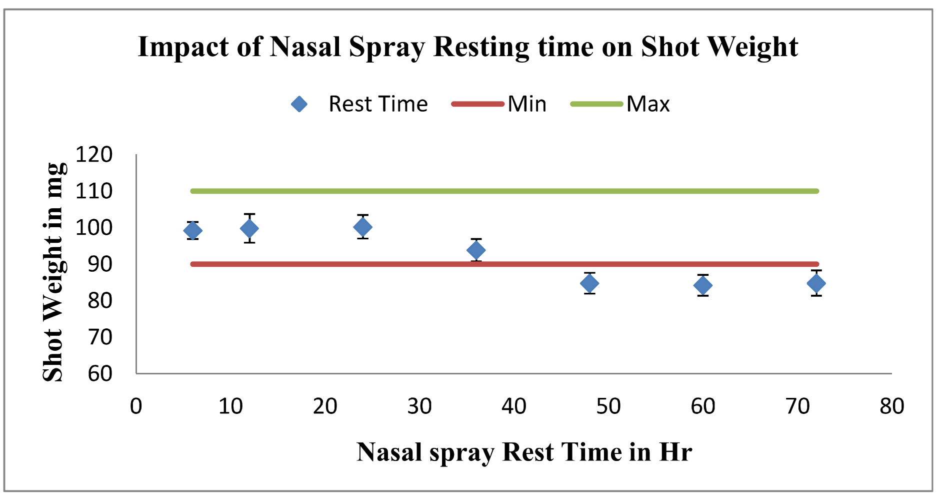


Figure 11.15 Impact of resting time on shot weight

From **Table 11.22**, it was observed that after initial priming, if the spray is used up to 24 hrs there is no need of repriming. But, if the spray bottle is kept unused for 36, 48, 60 or 72 hrs re-priming up to 2 sprays is required in order to achieve the required spray weight. Hence, the spray bottle should be labeled as to be primed if bottle is left unused for 24 hrs or more.

11.3.3.5. Impact of Temperature Cycling on DSD and shot weight

As the product was found to be stable at 2-8 ^{o}C, the effect of temperature variation on the quality and performance of the drug product was studied. The study included 12 hr cycle.

Procedure:

- The sample was kept at two different temperature conditions 2-8^{o}C and room temperature in a repeated 12 hr cycles for 3 days.
- The samples were analyzed for droplet size distribution, color, spray weight, pump clogging, and adherence of the drug to the walls of the container and functionality of pump components.

Table 11.23 Effect of temperature cycle on DSD

Cycle	Time (Hrs)	Temperature	Droplet Size Distribution (D 90 in µm)	Color	Spray Weight (mg)
1st	12	2-8 ^{o}C	99.11	Ok	106 ± 4.70
	12	RT	89.72	Ok	102 ± 3.05
2nd	12	2-8 ^{o}C	101.44	Ok	100 ± 1.64
	12	RT	94.60	Ok	98 ± 2.80
3rd	12	2-8 ^{o}C	102.38	Ok	101 ± 1.37
	12	RT	98.19	Ok	97 ± 3.26

We have also compared the effect of stroke length on shot weight **Figure 11.16**. The results for stroke length on shot weight are in accord with the results obtained by Guo and Doub (3), *i.e.*, the shot weight increases with increasing stroke length and required shot weight of 100 mg is achieved at the optimal stroke length of 4–5 mm for used nasal spray pump. RSD for stroke length settings of 1, 2, 3 and 6 mm was on higher depicting the variability in results and for stroke length of 4 and 5 mm was less than 5% depicting the reproducibility of results. No shot weight is outside the limit of ±15% of the average value at 4 -5 mm stroke length as shown in **Figure 11.16**. At stroke length of 6 mm, low shot weigh and variable results were obtained. It may be due to over actuation spray pump, which affected the normal working of the spray pump valve.

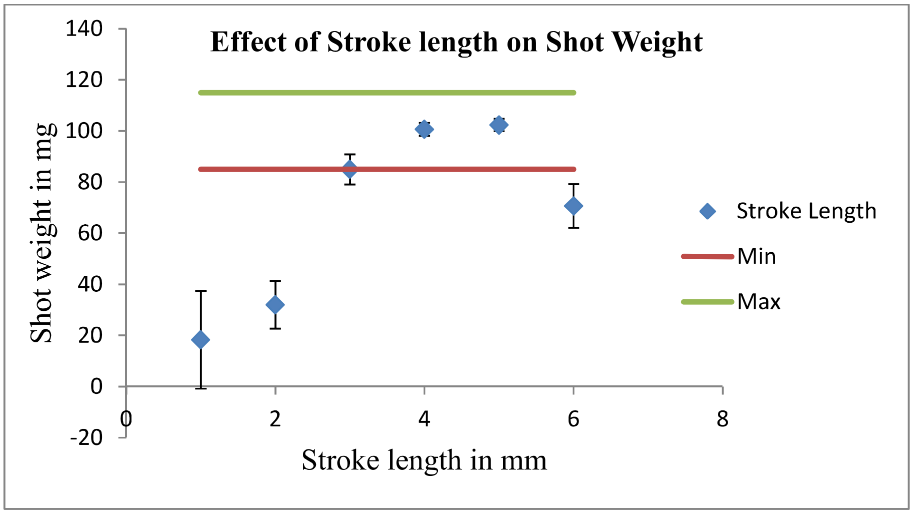


Figure 11.16 Plot showing effect of stroke length on shot weight

11.3.4. Statistical Analysis of Response 3 (Span)

p-values of different models, p-value for lack of fit in the models, Adjusted R^2 values and Predicted R^2 values of different models are shown in the **Table 11.24**.

Table 11.24 ANOVA analysis of Different Models Response 3 (Span)

Source	**Sequential p-value**	**Adjusted R-Squared**	**Predicted R-Squared**	
Linear	< 0.0001	0.9474	0.9425	
2FI	< 0.0001	0.9782	0.9752	
Quadratic	< 0.0001	0.9877	0.9847	Suggested
Cubic	< 0.0001	0.9932	0.9888	Aliased

Based on the p values and agreement between adjusted and predicted R^2 values, highest polynomial was chosen for response evaluation and prediction. Here quadratic model was found to best fit the experimental results. Cubic and higher models were found to be aliased (predicted results for one factor would be confounded by the other factors) and hence were left out. In order to improve the quadratic model, stepwise regression with alpha in value of 0.100 and alpha out value of 0.100 was performed. Prob> |t| is the probability of getting a ‘t’ value this large if the term does not actually have an effect. In order to keep the term in the model, this value must be less than or equal to the alpha in value and not larger than the alpha out value. Based on the results, reduced quadratic model was chosen.

Table 11.25 Factors Selected on Basis of Stepwise Regression Analysis Response 3 (Span)

Forced Terms Added	Intercept Coefficient Estimate	t for H0Coeff=0	Prob > |t|	R-Squared	MSE
C-Angle	0.70	17.37	<0.0001	0.7801	0.088
B-Force	-0.32	-16.17	<0.0001	0.9465	0.022
BC	-0.17	-10.59	<0.0001	0.9773	9.273E-003
B2	0.10	5.51	<0.0001	0.9834	6.853E-003
A-Distance	-0.046	-4.54	<0.0001	0.9868	5.528E-003
A2	-0.066	-4.45	<0.0001	0.9894	4.485E-003

Table 11.26 ANOVA Table for Response Surface Reduced Quadratic Model Response 3(Span)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Remark
Model	33.48	6	5.58	1244.11	< 0.0001	Significant
A-Distance	0.11	1	0.11	25.46	< 0.0001	Significant
B-Force	5.63	1	5.63	1255.48	< 0.0001	Significant
C-Angle	26.40	1	26.40	5885.61	< 0.0001	Significant
BC	1.04	1	1.04	231.95	< 0.0001	Significant
A2	0.089	1	0.089	19.83	< 0.0001	Significant
B2	0.24	1	0.24	53.22	< 0.0001	Significant
Residual	0.36	80	4.485E-003			
Lack of Fit	0.36	74	4.849E-003			
Pure Error	0.000	6	0.000			
Cor Total	33.84	86				

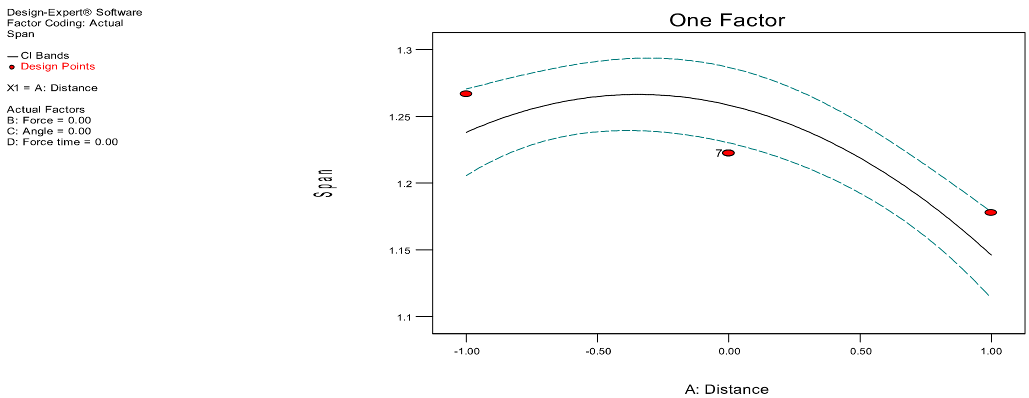
Table 11.26 shows the ANOVA analysis of chosen reduced quadratic model. The Model F-value of 1244.11 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, BC, A^2 and B^2 are significant model terms. Distance, Force and angle has significant impact on Span, while two factor interaction BC also have significant effect on span. Distance and Force have quadratic effect

on Span. Reduced model was fitting the data such that it eliminated the pure error and hence making the model significant and Lack of Fit non significant. Non-significant lack of fit shows that selected model is appropriate.

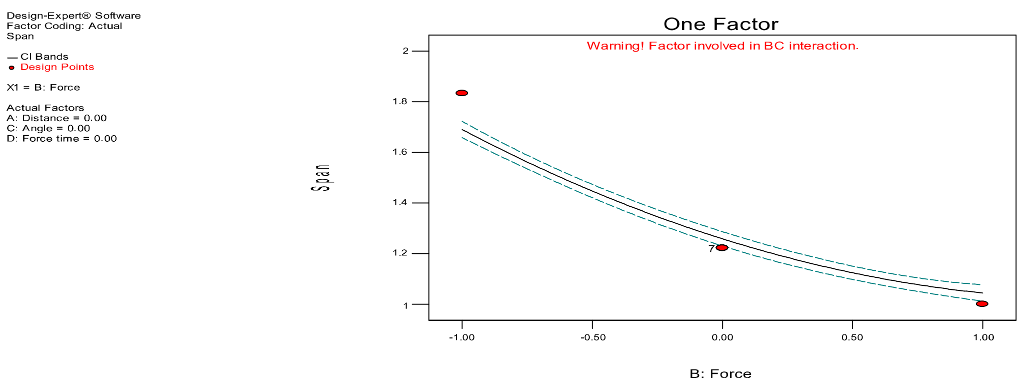
Table 11.27 shows summary of ANOVA results for reduced quadratic model. As it can be seen, the "Pred R-Squared" of 0.9872 is in reasonable agreement with the "Adj R-Squared" of 0.9886implying adequecy of selected model in predicting responses. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Obtained ratio of 113.523 for reduced quadratic model model indicates an adequate signal. Hence, selected reduced quadratic model can be used to navigate the design space.

Table 11.27 Summary of ANOVA Results of Reduced Quadratic Model response 3 – Span

Parameter	**Value**
Std. Dev.	0.067
Mean	1.28
C.V. %	5.21
PRESS	0.43
R-Squared	0.9894
Adj R-Squared	0.9886
Pred R-Squared	0.9872
Adeq Precision	113.523



17A.



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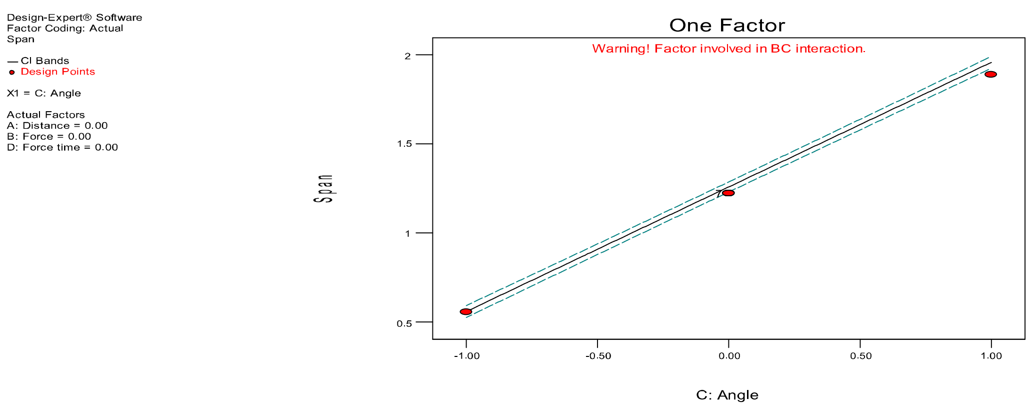


17C.

Figure 11.17 One factor response plots A. Distance Vs Span B. Force Vs Span C. Angle Vs Span

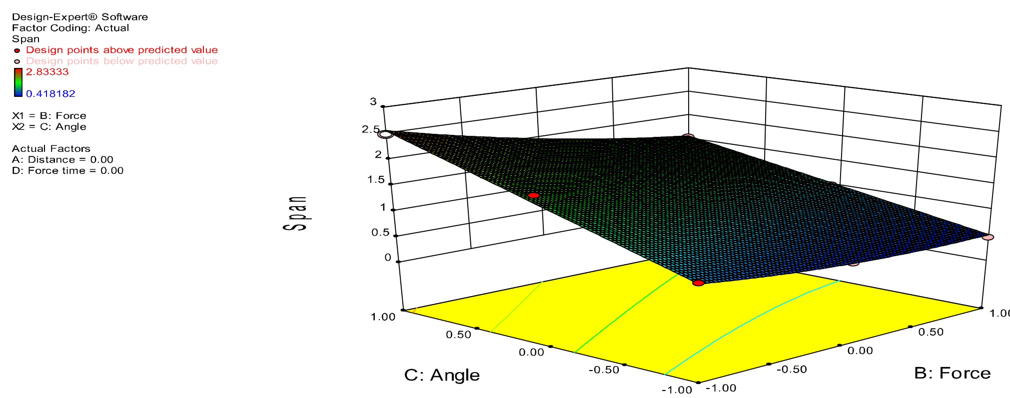


Figure 11.18 Response surface plot showing effect of Angle and Force on Span

Figure 11.17 shows one factor effect plots while **Figure 11.18** shows two factor response surface plot showing effect of force and angle. A positive sign indicates a synergistic effect, while a negative sign represents an antagonistic effect. Only plots showing effects of significant factors have been shown. Span value represent the width of the DSD from the median droplet range (D50) and, hence, a smaller the span value narrow is the DSD. Till date, there is no literature support on how span value affects nasal deposition and thereby nasal bioavailability. But, span values can be used to evaluate the quality of the spray. If the span value is large, it indicates that liquid dispersion has high surface tension, cohesiveness, and/or a relatively low amount of energy representing low dispersion efficiency of the nasal device. The span value is dependent upon on pump design and type of formulations (21). It can be seen that increase in distance and force showed quadratic effect while angle showed linear effect on span value. Increase in distance led to initial increase followed by prominent decrease in span. Increase in angle linearly and significantly caused rise in span. Highest span value has been predicted at lowest level of force 30 N i.e and highest level of angle 90. Span is a consolidated measure of broadness of the DSD. The span values are computed from the measured D10, D 50and D 90 values.

Predicted response at any factor level can be calculated using following equation:

Span=+1.25843

-0.045984*Distance

-0.32293*Force

+0.69919*Angle

-0.17000*Force*Angle

-0.066381*Distance^2

+0.10875 * Force^2

11.3.5 Statistical Analysis of Response 4 (Stable Phase)

p-values of different models, p-value for lack of fit in the models, Adjusted R^2 values and Predicted R^2 values of different models are shown in **Table 11.28**.

Table 11.28 ANOVA Analysis of Different Models on Response 4 (Stable Phase)

Source	Sequential p-value	Lack of Fit p-value	Adjusted R-Squared	Predicted R-Squared	
Linear	< 0.0001	0.3178	0.5946	0.5588	Suggested
2FI	0.0357	0.3729	0.6322	0.5576	Suggested
Quadratic	0.1043	0.4036	0.6505	0.5576	
Cubic	0.0183	0.5536	0.7216	0.5477	Aliased

Based on the p values and agreement between adjusted and predicted R^2 values, highest polynomial was chosen for response evaluation and prediction of response. Here 2FI model was found to best fit the experimental results. 2FI model was not chosen, due to inadequacy of sequential model p value (0.1043 vs 0.0357). Cubic and higher models were found to be aliased (predicted results for one factor would be confounded by the other factors) and hence were left out.

Table 11.29 Factors Selected on Basis of Stepwise Regression Analysis

Forced Terms Added	Intercept Coefficient Estimate	t for H_0Coeff=0	Prob > |t|	R-Squared	MSE
B-Force	-21.39	-10.56	<0.0001	0.5677	221.36
A-Distance	-5.61	-2.89	0.0049	0.6067	203.75
BD	5.06	2.17	0.0327	0.6279	195.12
AC	4.72	2.07	0.0418	0.6463	187.71

In order to improve the 2FI model prediction, stepwise regression with alpha in value of 0.100 and alpha out value of 0.100 was performed. Prob> |t| is the probability of getting a t value this large if the term does not actually have an effect. In order to keep the term in the model, this value must be less than or equal to the alpha in value and not larger than the alpha out value. Based on the results, reduced 2FI model was chosen. Apart from the terms selected based on stepwise regression analysis, term C (Angle) and D (Force time) were included in the final model to maintain the hierachy to satisfy the significant interaction term of BD and AC. **Table 11.29** shows the summary of ANOVA analysis of chosen reduced 2FI model. The Model F-value of 25.09 implies the model is significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, AC and BD are significant model terms. Distance and Force have significant impact on stable phase of actuation, while two factor interactions AC (Distance*Angle)and BD (Force*Force time)also have significant effect on stable phase of actuation. Non-significant lack of fit shows that selected model is appropriate.

Table 11.30 ANOVA table for Response Surface Reduced 2FI Model (Stable Phase)

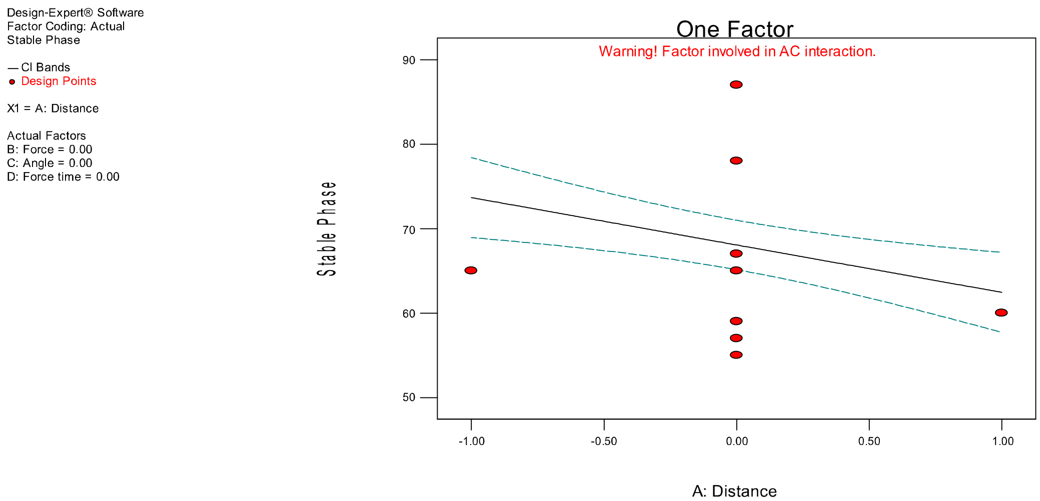
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	28419.24	6	4736.54	25.09	< 0.0001	Significant
A-Distance	1700.17	1	1700.17	9.01	0.0036	Significant
B-Force	24704.17	1	24704.17	130.88	< 0.0001	Significant
C-Angle	25.35	1	25.35	0.13	0.7150	
D-Force time	266.67	1	266.67	1.41	0.2381	
AC	802.78	1	802.78	4.25	0.0424	Significant
BD	920.11	1	920.11	4.87	0.0301	Significant
Residual	15100.35	80	188.75			
Lack of Fit	14267.49	74	192.80	1.39	0.3652	Non significant
Pure Error	832.86	6	138.81			
Cor Total	43519.59	86				

Table 11.31 Summary of ANOVA Results of Reduced 2FI Model (Stable Phase)

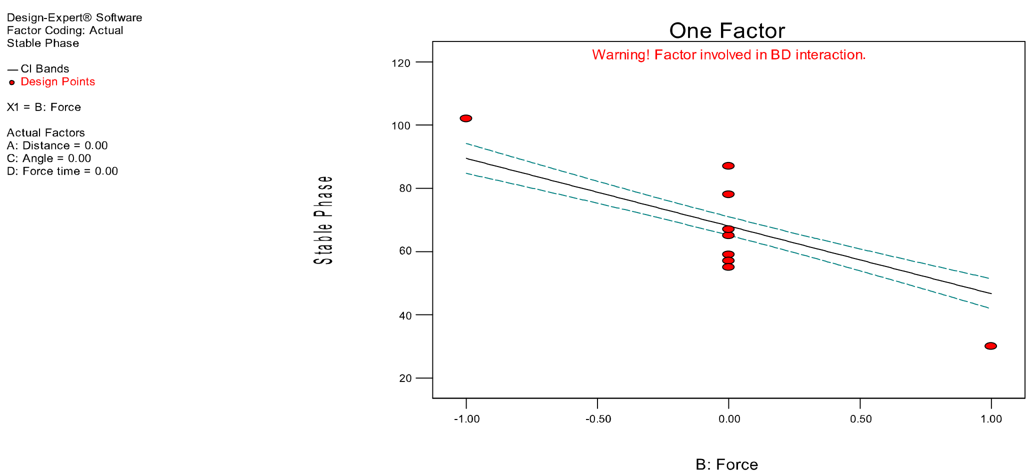
Parameter	**Value**
Std. Dev.	13.74
Mean	68.07
C.V. %	20.18
PRESS	18273.06
R-Squared	0.6530
Adj R-Squared	0.6270
Pred R-Squared	0.5801
Adeq Precision	18.875

Table 11.31 shows summary of ANOVA results for reduced 2FI model. As it can be seen, the "Pred R-Squared" of 0.5801 is in reasonable agreement with the "Adj R-Squared" of

0.6270 implying adequecy of selected model in predicting responses. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Obtained ratio of 18.875 for reduced 2FI model indicates an adequate signal. Hence, selected reduced 2FI model can be used to navigate the design space.



19A.



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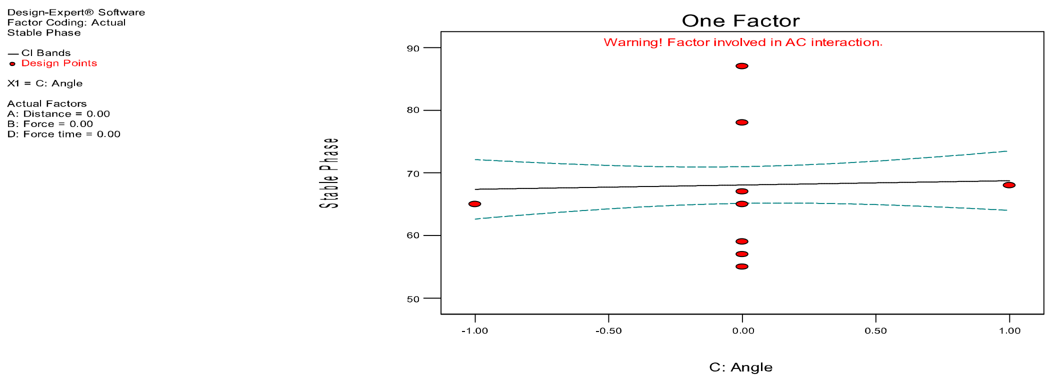

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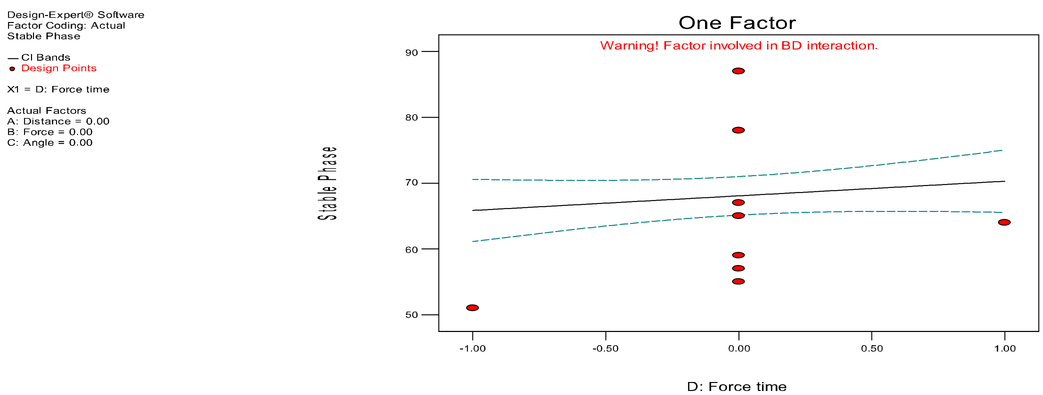
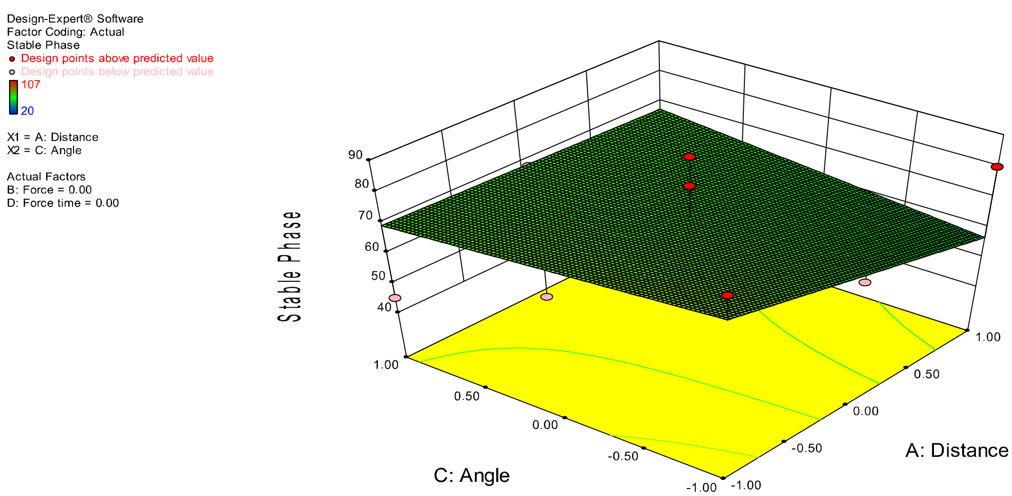


19D.

Figure 11.19 One factor response plots A. Distance Vs Stable Phase B. Force Vs Stable Phase C. Angle Vs Stable Phase D. Force Vs Stable Phase



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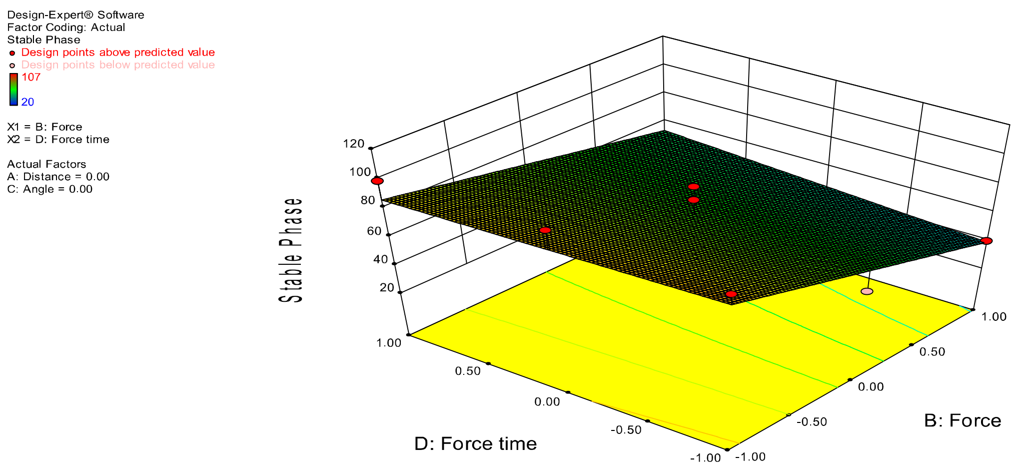

20B.

Figure 11.20 Two factor response surface plots A. Angle x distance Vs Stable phase B. Force time x Force Vs Stable Phase

Figure 11.19 shows one factor effect plots while **Figure 11.20** shows two factor response surface plots showing effect of force and angle. It can be seen that increase in distance and force showed linear effect on stable phase. Rise in these factors led to decline in stable phase. Angle and force time had no main effects on stable phase but were involved in significant two way interactions with distance and force respectively. Maximum stable phase was predicted from highest levels of angle (90^{o}) and distance (7 cm) as well as at highest levels of force (55 N) and force time (3 sec). The reduction in the duration of the stable phase as the force i.e velocity with which force is applied is increased, indicating a faster delivery of the dose.

Predicted response at any factor level can be calculated using following equation:

Stable Phase=+68.06897

-5.61111 * Distance

-21.38889 * Force

+0.68519 * Angle

+2.22222 * Force time

+4.72222 * Distance * Angle

+5.05556 * Force * Force time

11.3.5.1 Impact of force time on spray formation stages

Procedure: Phase of spray were determined form the Qt diagram depicted in **Figure 11.21**, which depict the all the three regions of nasal spray.

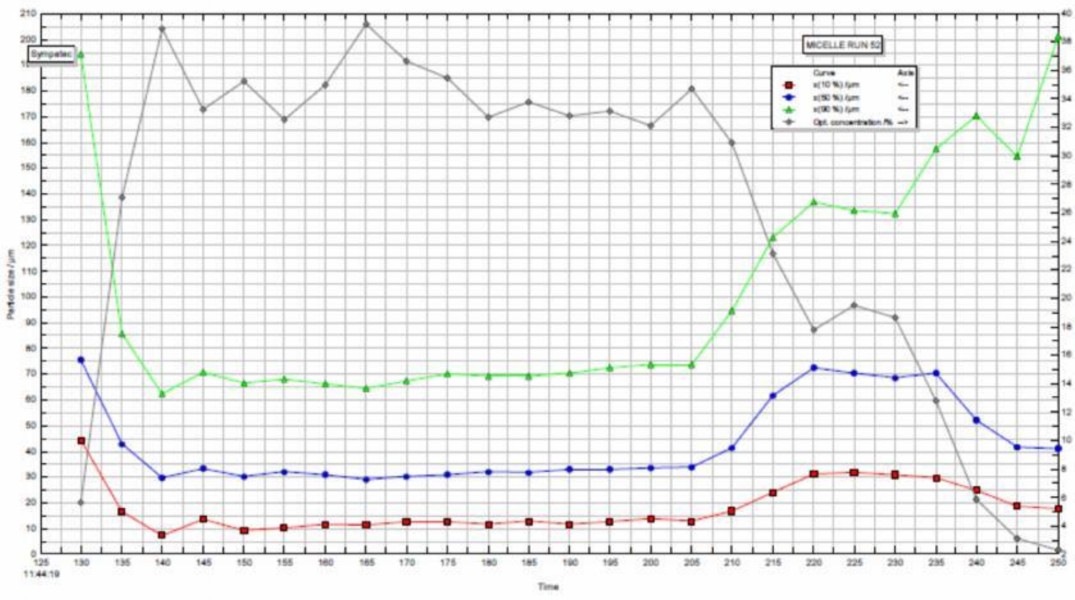

Figure 11.21 Qt diagram

Till date no one has studied the impact of force on stages of spray development. Impact of force on formation phase was found to be negligible as it remained constant throughout different force. A gradual decrease in stable phase and gradual increase in dissipation phase was observed (**Figure 11.22**). If force applied at the bottom of the nasal spray pump increased, time of dissipation phase increases leading to formation of large droplets. As force applied at the bottom of the nasal spray pump increases the stable phase time decreases i.e. total percentage or concentration of globule size decreases up to 50N force.

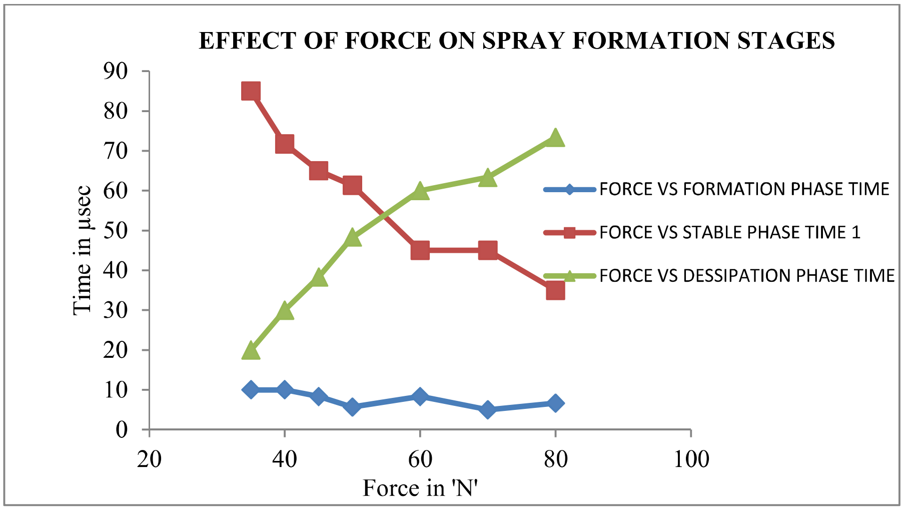

Figure 11.22 Plot showing effect of force on stages of spray development

11.4 Selection of Optimum Processing Parameters

Selection of optimum processing parameters in terms of distance from laser beam, force to be applied at the bottom of bottle, angle of spray pump and force time, each dependent factor as well as independent factor (D90, shot weight, span and stable phase) were applied with constraints. Constrains were chosen based on the requirement i.e., D90 was chosen to be between 10 to 200 microns, as at globule size of <10 micron, globules will reach the lungs along with the inhaled air and hence would not be of use in nose to brain delivery of drug while above 200 micron size, they will be trapped by nasal mucosa and due to larger size they will be cleared by mucocilliary clearance. Similarly shot weight of 100 mg specifies 100% assay of drug dose and hence shot weight was constrained within the limits of 90 to 100 mg based on the density of formulation. Span indicates uniformity of globule size in each spray and span value of 1 equals to uniform size distribution of the globules in the spray. As per regulatory guidelines span should range between 1 to 1.3 to ensure aforesaid uniformity of globule size in each spray.

Spray is divided in three phases namely first formation phase, middle stable phase and terminal dissipation phase. Ideally, larger the stable phase, higher will be the uniformity of globules in spray, i.e. stable phase gives indication of time period for which there was uniform distribution of globules within each spray. So stable phase constraints were selected to be between 90 sec to highest obtained in the design (107 sec).

Table 11.32 Constraints Applied for Selection of Optimized Batch

Name	Goal	Lower Limit	Upper Limit
A:Distance	is in range	-1	1
B:Force	is in range	-1	1
C:Angle	is in range	-1	1
D:Force time	is in range	-1	1
D90 (μ)	is in range	10	200
Shot weight (mg)	is in range	90	110
Span	is in range	1	1.3
Stable Phase (sec)	Maximize	90	107

Optimizatoin of process parameters was based on the desirability index which may range from 0 to 1 indicating worst fit of the dependent responses in specified goals (**Table 11.32**) to best best fit of the dependent responses in the specified goals.

11.5 Desirability Plot for Selection of Optimum Process Parameters

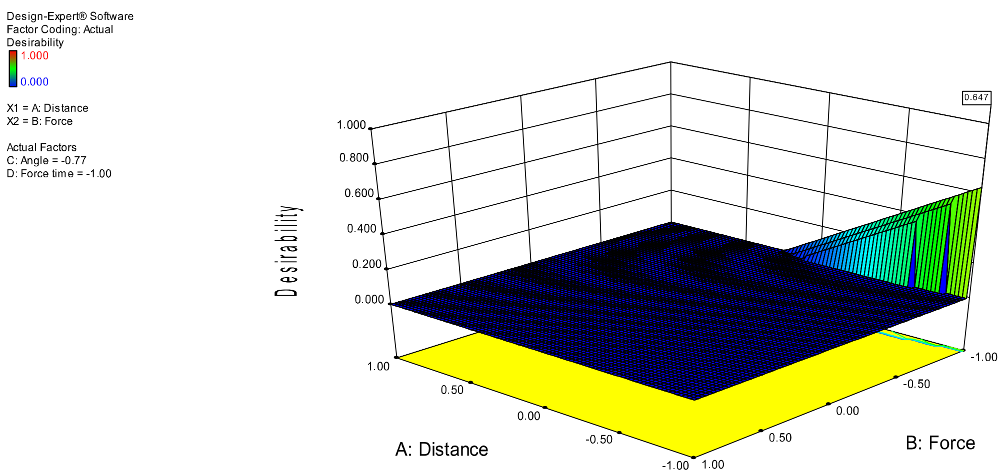

Figure 11.23 Desirability plot for selecting optimum parameters

Table 11.33 Selected Process Parameters based on Desirability

	Distance (cm)	**Force (N)**	**Angle (degree)**	**Force time (sec)**	**D90 (μ)**	**Shot weight (mg)**	**Span (μs)**	**Stable Phase (μs)**	**Desirability**
Coded level	-1.00	-1.00	-0.77	-1.00	76.36	106.26	1.00	101.01	0.647
Actual level	**3**	**35**	**26.95**	**1**					

Selected process parameters of distanec (3 cm), force (35 N), angle (26.95) and force time (1 sec). **Table 11.33** with desirability of 0.647 were further evaluated for expermimental confirmation.

11.6 Point Prediction and Confirmation

Table 11.34 shows predicted response for the selected process parameters along with the Standard deviation and 95 % confidence interval of the responses. Confirmation of the response was done by carrying out the experiment using the selected factor values in triplicate. Results shows and confirms that experimental and predicted values are in good agreement concluding the suitability of the selected models for optimization.

Table 11.34 Confirmation of Optimum Processing Parameters

Response	Prediction	Std. Dev.	95% Confidence Interval	Experimental Mean	Std. Dev.
D90 (μ)	76.3564	34.3572	54.3332-98.3796	75.35	21.35
Shot weight (mg)	106.261	4.77557	103.171-109.351	105.3	3.72
Span (Unit)	1.00005	0.0669725	0.960389-1.03971	1.02	0.01
Stable Phase (sec)	101.007	13.7388	91.4524-110.561	100.2	6.36

11.7 Conclusion

In this study, characterization of developed nasal drug products was carried taking into consideration factors such as DSD, spray pattern, plume geometry and the findings were in accordance with the previous results published. The results show that nasal spray characteristics are greatly affected by various factors such as formulation character, design of device, as well as the actuation parameters. The findings show that experimental set-ups of machine and formulation character in combination vary the results of DSD, plume geometry and spray pattern, and hence, set of parameters for spray for device development should be done parallel to formulation development. The findings suggest that along with drug product, experimental test method and the methodology, along with device actuation parameters can vary output. Spray pattern was analyzed means of automated machine and plume geometry was analyzed manually. Plume geometry as carried out manually it is subjected to operator bias. Therefore, automated analysis will help in getting reproducible results. During determination of the spray pattern and DSD, the distance between the nasal spray nozzle and the laser beam and the measuring zone of the laser diffractometer must be taken into consideration as it has an impact on the output and must be considered during nasal spray method development. Another factors affecting DSD and plume geometry are actuation parameters. Thus, regulatory bodies such as FDA and EMEA suggest use of an automated actuator. As observed stroke length, actuation forces the maximum influence on shot weights, DSD, plume geometry and spray pattern. As a result, these parameters should be chosen such that they will mimic hand actuation of target population group. In conclusion one can say that during development of method for characterization of nasal spray products in order to have consistent measurements to assure the quality of nasal drug products, critical parameters must be identified and evaluated.

11.8 References

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