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Assessment of the influence factors on in vitro testing of nasal sprays using Box-Behnken experimental design☆

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ABSTRACT

The purpose of the research was to investigate the influences of actuation parameters and formulation physical properties on nasal spray delivery performance using design of experiment (DOE) methodology. A 3-level, 4-factor Box-Behnken design with a total of 27 experimental runs was used in this study. Nine simulated aqueous formulations with different viscosities and surface tensions were prepared using carboxymethylcellulose sodium (CMC, gelling agent) and Tween80 (surfactant) each at three concentration levels. Four factors, actuation stroke length, actuation velocity, concentration of gelling agent, and concentration of surfactant were investigated for their influences on measured responses of shot weight, spray pattern, plume geometry and droplet size distribution (DSD). The models based on data from the DOE were then optimized by eliminating insignificant terms. Pfeiffer nasal spray pump units filled with the simulated formulations were used in the study. Nasal pump actuation stroke length exerts a strong, independent influence on shot weight, and also slightly affects spray pattern and plume geometry. Actuation velocity and concentration of gelling agent have significant effects on spray pattern, plume geometry and DSD, in a complicated manner through interaction terms. Concentration of surfactant has little, if any, influence on nasal spray characteristics. Results were fitted to quadratic models describing the inherent relationships between the four factors evaluated and nasal spray performance. The DOE study helped us to identify the source of variability in nasal spray product performance, and obtained better understanding in how to control the variability. Moreover, the quadratic models developed from the DOE study quantitatively describe the inherent relationships between the factors and nasal spray performance characteristics. With the assistance of the response surfaces developed from the DOE model, the time and labor in designing a nasal spray product to achieve desired product performance characteristics can be reduced.

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1. Introduction

A nasal spray product combines a therapeutic formulation and a delivery device, where formulation characteristics and device capabilities must be coordinated to accomplish consistent delivery into the nasal cavity. The drug delivery performance of aerosols introduced via the nasal cavity depends on many factors, such as the design of the pump, the shape of the orifice, physical properties of the formulation, and patient handling (Harris et al., 1988; Cheng et al., 2001; Suman et al., 2002; Dayal et al., 2004). Formulation and pump designs appropriate to achieve the desired nasal drug release characteristics are the key to development of a good nasal product.

Traditionally, during product development and testing, actuation of nasal spray devices is performed manually. However, operator dependent force profiles lead to poor reproducibility. Since actuation parameters are critical to the aerosolization process, this variability can lead to problems when a submission is made to the FDA, because broad product specifications must be adopted to allow for patient bias. The FDA draft guidance (U.S. Food and Drug Administration, 2003) recommends the use of an automated actuation system, which delivers reproducible actuation performance for in vitro testing. The guidance also recommends that actuation parameters for spray drug products should be relevant to proper usage of the product by the target patient population. Changes in actuation parameters, although within the proper working range of the device, may still lead to changes in test results.

A nasal spray formulation is typically a mixture of active pharmaceutical ingredients (API), polymers, surfactants, and excipients. Polymeric gel vehicles are normally used to improve nasal bioavailability by increasing nasal residence times and controlling the rate of drug absorption. Surfactants are mainly employed to stabilize or solubilize active formulation components. During nasal product formulation design, it is important to consider molecular interactions between these ingredients that affect the rheological and physicochemical properties of the solutions, which, in turn, affect the ability of the formulation to be aerosolized into appropriately sized droplets.

The combined variability from device and formulation makes the development of nasal spray products more complicated than traditional pharmaceutical products. The influences of actuation parameters and formulation physical properties on in vitro test results for nasal products have been investigated previously by Guo and Doub (2006) and Dayal et al. (2004, 2005), but in each study, targeted parameters were varied independently while keeping all other parameters constant.

Guo et al. used water to simulate nasal spray formulations, and actuation parameters were varied using an electronic automated actuation station (Guo and Doub, 2006). In that paper, the authors demonstrated that different actuation parameters affect the nasal spray characteristics in different ways and to different degrees. Among all the actuation parameters, stroke length and actuation velocity were shown to have significant effects on the nasal spray characteristics, while the other actuation parameters have little, if any, effect. Compared to spray pattern, plume geometry and DSD, shot weight provides very little characterization information.

Using various placebo solutions to simulate physical property changes in nasal spray formulations and controlling actuations via a pneumatic automated actuation station, Dayal et al. observed significant influence from actuation force and formulation viscosity (Dayal et al., 2004). Their spray pattern analysis revealed a power law relationship between viscosity and spray pattern area for CMC formulations. However, this relationship could not be obtained for carbopol formulations, which was attributed to differences in the rheological behavior of the two formulations. The addition of surfactant (0.5–5% Tween80) to a 2% CMC solution decreased the Dv50 values (16–26%) and altered the rheological properties. They concluded that the characteristic of nasal aerosol generation is dependent on a combination of actuation force, viscosity, surface tension and other rheological properties as well as pump design.

In another study, Dayal et al. used design of experiments (DOE) methodology to study the effects of formulation components (gelling agents and electrolytes) on formulation rheology, in vitro drug release, and droplet size distribution (DSD) generated from a high viscosity nasal pump using a 5-factor, 3-level Box-Behnken experimental design (Dayal et al., 2005). Gel formulations of hydroxyurea (HU) with surface-active polymers (hydroxyethylcellulose [HEC] and polyethylene-oxide [PEO]) and ionic excipients (sodium chloride and calcium chloride) were prepared using a Box-Behnken experimental design. The applications of Box-Behnken experimental design facilitated the prediction and identified major excipient influences on viscosity, DSD, and in vitro drug release.

While previous studies have identified the major factors that affect the physical properties of nasal sprays, their experimental designs were based on changing one variable at a time, and did not consider interactions between actuation parameters and formulation characteristics. DOE methodology can be used to improve understanding of the influence of actuation parameters, formulation characteristics and their interactions on nasal spray delivery performance. A properly designed set of experiments, in which all relevant factors are varied systematically can identify the factors having the greatest influence on the results, the existence of interactions between those factors, and the optimized factor values that yield the desired response.

The Box-Behnken design is one of the most efficient DOE methods (Ferreira et al., 2007). An advantage of the Box-Behnken design is that it does not contain combinations for which all factors are simultaneously at their highest or lowest levels. So these designs are useful in avoiding experiments performed under extreme conditions, for which unsatisfactory results are often obtained.

This paper describes experiments designed to elucidate interactions between four factors (actuation stroke length, actuation velocity, concentration of gelling agent, and concentration of surfactant) with respect to their influences on nasal spray shot weight, DSD, and spray pattern and plume geometry (angle and width) properties. Box-Benkhen methodology

was used to design a set of 27 experimental conditions that are capable of elucidating the influence of these factors on the nasal spray responses including second-order and interaction effects. The measured responses were fit to polynomial model functions, and an analysis of the polynomial coefficients and their standard errors was used to identify the factors and interaction terms that are statistically significant for each model.

2. Methods

2.1. Sample preparation

Pfeiffer (PFE) 0.10 mL nasal spray pumps (material number 62602, dip tube length 58 mm) and 20 mL bottles (material number 34473) were used in this project. Pump units are specified to deliver 100 μL of liquid per actuation (~100 mg for water). Each nasal spray unit was filled with 18 mL deionized water or simulated formulations prior to testing and the first six actuations were fired to waste as priming shots.

Extra-low-viscosity-grade carboxymethylcellulose (CMC) was kindly provided by Aqualon North America (Hercules Inc., Wilmington, Delaware). CMC is used as a gelling agent to increase solution viscosity.

Tween80 (Pharmacia, Piscataway, NJ) is used as surfactant to adjust the surface tension of solutions.

Formulation density was measured using 5 mL volumetric flasks, viscosity was measured using an Ostwald–Fenske viscometer (Fisher Scientific), and surface tension was measured using a Nima PS-4 surface pressure sensor (NIMA Technology Ltd., Coventry, England).

2.2. Design of experiments

JMP 5.1.1 software (SAS Institute, Cary, NC, USA) was used to generate the DOE matrix and analyze the response surface models. A 3-level, 4-factor Box-Behnken design was selected for this study, because it can evaluate quadratic interactions between pairs of factors while minimizing the number of required experiments. The influence and interactions of four factors were examined in this study: actuation stroke length, actuation velocity, concentration of CMC and concentration of Tween80. Ranges for these factors, based on previous studies (Dayal et al., 2004; Guo and Doub, 2006) are shown in Table 1. Nine formulations with different viscosities and surface tensions were prepared from aqueous mixtures of CMC and Tween80. A total of 27 experiments, with factor values as indicated in Table 1, were performed and the four responses (shot weight, spray pattern, plume geometry, and DSD) were measured for each experiment. The empirical relationships between the four input factors were evaluated from these results. The coded design patterns shown in Table 1 represent the scaled factor values (high (+), middle (0) and low (−)) used in each run, in the order of stroke length, velocity, concentration of CMC and Tween80, respectively.

Scaled factor values were used to develop the equations that predict responses from the input parameters. A scaled factor is mean-centered and scaled by range/2 and has values that range from −1 to 1:

$$F_{\text{scaled}} = \frac{2(F - F_{\text{center}})}{F_{\max} - F_{\min}} \quad (1)$$

In Eq. (1), F is the factor value, F_{scaled} is the scaled factor value, $F_{\max}$, $F_{\min}$ and F_{center} are the maximum, minimum and center point values used in the DOE. Factor scaling normalizes the influence of each factor on the prediction response. The scaled factors for the four factors used in this study are given by the following expressions:

$$S = \frac{2(\text{stroke length} - 4.4)}{5.3 - 3.5} \quad (2)$$

$$V = \frac{2(\text{velocity} - 50)}{70 - 30} \quad (3)$$

$$C = \frac{2(\text{CMC\_concentration} - 1\%)}{2\% - 0\%} \quad (4)$$

$$T = \frac{2(\text{Tween80\_concentration} - 2.5\%)}{5\% - 0\%} \quad (5)$$

S, V, C and T are the scaled factor values for stroke length, velocity, CMC concentration and Tween80 concentration, respectively.

2.2.1. Instrumentation

A SprayVIEWTM NSx automatic actuator (Proveris Scientific Corporation, Sudbury, MA) was used to actuate the nasal pump. The stroke length and actuation velocity varied according to the experiment design shown in Table 1, with the hold time and actuation acceleration values fixed at 200 ms and 2500 mm/s^2, respectively. Spray pattern and plume geometry responses were measured at 3 cm from the nozzle orifice using a SprayVIEW^{TM} NSP system (Proveris Scientific Corporation, Sudbury, MA) and analyzed by SprayVIEW^{TM} NSP software Version 4.4.2.

Shot weights were assessed by weighing the spray pumps prior to and after each actuation using a Mettler AE 240 analytical balance (Mettler-Toledo, Inc., Columbus, OH) having a maximum weighing capacity of 200 g with readability to 0.1 mg.

DSD was measured by laser light scattering using a Sympatec Helos system (Sympatec Inc., Clausthal-Zellerfeld, Germany) equipped with R4 range lens (size range from 0.5 to 350 μm). The nasal spray pump was positioned in such a manner that the laser beam intersected the center of the expanding spray cone 3 cm from the pump orifice. All actuations were fired upward, and a vacuum was applied above the sample to prevent droplet fallback. Time sliced measurements (5 ms per measurement) were performed using the 5% obscuration value as the starting and ending triggers. Time-sliced measurements with more than 90% of the maximum value in the obscuration vs. time profile were considered as the steady phase and used to calculate DSD.

Table 1 – DOE data table for the 3-level, 4-factor Box-Behnken design

Experiment #	Pattern	Stroke length (mm)	Velocity (mm/s)	CMC (%)	Tween80 (%)
1	00−−	4.4	50	0	0
2	0−0−	4.4	30	1	0
3	−00−	3.5	50	1	0
4	+00−	5.3	50	1	0
5	0+0−	4.4	70	1	0
6	00+−	4.4	50	2	0
7	0−−0	4.4	30	0	2.5
8	−0−0	3.5	50	0	2.5
9	+0−0	5.3	50	0	2.5
10	0+−0	4.4	70	0	2.5
11	−−00	3.5	30	1	2.5
12	+−00	5.3	30	1	2.5
13	0000	4.4	50	1	2.5
14	0000	4.4	50	1	2.5
15	0000	4.4	50	1	2.5
16	−+00	3.5	70	1	2.5
17	++00	5.3	70	1	2.5
18	0−+0	4.4	30	2	2.5
19	−0+0	3.5	50	2	2.5
20	+0+0	5.3	50	2	2.5
21	0++0	4.4	70	2	2.5
22	00−+	4.4	50	0	5
23	0−0+	4.4	30	1	5
24	−00+	3.5	50	1	5
25	+00+	5.3	50	1	5
26	0+0+	4.4	70	1	5
27	00++	4.4	50	2	5

3. Results and discussion

3.1. *Physical properties of the solutions*

Measured values of the formulation physical properties are shown in Table 2. Based on three replicates of each measurement, the standard deviations for density, viscosity, and surface tension are 0.001 g/mL, 0.1 centipoise, and 0.1 mN/m, respectively. The physical properties between different samples showed statistically significant difference ($p < 0.05$), although most of the changes are very small.

Quadratic models of formulation physical properties (density, viscosity and surface tension) have been developed on the basis of the data in Table 2, and have the form shown in the following equation:

$$R = a_0 + a_1C + a_2T + a_3CT + a_4C^2 + a_5T^2 \quad (6)$$

where R is the response and the a_i are regression parameters relating the response to the formulation composition factors. The regression parameters for each physical property are tabulated in Table 3. In all data treatments, probability values (p values) less than 0.05 are considered to be statistically significant. Performance properties of a nasal spray device are expected to be a function of many parameters including formulation density, viscosity and surface tension, geometric and physical properties of the nasal spray device, and parameters characterizing the actuation process. The estimated regression coefficients given in Table 3 offer insight into the relationship between formulation compositions and solution physical properties. The estimated coefficients are based on scaled factor values, and therefore the magnitude of each coefficient within each response model reflects the relative significance of each term in the model.

For the density model, the intercept term is dominant, reflecting the fact that all solution densities are very close

Table 2 – Physical properties of the simulated nasal spray formulations

Formulation	Density (g/mL)	Viscosity (centipoise)	Surface tension (mN/m)
Water	0.998	1.0	68.7
1% CMC	1.003	7.5	48.8
2% CMC	1.008	19.4	41.7
2.5% Tween	1.000	1.2	32.4
5.0% Tween	1.002	1.4	31.6
1% CMC + 2.5% Tween	1.004	8.3	32.8
1% CMC + 5.0% Tween	1.006	9.5	32.3
2% CMC + 2.5% Tween	1.008	26.0	32.0
2% CMC + 5.0% Tween	1.011	27.6	32.2

Table 3 – The coefficients and standard errors of coefficients of the quadratic models relating density, viscosity and surface tension to the CMC and Tween80 concentrations of the formulations used in this study

Regression coefficients		Responses								
Term	Regression coefficients	Density			Viscosity			Surface tension		
		Scaled estimate	Std. error	*p* value	Scaled estimate	Std. error	*p* value	Scaled estimate	Std. error	*p* value
Intercept	a_0	**1.0039**	**0.0001**	**<.0001**	**8.62**	**0.24**	**<.0001**	**32.00**	**0.9207**	**<.0001**
CMC	a_1	**0.0043**	**0.0001**	**<.0001**	**11.98**	**0.21**	**<.0001**	**−2.33**	**0.7974**	**0.0081**
Tween	a_2	**0.0016**	**0.0001**	**<.0001**	**1.38**	**0.21**	**<.0001**	**−9.38**	**0.7974**	**<.0001**
CMC × Tween	a_3	**−0.0003**	0.0001	0.0992	**1.95**	**0.36**	**<.0001**	**6.90**	**1.3811**	**<.0001**
CMC × CMC	a_4	0.0001	0.0001	0.3735	**4.70**	**0.29**	**<.0001**	0.90	1.0918	0.4190
Tween × Tween	a_5	**0.0006**	**0.0001**	**<.0001**	−0.40	0.29	0.1717	**9.25**	**1.0918**	**<.0001**

The coefficients are least squares estimates based on scaled factor values (scaled estimates). Terms showing significant influence are bolded.

to the density of water. The intercept, linear and quadratic CMC terms dominate the viscosity model, and a weak interaction effect between CMC and Tween80 is also evident. Similarly, the intercept, linear and quadratic Tween80 terms dominate the surface tension model, and a moderate interaction term also appears in the model. These features indicate that the concentrations of CMC and Tween80 influence the solution physical properties in a complex manner, and developing empirical models on the basis of solution physical properties is impractical. Therefore, the remainder of this paper examines empirical models that relate the formulation and actuation factors listed in Table 1 to the responses that characterize performance of the nasal spray delivery system.

3.2. *Nasal spray characteristics*

3.2.1. *Shot weight, spray pattern and plume geometry*

Table 4 shows nasal spray response values for shot weight, spray pattern, plume geometry and DSD under various experimental conditions as described in the Box-Behnken design. The coded design patterns are as described for Table 1. For shot weight, each value represents the average of three replicates. For spray pattern and plume geometry, in consideration of the higher variation for those measurements, an average of five replicates is provided. Quadratic models relating the four factors described in Table 1 to the nasal spray response values given in Table 4 have the form shown in the following Equation:

$$R = b_0 + b_1S + b_2V + b_3C + b_4T + b_5SV + b_6SC + b_7VC + b_8ST + b_9VT + b_{10}CT + b_{11}S^2 + b_{12}V^2 + b_{13}C^2 + b_{14}T^2 \quad (7)$$

R is the response and the b_i are scaled estimates of the regression coefficients (the coefficients corresponding to scaled factor values). The regression coefficients, standard errors and probability values for shot weight, spray pattern and plume geometry models are listed in Table 5. Each column of scaled estimates represents an empirical model for the given response. The magnitude of a scaled estimate within a model reflects the importance of that term relative to the other terms in the model. Terms composed of the products of two factors represent the interaction terms and terms with second-order factors indicate the nonlinear nature of the relationship between the response and the factor. A positive sign indicates a synergistic effect, while a negative sign represents an antagonistic effect.

For the shot weight model, only stroke length and the concentrations of CMC and Tween80 have statistically significant influence on the measured response, with no interactions between any pairs of the four factors. The model predicts a quadratic dependence of shot weight on stroke length, and the scaled estimates indicate that stroke length is the dominant influence on shot weight, while the concentration of CMC and Tween80 only slightly affect the shot weight. The stroke length affects the shot weight mainly by determining the volume of the formulation to be pulled into the dip tube, and subsequently, sprayed out of the unit, while the small contributions form CMC and Tween80 are primarily

Table 4 – Shot weight, spray pattern, plume geometry, and droplet size distribution data of the 27 Box-Behnken design experiment (spray pattern and plume geometry were measured at 3 cm from nozzle tip)

Experiment #	Pattern	Shot weight (mg)	Spray pattern		Plume geometry		Droplet size distribution			
			Area (mm^2)	Ovality	Width (mm)	Angle (°)	D10 (μm)	D50 (μm)	D90 (μm)	Span
1	00−−	97.6	655.9	1.22	34.6	60.0	11.13	24.24	43.95	1.35
2	0−0−	99.9	160.4	1.32	14.0	26.1	22.84	73.73	147.46	1.69
3	−00−	79.8	263.7	1.28	24.4	44.2	14.32	32.75	72.71	1.78
4	+00−	102.5	370.8	1.34	27.4	49.0	13.25	29.12	58.60	1.56
5	0+0−	100.0	393.9	1.30	28.8	51.3	10.63	25.70	50.32	1.54
6	00+−	101.0	218.3	1.26	23.3	42.3	16.24	36.94	87.97	1.94
7	0−−0	98.4	410.3	1.24	29.6	51.9	14.95	31.40	63.50	1.55
8	−0−0	75.7	741.8	1.19	39.5	66.3	11.03	24.17	44.50	1.38
9	+0−0	99.4	893.1	1.17	40.4	67.6	10.19	22.77	42.08	1.40
10	0+−0	96.4	962.5	1.18	43.1	71.1	8.78	21.20	40.33	1.49
11	−−00	82.1	142.1	1.55	13.8	25.7	25.43	80.15	162.39	1.71
12	+−00	102.8	152.2	1.28	13.4	24.9	24.57	74.03	155.82	1.77
13	0000	100.3	356.8	1.37	28.6	50.9	14.46	33.08	73.09	1.77
14	0000	100.3	353.1	1.35	28.3	50.4	13.97	32.49	72.09	1.79
15	0000	100.0	352.5	1.34	28.1	50.0	13.96	32.42	70.82	1.75
16	−+00	81.1	368.8	1.39	29.8	52.8	12.27	29.02	61.96	1.71
17	++00	100.8	527.3	1.34	34.1	59.1	9.92	24.69	50.50	1.64
18	0−+0	100.7	68.4	2.00	9.0	17.0	54.51	154.79	271.76	1.40
19	−0+0	83.5	157.5	1.30	21.7	39.8	21.20	57.22	125.98	1.83
20	+0+0	103.6	216.0	1.37	25.1	45.4	18.25	43.78	102.19	1.92
21	0++0	100.6	262.8	1.42	28.0	50.0	15.28	36.20	84.88	1.92
22	00−+	100.2	822.7	1.18	39.2	66.3	9.95	23.54	44.50	1.47
23	0−0+	100.8	135.3	1.39	13.2	24.8	26.57	83.19	168.46	1.71
24	−00+	82.7	281.7	1.33	25.5	46.0	16.29	37.98	87.84	1.88
25	+00+	104.6	360.6	1.40	27.5	49.1	13.84	32.71	71.24	1.75
26	0+0+	100.8	422.0	1.39	29.0	51.5	11.77	28.87	60.04	1.67
27	00++	101.2	211.7	1.31	21.7	39.7	20.45	53.52	124.40	1.94

due to their influence on the formulation density and viscosity.

Regression models for spray pattern area and plume geometry width and angle show similar effects from the input parameters. In each of the models, the intercept is the dominant term, and stroke length, velocity and CMC concentration all have statistically significant influences on the measured response via both first and second-order terms. These features indicate that increasing actuation velocity and/or decreasing concentration of gelling agent will lead to the production of a wider spray plume.

The interaction term of CMC and velocity also has significant influence on the spray pattern area, with a relatively large scaled estimate. Although the interaction term between CMC concentration and velocity in the plume geometry model is statistically significant, it can be neglected in an optimized model due to the relatively small-scaled estimate value.

Unlike the shot weight model, these metrics show no effect of Tween80 concentration except as an interaction term with CMC concentration, and then only for plume width and angle. The plume geometry models also show a second-order dependence on Tween80 concentration.

Spray pattern ovality describes the shape of a horizontal slice of the spray plume. The intercept dominates the ovality model, and only the linear CMC term shows significant influence. However, the scaled estimate for CMC term is less than 10% of the intercept; therefore, the effect of CMC has little importance and may be ignored.

3.3. *Droplet size distribution*

The DSD data (D10, D50, D90, and span) for the fully developed phase of the nasal spray at various experimental conditions from the Box-Behnken design are shown in Table 4. Each value is the average of three replicates. The regression coefficients and effect test results for the DSD model are listed in Table 6. As expected, all metrics for droplet size show similar patterns in effect tests, scaled estimates and prediction profiles. The *p* values for the DSD responses (D10, D50 and D90) indicate that each metric is significantly influenced by both actuation velocity and the concentration of CMC, but not by stroke length or concentration of Tween80. The intercept, linear and quadratic velocity terms, linear CMC term, and the CMC–velocity interaction term dominate the DSD models. These features indicate that increasing the concentration of gelling agent will lead to the production of larger droplets and that increasing actuation velocity will have the opposite effect. The interaction term of CMC and velocity has the same "negative" sign as does the velocity term.

Span is a consolidated measure of broadness of the DSD. The span values are computed from the measured D10, D50 and D90 values, and vary over the narrow range of 1.35–1.94. The intercept, linear CMC term, and the CMC–velocity interaction term dominate the span model, and no significant influence is observed from other terms. These features indicate that increasing concentration of gelling agent will cause wider DSD.

Table 5 – The coefficients of the quadratic model and effect tests for shot weight, spray pattern and plume geometry (Int is the intercept; *S*, *V*, *C* and *T* are the scaled factors for stroke length, velocity, CMC concentration and Tween80 concentration, respectively)

Regression coefficients		Shot weight (mg)			Spray pattern area (mm^2)			Spray pattern ovality			Plume width (mm)			Plume angle (°)		
Term	Coefficient	Scaled estimate	Std. error	*p* value	Scaled estimate	Std. error	*p* value	Scaled estimate	Std. error	*p* value	Scaled estimate	Std. error	*p* value	Scaled estimate	Std. error	*p* value
Int	b_0	**100.2**	**0.6**	**<.0001**	**354.1**	**23.6**	**<.0001**	**1.35**	**0.08**	**<.0001**	**28.3**	**0.6**	**<.0001**	**50.4**	**1.0**	**<.0001**
S	b_1	**10.7**	**0.3**	**<.0001**	**47.0**	**11.8**	**0.0018**	−0.01	0.04	0.7755	**1.1**	**0.3**	**0.0043**	**1.7**	**0.5**	**0.0073**
V	b_2	−0.4	0.3	0.1993	**155.7**	**11.8**	**<.0001**	−0.06	0.04	0.1393	**8.3**	**0.3**	**<.0001**	**13.8**	**0.5**	**<.0001**
C	b_3	**1.9**	**0.3**	**<.0001**	**−279.3**	**11.8**	**<.0001**	**0.12**	**0.04**	**0.0095**	**−8.1**	**0.3**	**<.0001**	**−12.4**	**0.5**	**<.0001**
T	b_4	**0.8**	**0.3**	**0.0241**	14.3	11.8	0.2500	0.02	0.04	0.5705	0.3	0.3	0.3581	0.4	0.5	0.4881
S × V	b_5	−0.3	0.5	0.6464	37.1	20.4	0.0942	0.06	0.07	0.4427	1.2	0.5	0.0516	1.8	0.9	0.0743
S × C	b_6	−0.9	0.5	0.1160	−23.2	20.4	0.2780	0.02	0.07	0.7510	0.6	0.5	0.2727	1.1	0.9	0.2594
V × C	b_7	0.5	0.5	0.3889	**−89.5**	**20.4**	**0.0009**	−0.13	0.07	0.0851	**1.4**	**0.5**	**0.0265**	**3.5**	**0.9**	**0.0025**
S × T	b_8	−0.2	0.5	0.7132	−7.1	20.4	0.7358	0.00	0.07	0.9718	−0.3	0.5	0.6539	−0.4	0.9	0.6481
V × T	b_9	−0.0	0.5	0.9632	13.3	20.4	0.5270	0.01	0.07	0.9437	0.3	0.5	0.6539	0.4	0.9	0.6869
C × T	b_{10}	−0.6	0.5	0.2808	−43.4	20.4	0.0552	0.02	0.07	0.7510	**−1.6**	**0.5**	**0.0146**	**−2.2**	**0.9**	**0.0306**
S × S	b_{11}	**−8.5**	**0.5**	**<.0001**	−0.9	17.7	0.9584	−0.02	0.06	0.6992	−0.3	0.5	0.4768	−0.7	0.8	0.3747
V × V	b_{12}	−0.2	0.5	0.6337	**−59.6**	**17.7**	**0.0055**	0.08	0.06	0.1881	**−4.9**	**0.5**	**<.0001**	**−8.7**	**0.8**	**<.0001**
C × C	b_{13}	−1.0	0.5	0.0530	**142.2**	**17.7**	**<.0001**	−0.04	0.06	0.5570	**3.7**	**0.5**	**<.0001**	**5.2**	**0.8**	**<.0001**
T × T	b_{14}	0.6	0.5	0.2079	−23.3	17.7	0.2128	−0.05	0.06	0.4098	**−2.1**	**0.5**	**0.0008**	**−3.2**	**0.8**	**0.0016**

Terms showing significant influence are bolded.

Table 6 – The coefficients of the quadratic model and effect tests for droplet size distribution for the fully developed phase of the nasal spray (Int is the intercept; S, V, C and T are the scaled factors for stroke length, velocity, CMC concentration and Tween80 concentration, respectively)

Regression coefficients		D10			D50			D90			Span		
Term	Coefficient	Scaled estimate	Std. error	*p* value	Scaled estimate	Std. error	*p* value	Scaled estimate	Std. error	*p* value	Scaled estimate	Std. error	*p* value
Int	b_0	**14.13**	**2.53**	**0.0001**	**32.66**	**6.58**	**0.0003**	**72.00**	**8.34**	**<.0001**	**1.77**	**0.07**	**<.0001**
S	b_1	−0.88	1.27	0.5021	−2.85	3.29	0.4037	−6.25	4.17	0.1599	−0.02	0.04	0.5850
V	b_2	**−8.35**	**1.27**	**<.0001**	**−27.63**	**3.29**	**<.0001**	**−51.78**	**4.17**	**<.0001**	0.01	0.04	0.7587
C	b_3	**6.66**	**1.27**	**0.0002**	**19.59**	**3.29**	**<.0001**	**43.19**	**4.17**	**<.0001**	**0.19**	**0.04**	**0.0002**
T	b_4	0.87	1.27	0.5045	3.11	3.29	0.3632	7.96	4.17	0.0805	0.05	0.04	0.2327
S × V	b_5	−0.37	2.19	0.8680	0.45	5.70	0.9387	−1.22	7.22	0.8684	−0.03	0.06	0.6224
S × C	b_6	−0.53	2.19	0.8141	−3.01	5.70	0.6071	−5.34	7.22	0.4736	0.02	0.06	0.7901
V × C	b_7	**−8.27**	**2.19**	**0.0027**	**−27.10**	**5.70**	**0.0005**	**−40.93**	**7.22**	**0.0001**	**0.15**	**0.06**	**0.0436**
S × T	b_8	−0.35	2.19	0.8777	−0.41	5.70	0.9439	−0.62	7.22	0.9327	0.02	0.06	0.7325
V × T	b_9	−0.65	2.19	0.7730	−1.57	5.70	0.7874	−2.82	7.22	0.7030	0.03	0.06	0.6765
C × T	b_{10}	1.35	2.19	0.5506	4.32	5.70	0.4632	8.97	7.22	0.2378	−0.03	0.06	0.6492
S × S	b_{11}	−0.48	1.90	0.8045	−0.35	4.94	0.9440	0.84	6.25	0.8959	−0.01	0.06	0.8547
V × V	b_{12}	**5.39**	**1.90**	**0.0150**	**21.47**	**4.94**	**0.0009**	**36.04**	**6.25**	**<.0001**	−0.08	0.06	0.1937
C × C	b_{13}	2.19	1.90	0.2707	4.82	4.94	0.3484	5.86	6.25	0.3668	−0.10	0.06	0.0894
T × T	b_{14}	−0.89	1.90	0.6476	−1.12	4.94	0.8249	−1.45	6.25	0.8200	−0.02	0.06	0.7698

Terms showing significant influence are bolded.

Table 7 – Optimized DOE models for nasal spray characteristics

Responses	Prediction equations	R^2	RMSE
Shot weight	$R = 99.88 + 10.73S - 8.33S^2$	0.96	1.87
Spray pattern area	$R = 337.99 + 47.03S + 155.72V - 279.30C - 89.45VC - 53.59V^2 + 148.29C^2$	0.97	45.66
Plume width	$R = 26.71 + 8.32V - 8.13C - 4.34V^2 + 4.34C^2$	0.96	1.89
Plume angle	$R = 47.83 + 13.78V - 12.42C - 7.71V^2 + 6.19C^2$	0.96	3.13
D10	$R = 14.57 - 8.35V + 6.66C - 8.27VC + 5.22V^2$	0.86	3.73
D50	$R = 34.45 - 27.63V + 19.59C - 27.10VC + 20.80V^2$	0.90	9.71
D90	$R = 74.80 - 51.78V + 43.19C - 40.93VC + 34.99V^2$	0.94	14.24
Span	$R = 1.68 + 0.19C - 0.15VC$	0.62	0.12

3.4. *Optimized DOE model for nasal spray characteristics*

Optimized DOE models for nasal spray characteristic were recalculated using JMP® software after eliminating all insignificant terms (p value > 0.05, or estimate value < 8% of the intercept). The optimized quadratic models for nasal spray characteristics are indicated in Table 7. Since different types of nasal spray pumps will produce different delivery performances and have different ranges of actuation velocity and stroke length, these equations are pump dependent and only apply to the pumps examined in this study.

The quadratic models show excellent fit for shot weight, spray pattern area, plume geometry and DSD, as demonstrated by the correlation coefficient (R^2) values. The simplified model for DSD span shows a poor fit, with $R^2 = 0.62$, down from 0.77 when all terms were retained in the model. However, this is not surprising in consideration of the narrow range of this parameter. Because the span is computed from D10, D50 and D90, the errors in these measurements will be amplified in the span response.

As shown in Table 7, optimized predictive models for all measured parameters are dominated by the influences of actuation velocity and CMC concentration. From the models, it is not possible to discern whether the primary effect of each factor is antagonistic or synergistic, but the influence of the interaction between these factors is apparent in the 3D response surface plots shown in Fig. 1. Fig. 1 also identifies the ranges over which each factor is synergistic or antagonistic with respect to each response. Stroke length is an independent influential factor in shot weight and spray pattern area, while the concentration of Tween80 has too little influence to be included in the optimized models.

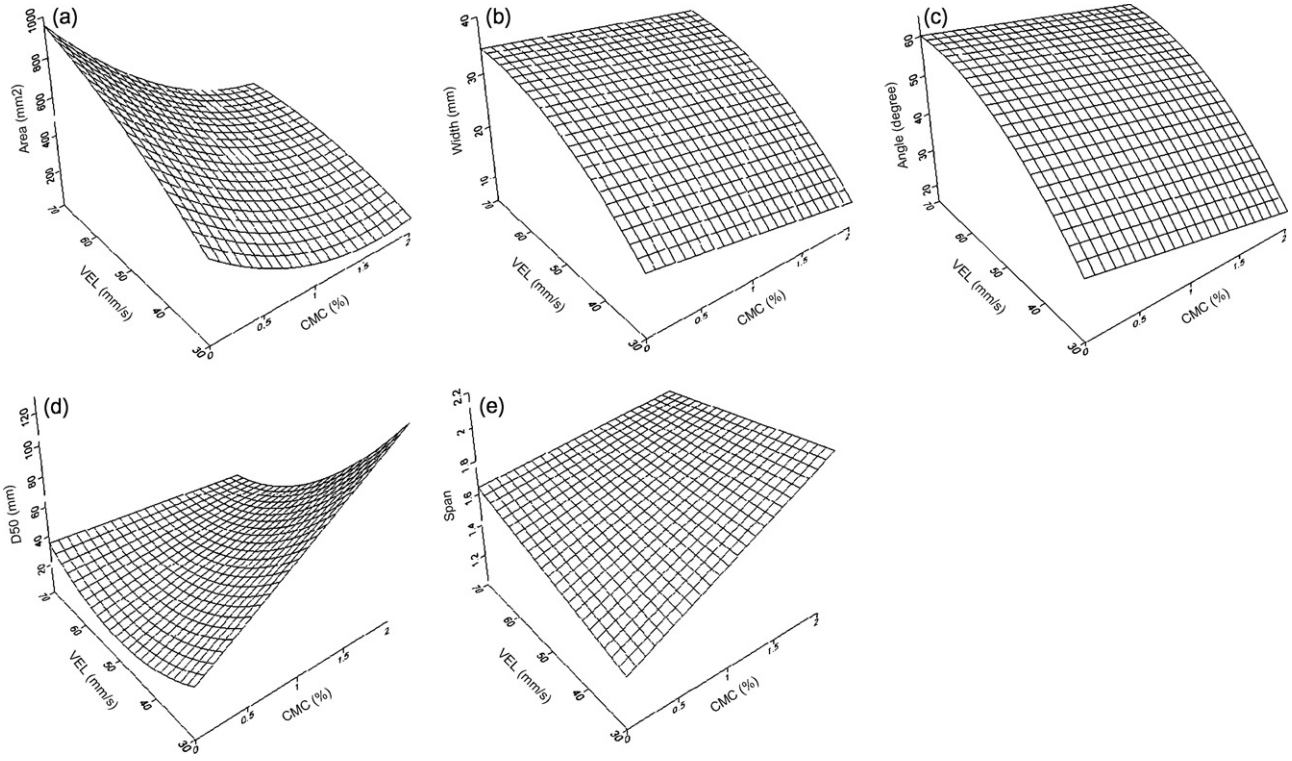

Fig. 1 – Response surface plots (3D) of the optimized DOE model showing the effect of actuation velocity and CMC concentration on various nasal spray characteristics: (a) spray pattern area; (b) plume geometry width; (c) plume geometry angle; (d) D50; (e) span.

4. Conclusion

In this study, the influence of four factors (actuation stroke length, actuation velocity, concentration of gelling agent and concentration of surfactant) on the in vitro characteristics of nasal sprays were investigated using a 3-level, 4-factor Box-Behnken design. The concentration of gelling agent (CMC) and surfactant (Tween80) are the dominant factors influencing the formulation viscosity and surface tension, respectively; therefore, their influences on nasal spray characteristics are most likely a result of their effects on these solution properties.

Of the factors studied, surfactant concentration has the least effect on nasal spray characteristics with very limited influence on shot weight, spray pattern and plume geometry, and no influence on DSD. The concentration of gelling agent has significant effects on most of the nasal spray characteristics, including spray pattern, plume geometry and DSD, but has little, if any, influence on shot weight. These results indicate that formulation viscosity affects nasal spray characteristics but formulation surface tension does not.

Actuation velocity also has similar significant effects as the gelling agent on nasal spray characteristics; however, the influence from these two factors is complicated in most responses by a significant interaction term. Actuation stroke length shows strong independent influence on shot weight, and slightly affects spray pattern and plume geometry, but has no influence on DSD.

Shot weight has strong response to stroke length changes, and is slightly affected by concentration of gelling agent and surfactant. Spray pattern, plume geometry and DSD are sensitive to changes in actuation velocity and concentration of gelling agent, but have little, if any, response to actuation stroke length or changes in the concentration of surfactant.

The Box-Behnken experimental design will be a useful tool for facilitating formulation development for a selected nasal spray pump to achieve desired drug release characteristics, and this is well demonstrated in this paper. A DOE study helps to identify the source of variability in nasal spray product performance, and thus obtain a better understanding of how to control the variability. For example, smaller droplet size may be obtained from a nasal spray product by decreasing the formulation viscosity; an increase in dosage per actuation may be obtained for a product via selection of a pump with a longer stroke length. Moreover, the quadratic models developed from the DOE study quantitatively describe the inherent relationships between the factors and nasal spray performance characteristics. With the assistance of the response surfaces developed from the DOE model, the time and labor in designing a nasal spray product to achieve desired product performance characteristics can be reduced.

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