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Statistical Modeling of Protein Spray Drying at the Lab Scale

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ABSTRACT The objective of this study was to examine the effects of formulation and process variables on particle size and other characteristics of a spray-dried model protein, bovine serum albumin (BSA), using a partial factorial design for experiments. Formulation variables tested include concentration and zinc:protein complexation ratio. Process variables explored were inlet temperature, liquid feed rate, drying air flow rate, and atomizing nitrogen pressure on a lab-scale spray dryer. Statistical data analysis was used to determine F ratios for each of the inputs, which provided a means of ranking the importance of variables relative to one another for each powder characteristic of interest. It was found that protein concentration and atomizing nitrogen pressure had the greatest effects on the particle size of the protein powder. For determining product yield, results showed that protein concentration was the critical variable. Finally, the outlet temperature was mostly influenced by inlet temperature and liquid feed rate. Mathematical models based on these input-output relationships were constructed; these models provide insight into some of the controllable variables of the spray-drying process.

Key Words: Bovine serum albumin (BSA), Particle size, Spray drying, Outlet temperature, Yield.

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INTRODUCTION

The purpose of these experiments was to study the relationship between some process and formulation variables and resulting powder characteristics using the spray-drying process. Spray drying is a common method for producing a dry powder from a liquid [1]. In this study, both protein solutions and zinc-complexed protein suspensions were spray dried. During the process, the liquid is initially atomized into a chamber of heated air, creating a spray of fine droplets. The solvent quickly evaporates under these conditions, forming dried particles. These particles are then separated, by means of a cyclone, into a collection container attached to the unit.

Some examples of the limitations of spray drying include problems with efficient particle collection and the potential instability of materials sensitive to high temperatures. Even so, a growing number of studies have confirmed the promise of spray drying to produce dried biopharmaceuticals [2-6]. Some advantages of spray drying include the ability to produce a dry powder rapidly (eg, compared to lyophilization) and the ability to control the particle size distribution.

Using a lab-scale apparatus, the effects of various spray-drying process and formulation variables on the product were examined using bovine serum albumin (BSA) as a model protein. Predicting how these parameters affect particle characteristics will be useful in future development of spray-dried biopharmaceuticals.

MATERIALS AND METHODS

Materials

BSA (A 7030; fatty acid-free, essentially γ-globulin-free) was purchased from Sigma (St Louis, MO).

Experimental Design

The effects of major inputs (including both process and formulation variables) were investigated. The process inputs were solution feed rate, atomizing nitrogen flow rate, drying air rate (or aspirator setting), and drying air temperature. Formulation variables included protein concentration and zinc:protein complexation ratio. The addition of zinc creates a suspension and increases the stability of BSA on spray-freeze drying [7]. Zinc complexation has also been shown to stabilize other pharmaceutical proteins upon drying, for instance, recombinant human growth hormone [8]. The effects of these inputs on particle size, yield, and outlet temperature were of primary importance to this study. Other outputs of interest were particle morphology and protein stability.

A factorial design to investigate the inputs was created using the software package JMP version 3.2.6 (SAS Institute, Inc, Cary, NC). A 6-factor, 2-level fractional factorial design was completed and constituted 16 of the experiments in this study. Ten additional runs were also carried out, and, because the data were useful with respect to model fitting, they were included in our models. These extra experiments comprised a few scouting runs (at operational inputs beyond the initial 16 runs) to confirm the operational phase space that would successfully yield spray-dried powders. Also, for conditions that were not spraying well (eg, excessive condensation in the drying chamber), runs were conducted at slightly different operational inputs from those dictated by the factorial design.

The JMP software was also used to determine which input variables were statistically significant in determining the spray-dried particle size, yield, and outlet temperature. To this end, an F ratio was calculated for each input variable, which provided a means of ranking variables relative to one another. Lastly, JMP was used to construct mathematical models for making output predictions for future experiments. Details of the statistical analysis can be found in the literature supplied with the JMP software.

Spray-Drying Procedure

The spray dryer employed herein was purchased from Büchi (model B-191; Flawil, Switzerland). Aqueous solutions of BSA and zinc-complexed suspensions of BSA were prepared at their given concentations in water. For runs that required complexation with zinc, zinc acetate was dissolved in water and added to the protein solution to satisfy a zinc:protein molar ratio of 50:1 and the specified final protein concentration (ie, for a 100 mg/mL protein concentration, 0.3 g of zinc acetate dissolved in 4 mL of water [1.5 mmol] was added to 2 g of BSA dissolved in 16 mL of water [0.03 mmol] yielding a 50:1 molar ratio of zinc:protein). The various spray-drying process conditions are listed in Table 1. To minimize protein denaturation, the solution or zinc-complexed suspension was kept on ice. Spray-dried powders were stored under desiccated conditions at room temperature prior to powder characterization.

Particle Size Measurement

At the end of the spray, the protein powder was collected from the spray dryer and then sized using a laser diffraction particle size analyzer (Coulter model LS130 Particle Size Analyzer, Beckman Coulter, Inc, Miami, FL). Acetone was used as the circulating fluid. Prior to measurement, each powder was sonicated for 4 minutes in methylene chloride at maximum output to de-aggregate the particles.

Scanning Electron Microscopy (SEM)

SEM images were obtained for all of the spray-dried powders to examine particle morphology. A JEOL model 6400 scanning electron microscope

Table 1 - Spray Drying Input and Output Values

Cond#	Input Values						Output Values				
	Liq. Feed (mL/min)	Atom. N_2 (L/h)	Asp. Setting (%)	T_{inlet} (°C)	BSA (mg/mL)	Zn: BSA (mol:mol)	T_{outlet} [a] (°C)	Yield (%)	$D_{v,50}$ [b] (µm)	Morphology[c]	Monomer loss (%)
1	20	900	100	130	100	0	36	20	2.6	I	1.7
2	20	900	100	180	100	0	54	34	3.5	I	1.3
3	20	900	100	180	100	50	45	38	5.2	ND[d]	0
4	5	500	50	110	100	50	49	37	6.2	II	0
5	15	900	75	220	2	0	62	8.4	2.3	ND	6.2
6	14	900	75	220	100	0	67	56	4.1	I	1.6
7	4	900	80	200	100	0	125	29	4.3	III	62
8	15	500	100	150	100	0	67	39	5.7	II	9.8
9	15	900	100	220	100	50	81	52	4.1	ND	0
10	3	500	80	150	100	50	81	64	7.8	III	0
11	15	500	80	220	2	50	79	31	2.9	IV	3.6
12	3	500	100	220	2	0	107	31	2.5	III	15
13	3	900	100	150	2	50	75	26	2.0	IV	11
14	6	900	100	120	2	0	66	14	2.0	ND	0
15	14	500	100	150	100	0	55	32	5.6	I	1.0
16	14	500	100	220	100	0	107	47	7.2	I	3.6
17	14	500	100	150	2	50	49	24	3.2	IV	3.5
18	13	900	100	150	100	50	41	32	3.8	II	0
19	4	500	80	220	100	50	117	34	9.8	III	4.3
20	4	500	100	150	100	50	83	36	7.8	III	0.20
21	15	900	100	220	2	0	79	8.4	2.5	IV	2.5
22	3	500	80	150	2	0	80	18	2.6	IV	1.9
23	4	900	100	150	2	0	46	7.6	2.4	III	4.7
24	4	900	100	150	100	0	75	54	4.7	I	0.10
25	3	900	80	150	2	50	74	10	2.2	IV	11
26	3	900	100	220	2	50	114	24	2.6	III	12

Note:Cond. = Experimental run, Liq. = Liquid, Atom. = Atomizing, Asp. = Aspirator, T_{inlet} = Inlet temperature, BSA = bovine serum albumin, Zn. = Zinc, T_{outlet} = Outlet temperature, $D_{v,50}$ = Volume median diameter.

[a]Average outlet temperature during the run.

[b]Geometric volume median particle size.

[c]Categories for particle morphology are described in the text.

[d]ND = Not determined.

(JEOL USA, Inc, Peabody, MA) was used for imaging after samples were sputter coated with Au.

Protein Aggregation

Soluble protein aggregates were monitored using size-exclusion chromatography (SEC) as described previously [7]. A G3000SW XL TSK Gel Column (Tosoh Biosep, Montgomeryville, PA) was used. Detection was by UV absorption at 214 nm. The data are reported as a percent loss in monomeric BSA compared with that in the protein prior to spray drying.

RESULTS AND DISCUSSION

Table 1 shows the input conditions and resulting output data for the 26 experimental runs completed during this study. The input conditions were selected on the basis of the partial factorial design discussed above. As reflected in the table, some input conditions were modified from the original design to reduce condensation in the drying chamber (ie, by decreasing the liquid feed rate, increasing the aspirator setting, and/or increasing the inlet temperature). Condensation inside the chamber causes a loss in yield because the dried particles stick to the chamber walls and therefore cannot be collected.

Particle Morphology

The morphologies of the different spray-dried powders were also examined by SEM. Various particle morphologies were observed, including (I) smooth spheres, (II) collapsed or dimpled particles, (III) particles with a "raisin-like" appearance, and (IV) highly crumpled, folded structures. Examples of these can be seen in the SEM images shown in Figure 1.

The particle morphology plays a profound role in the aerodynamic properties and performance of aerosol applications. For instance, having a porous, "crumpled" structure results in a much lower aerodynamic particle diameter compared with a dense particle [9].

Protein Integrity

Data for the loss of protein monomer are also presented in Table 1. The monomer loss ranged from 0% to 62%. It is expected that any degradation process would be accelerated by increasing the temperature. Among our data set it was observed

that the sample with the highest outlet temperature (125°C) had the greatest loss of monomer. However, no clear correlation was found between protein monomer loss upon spray drying and outlet temperature, or for any of the other process input or output variables. Even though there were trends observed that low liquid flow rate, low inlet temperature, and zinc complexation stabilized the protein, attempts at modeling resulted in a poor fit. Additional experiments (eg, using higher outlet temperatures) are necessary to further explore this question.

Geometric Particle Size

The geometric particle size was of primary interest to this study for the potential application of encapsulation in bioerodible polymers for sustained release. It is expected that the geometric particle size will affect the initial release. For instance, production of submicron particles by spray-freeze drying resulted in a substantial lowering of initial release for BSA microencapsulated in poly(lactide-co-glycolide) [7].

In the current study, production of submicron particles was not possible within the range of input conditions tested on the lab-scale instrument. The sprays resulted in a range of geometric particle sizes from 2 to 10 μm. The average particle size of all 26 runs was about 4 μm. Five different runs produced the smallest $D_{v,50}$ (volume median diameter) of about 2 μm, and the common input conditions for these runs were low protein concentration (2 mg/mL BSA) and high atomizing nitrogen flow rate (900 L/h). These results are logical because low

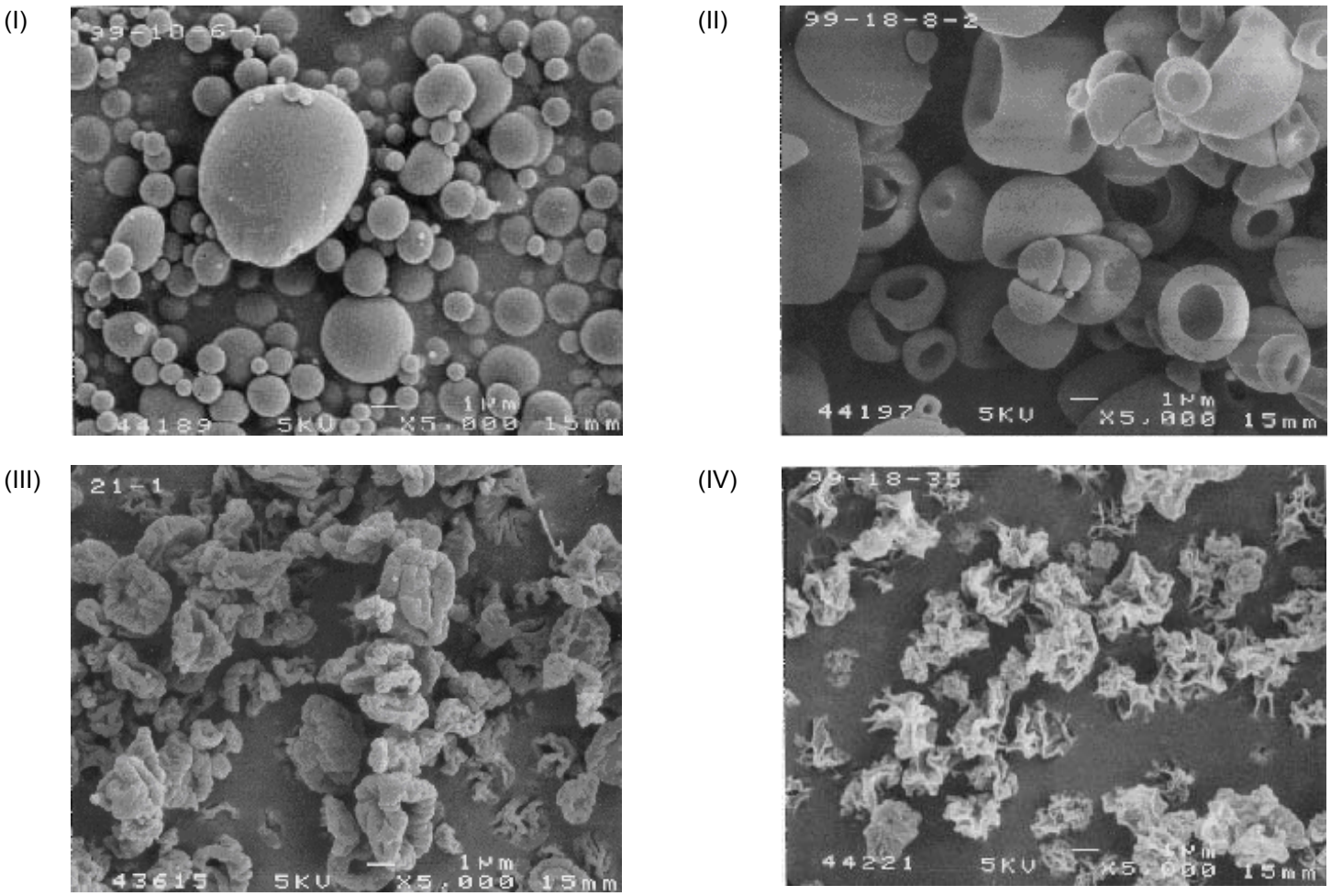

Figure 1 - SEM images of spray-dried BSA particles: (I) Type I, smooth spheres (see condition 1 in Table 1); (II) Type II, collapsed or dimpled (condition 4); (III) Type III, wrinkled or raisin-like (condition 12); (IV) Type IV, highly crumpled or folded (condition 17).

protein concentration decreases the amount of solid in each droplet exiting the nozzle. Therefore, when the water in the droplet evaporates, a smaller particle remains. Also, when the atomizing nitrogen flow rate is high, the drop exiting the nozzle tends to be smaller, so the resulting dried particle will be smaller. These trends are expected on the basis of established spray-drying theory [1,10].

In order to describe quantitatively how the various input variables affected geometric particle size in our system, an empirical statistical model was constructed. The model is the linear best-fit equation describing the particle size as the sum of an intercept and the product of the inputs and their fitted parameter estimates. Therefore, a positive parameter indicates that the output increases with increasing input variables. Conversely, the output decreases with increasing input when the parameter estimate is negative. A list of the parameter estimates for the particle size model is presented in Table 2, along with the F ratio (calculated using JMP) for each input variable. The F ratio is a quantitative means of comparing the significance of the input variables on particle size. Specifically, the greater the magnitude of the F ratio for a given input variable, the greater the influence of that variable on particle size.

From Table 2 it can be seen that for particle size, the most important input variables were the protein concentration (F ratio = 65) and atomizing nitrogen flow rate (F ratio = 24), which had parameter estimates that were positive and negative,

Table 2- Summary of Model Fit for Various Outputs

	Output: $D_{v,50}$		Output: T_{outlet}		Output: Yield	
Input in Model	Parameter Estimate	F ratio	Parameter Estimate	F ratio	Parameter Estimate	F ratio
Intercept	3.5	—	−1.9	—	4.2	—
Liquid feed rate	-9.5×10^{-2}	8.3	−2.8	57	-3.8×10^{-1}	1.0
Atomizing N_2	-4.5×10^{-3}	24	-2.4×10^{-2}	5.7	-1.2×10^{-2}	1.4
Aspirator setting	8.6×10^{-3}	0.31	3.1×10^{-1}	3.2	8.6×10^{-2}	0.24
Inlet temperature	1.1×10^{-2}	4.4	5.0×10^{-1}	79	8.8×10^{-2}	2.3
BSA concentration	3.3×10^{-2}	65	8.8×10^{-2}	3.9	2.4×10^{-1}	27
Zn:BSA	1.5×10^{-2}	3.9	-4.7×10^{-2}	0.32	9.7×10^{-2}	1.2

Note: $D_{v,50}$ = Volume median diameter, T_{outlet} = Outlet temtperature, Zn = Zinc, BSA = bovine serum albumin.

respectively. Therefore, particle size increases with increasing protein concentration and decreases with increasing atomizing nitrogen flow rate. These results are consistent with our experimental observations discussed above.

A plot of the experimental $D_{v,50}$ for each run versus the predicted value from the model fit is depicted in Figure 2. This plot shows that the measured particle size is similar to the particle size predicted by the model on the basis of a given set of input conditions. Specifically, the R^2 value is 0.85, indicating a reasonably good fit. An $R^2 = 1$ occurs when there is an exact fit (ie, the errors are all 0).

Outlet Temperature

Another critical output variable in spray drying is the outlet temperature. The range of outlet temperatures seen in this study was 36°C to 125°C. In the case of spray drying temperature-sensitive proteins and peptides, it is important that the outlet temperature is low to avoid product degradation.

Because the outlet temperature likely plays a role in the quality of the spray-dried product, another empirical model was developed.

The result of the model fit for outlet temperature is presented in Table 2. On the basis of the F ratios of various temperatures, the inlet air temperature was the parameter that most influenced outlet temperature (F ratio = 79), followed by the liquid feed rate (F ratio = 57). The atomizing nitrogen flow rate, protein concentration, and aspirator setting had lesser effects, and zinc:BSA ratio had no effect on the outlet temperature. Specifically, the outlet temperature increased with increasing inlet temperature and decreased with increasing liquid feed rate and atomizing nitrogen flow rate. These trends are consistent with the findings of others [10]. The model was reasonably accurate in predicting the experimental data (Figure 3) with an $R^2 = 0.86$.

Yield

Finally, the same approach was used to analyze data for product yield. Yields ranged from 8% to 64%. Most of the sprays resulted in low yields because of the difficulties in particle collection in spray drying, particularly when trying to produce small particles. Many of the smallest particles cannot be recovered in the lab-scale apparatus because they do not efficiently deposit in the cyclone and because their low masses cause them to be drawn up into the vacuum. As a result of such problems, only 4

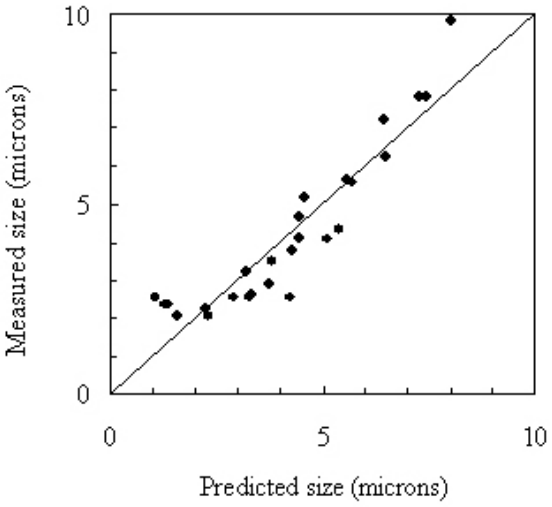

Figure 2 - Model fit for geometric volume median particle size ($D_{v,50}$) as a function of input variables compared with the experimental data for spray-drying runs (R^2 = 0.85).

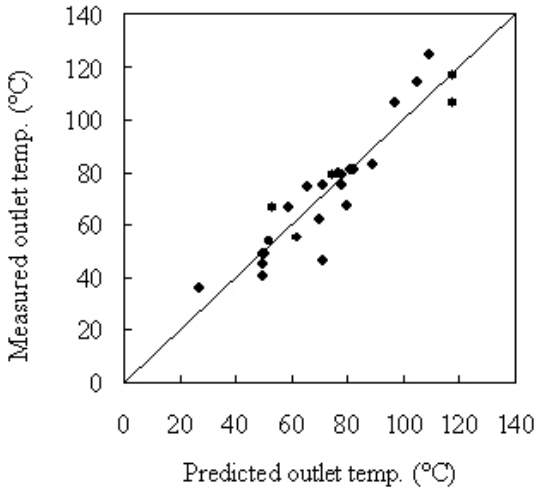

Figure 3 - Model fit for outlet temperature as a function of input variables compared with the experimental data for spray-drying runs (R^2 = 0.86).

batches had percentage yields greater than 50%. The only shared condition of these 4 runs was a high protein concentration (100 mg/mL). A high protein concentration results in an increase in particle size (discussed above), with the potential for more particles to be captured in the cyclone, therefore increasing the yield.

A model was fit for yield as a function of the input variables (with parameter estimates and F ratios presented in Table 2). As expected, the protein concentration had the highest F ratio of 27 and was therefore the most significant input condition in producing a high yield. The positive value for the parameter estimate from the model confirmed that the yield increased with increasing protein concentration. All of the other variables had F ratios in the range of 0 to 2 and were therefore less important.

Figure 4 compares experimental data for yield with the model fit. The model predicts values that are reasonably similar to the experimental data. The fit for yield (R^2 = 0.63) was not as good as the fits obtained for particle size (Figure 2) and outlet temperature (Figure 3). Even so, the model can be useful in predicting trends for yield as a function of input conditions.

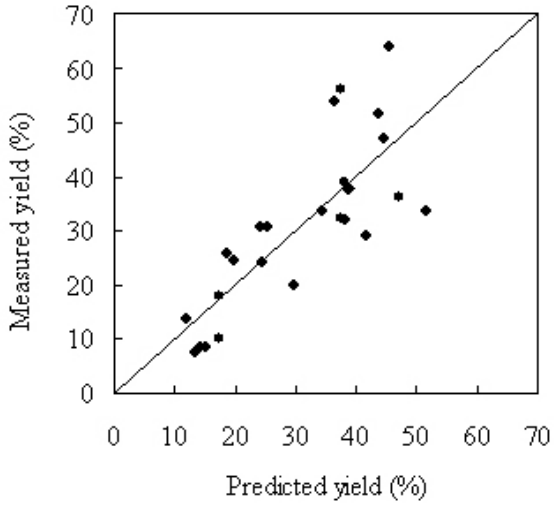

Figure 4 - Model fit for yield as a function of input variables compared with the experimental data for spray-drying runs (R^2 = 0.63).

CONCLUSION

Described herein are empirical statistical models that can be used to predict powder particle size ($D_{v,50}$), outlet temperature, and yield as functions of some formulation and process variables, with BSA as a model protein. These experiments were conducted using the lab-scale Büchi B-191 spray dryer. The statistical software package JMP was used to aid in the experimental design and data analysis.

To control the particle size of a spray-dried powder, it is important to keep the atomizing nitrogen flow rate relatively high and the protein concentration relatively low. Both of these conditions produced a smaller dried particle in this study. However, submicron particles were unattainable within the range of conditions tested on the lab-scale instrument; the smallest particle size obtained was 2 μm. To maximize powder yield, protein concentration should be high, which also tends to increase $D_{v,50}$. Finally, if the protein is particularly sensitive to degradation, the outlet temperature should be as low as possible. To this end, inlet temperature should be kept low and liquid feed rate should be relatively high. A variety of particle morphologies were observed in our study, ranging from smooth spheres to folded, crumpled structures, depending on the input conditions.

Using the mathematical models presented in this report, a rough estimate of an output value can be obtained on the basis of a selected set of input conditions for spray drying at the lab scale. Potential future work would include conducting additional runs to allow for construction of more robust models and testing the models' predictive abilities.

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