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Spray drying as a method of producing silk sericin powders

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ABSTRACT

Purpose: The purpose of paper is to analyse Spray drying as a method of producing silk sericin powders.
Design/methodology/approach: Aqueous sericin solutions were used as raw material for the production of dry powders using a lab-scale spray dryer. A linear regression analysis of agglomeration was employed, in addition to experimental designs at two levels with three factors for the analysis of three responses: moisture content, particle type and agglomeration degree. The process factors were the drying air temperature (120ºC and 160ºC), the feed rate (1.25 × 10-7 and 2.5 × 10-7 m³/s), and the concentration of sericin solutions of 10% and 30% (w/w) fed to the spray dryer.
Findings: The three responses were analyzed statistically to determine the effective parameters and it was concluded that moisture content depended on three factors--drying air temperature being the dominant parameter. Particle size and shape depended mainly on feed rate and agglomeration depended on the moisture content of the product.
Practical implications: As a result of the growing interest in drug delivery through a pulmonary route for local and systemic effects, the crucial physical characteristics of the spray-dried sericin influencing the dispersion and deposition behaviour including particle size, morphology, moisture content and agglomeration degree were examined for formulation and spray drying variables.
Originality/value: The most effective parameters on particle size and morphology were found to be the feed solution concentration and feed rate, while the temperature was an insignificant variable.
Keywords: Spray drying; Sericin; Pulmonary; Drug delivery

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1. Introduction

The Silk thread is produced by the domesticated silkworm, Bombyx mori; the thread is composed of two kinds of protein: a fibrous protein (fibroin) which forms the thread core, and a gum-like protein (sericin) that surrounds the fibroin fibres to join them together. Most of the sericin must be removed during raw silk production at the reeling mill, and during the other stages of silk processing. At present, most of the sericin is discarded in the silk processing wastewater. The recovery of sericin would not only reduce the environmental impact of silk manufacturing [21] but a significant economic and social benefit could be realized from the recovery and recycling of sericin protein.

For decades, much biochemical research has focused on the use of fibroin in biotechnological materials and biomedical applications [1,4]. Natural silk protein sericin can find applications in biomaterials as well. Sericin can impart useful and unusual properties to polymer gels, membranes, foams, fibres, and other composite materials [21]. It can be used to produce cryopreservatives, anticoagulants, and biocompatible materials.

The protein was also used as a coating material for cellulose fibre; the treated textile exhibited decreased free formaldehyde content, resistance to electricity, skin irritation and allergic reactions with increased water retention and a negligible decrease in the textile tensile strength [8]. It has excellent moisture absorption and release properties, it is antibacterial, UV resistant and it has pharmacological functions such as anticoagulation [12] anti-cancer and antioxidant activities [14, 15] with inhibitory action of tyrosinase [7].

Sericin, proposed as a candidate for pulmonary drug delivery in this study, may offer some additional advantages. Carbohydrates--especially lactose--are commonly used as pulmonary drug carriers since they have been approved by Food and Drug Administration due to their non-toxic and readily degradable properties after administration [9, 19] with its unique properties, Sericin constitutes a good alternative to carbohydrates. In addition to its antioxidant, antibacterial, anticancer and mucoadhesive properties, sericin is a water-soluble natural protein constituting 25%-30% of a versatile material like silk. Its molecular weight ranges widely from about 10 to over 300 kDa. The amino acids serine and aspartic acid constitute approximately 33.4% and 16.7% of sericin, respectively. In total, sericin contains 18 amino acids, most of which have strongly polar side groups such as hydroxyl, carboxyl, and amino groups. The protein can be cross-linked, copolymerized, and blended with other macromolecular materials to produce materials of improved properties [21, 22].

Considerable scientific research has demonstrated that improved therapeutic efficacy is achievable for carrier-based drug formulations by manipulation of the carrier particle size and morphology [6, 19]. Manufacturing of carrier-based dry powder formulations for pulmonary drug administration with desirable characteristics can be achieved by various methods, including milling, freeze-drying and spray drying [18]. Particles of different shape and morphology have been produced for various drug delivery systems, including conventional, encapsulated and porous particles, using the spray drying process and it has been confirmed that the powder characteristics can be controlled by spray drying conditions and powder formulations [2,3,16,13,12].

The advantages of the spray drying process include the negligible possibility of degradation of heat-sensitive molecules and rapid production of dry powders from the solution in one-step process with controlled particle characteristics; the disadvantages were reported to be the difficulty of obtaining a narrow size distribution and the tendency to agglomerate [5,18].

Optimisation and control of the powder dispersion and deposition properties are important phenomenon in the development of dry drug formulations for pulmonary delivery purposes [10, 11]. In this study, the physical characteristics including particle size distribution, shape and moisture content as well as agglomeration degree of the sericin powders produced using a lab-scale spray dryer were examined.

Since these characteristics are governed by formulation and manufacture variables, the significance of these variables, as well as interactions between them, was examined using a factorial experimental design and linear regression analysis. The formulation variable of sericin feed concentration, and input variables for the spray drying process including the solution feed rate and drying temperature were included in the study. The aim of this study is to estimate the output characteristics of the sericin powder produced using a lab-scale spray dryer under various conditions. This information will be useful for the optimisation of the spray drying manufacturing of sericin powders with different dispersion and deposition properties, proposed as a carrier for pulmonary drug delivery.

2. Material and method

2.1. Materials

Sericin was purchased in powder form from Silk Biochemical Co., Ltd. (Hangzhou, China).

2.2. Preparation of solutions

The sericin solutions were prepared by dissolving 10 g sericin powder in deionized water and stirring for 15 minutes to form solutions of 10% and 30% (w/w) concentration, respectively.

2.3. Spray drying

The feed solutions were spray dried with a laboratory-scale spray dryer (Büchi B-290 Mini Spray-Dryer). The solutions were pumped into the drying chamber at a rate of 1.25×10^{-7} and 2.5×10^{-7} m^3/s. Feed solutions were pneumatically atomized through a nozzle using compressed air at fixed pressure. Spray dry conditions, consisting of feed rate and inlet temperature, were set to values of each experiment. The air flow rate was fixed to 10^{-3} m^3/s. The powders were collected and moisture analyses were performed immediately. The particle size, shape and agglomeration analyses were performed within one week of production. This time the powder samples were stored in a molecular sieve 4A desiccator at ambient temperature in sealed sample cups.

2.4. Moisture content analysis

The moisture content measurements of the spray dried sericin powders were carried out using a Sartorious Electronic Moisture Analyzer (Model M-100). Each analysis was achieved by using samples of 1.5×10^{-3} kg for the duplicates. The powders were heated to the maximum temperature of 105°C and the percent weight losses were measured thermogravimetrically.

2.5. Particle shape and size analysis

In order to examine particle morphology, Scanning Electron Microscope (SEM) images were obtained for all spray-dried powders. The analyses were performed using a Philips XL 30S FEG in the Material Research Centre, Izmir Institute of Technology.

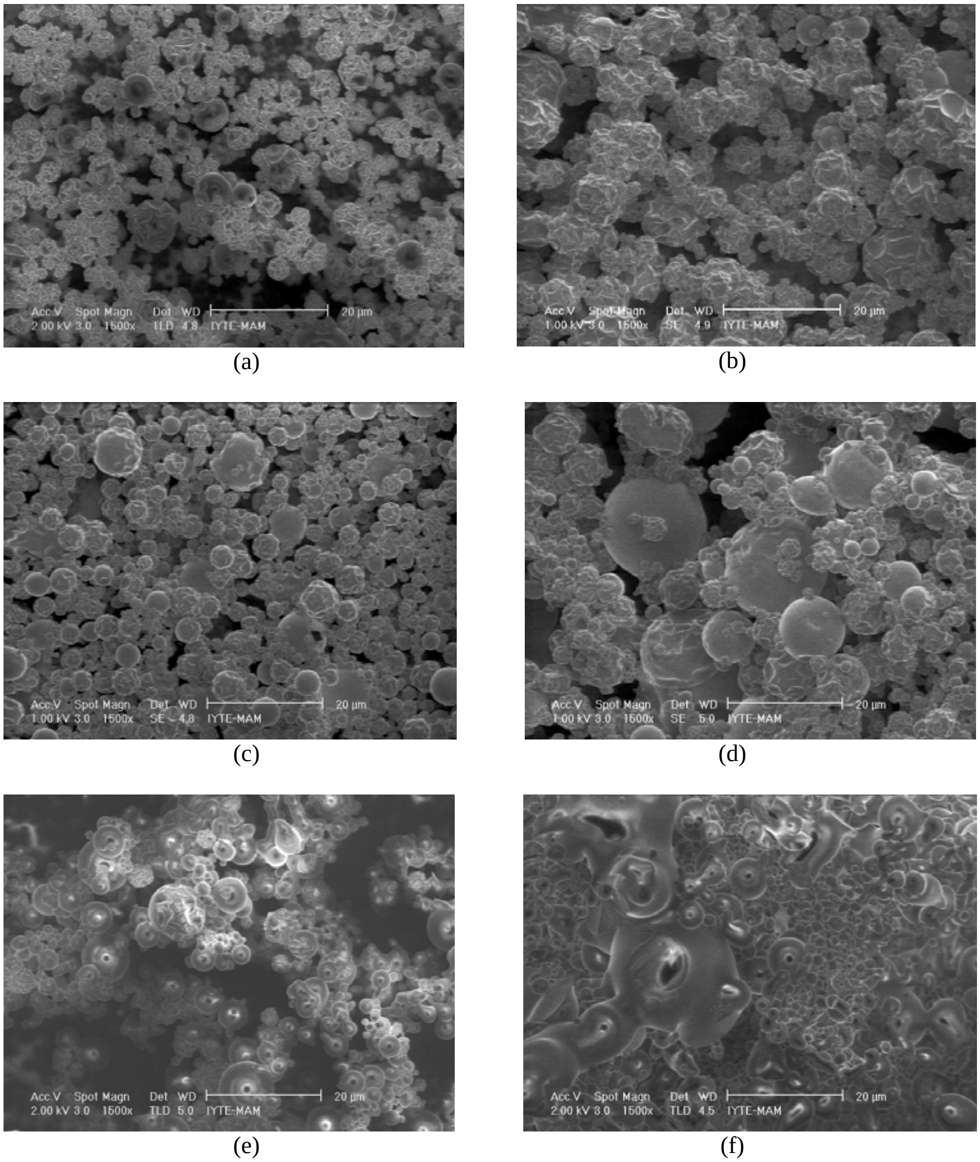


Fig. 1. SEM micrographs for the spray-dried particles with different morphologies: (a) Type 1, (b) Type 2, (c) Type 3, (d) Type 4, (e) Type 5 and (f) Type 6

2.6. Agglomeration analysis

The agglomeration degree of spray dried powders was analyzed by a stereomicroscope (Meiji EMZ-TR) in the Department of Mechanical Engineering, Izmir Institute of Technology.

2.7. Statistical analysis

The effects of major inputs on the responses including both process and formulation variables were investigated. A three-factor, two-level full factorial design with two replicates was completed on the sixteen randomized experiments in this study. The process inputs chosen were the solution feed rate and drying air temperature. The formulation variable was aqueous sericin concentration. The response variables were moisture content, particle morphology and agglomeration degree of spray-dried particles. The levels of the factors were coded as -1 and +1, corresponding to the low and high levels respectively. Actual values of the levels are 120°C and 160°C for drying air temperature, 1.25×10^{-7} and 2.5×10^{-7} m^3/s for the solution feed rate and 10% and 30% (w/w) for the concentration of the sericin

Table 1.
Categorization of the spray-dried particles

Category	Particle shape	Particle size	Particle size distribution
Type 1	wrinkled and smooth	1-5 µm	polydisperse
Type 2	wrinkled	1-5 µm	monodisperse
Type 3	wrinkled and smooth	1-5 µm	monodisperse
Type 4	wrinkled and smooth	5-20 µm	polydisperse
Type 5	mixed smooth and donut-shaped	1-5 µm	polydisperse
Type 6	mixed granules, smooth and sticky	5-20 µm	polydisperse

Table 2.
Definition and results of randomized experiments in coded factors

Run Order	Standard Order	Temperature	Feed Rate	Sericin Concentration	Moisture Content (% w)		Particle Type	Agglomerati on Degree	Run Order	Standard Order	Temperature	Feed Rate	Sericin Concentration	Moisture Content (% w)		Particle Type	Agglomerati on Degree
					D 1*	D 2*								D 1*	D 2*		
1	2	+1	-1	-1	4.06	3.60	1	1	9	12	+1	+1	-1	8.29	6.41	6	6
2	4	+1	+1	-1	5.89	5.65	5	3	10	6	+1	-1	+1	5.15	4.79	5	2
3	14	+1	-1	+1	4.49	4.12	5	2	11	16	+1	+1	+1	4.55	3.91	4	2
4	1	-1	-1	-1	7.26	6.38	1	4	12	11	-1	+1	-1	6.03	5.61	3	1
5	13	-1	-1	+1	5.96	5.76	5	4	13	5	-1	-1	+1	5.73	5.51	2	1
6	7	-1	+1	+1	6.29	6.00	5	4	14	15	-1	+1	+1	6.50	5.83	4	4
7	10	+1	-1	-1	4.46	3.96	1	2	15	8	+1	+1	+1	3.02	3.08	4	1
8	9	-1	-1	-1	6.62	6.09	1	3	16	3	-1	+1	-1	8.34	6.95	6	6

solutions. All statistical analyses of full factorial design and regression were performed by MINITAB Statistical Software, Release 14 for Windows, State College, Pennsylvania.

3. Results and discussion

3.1. Characterization of spray dried powders

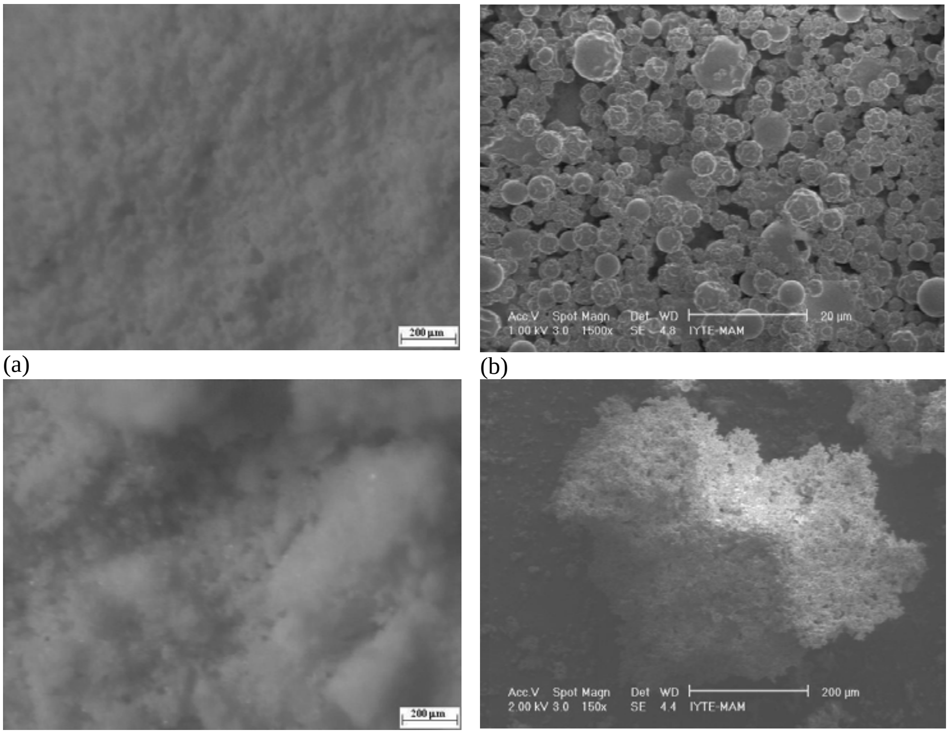
Spray drying of 10% and 30% (w/w) aqueous sericin solutions yielded light yellow powders and sticky agglomerates, depending on the formulation and drying process parameters. Morphology of sericin particles were investigated by scanning electron microscopy and it was observed that a variety of particles from hollow porous spheres to smooth or wrinkled nonporous particles coexisted after spray drying. Spray drying yielded powders with smooth and wrinkled particles, exhibiting either uniform or non-uniform size distributions with particle size ranges of 1-5 µm and 5-20 µm.

Scanning electron micrographs of the representative spray-dried particles are shown in Figure 1. The micrographs depicted different particle shapes, sizes, and size distribution of sericin powder, obtained at each run. Based on visual examination of the micrographs, the particles were graded in six categories (Table 1) according to their shapes (wrinkled, smooth, donut-shaped, straight or a mixture of all), sizes (1-5 µm, 5-20 µm) and particle size distributions (monodisperse or polydisperse).

The particles of diameter between 1-5 µm were termed "small" while those from 5 µm to 20 µm in diameter were called "large". The "type" categories indicate the adequacy of the particles for the specific use of pulmonary drug delivery. The particles manufactured for this purpose must be less than 5 µm and expected to have good aerodynamic properties. Some studies support the superiority of the spherical particles; others claim that wrinkling imparts better aerodynamic properties to the particles and more easily reach the innermost channels within lungs [3, 12, 13, 16].

In either case, the greatest problem in application is the tendency of small particles to agglomerate. Hence, in addition to surface properties, particle size and shape are vital for the practicability of the particles.

Moisture content, types of particle morphology and agglomeration degree are given in Table 2, in the run order of the



(a) (b) (c) (d)

Fig. 2. Representative stereo-microscope images of each category presenting the agglomeration degree; (a) no-agglomeration with agglomeration degree of ‘1’, (b) SEM image of non-agglomerated sample with x1500 magnification and (c) drastic agglomeration with agglomeration degree of ‘6 (d) the SEM image of a granule from the representative sample with x150 magnification

experiments. Moisture content of the particles is believed to be the most important variable for surface properties such as surface charge and stickiness. Moisture content was analyzed twice, immediately after drying to minimize experimental errors. The results of the two moisture analyses of the same sample were recorded as “duplicates” in Table 2.

The spray drying process also led to different agglomeration characteristics in the powder products. Agglomeration occurred for two reasons: Van der Waals forces between ultrafine particles and granulation due to high moisture content. It was predicted that interparticle attractions of submicron particles might cause the powder product to agglomerate, while insufficient drying caused a drastic agglomeration with formations so called granules as shown in Figure 2.

The agglomeration degree of spray-dried particles was examined in six categories, graded between the two cases which correspond to the observations of “no agglomeration” (1) and “drastic agglomeration”, leading to formation of large granules (6). The representative SEM micrographs and stereomicroscope images of the two extreme cases are shown in Figure 2.

The results were investigated by two level full factorial designs to observe the effects of process variables, and by regression analysis to observe the effect of response variables on each of the three responses; the results were then analyzed in detail.

3.2. Statistical analysis

Moisture content, particle morphology and agglomeration degree were taken as the critical properties of a spray-dried product. The models obtained by the statistical analysis of the experimental results showed the effects of the adjustable spray drying parameters on the responses. Each response was analysed to describe quantitatively how the various input variables affected the output in the system. The model is the linear best-fit equation, describing the response as the sum of an intercept and the product of the inputs and their fitted coefficient estimates for each factor. The p-value given in the analysis of the variance Table is a quantitative mean of comparing the significance of the input variables on the measured response. The parameters with a p-value over 0.05 were regarded as insignificant.

3.3. Moisture content

The ANOVA for moisture content in the drying air temperature, feed rate and feed concentration, as factors of a two level full factorial design, was performed and summarized in Table 3. Coefficient estimates and the p-values verified the significance of the main factors of temperature, concentration, second order interaction of feed rate and concentration, and the third order interaction of temperature, feed rate and concentration. Taking the terms into consideration with less than 0.05 p-value, the most effective parameters on moisture content were obviously temperature and concentration of the feed solution. Feed rate alone did not prove to be an effective parameter, but the interactions with concentration and temperature indicated significance.

The model assumptions were checked according to the normal probability plot of residuals, the predicted versus residual plot, and run order versus residuals plots. None of these plots implied a fault in the analysis, and the model assumption was confirmed.

The computer output gives model coefficient estimates and a final prediction equation for moisture content of the spray-dried particles in coded factors. The regression coefficients were calculated from the averages of corresponding effect estimates. The regression equation of the full model, Equation 1, with a regression coefficient R2 of 84.2 % is given below:

$$\hat{y}_1 = 5.5091 - 0.795x_1 + 0.263x_2 - 0.466x_3 + 0.123x_1x_2 - 0.110x_1x_3 - 0.408x_2x_3 - 0.476x_1x_2x_3 \quad (1)$$

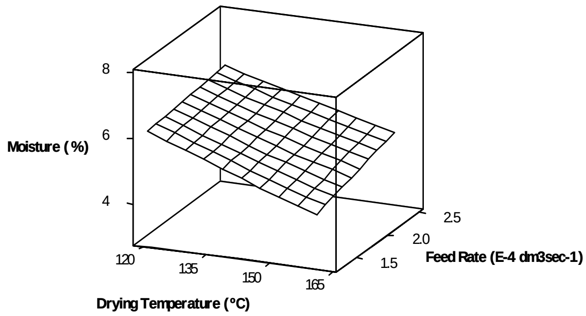
where ŷ1 represented estimated moisture percentages and x1, x2, x3 the factors, which were temperature, feed rate and the concentration respectively.

Response surface plots for each significant term in the model were analyzed. The response surface plots (Figure 3), shows the interaction of temperature-feed rate and temperature-concentration, respectively. Figure 3a shows that increasing the feed rate increases moisture content at every drying temperature value, but the variation is more significant at higher temperatures.

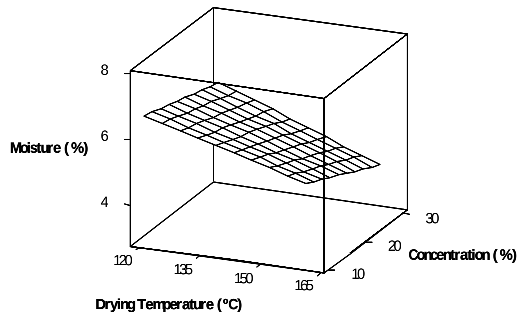
However, Figure 3b shows that increasing the concentration solution decreses moisture content at close levels at varying temperatures. The interaction of concentration and feed rate was negligibly small when compared to the effect of temperature. In both Figures (Figure 3a and 3b), the dominant effect of temperature is obvious.

Table 3.
ANOVA and the factor coefficients for moisture content (coded units)

Source	DF	Sum of Squares	P value	
Main Effects	3	14.683	0.05	*
2-way interactions	3	3.103	0.186	
3-way interactions	1	3.624	0.028	*
Pure Error	8	4.04		
Total	15	25.451		
Term	Coefficient		P value	
Constant	5.509		0.000	*
Temperature (x_1)	-0.795		0.002	*
Feed Rate (x_2)	0.263		0.177	
Concentration (x_3)	-0.466		0.031	*
Interaction $(x_1)(x_2)$	0.123		0.509	
Interaction $(x_1)(x_3)$	-0.110		0.554	
Interaction $(x_2)(x_3)$	-0.408		0.051	*
Interaction $(x_1)(x_2)(x_3)$	-0.476		0.028	*



(a)



(b)

Fig. 3. Response surface plots for the moisture content ($\hat{y}_1$) versus a) drying temperature and feed rate, b) drying temperature and sericin concentration

3.4. Particle morphology

Particle morphology categories were ordered according to particle size, particle shape and distributions. Type 1 represented the smallest particle size and a homogeneous size distribution. As the categorical number increased, particle size and size distribution range also increased. Generally two types of particle shape were observed--smooth spheres and wrinkled particles. Wrinkled particles were the result of collapsed hollow spherical structures due to fast evaporation. Lower concentrations of the feed solutions were expected to be the most significant parameter in particle size, and morphology for the solid content of the droplets seemed to be the determining parameter in particle formation. According to experimental analysis, it seems that the most important term was evaporation rate, which depended more on feed solution flow rate, interaction of feed rate and feed concentration. Also, a normal percent probability plot proved that temperature had no effect on particle size and morphology.

The software output for the statistical analysis of the reduced model for the response, particle morphology, is given in Table 4. The model is significant, with a p-value less than 0.0005 and R^2 value of 75.7%. Like the normal percent probability plot of the effects, analysis of variances indicated that x_2, x_3 and x_2 x_3 significantly affect the variability of the particle morphology.

Table 4.
ANOVA and the factor coefficients for the particle types (coded units)

Source	DF	Sum of Squares	P value
Main Effects	3	23.25	0.018 *
2-way interactions	3	16.25	0.043 *
3-way interactions	1	2.25	0.217
Pure Error	8	10.00	
Total	15	51.75	
Term	Coefficient		P value
Constant	3.625		0.000 *
Temperature (x_1)	0.250		0.002 *
Feed Rate (x_2)	1.000		0.177
Concentration (x_3)	0.625		0.031 *
Interaction (x_1)(x_2)	-0.125		0.509
Interaction (x_1)(x_3)	0.000		0.554
Interaction (x_2)(x_3)	-1.000		0.051 *
Interaction (x_1)(x_2)(x_3)	-0.375		0.028 *

*The terms and interactions with P value less than or equal to 0.05 have been considered as significant

Regarding the p-values given in the ANOVA output (Table 4), the feed rate and interaction of feed rate and concentration were equally significant with the highest effects and coefficient estimates. The resultant regression model is represented by Equation 2:

$$\hat{y}_2 = 3.625 - 0.25x_1 + x_2 + 0.625x_3 - 0.125x_1x_2 - x_2x_3 - 0.375x_1x_2x_3 \quad (2)$$

The response surface of particle type, with respect to two of the most effective parameters of feed rate and sericin concentration--given as Figure 4--can be examined to observe the serious interaction of these terms. The plot indicates that particle type does not change much at high feed concentrations. But the effect of feed rate becomes extremely important as the sericin concentration fed to the spray dryer decreases. And small particles (less than, or equal to 5 µm.), can only be produced at dilute feed solutions not exceeding 15%, and the feed rate should also be adjusted.

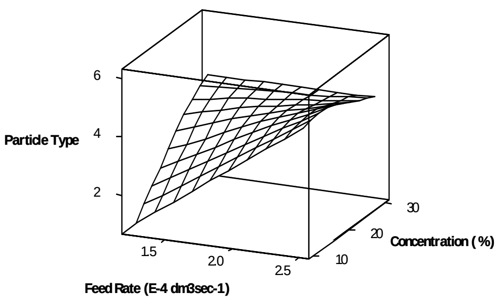


Fig. 4. Response surface plot for the particle type (ŷ$_2$) versus sericin concentration and feed rate

3.5. Agglomeration degree

The half normal and normal probability plots of effects for the response of agglomeration degree designated that none of the factors were significant. As a consequence, the agglomeration response data could not be analyzed by a factorial design procedure. But a stepwise linear regression analysis, including all possible factors of temperature, feed rate, feed concentration, moisture content and particle type, suggested that moisture content and particle type were the effective parameters on agglomeration.

Table 5.
Regression Analysis: Agglomeration versus moisture content (%) and particle type

Source	DF	Sum of Squares	P value
Regression	2	23.25	0.018 *
Residual Error	13	16.25	0.043 *
Total	15	2.25	0.217
Term	Coefficient		P value
Constant	-3.334		0.006 *
Moisture (y_1)	0.944		0.000 *
Particle Type (y_2)	0.278		0.048 *

*The terms and interactions with P value less than or equal to 0.05 have been considered as significant

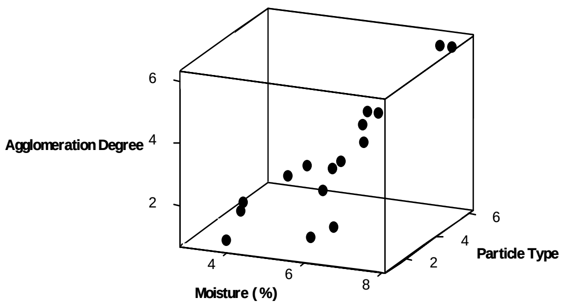


Fig. 5. Scatter plot of agglomeration (ŷ3) versus moisture content and particle type

Particle morphology requires a more sophisticated decision making process. The term "morphology" covers the entire particle size, particle shape and distribution characteristics; hence it is not an easy task to determine an ideal morphology. Different proposals were reported contrasting spherical with wrinkled particles. But taking the main constraint into account, we can state that particle size must be less than, or equal to, 5 µm, checking the characterisation results table (Table 1), the smallest particles of the type 1 agglomerated at even the lowest moisture contents.

This behaviour can be explained by Van der Waals forces, which have been the main cause of agglomeration of fine particle sizes as the magnitudes get closer to submicron scales the output for the linear regression analysis of the agglomeration data is given in Table 5. According to the results of the analysis the regression model is given as Equation 3:

$$\hat{y}_3 = -3.33 + 0.944\hat{y}_1 - 0.278\hat{y}_2 \quad (3)$$

The model explained 75.7 % of the data with respect to R^2 value. The plot of predicted versus observed agglomeration values approved the validity of the model and the plot of the residuals versus predicted values confirmed this result. The dependency of agglomeration degree, as a function of moisture and particle morphology, was given by the scatter plot of the agglomeration data with respect to the both factors of moisture content and particle morphology (Figure 5).

The dependency of particle morphology on mean agglomeration proves that there is a minimum degree of agglomeration range at intermediate values of particle types, which gets higher in number with increasing particle size. It can be stated that type 2 and type 3 (referring to Figures 1b and 1c), cause the minimum agglomeration with reasonable moisture levels. Hence an optimisation process which considers all moisture content, particle type and agglomeration degree is necessary.

The optimum conditions for the desired product must be decided by evaluating all the responses simultaneously. To be able to define the optimum conditions, the desired particle morphology, moisture and agglomeration degree must be described. Agglomeration is the most important response for application purposes and must be prevented; because the analysis proved that it depends mainly on moisture, moisture must be minimized.

4. Conclusions

As a result of the growing interest in drug delivery through a pulmonary route for local and systemic effects, the crucial physical characteristics of the spray-dried sericin influencing the dispersion and deposition behaviour including particle size, morphology, moisture content and agglomeration degree were examined for formulation and spray drying variables. The production of sericin powders of different characteristics was achieved using a lab-scale spray dryer and controlling the feed solution and operational parameters. Spray drying of the aqueous sericin solutions yielded light yellow powders and sticky agglomerates, depending on the formulation and drying process parameters. The spray drying process led to a particle size below 20 μm, either in the 1-5 μm or in the 5-20 μm range. The morphology of sericin particles changed from hollow porous spheres to smooth or wrinkled nonporous particles, exhibiting either monodisperse or polydisperse characteristics. The relative importance of the feed solution and spray dryer operational variables and their interactions were analyzed statistically using a full factorial two-level experimental design with three factors. The statistical analysis for the moisture content concluded that the temperature and feed solution concentration were significant factors, while the solution feed rate contributed the interaction terms. The most effective parameters on particle size and morphology were found to be the feed solution concentration and feed rate, while the temperature was an insignificant variable. The three variables examined had no significant effect on the agglomeration degree. A stepwise regression analysis then concluded that agglomeration strongly depended on particle size and type, in addition to moisture content.

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Additional information

The paper was published also in the Archives of Materials Science.

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