Application Note
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Sub-micron Bovine Serum Albumin particles

Nano Spray Dryer B-90 HP:
Innovative method for spray drying proteins with high yields.

1. Introduction

Spray-drying was shown to be a rapid, continuous, cost-effective, reproducible and scalable process for the production of dry powders from a liquid. Moreover, in the recent years, spray drying was identified by the pharmaceutical industry as a suitable method to produce protein particles used in pulmonary, nasal and oral delivery [1, 2].
Due to its property, bovine serum albumin (BSA) has many applications in life science disciplines, such as cell culture, in-vitro diagnostics, human and veterinary pharmaceuticals, molecular biology, serology and general research. It is also very well characterized and often used as a model protein in numerous biochemical applications [3].
The possibility to use the Nano Spray Dryer B-90 to produce protein sub-micron particles will be investigated here, using BSA as a model protein. The influence of the BSA concentration and pH on particles formation will also be studied.

2. Equipment

- Nano Spray Dryer B-90 HP
- JEOL 6380LVa type scanning electron microscope

3. Chemicals, Materials and Experimental

BSA solutions of 10 %, 1 % and 0.1 % were prepared by addition of lyophilized BSA powder (Sigma Aldrich, St Louis, MO, USA) into deionized water before being filtered through a glass fiber filter (Whatman GF/F) under vacuum in order to remove particles that can clog the system. Tween 80 was then added to the solution to a 0.05 % concentration and the pH of the solution was adjusted dropwise using acetic acid 5 % [V/V] and NaOH 5 % [V/V] (Table 1). The BSA solutions were then refrigerated before use. All solutions were prepared as % [w/V] solutions if not mentioned otherwise.

Microparticles were prepared by spray drying the BSA solutions using the tall set up of the BUCHI Nano Spray Dryer B-90 HP. During the process, the BSA solution was kept in an ice bath in order to cool it down and reduce the protein degradation by the heating system. The pump speed was set to reduce residency time around the heating system and therefore to lessen protein degradation. It is also recommended to shorten the tubing bringing the solution into the nebulizer as much as possible so that residence time around the heating is reduced.
The Nano Spray Dryer B-90 HP was operated in open loop and pressurized air with a pump speed of 90 %, the spray was set up at 80 % and the frequency at 125 kHz if not mentioned otherwise. Moreover, the experiments were run with an inlet temperature of 100 °C using a narrow spray nebulizer, a medium spray nebulizer and a large spray nebulizer. Parameters such as the inlet temperature, the outlet temperature and the gas flow rate were varying between experiments and recorded (Table 1).
The volume of the sprayed solution and the running time of the experiment were determined for each solution so that enough product can be recovered for analysis. When possible, the yield was calculated from the actually recovered powder amount in relation to the solid content of the used spray solution. The relative yield (%) was calculated by dividing the weight of the collected powder by the theoretical amount of sample sprayed, then multiplied by 100.

Images of the particles were obtained with scanning electronic microscopy (JEOL 6380LVa type scanning electron microscope), all samples were sputter-coated with gold before examination.

BSA particles were prepared with the Nano Spray Dryer B-90 HP using several experimental parameters. Those parameters are summarized in Table 1.

Table 1: Experimental parameters.

Experiment	Nebulizer	BSA concentration [% w/V]	Surfactant (Tween 80) concentration [%]	pH [-]	Gas flow rate [L/h]	Inlet temperature [°C]
A	Small	0.1	0.05	4.7	150	100
B	Small	1	0.05	4.7	150	100
C	Small	10	0.05	4.7	150	100
D	Medium	0.1	0.05	4.7	150	100
E	Medium	1	0.05	4.7	150	100
F	Medium	10	0.05	4.7	150	100
G	Large	0.1	0.05	4.7	150	100
H	Large	1	0.05	4.7	150	100
I	Large	10	0.05	4.7	150	100
J	Large	10	0.05	4.7	150	120
K	Small	1	0.05	7.2	150	100

4. Results and Discussion

BSA particles from 0.133 um to 6.34 um were produced using the Nano Spray Dryer B-90 HP with yields above 60 % (Table 2). Produced particles are mainly of spherical shape, however some donut shaped particles could also be observed (Figure 1).
In general, the particle size and size distribution appear to increase with nebulizer size and with the BSA concentration in the sample solution. This finding is in agreement with this reported by Arpagaus *et al*. (2012) [4].

Table 2: Experimentally measured values for the main results.

Experiment	Solution concentration [% w/V]	Throughput [mL/h]	Particle diameter estimation [um]	Nebulizer size
A	0.1	46.6	0.722 – 0.133	Small
B	1	24.5	1.61 - 0.185	
C	10	19.7	2.24 0.365	
D	0.1	23.58	0.803 – 0.192	Medium
E	1	23.31	2.31 – 0.317	
F	10	17.89	3.24 – 0.584	
G	0.1	113.3	3.14-0.300	Large
H	1	127.5	4.34 - 0.320	
I	10	70.3	6.34 – 0.528	
J	10	61.3	5.9 - 1.44	
K	1	25.46	1.3 - >0.5	Small

The drying temperature was set at 100 °C for all the samples except for sample J where it was set to 120°C. Except the temperature all settings were equal for experiments I and J. A higher drying temperature set in experiment J slightly narrowed the size distribution. Although the set temperatures were relatively high, protein degradation by the heat is unlikely to occur. The outlet temperatures (Table 2), that are equal to the maximum temperature the sample is exposed to, were recorded between 38°C and 61°C and are therefore favorable for spray drying heat sensitive biologicals [3].

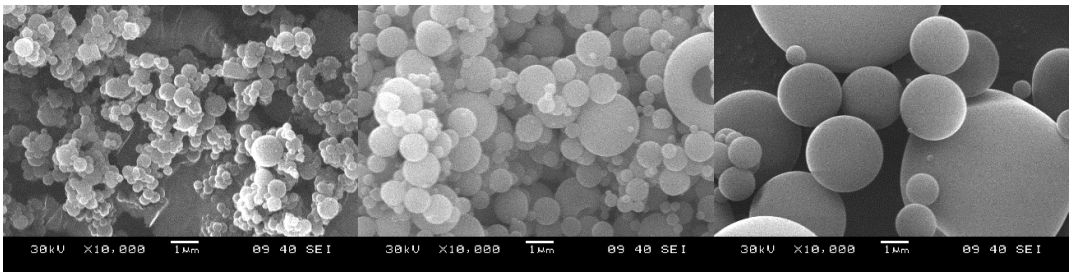

Figure 1: SEM photographs of particles produced with the small nebulizer and a 0.1 % BSA solution (left), the medium nebulizer and a 1 % BSA solution (middle) and the large nebulizer and a 10 % BSA solution (right).

It is, however, important to protect the recirculating solution from heat degradation by minimizing the residence time of the solution in the nebulizer and the heating system by reducing the tube length as much as possible and by setting a fast pump speed (90-100 %), moreover, the solution should be kept in an ice bath.
The influence of the pH on the product size was investigated by comparing experiments B and K. By increasing the pH the size distribution was slightly smaller (Table 2) and more experiments would be needed to confirm this effect.

5. Conclusion

BSA particles from 0.133 um to 6.34 um were produced with the Nano Spray Dryer B-90 HP using the parameter summarized in Table 3.

Table 3 : Parameters and results summary of BSA (0.1-10 %) spray drying.

Nebulizer size	Large	Medium	Small
Gas flow rate	144-148 L/min	142-145L/min	143-144 L/min
T inlet	100 °C	100 °C	100 °C
T outlet	44-50 °C	38 - 53 °C	51-61 °C
Spray rate	80 %	80 %	80 %
Pressure	65 - 68 hPa	66 - 75 hPa	64 - 65 hPa
Feed rate	90 % -100 %	90 %	90 %
Particle size	0.3- 6.34 um	0.192-3.24 um	0.133-2.24 um

Particle size and size distribution were increasing with nebulizer diameter and solution concentration. pH and the drying temperature did have minor influence the particle size, size distribution and shape that has to be further investigated.
Spherical particles were obtained using Tween 80 as a surfactant. Since donut shaped particles were also observed, an optimization of the surfactant concentration or type could be foreseen. Here applied process parameters may serve as starting values for process optimization and give a clear indication that the material can be successfully spray dried.

6. .References

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