NOZZLE SELECTION AND OPTIMISATION OF PROCESS CONDITIONS FOR THE SUPERCRITICAL-DRYING OF LYSOZYME

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In the perspective of production of dry therapeutic protein formulations, different supercritical spraying conditions were studied. Effects of the anti-solvent precipitation process conditions and nozzle system on the drying of hen egg-white lysozyme prepared in aqueous solution were investigated. Two types of nozzle systems were tested: a T-mixer impinging flow and a coaxial nozzle. Flow rates of CO_2 and ethanol were varied between 10-15 kg/hr and 20-25 ml/min, respectively. The particle morphology (scanning electron microscopy) and the water content (Karl-Fisher titration) were investigated for all conditions (37ºC, 100 bar, 2% (w/w) lysozyme).

Increasing the flow rate of ethanol resulted in a decrease of the residual water content of the powder. However, increasing the CO_2 flow rate influenced the residual water content in an intricate manner. Both the particles and clusters formed had different morphology with each nozzle and flow conditions. The T-mixer produced more or less fused primary particles forming porous spherical clusters or collapsed shells. Comparatively, the coaxial nozzle produced more or less porous spheroid primary particles forming loose clusters.

In conclusion, the particle morphology was sensitive to both the flow rates and the atomisation device. The ethanol flow rate significantly affected the residual water content.

INTRODUCTION

As the number of therapeutic proteins being introduced to the market is increasing, the need for appropriate stabilisation methods for these labile compounds is increasing as well [1]. Because proteins are often unstable in liquid formulations and traditional drying causes harmful stresses on them [2], alternative drying processes using supercritical fluids are being investigated.

Despite the poor solubility of water in supercritical carbon dioxide, the production of protein powders from aqueous solution is favoured over drying from organic solutions as organic solvents can affect the protein stability [3] and are poor at dissolving proteins.

Drying of aqueous protein solutions has been reported by several authors [4-9]. In these studies various type of nozzles were used, such as T-mixers [4], coaxial nozzles [5-7], and ultrasonic nozzles [8,9]. Appreciable results were obtained with each spray device and process conditions. However, none of these studies offers a comparison between both the process parameters and the atomisation device.

The aim of this study was to compare two atomisation devices – an impinging T-mixer and a coaxial nozzle – as well as the CO2 and ethanol flow rates, to determine their effect on the particle characteristics.

MATERIALS AND METHODS

Experimental set-up

The CO_2 was supplied by a diaphragm pump (Lewa) and mixed in a T-mixer with an ethanol flow added from a piston pump (Gilson). This mixture was then directly fed to the atomisation device, together with the protein solution that was added using a syringe pump (Isco). The pressure in the vessel and CO_2 flow rate were stabilised using the exit valves of the vessel. The dry protein powder was collected in a 4-litre vessel and recovered once the pressure was released. Operating conditions are described in the next section.

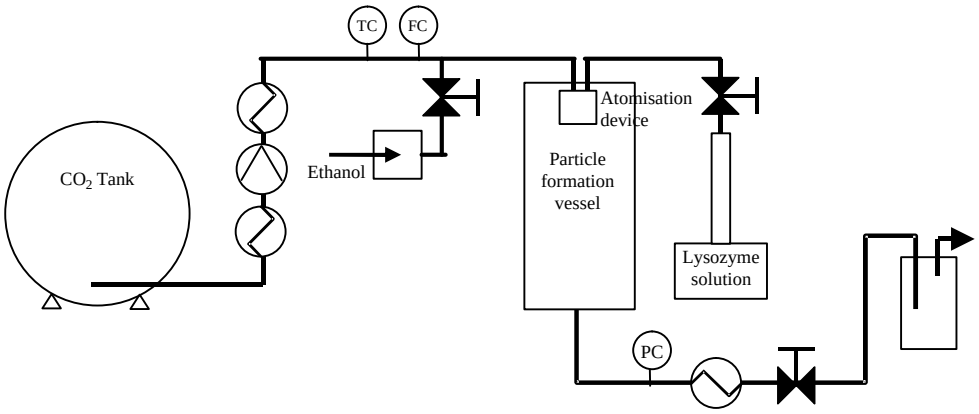

Figure 1 : Basic scheme of the experimental set-up

Materials

Lyophilised powder of lysozyme from chicken egg white (product code 62971; lot number 454137/1) was purchased from Fluka and conserved according to their instructions until use. Technical grade ethanol (100%) was used and carbon dioxide (grade 3.5) was purchased from Hoek Loos, Schiedam, The Netherlands.

Analytical techniques

Scanning electron microscopy (Jeol JSM-5400) images were used to examine the morphology of particles. Karl-Fisher titration (Metrohm 756KF) was performed as per manufacturer instructions.

Operating procedure

After the particle formation vessel was pressurised and stabilised at the desired temperature and pressure with the selected flow rates (CO_2 and ethanol, Table 1), the 20 ml of pressurised protein solution was sprayed into the vessel. After completion of the spraying process, the vessel was flushed with sufficient CO_2 to remove the residual ethanol before depressurisation and product recovery.

Nozzle assemblies
Two types of nozzles, a T-mixer and a coaxial nozzle (Figure 2 and 3), were used to compare their effect according to the residual water content and the particle morphology. The nozzle used in the T-mixer assembly (1/4") had an opening through which the lysozyme solution was injected. The coaxial nozzle had an inner and an outer concentric outlets and did not have an internal mixing chamber. The protein solution was injected through the inner tube.

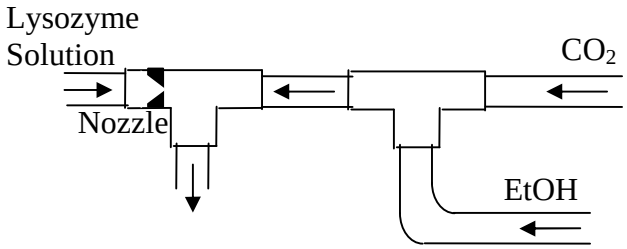

Figure 2 T-mixer

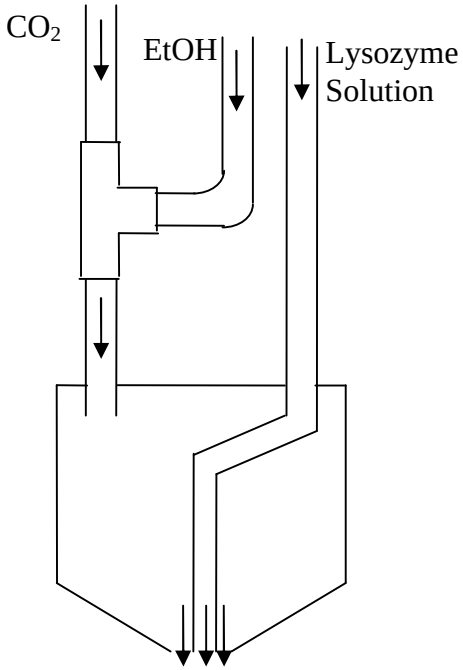

Figure 3 Coaxial nozzle

Operating conditions
In the experiments, the pressure, temperature and protein aqueous solution concentration and flow rate were kept constant at 100 bar, 37ºC, 2% (w/w) and 1 ml/min, respectively. The CO_2 and ethanol flow rates, and nozzle system were varied to identify their specific effect on the particle morphology (SEM), particle size (SEM), and residual water content (Karl Fisher).

RESULTS & DISCUSSION

Residual water content
The effect on the residual water content of the flow rates of ethanol and SC-CO_2 is shown in Table 1.

Increasing the ethanol flow rate consistently resulted in a significant diminution of the residual water content, both for the T-mixer and the coaxial nozzle. This result is conform expectations as increasing the ethanol-to-SC-CO_2 ratio causes an increase of the solubility of water in the SCF phase, and thus improves the water extraction.

Table 1 Residual water content (%) for the various process conditions

			T-mixer		Coaxial	
Conditions	CO_2 (kg/hr)	Ethanol (ml/min)	Average	Min-Max values (n=2)	Average	St Dev (n=3)
1	10	20	8.5	8.4-8.5	12.1	1.5
2	10	25	7.3	6.8-7.8	7.2	1.9
3	15	20	11.4	11.4-11.4	10.4	0.4
4	15	25	4.4	4.2-4.7	9.3	0.2

However, the effect of the CO_2 flow rate is more complex and no clear trend could be identified with the actual set of data. A number of factors can be brought up, leading to a hypothesis of the observed behaviour. On the one hand, the solubility of water in the SCF phase is decreased when the CO_2 flow is increased as the ethanol:CO_2 ratio is decreased. On the other hand, increasing the CO_2 flow rate causes a significant increase in mixing energy. For example, with either atomisation device, increasing the CO_2 flow rate from 10 kg/hr to 15 kg/hr is associated with a 3-fold increase of the kinetic energy. However, when considering the same CO_2 flow rate but comparing both spraying assemblies, the kinetic energy increases by more than 84-fold when selecting the coaxial nozzle. Also, higher flow rates are associated to higher refreshment rates which may lead to higher water uptake capacity. However, the higher velocity of the flows (1.5 fold from the lower to higher CO_2 flow rate limits, and 9-fold from the T-mixer to the coaxial nozzle) also results in a shorter residence time of the SCF phase through the vessel, and less time for the protein to precipitate before reaching the filter plate. The combination of these phenomena requires a closer investigation to identify the specific effect of the contribution of each of them on the final residual water content.

Particle morphology

All studied parameters affected the morphology of the primary particles as well as the clusters. Qualitative descriptions of the morphology and size approximation were done from SEM pictures (Figure 5 and 6, Table 2).

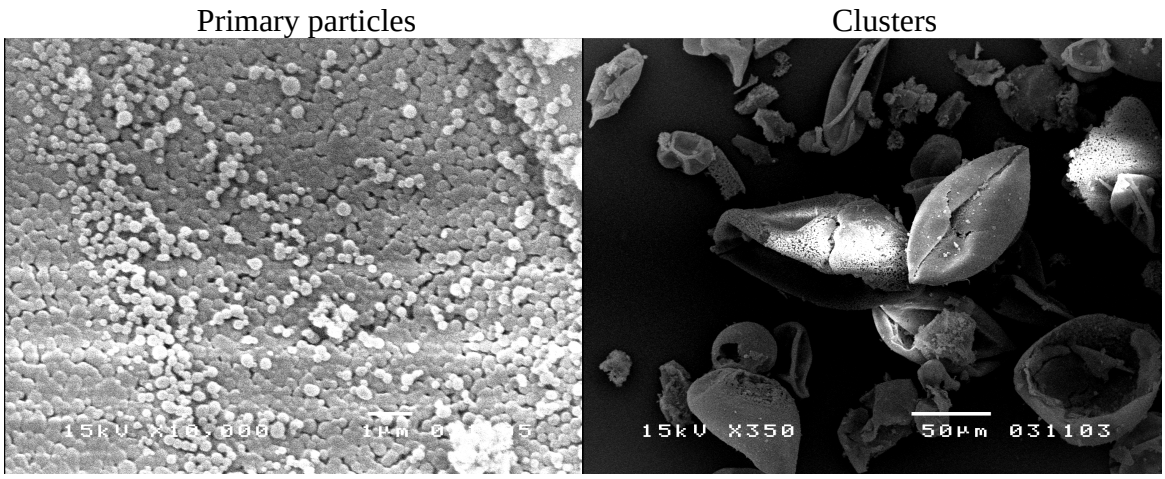

Figure 5 SEM pictures of primary particles (1 µm) and clusters (50 µm) produced the T-mixer, 10 kg/hr CO_2, 20 ml/min EtOH

Primary particles Clusters

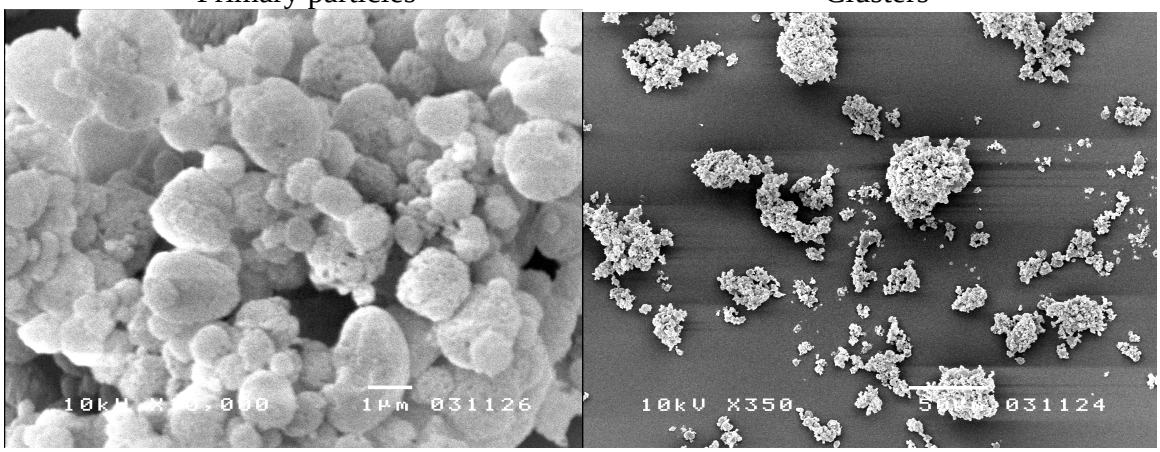

Figure 6 SEM pictures of primary particles (1 µm) and clusters (50 µm) produced with a coaxial nozzle, 15 kg/hr CO_2, 25 ml/min EtOH

Table 2 Particle morphology for the different process conditions using a T-mixer

	CO_2	10 (kg/hr)		15 (kg/hr)	
	Ethanol	Primary particle	Cluster	Primary particle	Cluster
T-mixer	20 (ml/min)	0.3 µm Spheres Fused (2D)	50 µm Folded hollow shell Discrete Smooth surface	0.3 µm Spheroids Fused (2D)	20-60 µm Spongy structure Agglomerates (<200 µm) Uneven surface with holes
	25 (ml/min)	0.2 µm Spheres Agglomerated (3D)	40-60 µm Collapsed hollow shell Discrete	0.3 µm Spheres Fused (3D) in loose network	20-50 µm Porous structure Rough surface
Coaxial	20 (ml/min)	0.2 µm Spheres Agglomerated (2D) >1 µm Porous spheroids	10-70 µm Loose aggregates Porous surface	0.5-2 µm Spheres and collapsed hollow shells Discrete	70-100 µm Loose aggregates of primary particles
	25 (ml/min)	>1 µm Porous spheroids Fused (3D)	200 µm Loose aggregates Porous surface	0.5-2 µm Spheres, Porous sponge, Discrete	<50 µm Loose aggregates of primary particles

Using the T-mixer, increasing the CO_2 flow rate resulted in an increased roughness and wider size distribution range of the clusters. However, minimal differences in primary particles were observed. Comparatively, the primary particles formed at low CO_2 flow rate with the coaxial nozzle were porous spheroids. At higher flow rates, the primary particles were more spherical and discrete with a better defined size range. Large clusters of loose aggregates were observed for all conditions, but the size increases proportionally with the flow rate.

With the T-mixer, the ethanol flow rate had a significant effect on the agglomeration of the primary particles as the low flow rate resulted in continuous surface of fused particles and high flow rate in agglomerated particles in an uneven surface. The higher solubility of water in an ethanol richer SCF phase explains the increased nucleation rate at higher ethanol flow rate, limiting the agglomeration with other particles. The higher diffusion rate of the water out of the droplets also affects the shape of the clusters by producing particles that are more collapsed or are more porous. At the contrary, variation of the ethanol flow rate had only a limited effect on the particle formation when using the coaxial nozzle. Clusters were larger when the higher ethanol flow rate was used. However, the low cohesion in between the primary particles made the clusters highly friable, such that the cluster size is not a good comparison basis.

CONCLUSION

Particle morphology has shown to be very sensitive to the process conditions. In consequence, criteria of particle and powder characteristics must be clearly defined to select both the appropriate atomisation device and flow rates. The residual water content could be significantly reduced by increasing the ethanol flow rate. The SC-CO_2 flow rate affected the particle morphology. However, its effect on the residual water content requires further investigation.

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