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Cyclone selection influences protein damage during drying in a mini spray-dryer

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ABSTRACT

The use of a small-dimensioned cyclone separator to spray-dry an aqueous solution of lysozyme on a mini spray-dryer produces consistently higher protein inactivation at all drying-air outlet temperatures examined between 50 °C and 105 °C. Differences in drying air flow rate through the machines will influence droplet/particle residence times within the drying chamber, but these are considered too small to explain the result. It appears more likely that a higher separation and retention of fines within the small cyclone causes higher measured protein inactivation. By virtue of their small size the fines have a greater specific surface area and suffer therefore a greater degree of protein damage when passing through the spray dryer from nozzle to collecting vessel. Although the dry powder yield is higher with the small-dimensioned cyclone than that obtained with the standard cyclone, the profile of residual moisture versus T_{outlet} is irregular in shape. A possible lack of equilibrium between the attributes of the protein particles and the exhaust air needs therefore to be considered.

1. Introduction

There are a number of mini spray-dryers available commercially for use in drug development research. The most widely used of these is the Büchi laboratory spray-dryer, currently available as its Model B-290. These bench top machines have proven themselves invaluable for spray-drying expensive materials available only in small quantities during the early stages of drug development. This is the case for pharmaceutical peptides and proteins which can be successfully spray-dried to yield amounts of <100 mg (Lee, 2002). Mini spray-dryers have, however, some drawbacks. First, the enthalpy loss through the glass drying chamber wall is high (Hanus and Langrish, 2007). Secondly, the small drying chamber volume, v_c, of <10 l produces a very short drying-gas residence time, τ. For the Büchi B-290 running at a drying-gas flow rate, Q_{da}, of 40 m^3/h, τ is equal to: 0.008 [m^3]/40 [m^3/h] = 0.72 s. This short τ limits kinetically the extent of drying (Langrish and Chiou, 2008) and only allows adequate drying of very small droplets generated by a two fluid nozzle, e.g. with a droplet diameter of <15 μm. Thirdly, the substantially longer τ in larger laboratory or pilot scale spray-dryers can result in differences in process-induced protein damage on scaling-up between mini and larger spray-dryers. These potential difficulties notwithstanding, mini spray-dryers are the units of choice for many applications in drug delivery research and development.

Mini spray-dryers can be fitted with cyclone separators of different sizes. We have previously calculated and demonstrated experimentally how a smaller dimensioned cyclone than that used routinely with the Büchi allows the retention of finer particles out of the exhaust gas (Maury et al., 2005). For the example of spray-dried trehalose the calculated lower separation limit with the small cyclone separator was <0.5 μm, less than half of that achieved with the larger standard cyclone. This makes the small cyclone separator very attractive for use to give a high dry powder yield when just milligram quantities of an expensive peptide or protein are spray-dried. Any differences in cyclone dimensions will, however, produce differences in the drying-gas flow rate through a spray-dryer (Masters, 1991), which may have consequences for the product attributes. In this study we have measured the affects of switching from the standard to a smaller cyclone on the properties of a spray-dried model protein, lysozyme. The results show a strong influence of cyclone size on the extent of protein damage occurring during drying in the Büchi. The use of a small cyclone separator to dry proteins in mini spray-dryers should therefore be viewed with some circumspection.

2. Materials and methods

2.1. Materials

A threefold-crystallized and lyophilized lysozyme powder from chicken egg white was obtained from Sigma Chemicals in Munich. Water was double-distilled from an all-glass apparatus.

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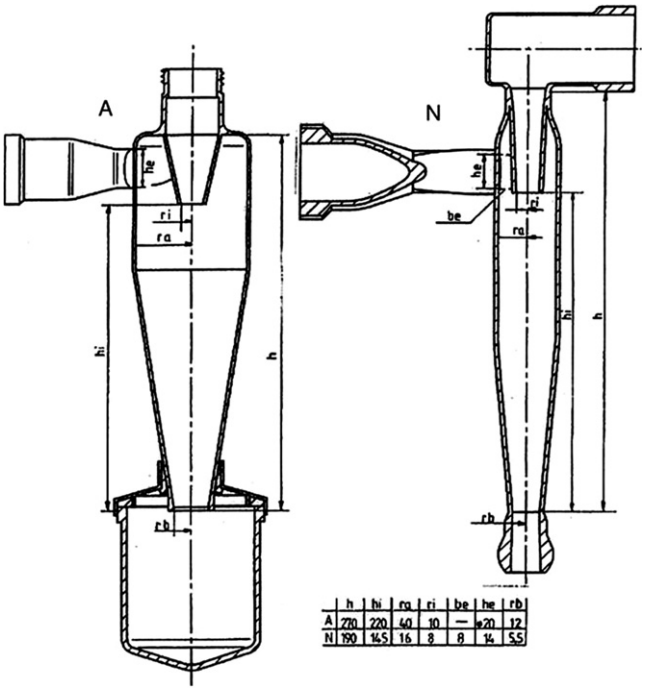

	h	hi	ra	ri	be	he	rb
A	270	220	40	10	—	20	12
N	190	145	16	8	8	14	5.5

Fig. 1. Construction of the standard Büchi cyclone (A) and the small-dimensioned cyclone (N). The dimensions are given in the table. Further information about the small cyclone is available in Maury et al., 2005.

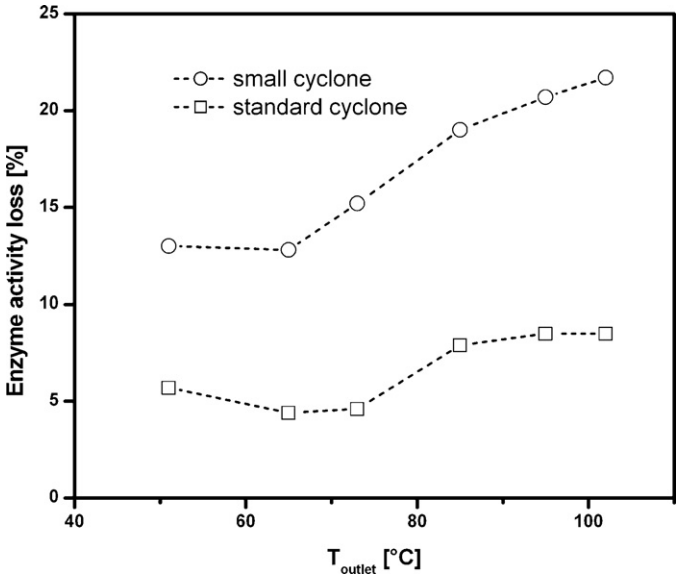

Fig. 2. Loss in enzymatic activity of spray-dried lysozyme in dependence of T_{outlet} when using the standard or small cyclone on a Büchi B-290 mini spray-dryer. Drying-gas flow rate Q_{da} = 65% aspirator rate; atomizing air flow rate Q_{aa} = 700 l/h; liquid feed flow rate Q_{lf} = 3 ml/min.

2.2. Methods

2.2.1. Spray drying

Spray-drying was performed on a Büchi B-290 mini spray-dryer equipped with either the Büchi's standard cyclone or a small cyclone (Fig. 1). 10% w/w solutions of lysozyme in water (with no further dissolved substances) were prepared and spray-dried at various drying air inlet and outlet temperatures, T_{inlet} and T_{outlet}, respectively. The two-fluid nozzle parameters were kept constant: liquid feed flow rate, Q_{lf} = 3 ml/min; atomizing air flow rate, Q_{aa} = 700 l/h. Under these process conditions the following values of $T_{inlet}/T_{outlet/standard}/T_{outlet/small}$ were obtained: 90 °C/56 °C/51 °C; 115 °C/73 °C/60 °C; 135 °C/86 °C/73 °C; 160 °C/103 °C/85 °C; 190 °C/115 °C/95 °C; 220 °C/134 °C/102 °C. In each experiment 10 ml of liquid feed were spray-dried; each experiment lasted therefore approx. 3 min and had a theoretical total dry powder yield of 1000 mg. The dried powder was removed from the collection vessel attached to the base of the cyclone immediately after completion of the spray-drying run. Each spray-drying experiment was performed in duplicate (n = 2). The drying-air volumetric flow rate through the spray-dryer, Q_{da}, was measured using an FA20R Flow Meter (Krohn, Germany) in dependence of the aspirator rate in %. It was attached by a length of rubber tubing to the exhaust-gas outlet.

2.2.2. Measurement of powder properties

The residual water content of each spray-dried sample was determined using Karl-Fischer titration on a Mitsubishi Moisture Meter CA-06 fitted with a Water Vaporizer VA-06. Samples of 80–150 mg of the spray-dried protein were heated to 140 °C. An initial rate of <15 μg water/min was used under a N_2 stream of 200 ml/min. All measurements were performed in duplicate. The amorphicity/crystallinity of each powder was examined using wide-angle X-ray diffraction on a Philips XPert MPD machine with CuK_α radiation at 40 kW and 40 mA. The powders were also viewed by scanning electron microscopy (SEC) on an Amray machine after Au sputtering. The enzymatic activity of the lysozyme was determined by using 2500 μl of a substrate solution comprising 0.015% micrococcus lysodeikticus cell suspension in 66 mM potassium phosphate buffer pH 6.24. This was added to 100 μl of the respective enzyme solution. The decrease in absorbance of this solution, $A_{450\,nm}$, was recorded over at least 5 min, and the activity of lysozyme in units/ml calculated from $\Delta A_{450\,nm}$/min using

$$\text{Units/ml} = \frac{(\Delta A_{450\,nm}/\text{min Sample} - \Delta A_{450\,nm}/\text{min Blank}) \cdot \text{df}}{0.001 \cdot 0.1}$$

df is the dilution factor, 0.001 is the change in absorbance per unit, and 0.1 is the volume in ml of enzyme solution. The resulting activity in units/ml was divided by that obtained for the untreated lysozyme (=100% activity). Each enzyme sample was examined in triplicate (n = 3).

3. Results and discussion

Fig. 2 shows a comparison of the loss in enzymatic activity incurred during spray-drying in dependence of T_{outlet} for both cyclone types. First, note that in both cases the activity loss increases sigmoidally with higher T_{outlet}. The plot for the small cyclone is, however, shifted to lower T_{outlet} compared with that for the standard cyclone. This means that the increase in damage to the lysozyme as temperature is raised sets in at lower T_{outlet} with the small cyclone. Secondly, consider that there is a substantially larger measured activity loss with the small cyclone when spray-drying under otherwise identical process conditions. This result also means greater damage to the lysozyme when using the small cyclone. A partial explanation of these results can be found by considering the measured drying-air flow rates, Q_{da}, shown in Fig. 3. The values are substantially lower with the small cyclone than with the standard cyclone. This is caused by the smaller radii of the cyclone chamber, r_a, and exit duct, r_i, of the small cyclone (see Fig. 1) which give greater resistance to gas flow passing through the machine. We also note that the values of Q_{da} in Fig. 3 for the B-290 are some 50% higher than those previously measured on the Büchi model B-190 (Maury et al., 2005), both with the standard cyclone. This is certainly caused by the larger diameter of the drying chamber of the B-290 which results in

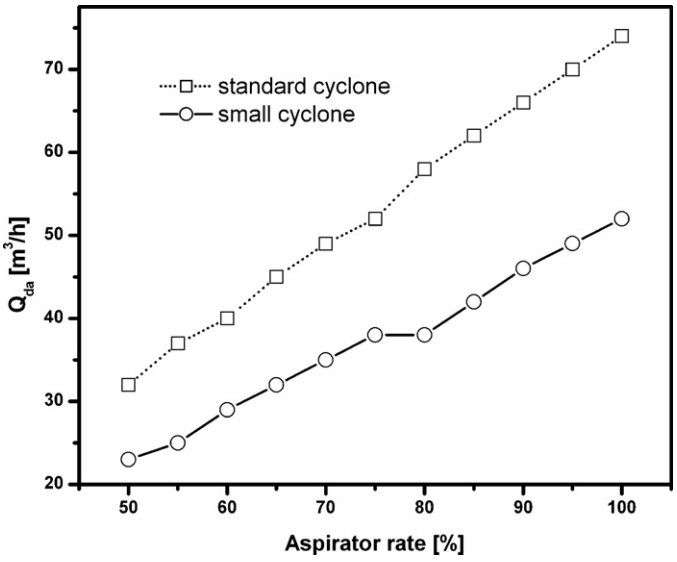

Fig. 3. Relation between measured drying air flow rate, Q_{da}, and aspirator rate in % on the Büchi B-290 mini spray-dryer for both standard and small cyclones.

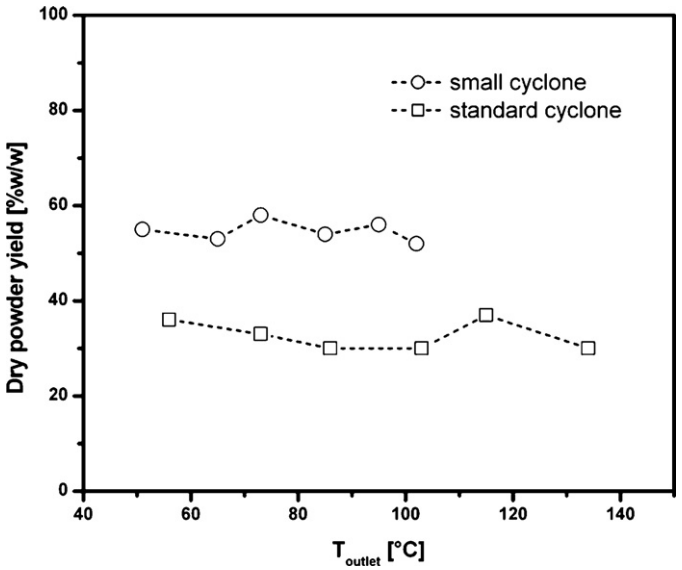

Fig. 4. Dry powder yield of spray-dried lysozyme in dependence of T_{outlet} when using the standard or small cyclones on a Büchi B-290 mini spray-dryer. The yield is defined as that powder that could be removed from the glass collecting vessel attached to the base of the cyclone.

lower resistance to gas flow. Turning back to Fig. 3, the lower drying-air flow rate through the small cyclone will increase the residence time of the droplet/particle, τ_d, in the drying chamber. At the aspirator rate of 65% used to dry the protein in this work the calculated values for τ_d (assuming it to be the same as τ) are $V_c/Q_{da} = 0.008\,m^3/45\,m^3/h = 0.64\,s$ and $=0.008\,m^3/32\,m^3/h = 0.95\,s$ for the standard and small cyclones, respectively. A larger τ_d will result in more prolonged thermal stress on the protein in the drying chamber with small cyclone, especially after the critical point of drying has been reached where the particle's temperature is rising rapidly from the wet-bulb towards T_{outlet} (Langrish, 2009). Although this should result in more damage to a thermolabile protein, it seems unlikely to be the major cause of the more than doubled inactivation with the small cyclone (cf. Fig. 2 Fig. 2). The difference in τ_d is very small compared with the residence time of the dry protein powder within the glass collecting vessel of either cyclone. This was at most 4 min (3 min process time plus 1 min disassembly and collecting time) during which time the protein powder is further exposed to a temperature of up to T_{outlet}. We need therefore to consider a more likely cause of the higher protein damage with the small cyclone.

Fig. 4 illustrates the substantially higher dry-powder yield obtained with the small cyclone at all T_{outlet}. This is certainly to a large degree a result of the lower separation limit of the particles from the drying air in the small cyclone which allows the collection of the fines that pass through the standard cyclone (Maury et al., 2005). Differences in product dryness can, however, also influence dry-powder yield (Langrish and Chiou, 2008); this will be discussed below. The collection of a larger amount of fines with the small cyclone is a likely cause of the higher degree of measured inactivation of the lysozyme. The fines will been produced from the smallest droplets within the spray emerging from the nozzle. These dry more rapidly to the critical point of drying than do the larger droplets and will therefore be exposed to a longer duration of thermal stress during the course of τ_d. Additionally, the small droplets will have a larger specific surface area than the larger ones which may allow enhanced protein adsorption and damage at the water/air interface. No formation of deposits was detected by eye anywhere in the spray-dryer with either cyclone type, thus ruling-out any deleterious effects of direct heat transfer from the glass walls to the particles. The larger specific surface area of the fines may also promote further protein damage during the residence time of the powder within the glass collecting vessel at approximately T_{outlet}.

Additionally we observed that the loss of powder when emptying the collection vessel of the small cyclone is less than that occurring with the large collection vessel attached to the standard cyclone (cf. Fig. 1). This is a further, but more minor, cause of the greater dry powder yield from the small cyclone and is especially important when the total mass of powder dried is as low as 100 mg. Clearly, when spray-drying only a small amount of an expensive material this higher yield of powder is of advantage. The small cyclone does, however, have a lower limit to the mass of powder it can collect per unit time than does the standard cyclone. When spraying a 35% w/w total solid's liquid feed, for example, the amount of powder passing through the small cyclone exceeds its through-put capacity and the cyclone becomes rapidly blocked. This was not the case with the standard cyclone. The 10% w/w solid's liquid feed examined in this work could, however, be readily collected on both cyclones.

With the standard cyclone the profile shape of residual moisture in the lysozyme powder versus T_{outlet} given in Fig. 5 is similar to that seen for spray-dried trehalose on a Büchi B-190 (Maury et al., 2005). Both of these spray-dried materials are fully amorphous (X-ray diffractograms not shown here, but were determined and have typical amorphous halo). Both the increasing activity loss and the decreasing residual moisture content alter therefore in a smooth, sigmoidal fashion with higher T_{outlet}. The profile for the small cyclone also shown in Fig. 5 differs, however, from that for the standard cyclone. There is no smooth sigmoid curve, but rather the residual moisture at low T_{outlet} is much higher than with the standard cyclone and is followed by a sharp fall to a plateau level between T_{outlet} of 68 °C and 73 °C. This irregular profile shape means that there is no sign of equilibrium between the moisture contents of the protein particles and the exhaust air. This is also indicated by the subsequent constant residual moisture at $T_{outlet} \geq 73$ °C. The lack of equilibrium between product and exhaust gas in mini spray dryers has been noted before (Chiou et al., 2008), although why this should only be the case with the small cyclone is unclear. Recall that τ_d is longer with the small cyclone than with the larger one. Whatever the cause of the irregular profile shape in Fig. 5 with the small cyclone it is evident that the higher yields obtained with the small

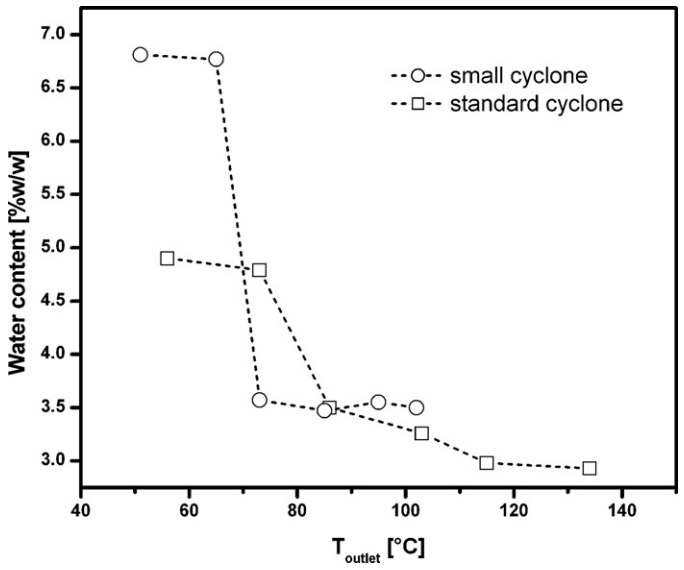

Fig. 5. Residual water content of spray-dried lysozyme in dependence of drying air outlet temperature, T_{outlet}, for the standard and small cyclones.

cyclone (cf. Fig. 4) are not caused by any differences in product dryness.

4. Conclusions

The small-dimensioned cyclone results in a substantial improvement in dry powder yield of lysozyme, but also a greatly increased enzymatic inactivation of the protein during spray-drying. The most likely cause appears to be a higher separation and retention of fines within the small cyclone which by virtue of their small size suffer a greater degree of protein damage. This phenomenon has clearly to be born in mind when using such a smaller cyclone with expensive proteins that only be spray-dried in very low quantities.

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