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Post-chamber inactivation of catalase powder during spray drying in bench-top machines

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ABSTRACT

The kinetics of inactivation of catalase during its residence time in the cyclone separator of two mini-scale spray dryers has been determined. The object was to determine if the catalase shows further inactivation after leaving the drying chamber and residing for up to 10 min in the glass collector until the run is complete. Substantial inactivation within the collector of both machines was detected, with an ultrasonic nozzle giving much greater post-chamber inactivation than a two-fluid nozzle. There was a correlation between inactivation rate and the differing drying-air outlet temperatures produced with the two nozzle types only within one machine and not between the two different machines. A kinetic model was derived that describes the degree of inactivation of the product powder in dependence of its residence time in the separator. Non-linear curve fitting of the model to the experimental data gave first-order half-lives on catalase inactivation in the collector of the Büchi of 5.1 and 15.8 min for the ultrasonic and two-fluid nozzles, respectively, and in the collector of the ProCept of 5.3 and 16.1 min. The lengthy process times required to spray dry large batches of a protein on these mini-scale machines should therefore be avoided.

1. Introduction

Pharmaceutical proteins can be readily spray dried to produce dry powders suitable for use as an inhalable form [1], in ballistic powder injectors [2] or as a storage-stable protein bulk alternative to freeze drying. Development work in this area is forced to start with a mini-scale, bench-top spray dryer, which can be operated with very small amounts of an expensive protein, e.g. <100 mg for a single run [3]. The subsequent issue of scale-up to a larger laboratory-size or pilot-size machine will involve inter alia considerations of different droplet size, altered residence time in the drying chamber, different size of the drying chamber, different enthalpy loss, etc.

Both small peptides and large proteins are damaged during spray drying on the bench-top scale [1], although usually to differing degrees. The damage is manifested as altered secondary structural elements detected by FT-IR [4], formation of soluble aggregates detected by chromatography [5] or insoluble aggregates by particle counting [6], or inactivation of enzymes detected by bioassay [7]. There are thought to be 2 major causes of this damage: adsorption of the peptide at the rapidly expanding liquid feed/drying gas interface during atomization [5], and heat transfer to the droplet/particle during its passage through the drying chamber. The droplet/particle residence time within the drying chamber of a bench-top machine such as the often-used Büchi B290 is 1–2 s [8]. During this time its surface temperature will be at the wet-bulb for only a few milliseconds [7] until the critical point of drying is reached, after which the surface temperature will increase to at most the temperature of the exhaust gas [9]. Yet the *total* residence time of the droplet/particle in the spray dryer will be much longer. After leaving the drying chamber the powder particles are detrained from the exhaust gas in a cyclone separator and then move down its inside conical wall to reach the collecting vessel mounted at the base of the cyclone. The powder remains in this collector for the further duration of the spray drying run, which depending on the volume of liquid feed to be sprayed and its flow rate through the nozzle can be 5–30 min or more. During this time the small mass of powder in the collector will be continually exposed to the temperature of the inside wall of the collector, which is approximately that of the exhaust gas. It is therefore possible that further damage occurs to the dried protein during its residence in the collector, i.e. after it has left the drying chamber.

In this paper we present our study of the inactivation of the model enzyme catalase collected in the collecting vessel of two bench-top spray dryers during the spray drying process. Our goal was to determine the extent of damage that occurs to the enzyme after the dried particles

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have passed through the drying chamber and reached the collection vessel.

2. Materials & methods

2.1. Materials

Catalase from bovine liver, 2000–5000 units per mg, was obtained as a lyophilized powder from Sigma Aldrich GmbH (Taufkirchen, Germany). The buffer salts, monobasic and dibasic potassium phosphate, were used as received from Carl Roth GmbH (Karlsruhe, Germany). Water was double-distilled from an all-glass apparatus.

2.2. Methods

The liquid feed was prepared by dissolving the catalase in aqueous phosphate buffer of pH 7.0 to a concentration of 10% w/w ≈ 100 mg/mL. In an initial experiment it was cold-atomized through each of the two nozzles to be used in the subsequent spray drying studies: the manufacturer's supplied two-fluid nozzle or a 25 kHz ultrasonic nozzle (Sonotek Corporation, Milton, NY, USA). 2 mL of liquid feed was atomized into a glass beaker at a flow rate of 1 mL/min, the solution then re-collected and measured for catalase activity using the bioassay cited below. Each experiment was performed individually three times (n = 3).

Spray drying of the liquid feed was done on both a Büchi B-290 Laboratory Spray Dryer (Büchi Labortechik GmbH, Flawil, Switzerland) and a ProCept 4M8 Spray Dryer (ProCept nv, Zelzate, Belgium), the latter machine fitted with a drying chamber length of 2.1 m. On both machines either the manufacturer's supplied two-fluid nozzle or a 25 kHz ultrasonic nozzle (Sonotek Corporation, Milton, NY, USA) were used. The ProCept's standard glass collecting vessel was used on both machines, attached to the base of the glass cyclone. The design and dimensions of both glass cyclones were very similar, as described for the Büchi in [8]. The process conditions were the same on both machines: drying gas (air) inlet temperature, T_{inlet}, = 141 °C; drying gas flow rate, Q_{da}, = 500 L/min; liquid feed flow rate, Q_{lf}, = 1 mL/min; atomizing air pressure = 2 bar ≈ 700 L/h; ultrasonic nozzle power = 2 W. The drying gas outlet temperature, T_{outlet}, depended on the nozzle type and the machine (see Section 3).

The following experimental technique was used to vary the residence time of the powder accumulating in the collecting vessel of the cyclone. We abandoned the idea of removing individual powder samples from the collector at different times during a single run, because it was not readily possible to ensure a uniform mixing of the complete powder-mass as it accumulated within the collector. Additionally the removal of samples would necessitate interrupting the spray drying run. Instead, the total process duration was varied between 2 min and 10 min to give differing maximum residence times of the powder batches in the collector. The spray dryer was first equilibrated on water until T_{out} was constant. Then the change-over to liquid feed took place and the machine run for a pre-set process time, $t_{process}$ = 2 min, 4 min, 6 min, 8 min, or 10 min, before changing back to water and stopping the run. The accumulated powder mass was then immediately removed from the collector into a small, screw-topped glass vial and mixed thoroughly by repeated inversion. In this fashion $t_{process}$ defines, for each run, the maximum residence time of the powder in the collecting vessel ($t_{max/res}$). It was thus possible to vary reliably the powder's $t_{max/res}$ in the collector and also to avoid the technical difficulty of removing a sample out of a uniformly mixed powder-mass contained in the collector during a run. Furthermore, $t_{max/res}$ is amenable to mathematical description (see Section 3).

Each run was performed three times (n = 3) with thorough cleaning of the spray dryer between each run. The dried powder recovered from each run was stored at −80°°C until assayed for enzymatic activity using the standard catalase bioassay fully described before [10].

The residual moisture content of each dried catalase powder was determined by Karl Fischer titration using a Methrom 831 Coulometer and 832 KP Thermoprep Oven (Methrom AG, Filderstadt, Germany). A sample of 20–30 mg was heated to 130 °C and the water vapour transferred in a dry nitrogen gas stream to the titrator. The water vapour was titrated to the level of 10 μg/min.

3. Results & discussion

3.1. Inactivation through atomization

Cold atomization of the catalase liquid feed through the ultrasonic nozzle produced substantially higher inactivation of the protein than with the two-fluid nozzle (Table 1). A high degree of protein damage after atomization through an ultrasonic nozzle has been reported before and attributed to nozzle heating [11] or cavitation within the liquid feed [12]. Since the object of this study was to detect any inactivation that occurs in the collector, the measured values for residual activity of the powders recovered from the collector, $a^{raw}(t)$, were first normalized by subtracting the value for nozzle-induced inactivation, ia^{noz}, given in Table 1: $a(t) = a^{raw}(t) - ia^{noz}$. In this way the measured inactivation of the powder recovered from the collector occurs post-atomization, i.e. in the drying chamber, cyclone or collector.

3.2. Inactivation of powders recovered in collector of Büchi B-290

For the Büchi B-290, Fig. 1 shows that the residual activity of the spray-dried catalase powders, $a(t)$, depends strongly on their maximum time, $t_{max/res}$, spent in the cyclone's collecting vessel. The first measured time-point is 2 min after switching from water to liquid feed and shows an activity-loss of approximately 10% with both nozzle types. At this time-point there are two possible sources of this inactivation: the drying process of the droplet/particle taking place in the drying chamber and cyclone, or heat transfer to the dried powder residing in the collector for up to $t_{max/res}$ = 2 min. Recall that the inactivation caused by atomization has already been accounted for in the values of $a(t)$ in Fig. 1. We cannot distinguish between the two causes at this first time-point. The subsequent further activity loss measured between 2 and 10 min can, however, be attributed to the powder's longer residence in the hot collecting vessel, $t_{max/res}$, with different total process times. We make here only a single assumption, i.e. that the residual activity of the powder entering the collector from the drying chamber and cyclone is unchanged with process time. This assumption is justified by the constant T_{outlet} when the spray dryer is running at equilibrium. A constant T_{outlet} means that the integral heat transfer to the droplet/particles during their residence time in the drying chamber and cyclone is constant [9]. This should produce a constant degree of inactivation of the protein during its passage through the drying chamber and cyclone. The two plots in Fig. 1 show that the longer the spray-drying run lasts, the greater is the inactivation of the peptide recovered from the collecting vessel. This inactivation occurs therefore by heat transfer during the residence of the powder in the collector.

This is supported by the data for residual moisture content, w(t), of the powders recovered from the Büchi B-290 (Fig. 2). The values with both nozzle types decrease with longer maximum residence time, $t_{max/res}$, confirming that the powder is dried further during its residence

Table 1
Inactivation of catalase by cold atomization through either the two-fluid nozzle (Q_{lf} = 1 mL/min, Q_{aa} = 700 L/h; 2 bar) or the ultrasonic nozzle (Q_{lf} = 1 mL/min; power = 2 W) into a glass beaker at room temperature (n = 3 individual experiments).

Nozzle type	Untreated	2-Fluid	Ultrasonic
Residual activity [%]	100.0 ± 2.5	98.6[a] ± 2.0	91.5[b] ± 3.0

[a] Not significantly different from 'untreated' at $p < 0.01$.
[b] Significantly different from 'untreated' at $p < 0.01$.